THERIO 2025

Annual Conference
Program and Proceedings

July 23-26 | Hyatt Regency

Sacramento

Society for Theriogenology | American College of Theriogenologists | theriogenology foundation

Message from the President

On behalf of the Society for Theriogenology, it is my honor to welcome you to the 2025 Annual Therio Conference.

We are thrilled to host you for three days of enriching continuing education and networking opportunities in the beautiful city of Sacramento, California. Our program features experts in the field from across the globe, more than a third of whom are ACT Diplomates. This year, we will feature more than 100 abstracts — presented as oral talks or posters, and back by popular demand, the 2nd Annual Therio News Hour (and a Half), Applied Research track, and half-day Technician Program.

We are also excited to present four symposia this year, generously hosted by Kokopelli Veterinary Clinic, UC Davis, and the Hyatt Regency. We extend our heartfelt thanks to Drs. Bruce Christensen, Kelley Thieman, and Pouya Dini for their efforts in curating a diverse and engaging lineup of programming for our attendees.

This conference would not be possible without the dedication of our many volunteers, whose thoughtful contributions have helped shape the Therio Conference over the last year, to reflect SFT's mission of promoting excellence in the field of theriogenology.

A special thank you goes to Dr. Heath King, our 2025 Conference Chair, for his leadership in keeping the program focused while ensuring an outstanding experience for all attendees. We are also grateful to our session chairs — Drs. Fernando Campos-Chillon, Autumn Davidson, Andrea Hesser, Darcie Sidelinger, and Jack Smith. I would like to recognize the ACT Scientific Information Committee, led by Dr. Karen Wolfsdorf, and the Student Abstract Committee, chaired by Dr. David Christiansen, who managed the impressive task of reviewing a near-record number of abstracts this year. We also appreciate the editorial team behind the Journal for Clinical Theriogenology — Drs. John Kastelic (copy editor), Michelle Kutzler (assistant editor), and Augustine Peter (editor) — for their work in maintaining the high standard and consistency of all accepted submissions.

I hope you will join me at the Theriogenology Foundation's "Soiree in Sacramento," Friday, July 25 at the California State Railroad Museum - an opportunity to visit with friends and colleagues in a unique setting. We appreciate the time and energy that TF volunteers put into making this event special. It is a highlight of the Therio Conference! Tickets are $85 and include a buffet dinner and one drink ticket. The best part – 100% of the proceeds from the Soiree benefit TF's grantmaking programs. **Learn more about the Soiree here** and check out the fabulous items in the **Online Auction Here.**

Lastly, we extend our deep gratitude to our program and event sponsors, and exhibitors. Your generous support is vital to the success of the 2025 Therio Conference, and we appreciate your continued partnership and commitment to our profession.

As both the Society and the American College of Theriogenologists continue to grow and evolve, your involvement and ongoing support remain essential. On behalf of SFT, thank you — and enjoy your time in Sacramento!

Candace Lyman

President

Society for Theriogenology

Acknowledgments

The Society for Theriogenology would like to acknowledge the following individuals for their contributions to the 2025 Therio Conference in Sacramento, California.

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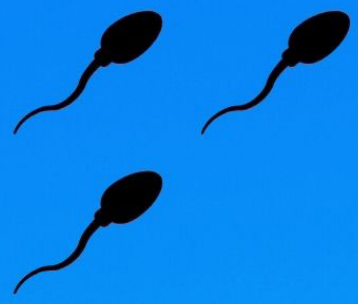
Therio Conference

See you at Booth #11 & 12



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Society for Theriogenology
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Overview

Registration Desk Hours

Wednesday	7:00am–9:00am/12:00–2:00pm/5:30pm–7:30pm
Thursday-Friday	7:00am–4:30pm
Saturday	7:00am–4:00pm

7.23 Wed

8:00 am — 12:00 pm
RFG Certification Training*
Regency Corridor & Regency D
Sponsored by the American Kennel Club and Royal Canin

8:00 am — 12:00 pm
ACT Exam Committee
Capitol Board Room

Canine Semen Freezing* Kokopelli Veterinary Clinic Sponsored by Minitube with support from Jorgensen Laboratories

1:00 pm — 5:00 pm
RFG Certification Training* Regency Corridor & Regency D Sponsored by the American Kennel Club and Royal Canin

Canine Semen Freezing* Kokopelli Veterinary Clinic Sponsored by Minitube with support from Jorgensen Laboratories

5:00 pm — 7:00 pm
SFT Board Meeting
Carmel A
ACT Board Meeting
Tahoe

7:00 pm — 8:30 pm
Theriogenology Foundation Board Meeting
Carmel A

(*) Pre-registration required. Additional symposia information available on page 13

7.24 Thur

7:00 am — 8:00 am
Continental Breakfast
Regency Foyer

8:00 am — 10:00 am
Dr. Jerry Rains Memorial Abstract Competition | Regency Ballroom ABC

10:00 am — 10:30 am
AM Break | Regency Foyer

10:30 am — 12:00 pm
Equine Track | Golden State
Small Animal Track | Regency Ballroom ABC
Sponsored by Royal Canin
Production Animal Track | Tahoe
Technician Track | Carmel A

12:00 pm — 1:30 pm
Lunch & Learn*
Regency Ballroom ABC
Sponsored by Royal Canin

1:30 pm — 3:30 pm
Equine Track | Golden State
Small Animal Track | Regency Ballroom ABC
Production Animal Track | Tahoe
Technician Track | Carmel A

3:30 pm — 5:00 pm
Therio News Hour (& a 1/2)
Regency Ballroom ABC

5:00 pm — 5:30 pm
VIP Cocktail Event (Invitation Only)
Regency Ballroom DEF

5:30 pm — 6:30 pm
Exhibitor Hall Opening Reception
Regency Ballroom DEF

6:30 pm — 8:00 pm
Trivia Night
Capitol View (15th Floor)

6:45 pm — 9:00 pm
ACT Exam Candidate Dinner
Dawson's Steak House at the Hyatt Regency

7.25 Fri

7:00 am — 8:00 am
Continental Breakfast with Exhibitors| Regency Ballroom DEF

8:00 am — 10:00 am
Dr. Jimmy Alexander Student Case Presentations
Regency Ballroom ABC
Sponsored by Lane Manufacturing

8:00 am — 10:00 am
ACT Business Meeting | Golden State

10:00 am — 10:30 am
AM Break | Regency Ballroom ABC

10:30 am – 11:30 am
Plenary Session
Regency Ballroom ABC
Sponsored by ACT

11:30 am — 12:00 pm
Bartlett Award and Theriogenologist of the Year Award Presentations
Regency Ballroom ABC
Sponsored by the Theriogenology Foundation and ACT

12:00 pm — 1:00 pm
SFT Business Meeting Luncheon
Meeting to begin at 12:15/ Steiner Award Presentation* | Regency Ballroom ABC

1:00 pm – 3:00 pm
Equine Track Dr. Michelle LeBlanc Memorial Lectures
Golden State
Small Animal Track
Regency Ballroom ABC
Sponsored by Royal Canin
Production Animal Track | Tahoe
Applied Research Track | Carmel A

3:00 pm – 4:00 pm
ACT and SFT Competitive Poster Session and Extended PM Break
Regency Foyer & Ballroom DEF

4:00 pm — 5:00 pm
Equine Track | Golden State
Small Animal Track
Regency Ballroom ABC
Production Animal Track | Tahoe
Applied Research Track | Carmel A

4:30 pm — 6:00 pm
Student Mentor Reception and Career and Training Fair
Capitol View

6:00 pm — 9:00 pm
Therio Foundation Soiree in Sacramento* (Limited tickets available on site)

7.26 Sat

7:00 am — 8:00 am
Continental Breakfast with Exhibitors| Regency Ballroom DEF

8:00 am — 9:00 am
ACT Competitive Case Reports
Regency Ballroom ABC

9:00 am — 10:00 am
Equine Track | Golden State
Small Animal Track
Regency Ballroom ABC
Production Animal Track | Tahoe

10:00 am — 10:30 am
AM Break| Regency Ballroom ABC

10:30 am – 12:00 pm
Non—Competitive Abstracts

Equine | Golden State
Small Animal| Regency Blrm ABC
Production Animal & Mixed Species | Tahoe
Case Reports | Carmel A

12:00 pm — 1:00 pm
Awards Presentation Lunch: ACT & SFT Competitive Abstract Awards and SCOTY Award*
SCOTY Award Sponsored by Lane Manufacturing
Regency Ballroom ABC

1:00 pm – 3:00 pm
Equine Track | Golden State
Small Animal Track
Regency Ballroom ABC
Production Animal Track | Tahoe
Applied Research Track | Carmel A

3:00 pm — 3:30 pm
PM Break | Regency Foyer

3:30 pm — 4:30 pm
Equine Track | Golden State
Small Animal Track
Regency Ballroom ABC
Production Animal Track | Tahoe
Applied Research Track | Carmel A

ACT American College of Theriogenologists — Our Science Comes to Life!

theriogenology foundation — The Future of Animal Reproduction

Society for Theriogenology — Veterinary Professionals Dedicated to Animal Reproduction

Competitive Abstract Presentations

DR. JERRY RAINS MEMORIAL ABSTRACT COMPETITION

Thursday, July 24 | 8 AM - 10 AM | Regency Ballroom ABC
Moderator: Dr. Karen Wolfsdorf

8:00 AM **Samantha McCarter, DVM**
Comparing neonatal puppy growth between overweight and lean bitches

8:15 AM **Gabriela Carneiro de Sousa, DVM**
The uterine transcriptome during parturition in the bitch: preliminary results

8:30 AM **Joy Ledeck, DVM**
Unlocking the future of equine fetal sexing: mass spectrometry analysis of maternal conjugated estrogens in serum

8:45 AM **Leslie Alejandra Sandoval Rosales, DVM**
Comparison of the effect of motility stimulants on frozen-thawed semen in stallions

9:00 AM **Victoria Lindsay (Oklahoma State University)**
Effects of firocoxib on oocyte quality in mares undergoing repeated TVA procedures: a preliminary study

9:15 AM **Daniela Cortes, DVM**
Analytical validation of different diagnostic tests for the detection of leucocytes in canine semen

9:30 AM **Daniel Gomes, DVM**
Effect of supplementation of donor mares with altrenogest on embryo recovery and size

9:45 AM **Sabrina Bellaver Cousseau, DVM**
Transcriptomic profile of single immature and in vitro matured equine oocytes after holding

COMPETITIVE POSTER PRESENTATIONS

Friday, July 25 | 3 PM - 4 PM | Regency Foyer
Judged by the ACT Scientific Information Committee

3:00 PM **Hugo Monteiro, DVM, PhD**
A detailed characterization of Streptococcus zooepidemicus mechanism of infection during equine placentitis

3:00 PM **Margo Verstraete, DVM**
Exploring the potential of equine endometrial organoids: tissue similarities, cycle-stage influence, and long-term stability

3:25 PM **Hayley Moore, DVM**
Ovarian remnant syndrome in a 2-year-old cat with inconclusive diagnostics

3:25 PM **Josefina Ghersa, DVM**
Ultrastructural features and prostaglandin E secretion by equine trophoblastic vesicles

3:45 PM **Jaden Thompson, MS**
Immunological and Transcriptomic Insights into Equine Persistent Breeding-Induced Endometritis (PBIE)

DR. JIMMY ALEXANDER STUDENT CASE REPORT COMPETITION

Friday, July 25 | 8 AM - 10 AM | Regency Ballroom ABC
Moderator: Dr. David Christiansen

8:00 AM **Kayleigh Moore (Washington State University)**
Peri-hock umbilical cord entanglement in an Angus calf

8:15 AM **Baker White (Virginia-Maryland)**
Management of unilaterally fixed twins in a multiparous American Quarter Horse mare

8:30 AM **Alysa Giudici (Cornell)**
Testicular neoplasm associated polyostotic hyperostosis in a male Budgerigar

8:45 AM **Dylan Desosa (Texas A & M)**
Clinical workup of a hyperechoic structure in the uterus of a chronically infertile mare

9:00 AM **Cameron Nau (The Ohio State University)**
Granulosa-cell Tumor Angus Heifer

9:15 AM **Reagan Stephens (Texas A & M)**
Metritis in a postpartum mare associated with a retained hippomane

9:30 AM **Jack Detten (Texas A & M)**
Chronic balanoposthitis and urethritis secondary to phimosis in a Tennessee Walking Horse gelding

9:45 AM **Noah Ennis (University of Missouri)**
Concurrent cystic endometrial hyperplasia, leiomyosarcoma, and pituitary adenoma in a 12 year old Nigerian Dwarf goat

COMPETITIVE CASE REPORTS

Friday, July 25 | 8 AM - 9 AM | Regency Ballroom ABC
Moderator: Dr. Roberto Palomares

8:00 AM **Jennifer Attridge, DVM**
Sperm peritonitis following trans-cervical insemination in healthy bitches

8:15 AM **Valentine Prié, DVM**
Postmortem ovary harvest following intrathecal lidocaine hydrochloride injection in a Warmblood mare

8:30 AM **Joshua Trumble, DVM**
Unilateral cryptorchidism with persistent paramesonephric duct remnants in a Mustang stallion

8:45 AM **Morgan Flanders, DVM**
Colloidal silver effects on semen parameters in canine

DR. JIMMY ALEXANDER STUDENT RESEARCH POSTER COMPETITION

Friday, July 25 | 3 PM - 4 PM | Regency Foyer
Judged by the SFT Student Abstract Committee

3:00 PM **Isabella Gange (Virginia-Maryland)**
Geographical differences in prevalence and antimicrobial resistance of Escherichia coli isolates from bitches with pyometra

3:15 PM **Carla Joseph (Ross University)**
Comparative post-breeding outcomes in jennies inseminated with cryopreserved semen re-extended in seminal plasma or treated with platelet-rich plasma

3:30 PM **Autumn Mendoza (Michigan State University)**
Impact of canine obesity on maternal insulin resistance and fetal metabolic profile

3:40 PM **Eden Manuel (Oklahoma State University)**
Delineation of miRNAs as biomarkers in equine chronic endometritis during different phases of the estrous cycle

Non-Competitive Abstract Presentations

ACT POSTERS
Friday, July 25th | 3:00-4:00 PM | Regency Foyer

Vulvar injection of 2.5% iPAAG Hydrogel to improve perineal conformation
Lauren Pasch, DVM, DACT

Impact of pituitary pars intermedia disorder (PPID) on the equine endometrium | **Carleigh Fedorka, PhD**

Phenotypic variation in female caprine XX/XY hematopoietic chimeras, a case report | **Adamarys Ruelas**

The effect of endometrial cyst removal via laser on pregnancy rates in the mare | **Karen Wolfsdorf, DVM, DACT**

Chronic endometritis in a Thoroughbred mare with Bordetella bronchiseptica | **Hayley Rossiano, DVM**

Bilateral uterine horn segmental aplasia in a doe
Adriana Garzon, DVM, MS, PhD

Glucuronide estrone and estradiol secretion in pregnant mare serum: insight from a liquid chromatography tandem mass spectrometry analysis
Jérôme Ponthier, DVM, MSc, PhD, Dipl. ECAR

Follicular dynamics in insulin resistant mares | **Eduardo Prado, DVM**

The unique framework of the equine fetal gonad for the synthesis of estrogen precursors | **Katarzyna Malin, DVM**

Endometrial polyp identified as a potential cause of infertility in a mare
Helen Brown, DVM

Alpaca with a history of multiple dystocias | **Nathaniel Ward**

Estradiol cypionate-sulpiride administration to seasonally non-cycling mares: the endocrine response/Estradiol cypionate-sulpiride administration to seasonally non-cycling mares: the physiological response | **Erin Oberhaus, MSc, PhD and Jenny Sones, DVM, PhD, DACT**

Clinical management practices of equine endometritis in India
Afroza Khanam, MSc, MPH, PhD

Assessment of Fetal Ultrasound Parameters for Predicting Parturition Date in Mares | **Kornelia Omyla, Med Vet, MRCVS**

The uterine environment reduces the pluripotency of the in vitro-produced blastocyst | **Alejandro de la Fuente, DVM, MSc, PhD**

Delayed embryonic development or long sperm survival in an embryo donor mare | **Javier Funes, DVM**

Effects of Sinigrin in Combination with a Low Iodine Diet on Equine Fetal Development and Urine Iodine Concentration | **Claire Card, DVM, PhD**

Effect of intrauterine ozone therapy on the post-breeding inflammatory response in mares: preliminary results | **Alexandra Grillos, DVM**

Microbiota-Immune Interactions in the Vaginal and Uterine Environment from Late Gestation to Early Postpartum in Dairy Cows with Endometritis
Ali Bazzazan, PhD

The seminal microbiome and its influence on the mare uterine microbiome
Giorgia Podico, DVM, MSc, DACT, PhD

Megestrol acetate medication error induced diabetes mellitus in a cat
Audrey Kelleman, DVM, DACT

Prenatal ultrasonographic diagnosis of kidney abnormality in a 4-month-old equine fetus | **Nayara Laquiz, DVM**

The effect of FSH commercial source, and in vitro-maturation medium formulation, on maturation, cleavage, and blastocyst rates after Intracytoplasmic Sperm Injection (ICSI) in equine oocytes | **Luisa Ramirez-Agamez, DVM, PhD**

Factors affecting lactate-induced acrosomal exocytosis in viable frozen/thawed stallion sperm | **Camilo Hernandez-Aviles, DVM, PhD, DACT**

Equine dystocia managed with assisted and controlled vaginal delivery
Karis Blankenship, DVM

Development of a diagnostic test for Nocardioform placentitis
Shavahn Loux, PhD

Extracellular vesicles secreted by mouse oviductal organoids: a model for contraceptive development | **Riley Thompson, DVM, PhD, DACT**

SFT STUDENT POSTERS
Friday, July 25th | 3:00-4:00 PM | Regency Foyer

Twin pregnancy in a southern tamandua (Tamandua tetradactyla)
Taylor King (Western CVM University of Saskatchewan)

Femoral dysgenesis in a neonatal Weimaraner puppy
Acadia Parker (Virginia – Maryland)

Investigation into hypochlorous acid as a treatment for bacterial endometritis in the mare | **Kayleigh Moore (Washington State University)**

First documented case of fetal anasarca in a Karst Shepherd dog: a detailed ultrasonographic progression from onset to outcome
Ana Andrejašič (University of Ljubljana, Slovenia)

Hemospermia secondary to cutaneous habronemiasis of the urethral process | **Ellie Sandt (University of Pennsylvania SVM)**

Adhesion induced pyometra and subsequent peritonitis in a Miniature Horse
Mikayla Reynaud (Oklahoma State University)

Testicular asymmetry reported after vasectomy in a one-year-old Miniature Pinscher dog | **Ammasie Allred (Washington State University)**

Validation of a portable Computer Assisted Semen Analysis (CASA) system for evaluating progressive motility and concentration of stallion and bull sperm in field and laboratory settings | **Georgia Lefaivre (University of Calgary)**

Presumed fetal anasarca dystocia in a Guernsey goat | **Morgan Simpson (Auburn University)**

Polycystic/fibrocystic mastopathy following ovariohysterectomy in a diestrous bitch | **Kara Mosier (Michigan State University)**

Paraphimosis in a Thoroughbred gelding | **Katherine Dickinson (Auburn University)**

Successful uterine prolapse replacement of extended duration in a Hereford cross cow | **Cody Davis (Auburn University)**

Pregnancy toxemia in the bitch | **Sara Bucher (Louisiana State University)**

Callicrate Banding Failure Leading to Surgical Corrective Castration
Taylor Yenrick (Auburn University)

Effect of knockout serum replacement supplementation in culture medium on bovine blastocyst gene expression after cryopreservation
Erika Mackenzie (St. Georges University)

Gangrenous mastitis in an English bulldog post-Caesarian section
Bryson Jacobs (Oklahoma State University)

Large offspring syndrome in cattle | **Maya Zinke (University of Illinois)**

Caruncular edema and torsion in an Icelandic ewe | **Rachael O'Connell (Cummings School of Veterinary Medicine at Tufts University)**

Congenital encephalocele in a live Friesian foal | **Haylee Hutchins (University of Georgia)**

Decoding Hormonal Effects in Bovine Oviductal Organoids: A New Era in Reproductive Research| **Brandi Dunn (Colorado State University)**

EQUINE ABSTRACTS
Saturday, July 26 | 10:30 AM - 12 PM | Golden State
Moderator: Dr. Callum Donnelly

10:30 AM **Camilla Scott, BVetMed, CertAVP (ESM), DACT, MRCVS**
Association between endometrial swab bacteriology and cytology and live foal rates in Thoroughbred broodmares in the United Kingdom

10:45 AM **Firdous Khan, BVSc, MVSc, DVSc, DACT**
Effect of N-acetyl cysteine treatment on uterine cytokine and chemokine profiles in mares with persistent breeding-induced endometritis

11:00 AM **Camilo Hernandez-Aviles, DVM, PhD, DACT**
The effect of cryoprotectant type at different steps of the cryopreservation process of stallion sperm

11:15 AM **Soon Hon Cheong, PhD, DVM, DACT**
Rheotaxis-based sperm separation of frozen-thawed equine semen using microfluidics

11:30 AM **Shavahn Loux, PhD**
Vitamin D3 as a Novel Treatment for Equine Persistent Breeding-Induced Endometritis

11:45 AM **Erin Oberhaus, MSc, PhD**
Estradiol cypionate-sulpiride administration to seasonally non-cycling mares: the endocrine response
Jenny Sones, DVM, PhD, DACT
Estradiol cypionate-sulpiride administration to seasonally non-cycling mares: the physiological response

SMALL ANIMAL ABSTRACTS
Saturday, July 26 | 10:30 AM - 12 PM
Regency Ballroom ABC
Moderator: Dr. José Len

10:30 AM **José Len Yin, MVZ, MS, PhD, DACT**
Effect of FSH and P4 on canine cumulus-oocyte complexes (COCs) metabolism

10:45 AM **Jamie Douglas, DVM, MS**
Cataloguing G-protein coupled receptors expressed in the canine placenta

11:00 AM **Meera Gatlin, DVM, MPH, DACVPM**
Assessing the impact of dog breeder mentorship and experiential learning on student knowledge and attitudes toward dog breeding

11:15 AM **Sandra Ayres, DVM, MA, DACT**
Spontaneous Ovulations in Cats Used in a Non-surgical Spay Study

11:30 AM **Bruce Christensen, DVM, MS, DACT**
Disagreement between claimed and actual quality of shipped frozen canine semen

11:45 AM **Samantha McCarter, DVM**
Habitual physical activity of lean and overweight bitches throughout gestation measured with a triaxial accelerometer: preliminary results

PRODUCTION ANIMAL AND MIXED SPECIES ABSTRACTS
Saturday, July 26 | 10:30 AM - 12 PM | Tahoe
Moderator: Dr. Jessica Klabnik

10:30 AM **Dane Schwartz, DVM**
Comparing vaginal douche, cervicovaginal mucus, and uterine lavage for diagnosis of Tritrichomonas foetus in naive heifers exposed to a naturally infected bull

10:45 AM **Soon Hon Cheong, PhD, DVM, DACT**
Cooling rate effect on post-warming outcomes from bovine embryo vitrification

11:00 AM **Rusty Stott, DVM**
Effect of parturition induction methods on delivery of cloned lambs

11:15 AM **Adam Ward, DVM**
Granulosa cell tumor in a caprine ovotestis

11:30 AM **Sai Kumar B.A.A.**
Changes in body condition score, trace minerals by parity, physiological state, and their influence on postpartum resumption of estrous cyclicity in beef cattle

11:45 AM **Maria Julia Wollentarski, DVM**
Effects of feeding rumen-protected choline 21 days prepartum to 100 days postpartum on health and reproduction of Holstein dairy cows

CASE REPORTS
Saturday, July 26 | 10:30 AM - 12 PM | Carmel A
Moderator: Dr. Katelyn Waters

10:30 AM **Alyssa Shelby, DVM**
Granulosa cell tumor in a midgestational Boston terrier bitch

10:45 AM **Gabriela Carneiro de Sousa, DVM**
Rectovaginal fistula in an adult English bulldog

11:00 AM **Leah Ramsaran, DVM**
Severe uterine torsion and moderate anemia in a late-term gestation Maine Coon

11:15 AM **Jessica Rush, DVM**
Ovine fetal deformities due to Cache Valley Virus in the Southeast

11:30 AM **Brittany Middlebrooks, DVM, DACT, MS**
Identification and management of an atypical granulosa cell tumor in a pregnant mare

11:45 AM **Tressa Reiner, DVM**
Schistosomus reflexus as a cause of cesarean section in a Labrador Retriever

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Conference Schedule

Thursday, July 24

7:00 am-4:30 pm Registration Open | Regency Foyer

7:00 am-8:00 am Continental Breakfast | Regency Foyer

7:15 am Bus Departs for UC Davis Student and ART Symposia

8:00 am-10:00 am General Session: Dr. Jerry Rains Memorial Abstract Competition | Regency Ballroom ABC

10:00 am - 10:30 am Morning Refreshments | Regency Foyer

	Equine Track Golden State	Small Animal Track Regency Ballroom ABC Sponsored by ROYAL CANIN	Production Animal Track Tahoe	Technician Track Carmel A
10:30 am - 11:00 am	Katrin Hinrichs, DVM, PhD Follicular dynamics and oocyte developmental competence	Sabine Schäfer-Somi, DVM, Dipl ECAR The Delay of Puberty with Deslorelin	Jennifer Koziol, DVM, MS, DACT Keys to Successful Bull BSES	Samantha Snyder, BS The Art of Breeder-Client Communications for Support Staff
11:00 am - 11:30 am	Katrin Hinrichs, DVM, PhD Follicular dynamics and oocyte developmental competence	Sabine Schäfer-Somi, DVM, Dipl ECAR Melatonin Treatment in Cats	Jennifer Koziol, DVM, MS, DACT Review of semen evaluation	Samantha Snyder, BS The Dream Team: How to be an Asset to Your Repro Vet
11:30 am - 12:00 pm	Fernando Campos-Chillon, MS, DVM, PhD, DACT The Secret Life of Equine Oocytes: Maturation Dynamics	Sabine Schäfer-Somi, DVM, Dipl ECAR Mycoplasmosis in Dogs	Jennifer Koziol, DVM, MS, DACT Navigating Dystocias and Fetotomies	Samantha Snyder, BS The Dream Team: How to be an Asset to Your Repro Vet
12:00 pm - 1:30 pm	**Optional Lunch & Learn *(pre-registration required)*: The Nutrition-Reproduction Connection with Emmanuel Fontaine, DVM, MS, PhD | Sponsored by ROYAL CANIN Or Lunch On Your Own**			
1:30 pm - 2:30 pm	Beatriz Macias-Garcia, DVM, PhD Optimizing Equine Oocyte Maturation In Vitro: Insights from the Follicular Environment	Jamie Burkitt, DVM, DACVECC Update: The RECOVER Newborn Resuscitation Guidelines	Jennifer Koziol, DVM, MS, DACT Bull Reproductive Surgeries	Kara Kolster, DVM, DACT Semen Evaluation Techniques
2:30 pm - 3:30 pm	Fernando Campos-Chillon, MS, DVM, PhD, DACT The Delicate Dance: Intricacies and Challenges in Equine ICSI and Embryo Cryopreservation	Cheryl Lopate, MS, DVM, DACT Diagnosis and treatment of inflammatory endometrial disorders in the bitch	Bret McNabb, DVM, DABVP, DACT Epizootic Bovine Abortion (aka Foothill Abortion) - A West Coast Story	Kara Kolster, DVM, DACT Chill processing, Freeze processing, +/- Managing frozen semen/tanks
3:30 pm - 5:00 pm	**General Session: Therio News Hour (and a 1/2) | Regency Ballroom ABC**			

5:00 pm-5:30 pm VIP Cocktail Event *(By Invitation Only)* | Regency Ballroom DEF

5:30 pm-6:30 pm Exhibitor Hall Opening Reception | Regency Ballroom DEF

6:30 pm-8:00 pm Trivia Night | Capitol View (15th Floor)

Please note that the speaker schedule and room locations are subject to change.
Video and audio recording is strictly prohibited during any session.

Conference Schedule
FRIDAY, July 25

7:00 am-8:00 am Continental Breakfast with Exhibitors| Regency Ballroom DEF

8:00 am-10:00 am General Session: Dr. Jimmy Alexander Student Case Presentations | Regency Ballroom ABC

8:00 am-10:00 am ACT Business Meeting | Golden State

10:00 am - 10:30 am Morning Refreshments in Exhibit Hall

10:30 am - 11:30 am General Session: ACT Plenary Speaker Daniel Givens, DVM, PhD, DACT, ACVM (Virology) - The changing attributes of veterinary medical education and the resulting impacts on your future colleague | Regency Ballroom ABC

11:30 am - 12:00 pm General Session: David E. Bartlett and Theriogenologist of the Year Award Presentations | Regency Ballroom ABC

12:00 pm - 1:00 pm **SFT Business Meeting Luncheon (Advance registration required) Steiner Award Presentation**
Meeting to begin at 12:15 | Regency Ballroom ABC

	Equine Track Golden State	Small Animal Track Regency Ballroom ABC Sponsored by ROYAL CANIN	Production Animal Track Tahoe	Applied Research Track (ART) Carmel A
1:00 pm - 2:00 pm	The Theriogenology Foundation's Dr. Michelle LeBlanc Memorial Equine Lecturer: Katrin Hinrichs, DVM, PhD The Promise of IVF in Horses (2 hour lecture)	Janice Cain, DVM, DACVIM (SAIM) Cesarian Section: Emergency vs Elective: how and when to plan for an elective C-section and how to select the correct date for surgery	Bret McNabb, DVM, DABVP, DACT Brucella Ovis: Diagnosis and Control Strategies	Sabine Schäfer-Somi, DVM, Dipl ECAR Semen Evaluation, Conservation and Transport
2:00 pm - 3:00 pm		Janice Cain, DVM, DACVIM (SAIM) Cesarian Section Logistics: Anesthesia, Prep, Surgical Tidbits, and Post-partum Early Newborn Care for the Best Success	Fauna Smith, DVM, PhD Non-Infectious Reproductive Disorders of the Female Small Ruminant	Ky Pohler, MS, PhD Reproductive Biotechnology Research

3:00 pm - 4:00 pm **General Session: ACT and SFT Student Poster Competitions and Extended Afternoon Refreshments**
Regency Foyer & Ballroom DEF

4:00 pm - 5:00 pm	Raul Gonzalez Castro, DVM, MS, PhD Sperm's Vital Role: A Functional Perspective on Fertilization	Lacey Rosenberg, DVM, DACT Reproductive Challenges and Jenna Dockweiler, DVM, DACT, Preservation of Historical Dog Breeds	Fauna Smith, DVM, PhD Infectious Reproductive Disease of Female Small Ruminants	Beatriz Macias-Garcia, DVM, PhD The Secretome, A Promising Tool in Equine Assisted Reproduction

4:30 pm-6:00 pm Student Mentor Reception and Career and Training Fair | Capitol View (15th Floor)

6:00 pm-9:00 pm Therio Foundation Soiree in Sacramento (Advance Ticket Purchase Required) | California State Railroad Museum
Limited tickets available onsite.

Please note that the speaker schedule and room locations are subject to change.
Video and audio recording is strictly prohibited during any session.

Conference Schedule
SATURDAY, July 26

7:00 am-8:00 am Continental Breakfast with Exhibitors | Regency Ballroom DEF

8:00 am-9:00 am General Session: ACT Competitive Case Reports | Regency Ballroom ABC

	Equine Track Golden State	Small Animal Track Regency Ballroom ABC Sponsored by ROYAL CANIN	Production Animal Track Tahoe	Applied Research Track (ART) Carmel A
9:00 am - 10:00 am	Pablo Ross, DVM, MS, PhD Innovations in Equine Theriogenology: Stallion Sperm Sex Sorting for ICSI	James Lavely, DVM, DACVIM (Neurology) It's been 4 hours now what!? Priapism, Phimosis and Paraphimosis in the Dog & Cat	Jason Coe, DVM Principles of Semen Cryopreservation in Cervids	
10:00 am - 10:30 am	**Extended AM Break In Regency Ballroom DEF**			
10:30 am - 12:00 pm	Abstract Presentations: Equine Non-Competitive	Abstract Presentations: Small Animal Non-Competitive	Abstract Presentations: Production Animal and Mixed Species	Abstract Presentations: Case Reports Non-Competitive
12:00 pm - 1:00 pm	**Award Presentations Lunch: ACT and SFT Competitive Abstract Awards and SCOTY Award (Advance Lunch Registration Required) | Regency Ballroom ABC**			
1:00 pm - 2:00 pm	Carleigh Fedorka, PhD What's Good for Sperm Isn't Always Good for Pregnancy: The Seminal Plasma Paradox	James Lavely, DVM, DACVIM (Neurology) The Pediatric Neurologic Examination and Case Highlights	Jason Coe, DVM Multiple Ovulation Embryo Transfer in Cervids	Virginie Gaillard, PharmD, PhD Beyond Gut Microbiota, A Pleiotropic Probiotic for the Perinatal Period of dogs and cats
2:00 pm - 3:00 pm	Robert Foss, DVM Embryo Recipient Management in the Era of Art; What Have We Learned?	Kristin MacDonald, DVM, PhD, DACVIM (Cardiology) Cardiology Essentials for the Theriogenologist- Part I: Murmurs in Puppies & Kittens	Ky Pohler, MS, PhD Causes and Solutions of Reproductive Inefficiency in Cattle Part 1	Pablo Ross, DVM, MS, PhD Bovine Sperm Sex Sorting: Advancing Beyond Conventional Semen Fertility
3:00 pm - 3:30 pm	**Afternoon Refreshments | Regency Foyer**			
3:30 pm - 4:30 pm	K. Gary Magdesian, DVM, Dipl. ACVIM, ACVECC, ACVCP Early antibiotic and IV fluid therapy of the critical neonatal foal	Kristin MacDonald, DVM, PhD, DACVIM (Cardiology) Cardiology Essentials for the Theriologist- Part II: Acquired Heart Disease	Ky Pohler, MS, PhD Causes and Solutions of Reproductive Inefficiency in Cattle Part 2	Raul Gonzalez Castro, DVM, MS, PhD Evaluating Sperm Fertilizing Potential: What Can We Predict?

Please note that the speaker schedule and room locations are subject to change.
Video and audio recording is strictly prohibited during any session.

2025 Symposia Offerings

Wednesday, July 24

Canine Semen Freezing Course

Wednesday, July 24 | 8:00 AM – 12:00 PM / 1:00 PM – 5:00 PM | Kokopelli Veterinary Clinic, 1420 Fulton Ave, Sacramento, CA
Instructors: Drs. Bruce Christensen and Kris Gonzales | 5 CE Credit Hours

Participants will be divided into small groups and assigned to an experienced technician. All groups will be supervised by the two instructors. Each group will collect semen from dogs and physically go through all the steps of semen evaluation and semen freezing, including:

- Fractionation of the ejaculate
- Alternate ways to evaluate morphology
- Basic semen freezing protocols, including different variations
- Semen thaw and evaluation protocols
- Objective motility, concentration, and morphology evaluation using a CASA system
- Use of different freezing extenders (including both one- and two-step options)
- Two-ejaculate method of semen freezing
- Appropriate insemination dose determination

The lab will be hands-on, with each participant performing all the tasks under supervision. Information will be provided on equipment needed to get started. Participants should leave with all the knowledge and skills to maintain an active, accurate, effective frozen semen service.

Thank you to Sponsor Minitube and to Jorgensen Laboratories for their additional support.

Respiratory Function Grading (RFG) Certification Training for Brachycephalic Obstructive Airway Syndrome (BOAS)

Wednesday, July 24 | 8:00 AM – 12:00 PM / 1:00 PM – 5:00 PM | Regency Ballroom Corridor, Ballroom D, and Innovation Room
Instructors: Drs. Kelley Thieman, Kat Ham, and Carrie Stefaniak | 5 CE Credit Hours

This laboratory will train veterinarians to perform OFA Respiratory Function Grading Scheme (RFG). The RFG is a non-invasive scoring system that was developed to provide an objective test to measure the severity of brachycephalic obstructive airway syndrome, thereby helping breeders and veterinarians to evaluate dogs for breeding, and ultimately to produce healthier brachycephalic dogs. The laboratory will cover how to perform the RFG scoring and is designed to result in OFA approval.

Thank you to the American Kennel Club and to Royal Canin for their support.

2025 Therio Conference Student and Art Symposia

Thursday, July 25 | UC Davis

Veterinary Student Dystocia Management

Thursday, July 24th | 8:00 AM – 12:00 PM | Gourley Clinical Teaching Center
Instructors: Drs. Bret McNabb and Daniela Orellana Guerrero

This lab teaches students to identify normal parturition positions and presentations as well as delivery in large animals. It also provides hands-on experience diagnosing and correcting dystocia cases. Students will practice assisted vaginal deliveries and learn proper use of obstetrical instruments. The lab emphasizes recognizing complications and applying appropriate techniques to ensure safe and effective intervention during difficult parturitions.

ART Wet Lab for Bovine IVF and Demonstration of the Equine ICSI Procedure

Thursday, July 24th | 8:00 AM – 4:00 PM | 1089 Veterinary Medicine Dr., VetMed 3B (VM3B building)
Instructors: Drs. Alejandro de la Fuente, Kazuki Takahashi, Soledad Martin-Pelaez, Katarzyna Malin, Margo Verstraete, and Pouya Dini
7 CE Credit Hours

Wet lab for Bovine IVF and demonstration of the Equine ICSI procedure. This workshop will consist of four stations with participants rotating through the stations throughout the day. Participants are required to review the study materials before the lab.

Review of ART in Domestic Animals Lecture and Lab:

Station 1: Aspirating bovine oocytes from slaughterhouse materials, then filtering and searching for COCs. The methods for searching and manipulating equine and small ruminant oocytes will be compared highlighted.
Station 2: Bovine semen preparation and in vitro fertilization. Methods for semen preparation for equine ICSI will be highlighted.
Station 3: Equine ICSI demonstration, covering all steps from oocyte searching to the ICSI and culture procedure.
Station 4: Equine embryo thawing and preparation for transfer.

The Society for Theriogenology would like to thank host UC Davis and faculty instructors for their contributions.

Thursday, July 24 | 12:00 pm - 1:30 pm

Lunch & Learn *(Optional Session)*: The Nutrition-Reproduction Connection with Emmanuel Fontaine, DVM, MS, PhD.

1.0 CE Credit Hour

Additional fee and advance registration required

Gestation, lactation, and growth are distinct physiological stages during which the nutrition received by the mother and her offspring will play a crucial role in their harmonious development. In dogs and cats, many errors are, however, made during this period. Some of these errors can have consequences on the health of the mother and/or her offspring, and on fertility as well. This presentation will revisit the essential points to know on this topic and provide practical information on how to optimize nutrition during these specific life stages.

About the Speaker: Dr. Emmanuel Fontaine is a veterinary professional specializing in canine and feline reproduction, with a strong academic foundation shaped by seven years at the esteemed Veterinary School of Alfort in Paris. He is committed to the dissemination of scientific knowledge by sharing the latest research, techniques and best practices through his online courses and blog. Now continuing his professional path in Canada, Emmanuel remains actively engaged in the academic and veterinary communities, sharing his expertise and learning from global peers.

Lunch & Learn generously sponsored by

ROYAL CANIN

Looking for a Quiet Space or Lactation Room During the Therio Conference?

Please inquire at the registration desk for information. Room will be available to Therio Conference attendees Wednesday, July 23- Saturday, July 26

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BIDDING OPEN

Wednesday, July 23, 9 am PST
Saturday, July 26 at Noon PST

Even if you aren't attending the Soiree, you can still be part of the fun – anyone, anywhere can participate!

All items will be shipped to winning bidders after the Therio Conference.

SNEAK PEEK

Framed Engraving of a Bull Elk
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National Parks Handknit Hat
Donated by Dr. Gail Colbern

Nandi Cow and Calf Sculpture
Donated by Dr. Jim Floyd

...And More!

SCAN TO PREVIEW AND BID

NANDI THERIOGENOLOGY SCHOLARS

CLASS OF 2025

The Theriogenology Foundation is pleased to announce the selection of four senior veterinary students as their 2025 Nandi Theriogenology Scholars. This fifth class of Nandi Scholars was selected from a pool of 20 applicants representing 14 different veterinary schools. They will be recognized and receive their awards of $7,500 each at the Annual Therio Conference in Sacramento, CA on Friday, July 25, 2025.

Thank you to the 23 dedicated reviewers who selected these four outstanding students, and to all who have donated to the Foundation to make these awards possible.

In the alphabetical order of their schools, the 2025 Nandi Scholars:

Liz Patton
Colorado State University

Liz Patton was first drawn to theriogenology on a tour of the Equine Reproduction Laboratory at Colorado State University with her 4-H club in high school. As an undergraduate, she worked as an intern at Sunup Ranch Quarter Horses breeding Quarter Horses and helping with the management of grass fed Angus beef cattle. Liz has spent time volunteering for Can Do Canines while in college to help train and socialize service dogs as well as learn about their breeding program. After graduation, Liz will be moving to Lexington, Kentucky to complete an equine field internship at Hagyard. Liz hopes to work at a mixed animal clinic and to pursue a residency in theriogenology via the alternate route.

Devon Anderson
Purdue University

Devon Anderson is an Indiana native who attended Purdue University for both his undergraduate and veterinary degrees.

Growing up, Devon was heavily involved in 4-H and raised rabbits and poultry. During his undergraduate career, he was an Animal Sciences Ambassador and participated in poultry, swine, and bovine research sparking his interest in reproduction. In veterinary school he held numerous leadership positions, including service as President of Purdue's SAVMA Chapter, Curriculum Review Committee Student Representative, President of Purdue's student chapter of Society for Theriogenology, and the sole student representative on Purdue's Veterinary Dean Search Committee.

After veterinary school, Devon will be starting a Large Animal Rotating Internship at Purdue University and plans to apply for an academic theriogenology residency program.

Kassie Crissman
Louisiana State University

Kassie Crissman has spent nearly 10 years showing Arabian horses, a passion that evolved into an interest in theriogenology. She is currently preparing for her own foaling season and looks forward to sharing the experience of breeding and raising horses with future clients.

Kassie is currently working towards publishing research as a 2023 Veterinary Fellow for the Foundation for Food and Agriculture Research. She has twice presented at the annual Therio Conference and was honored with a 2nd place award for her endometrial cup research. After graduation, Kassie will begin a Small Animal Rotating Internship at Virginia-Maryland, where she plans to continue her involvement in research.

Taythen Larson
Washington State University

Taythen Larson grew up in a ranching community, helping his family, friends, and neighbors with all aspects of cattle production. In high school, Taythen became a certified artificial insemination technician through a Future Farmers of America program and spent summers working with a local veterinarian. While at WSU, Taythen continued to be involved with theriogenology work such as gaining experience with obstetrics, ultrasonography, and palpation and participating in the Northwest Bovine Veterinary Exchange Program in helping with artificial insemination, embryo transfer on several thousand head of beef cattle in Montana. After graduation, Taythen plans to return to southern Idaho and join a large animal general practice. Taythen intends to pursue certification through the American College of Theriogenology by achieving diplomate status through the non-traditional route.

Guide to 2025 Exhibitors

Exhibit Hall Hours

Thursday	5:00 pm - 6:30 pm
Friday	7:00 am - 5:00 pm
Saturday	7:00 am - 10:30 am

The American Kennel Club, Inc. - Booth 4
The AKC Veterinary Outreach Program serves as a bridge between the American Kennel Club and the veterinary community.

Aurora Pharmaceutical, Inc. - Booth 14
Aurora Pharmaceutical is a leading manufacturer of veterinary medications and wellness products, committed to improving animal health and performance. We specialize in developing high-quality, FDA-approved solutions for livestock, companion animals, and equine care. Our innovative approach, rigorous quality standards, and focus on customer needs make us a trusted partner for veterinarians, producers, and pet owners nationwide. Aurora Pharmaceutical is dedicated to advancing animal health through science and innovation.

Botupharma USA - Booth 33
Botupharma USA exists to provide veterinarians, breeders, exhibitors, and owners with industry-leading reproduction supplies and performance supplements. An unwavering commitment to quality, customer service, and education paired with vendor partnerships across the U.S. helps ensure you have what you need, when you need it.

Creative Science - Booth 18
Creative Science is a veterinary-trusted leader in animal health, formed by the merger of Arenus Animal Health, Kinetic Vet, Equine Medical & Surgical Associates, Breeder's Choice, Exodus Breeders, and Banixx. Dedicated to innovation, we provide scientifically backed solutions that fill critical gaps in pet and equine care.

GeneTech Animal Reproduction - Booth 10
GeneTech is an advanced center for equine reproduction specializing in Intracytoplasmic Sperm Injection (ICSI) and advanced semen analysis services. Utilizing cutting-edge technology, highly skilled veterinary practitioners and embryologists, and a deep understanding of equine genetics, GeneTech is setting new benchmarks in the equine breeding industry. Their commitment to quality and innovation is evident through their comprehensive suite of services that encompass every aspect of the ICSI process in addition to their continued commitment to in-house research. At the heart of GeneTech's success is their team of experienced embryologists and reproductive specialists. These experts meticulously analyze semen samples to identify the most optimal sperm candidates for the ICSI procedure. Using state-of-the-art equipment, technology and high-specific protocols, their team maximizes the chances of successful embryo development.

Healvet USA, Inc. - Booth 13
Healvet USA, Inc. specializes in advanced veterinary blood analyzers and test kits. Our easy-to-use, reliable diagnostic solutions help veterinarians and veterinary professionals achieve fast, accurate results. With a focus on innovation and quality, we are committed to improving animal health through accessible, high-performance tools for modern veterinary practices.

IMV Technologies - Booth 23
IMV Technologies provides a wide range of ultrasound scanners and digital radiography systems for the small animal, farm animal and equine markets.

Jorgensen Laboratories, LLC - Booth 1
Jorgensen Laboratories has supplied the veterinarian with specialized equipment for over 50 years, and the JORVET trademark is synonymous with high quality products for the veterinary profession. Please stop by our booth and see the latest innovations in veterinary equipment for the progressive veterinary practitioner.

KARL STORZ Veterinary Endoscopy America, Inc. - Booth 5
KARL STORZ Veterinary Endoscopy is the only manufacturer of minimally invasive surgical and endoscopy equipment with a division dedicated to veterinary professionals. Your success in MIS depends on high quality imaging, instrumentation, and educational support from top specialists and highly trained staff provided by KARL STORZ.

Lane Manufacturing, Inc. - Booths 16 and 17
Lane Manufacturing, Inc. has been manufacturing semen evaluation equipment for over 40 years. The Pulsator IV electronic ejaculator is known around the world as the gentlest and the most user friendly of all ejaculators commercially manufactured. We strive to be the best in manufacturing quality, durable and efficient semen collection equipment, because this is our only business!

MAI Animal Health - Booth 2
MAI Animal Health™ is THE SOURCE for solutions in animal healthcare. With extensive experience and expertise across multiple veterinary disciplines and species, we manufacture and supply a vast array of innovative, practical products in categories including Reproduction, Containers, Dental, Specialty, and Instruments. Veterinarian owned and trusted globally for over 40 years.

Merck Animal Health - Booth 20
Our passion is simple: do what's right for the horse. It's our unconditional commitment to work tirelessly toward that goal by continually innovating and improving the products and programs that impact the health and well-being of horses and those who care for them.

Minitube USA - Booths 11 and 12
Minitube is the world-wide leading supplier of systems for the field of assisted animal reproduction. With our comprehensive range of products and services for artificial insemination, embryo transfer and related biotechnologies, we effectively support our customers in animal breeding, veterinary medicine and research worldwide. Our team of specialists is continuously working on the improvement and development of reproductive technologies.

Nexpring Health - Booth 32
Nexpring Health brings together industry leaders: including Cook Reproductive Health, Hamilton Thorne Inc, Planer, IVFtech, Gynemed, Gynétics, Microptic, TekEvent, and Embryotech into one powerful global force. Dedicated to redefining the future of ART by putting embryologists, clinicians, and fertility clinics at the center of everything we do.

Platinum Performance - Booth 34
For nearly 30 years, Platinum Performance® has been proud to stand beside veterinarians, driving innovation in advanced nutrition to support optimal health, performance, and reproductive health in animals. At Platinum Performance® we believe that good nutrition is good. For more information about Platinum Performance® formulas, call a Platinum Advisor at 866-553-2400 or visit www.PlatinumPerformance.com. HALL HOURS!

Professional Embryo Transfer Supply (PETS), Inc - Booth 3
For over three decades, PETS, Inc. has been a global leader in the embryo transfer industry. We strive to continue this by offering quality products from various manufacturers and exceptional customer service. We look forward to seeing everyone at this year's Therio meeting.

Royal Canin - Booths 29 and 30
At Royal Canin, we know health is something different for every pet. We create tailored nutrition that helps cats and dogs live their healthiest lives. At Royal Canin, we invest in the health of pets starting at conception through the rest of their lives. Royal Canin is the only company to offer diets specifically designed for reproduction and lactation for dogs and cats.

Universal Imaging, Inc. - Booth 6
Ultrasound and Digital Radiography Veterinary Solutions Leading the diagnostic imaging industry for 49 years, Universal Imaging offers cutting edge Ultrasound, Digital Radiography, CT, and Cloud Solutions for today's veterinarian. Offering superior technology, service and education, we're committed to meeting the needs of our customers, and their patients. www.univesalimaginginc.com.

VetMotl, Inc. - Booths 24 and 35
VetMotl, Inc. is commercializing worldwide the VetMotl™ Sperm Separation Devices, first-of-their-kind devices for use in veterinary assisted reproductive

technology (ART) procedures. VetMotl devices deliver increased efficiencies in blast development and viable implantable embryos in fertility procedures. VetMotl devices provide considerable time savings and standardization over traditional methods.

Veterinary Books by Success Concepts - Booth 8

Veterinary Books by Success Concepts is your one-stop for all your Veterinary book needs.

Veterinary Reproduction Innovations, APC - Booth 7

Veterinary Reproduction Innovations (VRI) was established to offer state-of-the-art technology and the most advanced assisted reproduction procedures to provide the next generation of phenomenons in the many genres of the equine and bovine industries. As health care professionals, we also recognize the need for optimal care not only for reproductive efficiency but for the well-being of the patients entrusted to us. Our team is devoted to giving quality, specialized and compassionate care to our patients, keeping in mind that the reproduction equation is only solved with overall health.

Wise Option - Booth 31

Wise Option is a tailored, cloud-based management software platform for veterinary practices and equine businesses. We simplify complex record-keeping and streamline operations, helping practitioners efficiently and precisely manage health records, billing, and reproduction data.

THANK YOU!

The Therio Conference would not be possible without the support of our exhibitors and sponsors. Make sure to visit them in the Exhibit Hall to show your appreciation!

2025 Therio Conference Plenary Speaker

The American College of Theriogenology is pleased to share the 2025 Plenary Speaker is Dr. Dan Givens, presenting ***The changing attributes of veterinary medical education and veterinary employment and resulting impacts on our future colleagues*** on July 25th, 10:30 -11:30 am in Regency Ballroom ABC.

M. Daniel Givens,

DVM, PhD, DACT, ACVM (Virology)

Dr. Dan Givens practiced veterinary medicine for both food animals and companion animals in Campbellsville, Kentucky. He then went on to complete a residency, a PhD in biomedical science and NIH-funded post-doctoral research at Auburn University. A diplomate of both the American College of Veterinary Microbiologists (virology specialty) and the American College of Theriogenologists, Dr. Givens has conducted applied research in infectious diseases that affect reproduction of cattle. This work focuses on the diagnosis of uniquely localized viral infections and the prevention of viral transmission.

Throughout 28 years as a faculty member, Dr. Givens has interacted with students in classrooms, laboratories, and clinical rotations and assisted clients through clinical service and consultations. After serving as a faculty member, director of an agricultural experiment station, acting department head, and associate dean for academic affairs at Auburn, Dr. Dan Givens became the fifth dean of Virginia-Maryland College of Veterinary Medicine at Virginia Tech. Dr. Givens focuses on a timely, bold, strategic vision to strengthen the college's One Health approach to solving complex problems.

Dr. Givens recently served on the AVMA Council on Education (COE) Strategic Planning Steering Committee and currently serves as an accreditation site visitor for the AVMA COE and is a member of the American Association for the Advancement of Science and the Virginia Academy of Science, Engineering, and Medicine.

Save the Date!
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CONGRATULATIONS!

2025 Theriogenologist of the Year

Igor Canisso,

DVM, MS, PhD, DACT, DECAR
(Equine Reproduction)

Dr. Canisso grew up working with his late father on their family ranch in Birigui, São Paulo, where he bred and raised livestock and horses. This upbringing instilled in him a passion for a career in veterinary medicine focused on animal reproduction. After graduating with a degree in Veterinary Medicine from the Federal University of Paraná Campus Palotina, he practiced as a large animal veterinarian for three years. While pursuing his master's degree at the Federal University of Viçosa, he worked as a veterinarian on a private mule-producing farm and in the draft horse breeding program at the university. After completing his MS, he moved to Britain as a visiting scholar and fellow in equine reproduction at Aberystwyth University. He then finished a residency in Theriogenology at Cornell University and obtained his PhD in Equine Reproduction from the University of Kentucky. After Kentucky, he was appointed Assistant Professor of Theriogenology at the University of Illinois College of Veterinary Medicine, where he is currently a tenured Associate Professor. Over the years, Dr. Canisso has provided advanced reproductive services at the Illinois Veterinary Teaching Hospital, on horse farms, and at breeding centers statewide. His research program emphasizes equine perinatology and the subfertility of mares and stallions, and he has published over 150 manuscripts. Dr. Canisso is proud of his strong track record in training exceptional house officers and graduate students. He offers continuing education globally to veterinarians, leveraging the world stage to share knowledge and technology with the horse industry. Additionally, he provides consulting services to breeders and veterinarians both domestically and internationally, focusing on Arabian and Quarter Horses. He chaired the 7th International Symposium on Stallion Reproduction and the International Symposium on Donkey Science. Dr. Canisso is board-certified by the American College of Theriogenologists and the European College of Animal Reproduction (Equine Reproduction).

2025 Dr. David E. Bartlett Award for Lifetime Achievement in Theriogenology

John Kastelic,

DVM, PhD, Dip, ACT

Dr. John Kastelic was raised on a dairy near Edmonton, Alberta, Canada. After completing his DVM (University of Saskatchewan, 1982), he spent 2 years in practice, 1 year in a theriogenology residency, then earned an MS (1988) and PhD (1990) with Dr. OJ Ginther at the University of Wisconsin-Madison. Dr. Kastelic was a Research Scientist at Lethbridge Research Centre (1990-2012) and Professor of Theriogenology at the University of Calgary, Faculty of Veterinary Medicine (2012-2025). In heifers, Dr. Kastelic used transrectal ultrasonography to characterize ovarian follicular development and CL function (and control them for fixed-time AI) and studied embryonic loss and diagnosis of pregnancy and fetal sex. In bulls, he determined effects of early-life nutrition on puberty and reproductive development, evaluated breeding soundness, and studied scrotal/testicular thermoregulation, demonstrating that increased testicular temperature affected sperm directly (not secondary to hypoxia). Dr. Kastelic has published ~350 peer-reviewed papers, 7 book chapters, and has >15K citations.

Dr. Kastelic was Co-Editor of Theriogenology (2003 to 2013), has been Copy Editor for Clinical Theriogenology since 2018 and Co-Editor of The Canadian Veterinary Journal since 2020. He has held presentations and workshops on science and scientific writing in 20 countries and taught many DVM and graduate students.

A Diplomate of the ACT since 1994, Dr. Kastelic served on the Examination Committee (2001 to 2008), mentored 2 private practitioners who became diplomates, was Vice-President, President and Past-President, and recently chaired the Exam Process Committee. Dr. Kastelic was ACT Theriogenologist of the Year in 2009 and is enormously grateful and humbled to be receiving the 2025 Dr. David E. Bartlett Award.

BUILDING A CAREER IN THERIOGENOLOGY PROFESSIONAL PATHWAYS & TRAINING OPPORTUNITIES

When: Friday, July 25 4:30-6:00pm
Where: Capitol View Room (15th Floor)

This networking event will be informative and fun no matter where you are in your educational path.

Veterinary students and recent graduates are invited to attend this year's career and training Fair as an extension of the Student Mentor Reception. Veterinary schools, training programs and corporate partners will be available to connect with you to discuss their work, share information and introduce you to the range of opportunities available for young professionals – and the future leaders in the field of theriogenology.

2026 AMERICAN COLLEGE OF THERIOGENOLOGY CERTIFYING EXAM

IMPORTANT DATES

Monday, December 1, 2025 - ACT Diplomate Candidacy Applications (Credentials Packets) Due

Wednesday, April 15, 2026 - Exam Registration Fee Due (for approved candidates)

Tuesday, July 14 and Wednesday, July 15, 2026
ACT Certifying Exam

Please check additional requirements for Becoming A Diplomate at theriogeniology.org/page/requirements

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2024 John Steiner Award for Practitioner Excellence

Dr. Jill Colloton

In January 2009, the SFT Board established the Dr. John Steiner Award for Practitioner Excellence, recognizing veterinarians who embody the Society's mission to promote excellence, share emerging knowledge, foster global scientific exchange, support client education, and build professional networks.

Dr. Jill Colloton, the 2024 recipient of the John Steiner Award for Practitioner Excellence, was nominated by Dr. Marthina Greer. Dr. Greer presented the award at the Therio Conference in Oklahoma City and did not reveal Dr. Colloton's identity until the end of her comments.

Like many of you, this person has mentored veterinarians and veterinary students alike. Unlike many of you, this person has achieved the ultimate in work-life balance, working 3 days a week. This has allowed ample time to develop new techniques and spend time training and raising dogs. This individual has been active in working with the veterinary students interested in attending the Therio conferences, pairing them with practitioners and others to bring them into the fold and foster their interest in our organization.

I have admired this individual's ability to champion the use of transrectal ultrasound for dairy cattle, revolutionizing how pregnancies are diagnosed. Additionally, this person has used ultrasound for fetal sexing in bovine. She teaches ultrasound techniques to veterinarians around the world.

This person has developed techniques as requested by her clients to use ultrasound for: accurate and early diagnosis of early pregnancy or failure to conceive; assessment of fetal viability and twinning; ovarian assessment for synchronization protocols and embryo transfer; fetal sexing for cull and sale decisions; diagnosis of pathology; developing treatment plans to allow for minimal hormonal interventions; early diagnosis and intervention for management problems such as diseases or nutritional concerns.

As you likely know by now, she is a 1988 graduate from the University of Illinois College of Veterinary Medicine. She has active practice in Wisconsin, limited to ultrasound services to dairy producers.

She is a member of the Society for Theriogenology and has been active in their student relations. She is an honorary member of the American College of Theriogenologists. She is a contributing author to multiple bovine reproductive textbooks. She and her husband raise, train and hunt with their Small Munsterlander dogs.

Please help me honor the 2024 John Steiner Practitioner of the Year recipient, Dr. Jill Colloton.

Who will receive the 2025 John Steiner Award for Practitioner Excellence?

Find out Friday, July 26th at 12:00pm at the SFT Business Meeting!

HONORING DR. MICHELLE LEBLANC

Michelle LeBlanc, 1954-2013

Dr. Michelle LeBlanc received her DVM from Michigan State University in 1977 and became a Diplomate of the American College of Theriogenologists in 1982. She served on the faculty of the University of Florida, College of Veterinary Medicine for 22 years prior to practicing at Rood and Riddle Equine Hospital in Lexington, KY, where the LeBlanc Reproduction Center was named in her honor.

Dr. LeBlanc made major contributions to the field of theriogenology through her research, teaching, and service and was widely recognized as being at the forefront of new technologies in equine reproduction. Her extensive list of publications and her active participation in numerous national and international conferences are a testament to her expertise and her global reputation as an authority in equine reproduction.

Several key innovations highlight the creativity and dedication she brought to her work, including the development of the equine colostrometer, advancements in the treatment of endometritis, the introduction of the concept of failure of uterine clearance in subfertile mares, and improvements in the management of high-risk pregnancies, placentitis, and neonatal septicemia — all of which stand as milestones in the advancement of equine theriogenology.

Michelle received numerous awards, including the World Equine Veterinary Association Lifetime Achievement Award and the Carl J. Norden Distinguished Teacher Award; she was named 2000 Theriogenologist of the Year by the ACT; and in 2007, Michigan State University awarded her the Distinguished Veterinary Alumnus Award/Practitioner. ***She was honored by SFT with the David E. Bartlett Lifetime Achievement Award in 2013.***

Dr. LeBlanc's family established a fund in her memory at the Theriogenology Foundation to support the Equine program at the annual Therio Conference. Each year, the Theriogenology Foundation designates a speaker in Equine Track as the Dr. Michelle LeBlanc Memorial Equine Lecturer and is invited to provide a two-hour presentation during the conference. This honor is awarded to the individual who best exemplifies the innovative work, dedication to theriogenology, and commitment to mentorship that defined Dr. LeBlanc's remarkable career

2025 Dr. Michelle LeBlanc Memorial Equine Lecturer

The 2025 Dr. Michelle LeBlanc Memorial Equine Lecturer is Dr. Katrin Hinrichs. ***She will present The Promise of IVF in Horses under the LeBlanc designation on Friday, July 25th from 1:00 – 3:00 pm.***

Dr. Hinrichs is the Harry Werner Endowed Professor of Equine Medicine at the School of Veterinary Medicine, University of Pennsylvania. She obtained her DVM from the University of California, Davis and her PhD from the University of Pennsylvania. She served on the faculty at Tufts University and Texas A&M University before returning to Penn, where she currently is Chair of the Department of Clinical Studies – New Bolton Center. Her laboratory has pioneered research in equine assisted reproduction, including cloning, embryo biopsy and embryo vitrification, and developed methods for clinical application of ICSI that are now utilized worldwide. Most recently, her laboratory published the first efficient protocol for standard equine IVF. Dr. Hinrichs' honors include Theriogenologist of the Year, honorary doctorates from the University of Copenhagen and the University of Ghent, and the Simmet Prize for Assisted Reproduction. She was inducted into the Equine Research Hall of Fame in 2022.

On receiving this designation, Dr. Hinrichs shared:

I am honored to have been designated the Michelle LeBlanc Memorial Equine Lecturer for 2025. Michelle and I never had the opportunity to work together directly, but we often found ourselves at the same meetings and workshops and I was lucky to be able to spend some memorable times with her. She was a force of nature, and she has an enduring legacy in equine reproduction for her clinical work, her research and especially her impact as a colleague and mentor. She made friends, collaborators, and acolytes wherever she went, and served as an incredible ambassador for our specialty. Michelle is held in the highest regard by the Society and College, and I feel privileged to have been selected for this award in her name.

Michelle LeBlanc was a consummate professional, with high standards for herself and those around her. She loved her life and her work. Her dedication to the field was evident in her research, which addressed some of the most pressing issues in mare reproductive health. Her studies on improving clinical practices related to placentitis, as well as the management of endometrial infection and inflammation in mares, have been translated into standard clinical procedure worldwide. Michelle's approach to research was always practical and clinically focused, asking and answering questions that directly related to improving the care of the amazing animals we work with. Her ability to bridge the gap between academia and real-world veterinary medicine serves as an example for all of us in the profession.

To me, this award is a reminder of the importance of innovation and leadership in our profession, but also a reminder of the value of being a colleague and friend. I am proud to be part of this lasting tribute to Michelle's life and work.

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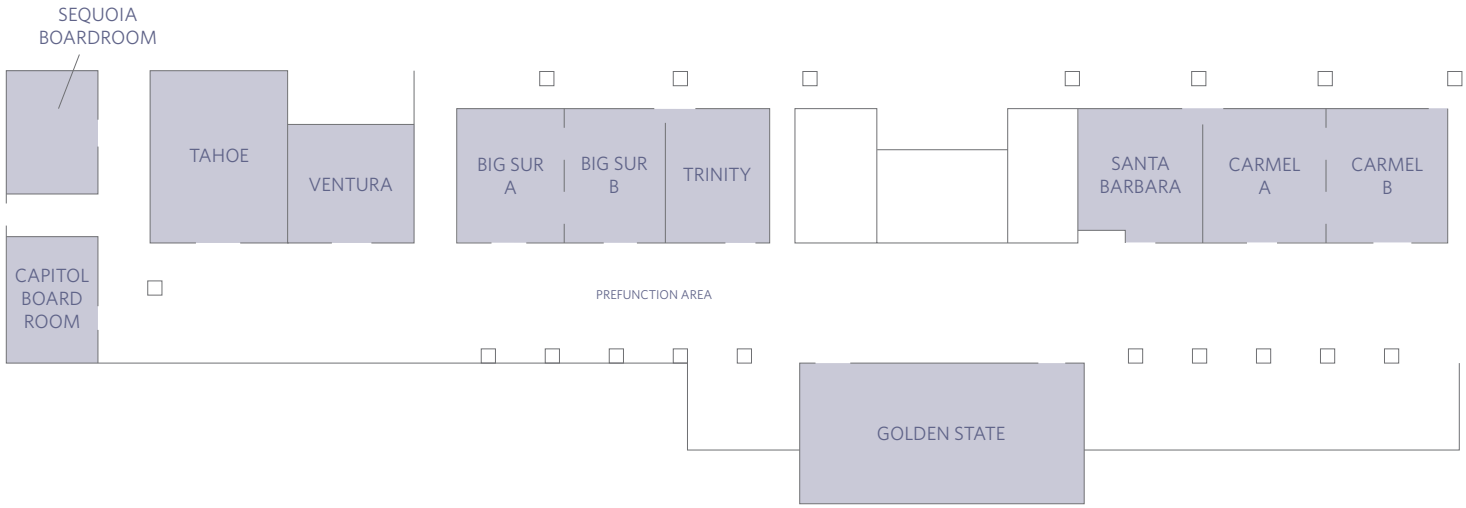

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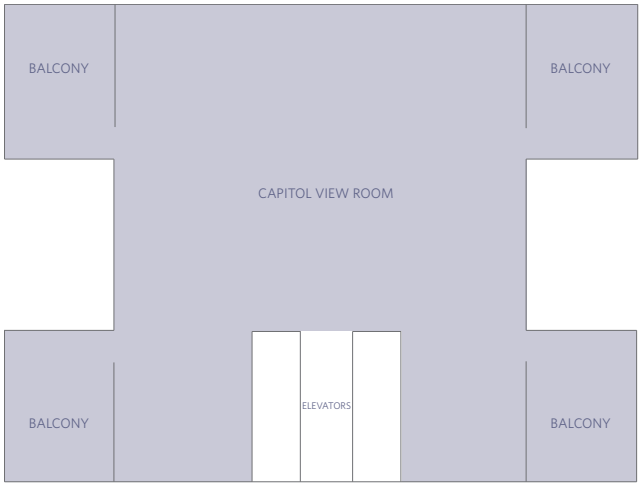

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The changing attributes of veterinary medical education and veterinary employment and resulting impacts on our future colleagues

M. Daniel Givens, DVM, PhD, DACT, DACVM

Virginia-Maryland College of Veterinary Medicine, Virginia Tech

Abstract: Changes have occurred and will continue to occur in the demand for veterinary medical education and the resulting provision of veterinary care. Changes in veterinary medical education are associated with variation in accessibility, cost, and methods of educational delivery. These changes occur alongside changes in the benefits that result from gaining a veterinary medical education. The varied benefits impact social, emotional, physical, and intellectual as well as financial health. The described changing attributes associated with this education and the changing impacts of gaining this education will mold and form our future veterinary colleagues. Through a clearer understanding of these changes over time, we might develop a healthier and more productive relationship with our future veterinary colleagues.

Changing attributes of veterinary medical education

Clearly, over the last three decades, the number of students in the United States being educated as veterinarians has significantly increased. Over the last 30 years enrollment at United States Colleges of Veterinary Medicine increased by 82% (7,262 students) from the 8,881 students enrolled in 1995. Similarly, from 1995 to 2024, the number of veterinary graduates at United States Colleges of Veterinary Medicine increased by 72% (1,525 graduates) from the 2,130 veterinarians graduating in 1995.

Between 1980 and 1998, the number of veterinary medical education programs in North America remained static. Since then, the number of colleges and schools of veterinary medicine has increased. Between 1998 and 2018, five new US programs and one Canadian program pursued accreditation by the AVMA Council on Education (COE). The educational foundation and approach of these six newly established colleges of veterinary medicine transitioned away from land grant university programs with the majority of clinical experiential training in academic teaching hospitals to non-land grant and private university programs using primarily distributed sites for clinical experiential training. As of March 2025, the AVMA COE accredits 53 veterinary colleges: 30 in the US, 18 internationally, and five in Canada. Additionally, there are five provisionally accredited US veterinary colleges and nine currently proposed programs working toward accreditation.[1] Of the proposed programs, Rowan University and Utah State

University have been granted letters of reasonable assurance and plan to admit first classes in Fall 2025. Clemson University College of Veterinary Medicine has a comprehensive site visit scheduled for June 2025 in anticipation of admitting a first class in Fall 2026.

Changing attributes of veterinary employment

Despite an increase in the number of veterinarians graduating and the proposed ongoing increase in the number of colleges of veterinary medicine, critical and routine veterinary services are not available or in short supply in many areas.[2] This critical shortage has been attributed to increased demand for veterinary services with the adoption of pets during the pandemic, veterinarians and veterinary technicians leaving the field as work-related stress increased, and low pay for veterinary technicians.

At the end of 2020, 45% of households owned dogs, up from 38% at year-end 2016. The population of pet dogs was estimated to be between 83.7 million and 88.9 million in 2020, up 9 to 16% from year-end 2016.[3] The average annual turnover rate among veterinary team members in various roles within small animal practice was 23% in 2023 and increasing each year.[4]

Based on data from 2024, new veterinary graduates were able to secure higher starting salaries (mean around $130,000) compared to prior years while equivalent salary increases were not received by more experienced veterinarians (mean around $150,000).[5]

Private equity funneled $51.6 billion into the veterinary sector from 2017 to 2023.[6] In the first four months of 2024, private equity invested $9.3 billion into the veterinary sector. Financial advisors have singled out the industry for its higher-than-average rate of return on investment. In the United States, corporations and private-equity funds have been rolling up smaller chains and previously independent practices at a rapid pace. Mars Inc., operates more than 2,000 practices under the names Banfield, VCA, and BluePearl. JAB Holding Company, owns more than 1,000 National Veterinary Associates hospitals. In the United States, 25 to 30% of companion animal veterinary practices are estimated to be under large corporate umbrellas, up from 8% in 2011. Based on a reported case study in Arizona, fewer than 15% of corporate-owned practices added brand identity to the practices that they own; most keeping the original practice name, leaving clients with the perception of local ownership.[7]

The price for veterinary services is increasing at a rapid pace compared to other costs. In March 2024, the Consumer Price Index for urban consumers increased 3.5% year over year; the veterinary services category increased 9.6%.

Hypothesized impacts

At times, alignment exists between two values to direct a clear approach and pursue a positive outcome. As an example, the presentation of a known pet by a trusting and beloved client can align relational and transactional values to create a positive experience for all. However, in other circumstances, the attributes of a relational approach and a transactional approach can be experienced as two poles on the spectrum of how to most appropriately approach an interaction. In situations where two values do not naturally align, an individual is tasked with the prioritization of values to select the most appropriate action. When valued principles do not align, how do you and the veterinarians around you prioritize the following related values? How has your tendency for prioritizing each value within the following couplet been impacted by your experience in veterinary medical education and your experience as a veterinarian?

1. Relational versus Transactional
2. Principled/Brave versus Pragmatic/Effective
3. Focused on short-term benefits versus Focused on long term benefits
4. Disruptive change versus Productive constancy
5. Personal responsibility for well-being versus Community responsibility for well-being
6. Learning in advance versus Learning at the point of needing to know
7. Negotiating versus Understanding and acceptance of nonnegotiables

Applications of hypotheses

Based on experienced interactions in the course of life, in the process of veterinary medical education, and in the practice of the veterinary profession, we develop patterns of prioritizing values to approach interactions. To better understand ourselves and our veterinary colleagues and, consequently, to strengthen our professional relationships, we are likely to be well-served to discuss scenarios where the described couplets do not naturally align. What are our tendencies in prioritizing these related values? How do those tendencies differ from those who surround us during our day-to-day work activities?

In conclusion, the last three decades have ushered in many notable and significant changes in veterinary medical education and in veterinary employment. It is not rational to consider that these changes would not impact the tendency of veterinarians to prioritize values as they approach challenging interactions. By understanding (a) our own tendencies, (b) the tendencies of our colleagues, and (c) the

precedents that shape those tendencies, we are likely to develop shared understandings of how challenging interactions are likely to be approached and anticipate pathways to optimal outcomes.

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Update: The RECOVER Newborn Resuscitation Guidelines

Jamie M. Burkitt, DVM, DACVECC
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University of California, Davis
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The newborn puppy or kitten must rapidly transition its physiology to survive in the extra-uterine environment. Perinatal mortality is high, with studies showing 6% of puppies born naturally, 11% of puppies born after dystocia, and 8-12% of puppies born via C-Section being stillborn. Among C-section puppies that survive the intra- to extra-uterine transitional period ("transition"), up to 13% die in the first 2 hours of life. The first few minutes of life may be the most life-threatening in dogs and cats. The RECOVER Initiative uses the term "newborn" for a dog or cat during the first few hours of life during which the animal is transitioning from intra- to extra-uterine physiology. During this period, the placenta is removed, and the lungs fill with air for the first time.

During transition pulmonary blood flow increases dramatically. In addition, systemic arterial pressure and left atrial pressure increase. Conversely, right-sided heart pressure and pulmonary arterial pressure drop significantly. Closure of the foramen ovale, a normal opening in the fetal atrial septum, occurs when left atrial pressure surpasses right atrial pressure, and for the first time, venous blood from the cranial and caudal vena cava pass through the right atrium into the right ventricle to enter the pulmonary circulation via the pulmonary artery. Closure of the ductus arteriosus occurs when systemic arterial pressure increases and pulmonary arterial pressure decreases, leading to left-to-right blood flow followed by constriction and closure. The combination of the drastic change in pulmonary and hemodynamic function makes transition tenuous and explains in part the high mortality noted during this time.

TERMINOLOGY

The RECOVER Initiative has adopted specific terminology demarcating the various periods of development of the puppy and kitten from birth to sexual maturity. Because of the rapid and extensive changes in physiology, consistency in terminology and hence the ability to apply clinical guidelines correctly is of great importance. The RECOVER **Newborn** guidelines target the management of dogs and cats during the previously described transition phase, which occurs from birth to the first few hours of life. The term "**neonatal**" includes this newborn period and beyond, up to independent urination, defecation, locomotion, and initiation of weaning, which is ~3-4 weeks of age in puppies and kittens. Because of the drastic changes in physiology and metabolism through the neonatal period, it is important to understand that the Newborn RECOVER Guidelines described in this lecture do not apply throughout the neonatal period. The term "**pediatric**" is used to describe dogs and cats from the time of weaning to the time of sexual maturity, roughly from 4 weeks of age until 6 months of age. The term "**adult**" is used to describe dogs and cats that have reached sexual maturity.

IDENTIFICATION OF NEWBORNS IN NEED OF RESUSCITATION

Adult dogs and cats require cardiopulmonary resuscitation (CPR) when they are unresponsive and apneic unless a do-not-resuscitate order is in place. Given the precarious physiologic changes in the newborn, resuscitative efforts may be required before apnea and unconsciousness have occurred. Patient selection in this population is thus based on identifying those not in need of CPR.

- The recommended criteria to withhold resuscitative efforts from a newborn include presence of all of the following three conditions:
  (1) normal parturition;
  (2) mother able to provide care;
  (3) vigorous: breathing (RR > 15 breaths / minute), clear vocalization and a vigorous response when testing for reflex irritability.

Any newborn dogs and cats not exhibiting all 3 of these criteria may require resuscitative measures, even if they are breathing and have an obvious heartbeat and/or pulse. By these conditions, all animals born by C-section require resuscitative efforts.

THE FIRST 1-2 MINUTES

Supporting oxygenation in transition is critically important in the newborn and includes the following therapeutic steps, performed roughly contemporaneously immediately at birth:

- Establish a patent airway
- Dry newborn, provide heat support, and tactile stimulation

- Supplement oxygen (select cases)
- Ensure adequate ventilation, using positive pressure if needed

Establish a Patent Airway: Fetal membranes should be removed immediately. The airway should be cleared with a clean cloth around the nostrils and mouth in vigorous newborns and by gentle aspiration using a suction bulb or DeLee suction catheter in non-vigorous newborns.

A note about "swinging": Acceleration and deceleration in a head-down position (i.e., "swinging") to clear airways of fluid removal was used in human infants more than 100 years ago. Swinging carries meaningful risk of intracranial hemorrhage, trauma, and aspiration of gastric contents, and also delays the initiation of more effective resuscitative interventions. Swinging or other acceleration / deceleration techniques should not be used in newborns.

Dry newborn, provide heat support, and tactile stimulation: Tactile stimulation accomplished by rubbing the animal with a warm towel may improve ventilation and circulation, and should be initiated as early as possible. Newborn puppies and kittens are at high risk of hypothermia, and maintenance of normothermia using heat support is important. Warming lamps are ideal because they allow for concurrent support measures; other methods include an enclosed incubator, warming discs or heated bags of grain with ample physical barrier to prevent overheating or burns.

Oxygen supplementation in select cases: Oxygen should be supplemented if the patient is cyanotic or severely bradycardic (HR < 50 beats / minute), but routine administration of 100% oxygen is currently not recommended in newborns due to the associated harm, including reduced survival rates in oxygen-supplemented newborns compared to those breathing room air. In veterinary medicine, it is reasonable to administer flow-by oxygen as needed if respiratory issues persist after airway clearance while other support measures are performed.

Ventilation, including positive pressure in select cases:

- Apnea or gasping: If the patient is apneic or gasping, ventilation should be actively supported by administering positive pressure ventilation (PPV) breaths with a tight-fitting face mask at a rate of 20-30 breaths per minute, regardless of heart rate.
- Bradycardic newborns, including those with regular breathing pattern: In all newborns, the heart rate is used to guide respiratory support measures since bradycardia is most commonly due to hypoxemia. Intervention beyond drying, warming, and stimulation is recommended in newborn puppies and kittens with bradycardia (e.g., < 120 bpm). Such interventions include PPV with a tight-fitting mask, more aggressive oxygen supplementation, and use of a continuous heart rate monitor to assess for response to therapy.
    - Atropine is likely not effective as bradycardia is the consequence of hypoxemia rather than high vagal tone.
    - Doxapram may be considered in addition to other supportive measures for puppies and kittens that are bradypneic. For puppies and kittens requiring PPV, we recommend against routine doxapram administration since the therapeutic focus should remain on PPV and other supportive measures as detailed here.
    - Some authors recommend GV26 (Jen Chung acupuncture point) stimulation in attempts to improve ventilation in bradypneic newborns. However, published canine and feline reports have been small and lack control groups, and thus evidence of efficacy is lacking. Additionally, the location of the needle prevents prompt application of PPV with a tight-fitting facemask, which is a proven effective measure. Thus, we do not recommend routine needle insertion at GV26 in newborn kittens and puppies with inadequate breathing efforts at birth when PPV is available.
    - Reversals should be administered if the dam/queen received an opioid, a benzodiazepine, or an α_2-adrenoceptor agonist prior to delivery of the newborn.
        - N*aloxone* – 4 µg / 100 grams IV or intraosseous (IO) [IV or IO preferred if possible], SC, or IM; if given by a transmucosal route, we recommend 100 µg total dose per newborn puppy or kitten.
        - Atipamezole – 5 µg / 100 grams IV or IO [IV or IO preferred if possible] or IM; if given transmucosally, 10 µg / 100 grams of body weight.
        - Flumazenil – 2 µg / 100 grams IV, IO, IM, or transmucosally. As benzodiazepines have very long half-life in newborns, a continuous rate infusion may be needed at 1 µg / 100 grams body weight / hour either IV or IO.

RESUSCITATION AFTER THE FIRST 1-2 MINUTES

Resuscitation in newborns is fundamentally different than in older patients in that effective ventilation, as opposed to chest compressions (as recommended in adults), has primacy. **Endotracheal intubation** can be challenging but can be accomplished with small uncuffed endotracheal tubes or venous catheters. It is reasonable to deliver 20-40 short breaths per minute (e.g., 1 breath every 2 seconds) with chest excursion commensurate to the size of the animal to address clinical hypoxemia.

For more **severe bradycardia (heart rate <50 bpm)** despite optimal ventilation by PPV, **chest compressions** should be initiated by positioning the thumb and index fingers of one hand on opposite sides of the chest just over the heart and compressing approximately 30-50% of the chest width. There are two fundamentally different aspects from adult CPR: (1) chest compressions are initiated during bradycardia despite presence of pulses, (2) effective ventilation in newborns precludes concurrent chest compression and thus PPV breaths should be delivered during a very brief pause in chest compressions. It is recommended that compressions and ventilations be delivered at a ratio of 4 compressions : 1 ventilation (4:1), administered at a rate such that 120 chest compressions and 30 breaths can be delivered in a minute (i.e., 150 events per minute).

Epinephrine is less important, as the core issue is asphyxiation. However, if PPV and chest compressions have continued for > 60 seconds without an increase in heart rate, IV or IO epinephrine should be considered (1 – 3 µg / 100 grams given IV or IO) every 3 – 5 minutes as in adult CPR. If the IV and IO routes are not available, a single, higher dose of epinephrine can be given intratracheally (5 – 10 µg / 100 grams). Hypoglycemia can occur during prolonged resuscitation and should be addressed if identified.

NEWBORN CPR ALGORITHM

The RECOVER Newborn CPR guideline process is, at time of writing, in the consensus stage with subject matter experts; thus, the recommendations discussed in this manuscript may change as consensus is reached on each of the 28 questions evaluated during the process. It should be noted that these guidelines are the first evidence-based, consensus newborn CPR guidelines in veterinary medicine, and they represent the work of 3 executive committee members, 3 domain chairs, 3 information specialists who completed all of the searches for the PICO questions, and 56 evidence evaluators (2 per PICO question) who read the primary literature identified and critically evaluated the evidence used to generate the guidelines. A draft algorithm is presented here (Figure 1) that summarizes the current preliminary newborn resuscitation guidelines. Be aware that some changes to these guidelines are likely after the consensus process.

Initial Assessment

As described above, the initial assessment attempts to identify patients not in need of resuscitation, including patients with normal parturition, a dam able to care for the newborn, and signs of being vigorous, breathing normally, and vocalizing. If all of these are present, the newborn should be placed with the dam, kept warm, and monitored with Apgar scoring for the first hour. Patients that do not meet these criteria should have the airway cleared gently, and be actively warmed, dried, and stimulated.

Reassessment

Once initial interventions are complete, assessment of heart rate, as a marker of hypoxia, is the next critical step. Any newborn with a heart rate < 120 beats / minute should have more aggressive respiratory support, including PPV with a tight-fitting mask, oxygen supplementation, and reversal drug administration if appropriate. If the heart rate remains below 120 per minute, intubation should be attempted if possible and the newborn ventilated with 100% oxygen. If despite these interventions the heart rate continues to drop to ≤ 50 beats / minute, interposed chest compressions and ventilations as described above should be initiated, as well as epinephrine therapy if there is not a response to CPR within ~ 1 minute.

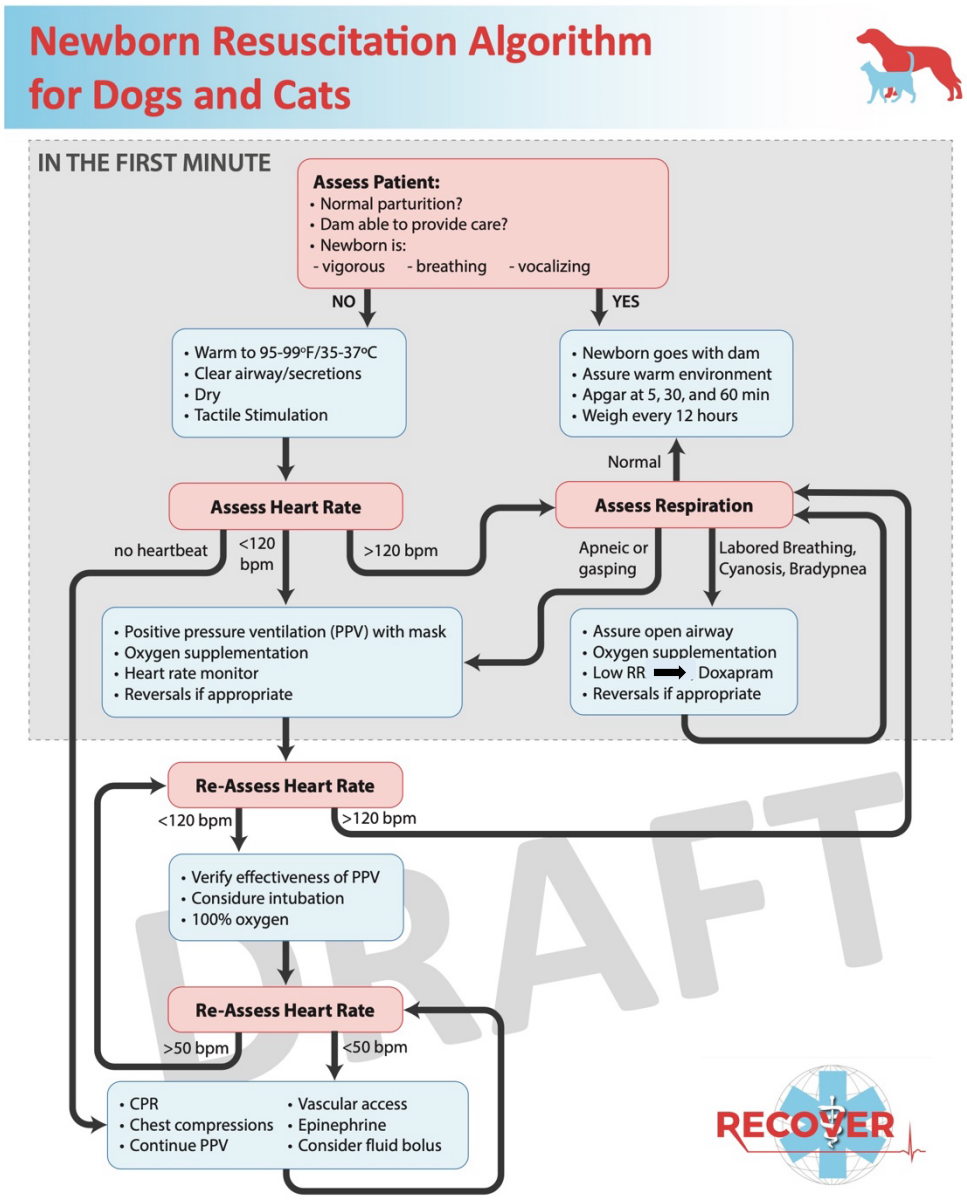
Stabilization

Resuscitation attempts may be de-escalated or stopped when the heart rate remains consistently above 120 bpm and the patient has normal respiratory effort and effectiveness. They should be monitored closely using Apgar scoring and returned to the dam as soon as reasonable.

CONCLUSIONS

Resuscitation of the newborn puppy or kitten is fundamentally different from CPR in the dog and cat. A focus on managing asphyxia is key, even if the clinical signs are primarily bradycardia. This is because of the physiology of the transitional period, which is fundamentally different that that in older neonates and pediatric dogs and cats. Early intervention to support respiration is essential to newborn resuscitation.

Figure 1: DRAFT RECOVER Newborn Resuscitation algorithm. Note that this algorithm has not yet completed the consensus process and is likely to change before being finalized.



Canine Cesarean Section: Emergency vs. Elective
How to plan and prepare

Janice Cain, DVM, DACVIM

Canine cesarean section (CS) is a subject well covered in the literature and discussed often at meetings and forums.[1] The aim of this lecture is to briefly review the indications/contraindications for emergency CS (Em-CS) and then to discuss in depth the concept/indications and procedures required for a successful planned, elective CS (El-CS). The first important concept is to emphasize that in most cases, CS is for the benefit and higher survival outcomes for the puppies. Other than in some brachycephalic breeds and individuals for which natural whelping can be quite physically difficult for the dam, the choice for CS is made primarily for puppy survival.

Emergency Cesarean Section

During a dystocia situation, the decision of whether to continue medical management or perform an emergency CS (Em-CS) is not always easy and often a matter of opinion or choice of both the attending veterinarian and owner. This subject has been recently reviewed.[2] Other than a brachycephalic dam in distress with non-progressive labor or in cases of obvious fetal obstruction, most dystocia occurrences are due to inertia. Most commonly the problem is secondary inertia: some pups delivered but then labor does not productively continue and can be refractory to medical intervention.

Presentation of such a case requires thorough assessment of the bitch for hypovolemia/dehydration, hypocalcemia or other underlying health conditions; the correction of which might restart progression of labor. Vaginal palpation to determine if a fetus is within the pelvis or vaginal canal can give additional information. Assessment of fetal well-being is necessary to make informed decisions. This is done ideally with ultrasonography to determine the number and viability of remaining fetuses. Assessment of fetal heart rate (FHR) is essential to determine whether any fetus is at eminent risk. The normal FHR is 180-200 bpm, with some transient decrease at the onset of labor and during contractions. Persistent FHR in the 140-160 bpm range, however, indicates fetal distress. The use of radiography at this stage will not assess fetal viability, is limited in aid to determine fetal obstruction, and is largely not helpful.

If the goal is to save the pup/puppies with low FHR, then movement to Em-CS is indicated ASAP. Time is critical in that situation: any delay or continued attempt at medial treatment for dystocia can lead to fetal demise. In one report, the overall fetal mortality was higher with Em-CS after at least one pup was vaginally delivered, as compared to Em-CS prior to any births.[3] The point of this is to show that with dystocia, waiting is not generally favorable to newborn survival. Survival of the bitch is of primary concern, however; the need to stabilize prior to anesthesia is obvious, but then moving to surgery quickly is ideal.

Another concept to consider is when NOT to do Em-CS. As above, the health outcome of the bitch is our primary concern. Every attempt is made to prevent an anesthetic related incident. If the dam is too weak or compromised but is otherwise not in distress due to fetal obstruction or uterine trauma (rare), then the correct decision is to wait, accepting that fetal loss *in utero* might occur. An important point to emphasize: *deceased fetuses within an intact uterus do not pose a health risk to*

the dam. There is no increase in post-partum metritis, sepsis, toxicity or other complications due to decaying fetuses and placenta material that is not removed at time of labor. Fetal material will pass through the birth canal within a few days, in most cases. While we do observe the dam for any sign of declining health, it is rare to have an issue with this approach. It is *absolutely* not indicated to do an Em-CS to remove deceased fetuses. Continued observation and recheck by US in a few days to a week or so post-partum is done to determine if any fetal material remains. Eventually (weeks later when the dam is stable and not as an emergency), hysterotomy can be performed to remove any fetal remains that do not pass, but in the author's experience, this is very rarely needed. Another concept: if there is a relatively large litter and 1-2 pups remain unborn, especially at the end of a long labor or after a good attempt at medical management to enhance chance for vaginal delivery, it is permissible to do nothing further! The need for Em-CS is a choice at that point for the owner and veterinarian to make. If the owner accepts the newborn survival outcome as it stands, an Em-CS can be declined. Some pups will be born alive after a considerable interval, but others might die *in utero* and pass later.

Elective Cesarean Section

An alternative to management of dystocia and potential Em-Cs is a planned, elective Cesarean section (El-CS). This approach to canine delivery has gained acceptance among breeders and veterinarians that routinely practice reproductive medicine. The primary advantage to El-CS is improved newborn survival. For example, in one report, all pups from El-CS survived, whereas newborn death rate ranged from 3% to 20% in litters born from both natural whelping or Em-CS.[4] Others have cited similar findings indicating improved newborn survival with El-CS compared to vaginal delivery or Em-CS due to dystocia.[5,6]

Some indications and considerations for an El-CS:

1. Indication based on breed: brachycephalic, "bully" breeds, some of the Molosser type and other giant breeds, and other breeds with known poor whelping success (poor newborn survival) such as Pembroke Welsh Corgis and Scottish Terriers.
2. Indication based large litter size and therefore risk of secondary inertia.
3. Indication based on 1-2 pup litter size and potential for delayed or lack of normal labor (primary inertia).
4. Indication based on anatomy: such as historical pelvic trauma or unremovable vaginal septum.
5. If tocolytic drugs are used to prevent pre-term labor, poor whelping ability can result.
6. Owner preference due to several reasons: concern for lack of a reliable emergency availability if an Em-CS is needed; preference for consistent veterinary care/staff relationship; prior poor outcome with this dam or another due to Em-CS or difficult whelping; concerns regarding cost difference between El-CS and Em-CS.

Additionally, it is important to also consider potential negative aspects of the idea for planned El-CS:

1. Any anesthetic/surgical procedure has inherent, unavoidable degree of risk.
2. Pain/discomfort post-surgery and disorientation at time of anesthesia recovery.
3. Potential complications of wound healing, risk of infection, adhesions.
4. Possible delay in mothering behavior by the dam after anesthesia/surgery.
5. Possible decrease in milk production/milk letdown.

6. More work for the owner to care for pups and strictly observe dam/pups for several days after surgery.
7. Cost to owner as opposed to uneventful natural whelping.

Planning the elective Cesarean section

Once the expected due date (EDD) is established, a planned El-CS can be scheduled for 1 or 2 days before the EDD. Two days before the EDD is the choice for dams with a large litter because early onset of normal labor is possible. Also, consider familial history or specific history of prior litters for this dam, when possible. As well, consider the tolerance and service availability for the owner to seek Em-CS if labor starts before the planned El-CS date. One day before the EDD is routine for small to medium sized litters. Surgery can be scheduled on the EDD for a dam with a single fetus since they typically do not go into labor early or at all (lack of fetal stimulation to initiate labor due to only a singleton).

It is important to inform the client that the planned El-CS in advance of the onset of labor can be a bit unsettling at first! Usually, there are no signs of labor, the dam is comfortable and would eat if allowed, no nesting or other signs of discomfort. Measurement of serum progesterone level at this time can be above 2 ng/ml (the level consistent with labor initiation) and likewise, no drop in body temperature. Typically, colostrum production is adequate.

Establishing the EDD is largely dependent on accurate determination of the date of ovulation. The EDD is 63 days (+/- 1 day) from ovulation. Some breeds will vary, including Cavalier King Charles Spaniels (slightly shorter gestational length) and Greyhounds (slightly longer gestational length).[7,8] Accurate determination of ovulation date is the key! Assessment of ovulation in the bitch is done indirectly, by the evaluation of parameters concurrent with ovulation, rather than direct determination of ovulation by ultrasonographic inspection or palpation.

Ovulation is triggered by release of LH (typically a 2-day lag for ovulation after the LH peak). Measurement of serum LH can be used, but not as the sole factor since the peak can be missed even with daily blood sampling. Most commonly, serial measurement of serum progesterone levels is used since the initial rise from basal levels is consistent with the LH peak.[9] Interpretation depends on the assay system used (reference laboratory chemiluminescence or in-clinic assay systems) since the value obtained by these different systems will vary. In general, interpretation of the progesterone curve can be as follows:

Basal value: <1.5 ng/ml
LH peak: 1.6 to 2.5 ng/ml
Ovulation: 3.8 to 6 ng/ml
Mature ova: 6 to 20 ng/ml

It is best to start progesterone testing relatively early (day 5-7 after the onset of proestrus) and then repeat testing at least every other day. If concurrent LH testing is planned, then daily serum samples can be frozen and later measured for LH to better confirm the LH peak.

If the goal is to plan an El-CS, but accurate determination of ovulation date was not done prior to breeding, it is very difficult to select the correct surgery date in advance. Some other methods to help determine gestational age are variable by breed and not as reliable, but include:[10-14]

1. Ultrasonography (US) 23-28 days after breeding and measurement of vesicle size. Other US measurements mid-gestation. All can be variable.
2. Determination of the end of estrus by vaginal cytology. Gestational length after the onset of diestrus is 57 +/- 4 days.

Other parameters used very close to term but can vary by several days. Note: When using the parameters below, the ability to plan in advance is difficult and could require the veterinary staff to be available with little notice.

3. Radiography to assess tooth bud formation and ossification of distal extremities.
4. US to assess fetal GI motility and mature appearance of kidneys.
5. Evaluation of declining progesterone (typically <2 ng/ml) indicates labor is due to start within 1-48 hours (aka "reverse" progesterone testing). Note: this is not reliable in all pregnancies and the progesterone level can drop precipitously (within hours) from last measurement. Progesterone might not drop at all when a single fetus is at term.
6. Evaluation of body temperature (measured rectally) tends to fall to levels under 99F at time of progesterone decline (inconsistent).
7. Tocodynamometry to determine onset of contractions.

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Canine Cesarean Section:
Anesthesia, Surgical tidbits and newborn recovery tips

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Canine Cesarean section (CS) is commonly performed by specialists, emergency clinic practitioners, and those with a special interest in canine reproduction. A variety of methods and preferences exist for many aspects of the CS. The purpose of this discussion is to present ideas for anesthesia protocols, surgical technique, and highlights of newborn recovery. Continuing from the previous lecture, Canine Cesarean Section: Emergency vs. Elective, this discussion will emphasize cases for which elective C-section (El-CS) is planned. These recommendations can also be used for emergency C-section (Em-CS) cases; the difference concerns timing for surgical preparation and stabilization of the dam if presented for Em-CS due to dystocia.

Anesthesia for the Canine Cesarean Section

The goal of any anesthetic protocol is to provide humane unconsciousness, limit or block pain sensation, and a comfortable recovery. Added to this for a CS are the factors for newborn revival and speedy recovery of the dam so she can attend to pups in a reasonable timeframe. Anesthetic considerations and protocols for canine CS were recently reviewed.[1]

Physiological factors due to pregnancy and outcomes that can affect anesthesia include:

1. Anemia of pregnancy is expected. A normal hematocrit can indicate dehydration.
2. Decreased vascular resistance with increased cardiac output typically results in a normo-tensive patient. If hypotension persists after appropriate fluid treatment, consider ephedrine as a pressor agent.[2]
3. Increase in oxygen demand coupled with reduced respiratory volume. Pre-oxygenate prior to induction, intubate quickly and monitor closely for apnea.
4. Decreased tone of the lower esophageal sphincter coupled with increased abdominal pressure increases the risk for regurgitation. Be sure patient is adequately fasted if an El-CS. Intubate quickly and extubate with care and attention.

Pre-anesthesia sedatives/antinociceptive agents

The use of a pre-anesthesia sedative can lower the induction drug dose, calm the patient prior to anesthesia, and is recommended. Some published studies report avoidance of any pre-anesthesia sedative or pain control medication. This could be due to the nature of the study design to measure the effects of induction agents and, in the author's opinion, is not ideal.

Opioids[3-5] The use of an opioid prior to anesthetic induction is recommended and administered approximately 15 minutes prior to induction. If newborns seem sluggish at delivery, reversal with naloxone is possible: 1 drop (0.1ml) sublingual (or 0.02mg/kg IM).

1. Morphine: low lipid solubility (0.1-0.3 mg/kg IV)
2. Methadone: similar to morphine, less sedative (0.1-0.2 mg/kg IV)
3. Hydromorphone: relatively medium level of solubility (0.05-0.075 mg/kg IV)
4. Fentanyl: highly soluble and crosses placenta at higher dose: not recommended
5. Buprenorphine: mixed mu antagonist and not fully reversible, not recommended

Dexmedetomidine[6,7] For a stable patient (as in El-CS cases), low doses can be very helpful to sedate a highly-reactive dam with little to no effect on newborn recovery. Dose: 2-4 mcg/kg (0.002-0.004 mg/kg) IV or IM if necessary. Can be reversed in both dam and newborns with atipamezole if needed.

Phenothiazines and benzodiazepines[8,9] are not recommended due to prolonged newborn recovery.

Anticholinergic agents

Bradycardia can occur secondary to Increased vagal tone induced by intubation and handling of the gravid uterus. Atropine is avoided due to placental transfer that can increase the HR of fetuses. Low fetal HR is primarily due to hypoxemia and an indicator of stress. Artificial increase in fetal HR, without correction of the underlying cause of bradycardia (e.g. low uterine blood flow), could be contraindicated. The use of glycopyrrolate (0.005–0.01 mg/kg IV) is a preferred to maintain normal HR in the dam while not affecting fetal HR; its large polar structure and low lipid solubility prevents significant placental transfer.[10] Since anticholinergics can also cause gastric stasis, the author prefers to use glycopyrrolate only if indicated to treat maternal bradycardia rather than as part of the routine pre-anesthetic regimen.

Induction agents

The goal is to provide rapid, safe intubation and oxygen support and a level of anesthesia that will alleviate stress and reactivity.

Propofol[2,5, 6, 11-13] Introduction of propofol into the veterinary toolbox greatly changed the entire protocol for CS. The drug is rapidly cleared from maternal and fetal circulation and CNS impact by rapid redistribution and hepatic metabolism. Additionally, return to maternal circulation from the fetus is possible. Patients can be induced safely and quickly, intubated rapidly and little impact is seen in newborn recovery. Recovery is typically smooth and rapid. The standard protocol for this author, is to induce with a titrated dose of propofol to effect (safely, comfortably intubated); typically 4-6 mg/kg IV).

Alfaxalone[14,15] Another good choice due to low cardiovascular depressant effects and preferred by some individuals, especially for patients that are not entirely stable and at a higher risk of anesthesia complications. Also, minimal impact on newborn recovery is seen. Possible short-term tremors or excitement upon recovery, especially since benzodiazepine use is contraindicated for CS. Dose range 1.6-3 mg/kg IV and can be repeated as needed for intubation.

Ketamine and barbiturates[16,17] not recommended for CS. High CNS depression and potential for prolonged depression in newborns.

Inhalant agents: Not recommended for induction since rapid control of airway is needed.

Anesthesia maintenance

Propofol or Alfaxalone, continued titration or CRI: Several protocols have evaluated the use of either propofol or Alfaxalone administered by constant rate infusion (CRI) for anesthesia maintenance with varied results.[4,15,18] Several studies were underpowered as regards number of cases, such that conclusions are difficult. This author finds the best result when propofol is *titrated to effect by intermittent boluses* to maintain anesthesia between time of

induction and complete newborn delivery (15-20 minutes) as opposed to the CRI in those reports. Intermittent boluses of 1-3 ml IV (0.5-2 mg/kg) are given to maintain a light but humane level of maintenance anesthesia. All newborns are quickly delivered, and then inhalant anesthesia is started and continued for the remainer of surgery.

Inhalants [2,11,19] Fetuses exposed to inhalant anesthesia can have delayed recovery. These agents can rapidly cross the placenta and lead to both decreased blood pressure and respiratory depression at time of newborn resuscitation. As well, due physiological effects of pregnancy, the minimum alveolar concentration dose is greatly reduced.[20] For these reasons, it is ideal to use very low dose setting or to delay use of inhalant agents until after delivery of all pups.

Local or regional anesthesia

Epidural. Protocols have been described using bupivacaine or lidocaine often mixed with an opioid.[4,21,22] Advantages of an epidural include good pain control and possibly lower doses of other anesthetic agents (induction and maintenance). Good newborn recovery is expected. Disadvantages are mostly based on operator expertise that will affect time and proper dosing. If it takes too long to accomplish, then the benefit is greatly outweighed. Also, prolonged recovery of the bitch to the point of normal ambulatory and maternal ability is possible.

Local: A rapid, effective agent such as lidocaine injected by an infiltrative method into the incisional area (superficial and deep) can help with pain management. This can reduce the total dose of anesthesia needed for CS and help with post-operative pain control. Bupivacaine (short or long acting) can also be considered.

Other peri-anesthetic considerations:

1. IV fluid support during surgery is routine.
2. Prevention of reflux/regurgitation/aspiration. A considerable risk is aspiration of gastric fluid due to its acidic nature and consequent Mendelson syndrome (fatal pneumonitis). Consider a proton pump inhibitor and or metoclopramide.[23]
3. Infection prevention. A single dose of a broad-spectrum antibiotic (e.g. cefazolin) prior to the start of surgery can be considered. Repeated or post-op antibiotics are not indicated.
4. Promotion of fetal maturity. When planning the El-CS properly, there is no concern for fetal maturity. Reports using both aglepristone (a progesterone antagonist) and dexamethasone to theoretically improve surfactant production have been either inconclusive or do not support these protocols.[24,25]
5. Oxytocin to increase uterine contractions and milk let-down. This can be started once pups delivered. 1-2 IU SQ and repeated in 15-20 minutes.
6. Post-operative pain control for the dam. Most commonly NSAIDS are used immediately post-operatively and then as needed for 1-3 days. Low concerns for passage into milk, especially early in lactation.[26,27]

Surgical Tidbits and Advice

Detailed CS surgical procedures have been described in a variety of veterinary texts and on-line resources. A few ideas and areas to emphasize:

1. Speed can be helpful to decrease exposure of the fetuses to anesthetic agents: limit the time between induction and onset of surgery. All should be in place and surgeon scrubbed and ready. Preparation of the surgical area with the appropriate equipment for surgery, monitoring and post-delivery newborn care is essential.
2. Dorsal recumbency (tilting is not needed) and rapid laparotomy incision. Typically quite easy due to abdominal distention and thinning of the subcutaneous tissue in the linea area.
3. Only expose the portion of the uterus at a time. Exposing only a portion of the uterus will enhance care of the sterile field and help with temperature support.
4. Try to find the bifurcation and incise on either side. Usually the intercornual septum (between the uterine body and horns) can be gently stretched to allow delivery of all pups through one incision. Sometimes this is not possible and another incision in the opposite horn will be needed. The author does not recommend an *en bloc* hysterectomy for puppy delivery.
5. Each pup is worked to the hysterotomy incision and delivered. The surgeon can use a laparotomy sponge to remove amniotic membranes from the newborns' face/muzzle. The newborn will often start to breath at this time.
6. The umbilicus is clamped (a non-traumatic clamp is ideal) and the cord cut on the maternal side of the clamp. An additional clamp (maternal side) is not needed.
7. The pup is delivered to the resuscitation team in a manner to maintain the surgeon's sterile field. The author prefers to deposit the pup, on a surgical towel, on an adjacent mayo-stand.
8. The placental unit is removed with gentle traction and wiping using a laparotomy sponge.
9. Repeated until all pups are delivered. Very important to palpate to both utero-tubular junctions/ovaries and down into pelvic canal to be sure that no pups remain!
10. Inspection at this time of the entire endometrial surface (done by inverting each horn) to remove any remaining placental tissue and decayed material from fetal resorptive sites. Not an indication for hysterectomy.
11. Note: the author does not support OVH at the time of CS. Ovariectomy (OE) can be considered but post-op hemorrhage is still of concern. The author typically sends dam/pups home 60-90 minutes after completion of surgery and thus does not perform OE (would need to monitor longer for potential hemorrhage). If the dam/pups are kept at the veterinary facility longer, staffing must be adequate to start/supervise nursing and care for all.

Early Newborn Recovery

When performing an El-CS, newborn recovery is typically quite successful. When the timing has been conducted properly to know the EDD and therefore the correct surgical date, and the anesthesia performed in such a way to minimize fetal exposure, pups are typically very strong and easy to recover. Some areas of advice include[28]:

1. Stimulation by drying and briskly rubbing will typically stimulate crying.
2. Aspiration of amnionic fluid from pharynx by gentle suction using a newborn bulb syringe or DeLee mucus trap-suction device.
3. Continued temperature support is essential. Newborns can chill rapidly.
4. Ligate umbilical cords and treat umbilical stump with an antiseptic agent (dilute chlorhexidine or iodine solution).

5. If a newborn does not respond positively to the above, institute naloxone (0.1ml sublingually) then immediately provide breathing support. Various devices are available to aid newborn breathing (puppy AMBU-bag) and oxygen support.
6. If HR is slow or non-existent, can try 0.01 ml of epinephrine sublingually. Gentle chest compressions can be started.
7. Use of the Jen Chung, acupuncture GV 27 needle placement (nasal philtrum) can be added.
8. If prolonged recovery, use of an intraosseous catheter can be considered. Ultimately intracardiac injection can be attempted, but the prognosis worsens if at this state.
9. Pups are not swung, shaken or briskly inverted. Cerebral hemorrhage an occur.
10. Use of doxapram to stimulate respiration is controversial in human medicine. Increased cerebral oxygen demand can counteract any observed benefit.[29] The author does not recommend.
11. Dextrose (50%) can be applied to oral mucus membranes in a pup that is too weak to nurse.
12. Caffeine is used in human infants with apnea of prematurity. Extrapolating the dose: 10 mg/kg orally (sublingual) might help with a pup that is not thriving, but its use in veterinary medicine has not been assessed.

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Genetic Counseling and Genetic Diversity

Reproductive Challenges and Preservation of Historical Dog Breeds

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Basic Genetics Terms and Concepts

The **gene** is the basic unit of inheritance. Genes are passed from parents to their offspring and encode the information needed for physical and biological functions. A **genetic mutation** or **variant** is a permanent change in the **DNA sequence** (or the order of the **nucleotides**: **adenine**, **cytosine**, **guanine**, and **thymine**) that makes up a gene. While the terms “mutation” and “variant” are used fairly interchangeably in the literature, “variant” is more inclusive as not all of these changes are deleterious. The **genotype** of an organism is its complete set of genetic material. This term can also be used to refer to the allele an individual carries at a particular genetic location, or **locus**. An **allele** is a form of a gene; this may be **wild-type** (the naturally occurring form of that gene in nature) or **variant/mutant-type**.

Ploidy refers to the number of sets of chromosomes in an organism. Dogs (and most other mammals) are **diploid**, meaning they have two copies of each chromosome. This also means dogs have two copies of an allele (either wild-type or variant/mutant-type) at any given locus, one of which is inherited from the sire while the other is inherited from the dam. A dog may therefore be **homozygous**, or possess two identical alleles at a particular locus, or **heterozygous** (possessing two different alleles at a particular locus). **Autosomes** are the numbered chromosomes; dogs have 38 pairs of autosomes and one pair of **sex chromosomes** (XX - female or XY - male).

Phenotype refers to the set of observable characteristics of an organism resulting from the interaction of its genotype with the environment. In the dog, this term may refer to disease states, behavior, physical traits, or laboratory values, among others.

The **mode of inheritance** refers to how a variant is passed down to the next generation. This concept can also be thought of as the number of copies of a variant required to be at-risk for the associated disease or to possess the associated trait. The following modes of inheritance are most common and typically refer to simple/Mendelian traits and diseases:

- **Autosomal dominant** - one copy of the associated variant is needed to be at-risk for a disease or possess a trait. At least one parent will also be at-risk or possess the associated trait. The disease or trait is typically present in every generation.
- **Autosomal recessive** - two copies of the associated variant are needed to be at-risk for a disease or possess a trait. The parents may or may not have also been at-risk or possess the trait, depending on their genotypes. If they were not at-risk, both parents must have been **carriers** of the variant. The disease or trait is not typically present in every generation.
- **X-linked recessive** - because male animals only possess a single X chromosome, males need only one copy of the associated variant to be at-risk for a disease or possess a trait. Males are more commonly affected and may appear in every generation. Female animals typically require two copies, though this is not a perfect science due to a phenomenon called **X-inactivation**. X-inactivation refers to the random process by which one X chromosome is inactivated during embryonic development. Both the maternal and the paternal X chromosome have an equal chance of inactivation. Females may display a phenotype ranging from clinically unaffected to fully affected, depending on the disease in question, the pattern of X-inactivation, and the individual.

- **Codominant** - with this inheritance pattern, both alleles are expressed rather than one over the other. For example, a variant in the *ADAMTS17* gene is associated with primary lens luxation in several dog breeds (Farias et al. 2010). Most individuals homozygous for this variant ultimately develop lens luxation (Gould et al. 2011); however, heterozygous individuals also have a small chance of developing this condition (Elston et al. 2012).

Polygenic conditions refer to diseases or traits that are controlled by two or more genetic variants and are therefore not inherited in a simple Mendelian fashion. **Complex or multifactorial conditions** are diseases or traits that are controlled by inherited factors as well as environmental factors. Polygenic and complex/multifactorial diseases and traits are not easily genetically tested.

The **allele frequency** refers to the proportion of a variant allele at a specific locus, usually represented as a percentage or a decimal. The allele frequency will range from 0.00 to 1.00, with 0.00 meaning no dogs within the population possess any copies of the variant allele and 1.00 meaning all individuals within the population possess two copies of the variant allele (Rezaei et al. 2013).

Penetrance refers to the proportion of individuals possessing the genotype that go on to display the associated phenotype (Zangerl et al. 2006). For example, progressive rod-cone degeneration, a type of progressive retinal atrophy in the dog, has near-complete penetrance (Zangerl et al. 2006). This means almost all individuals with two copies of the associated autosomal recessive variant in the *PRCD* gene will experience vision loss. Penetrance is variant-dependent.

Variable expression refers to the varying symptoms that may be present in different individuals with the same genotype. For example, ichthyosis caused by an autosomal recessive variant in the *PNPLA1* gene tends to have extremely variable expression; some dogs experience severe flaking of the skin that is minimally responsive to treatment, while others experience few symptoms (if any). Expression is individual-dependent (Grall et al. 2012).

Genetic screening refers to the practice of testing a population for known inherited disease-associated alleles to identify individuals that are at-risk for the associated diseases, and to identify individuals with the potential to pass disease-associated alleles to their offspring. The results of a genetic test are prognostic, rather than diagnostic; the genotype of an individual is able to determine what known diseases a dog may be at-risk of developing or passing on. The likelihood of disease development depends on the penetrance of the variant and the expression of the individual.

Genetic Health Testing

There are two basic types of genetic variant testing: **direct variant testing** and **linkage-based testing**. Direct variant tests assay the exact disease-associated or trait-associated gene and determine the genotype at that locus. Linkage tests do not test for a variant directly; rather, this type of test detects a gene or genes that are physically very close to the variant of interest on the chromosome. These genes remain in close proximity during meiosis, and therefore they are expected to be inherited together; the presence of a linked gene infers the presence of the variant of interest. Linkage tests may have slightly lower accuracy than direct variant testing, though predictive accuracy for this type of test remains high (Pulst et al. 1999).

Polygenic conditions may be assessed by a **polygenic risk score** (PRS) in some cases. A PRS is determined by utilizing several different genetic variants (which are known to have a collective influence) to assess the risk of a certain condition. For example, a genome-wide association study in the Labrador retriever found 99 regions of association with cranial cruciate ligament rupture (Baker et al. 2017). These regions explained 48-56% of the phenotypic variation observed, suggesting that cranial cruciate ligament rupture in this breed is both highly polygenic and moderately heritable. Polygenic risk scores remain in their infancy in the dog, though they will likely become more common in the future.

The accuracy of disease-associated allele testing is typically high for most reputable genetics companies and university laboratories, regardless of the technology utilized. Though not exhaustive, the Orthopedic Foundation for Animals maintains a list of laboratories appropriate for use in canines. There is no required regulatory oversight for veterinary genetic testing in North America; therefore, selection of a reputable company is imperative.

Genetic Diversity

The **coefficient of inbreeding** (COI) is defined as the likelihood of inheriting two identical alleles from each parent. It is typically expressed as a percentage and represents the fraction of all genes that are expected to be homozygous in an individual. For example, an individual with a COI of 20% would have a one in five chance of homozygosity at a particular locus, and 20% of the loci in that individual would be expected to be homozygous. The COI of an individual increases with increased relatedness of its parents. Some degree of inbreeding is necessary to maintain the desirable, consistent characteristics and traits of a breed (Meyers-Wallen et al. 2003).

The COI can be estimated by using pedigree analysis or by genomic measurements based on actual homozygosity. Pedigree-based COIs depend heavily on recordkeeping accuracy and can deviate substantially from genomic-based COIs due to recombination, segregation, and relatedness of founding members of a breed (Chu et al. 2019).

Inbreeding depression describes the reduced fitness and fecundity found in the offspring of related individuals (Charlesworth et al. 2009). This phenomenon has been found to occur in humans, animals, and plants and is predominantly caused by increased homozygosity of individuals and the presence of deleterious recessive variants within the population. Inbreeding depression has also been identified in the dog (Leroy et al. 2015; Bannasch et al. 2021; Yordy et al. 2020), where reduced fecundity, shortened lifespan, reduced neonatal survival, and increased morbidity have been associated with an increased measure of inbreeding (Chu et al. 2019). Each 10% increase in genomic COI in the Golden Retriever resulted in one fewer puppy per litter (Chu et al. 2019). Additionally, an increased deleterious variant load has been associated with an increased measure of inbreeding (Donner et al. 2023). Recent inbreeding (compared to historic inbreeding) may have a more detrimental effect, as there is less time for natural or artificial selection to remove potentially deleterious recessive alleles brought together by the recent common ancestor (Yordy et al. 2020).

Hybrid vigor, also called **heterosis**, has not been well-characterized in the dog. This term refers to the improved average fitness for a given trait of cross-bred first-generation offspring when compared to the average fitness of either parent strain or breed. Traits with lower heritability are expected to benefit most from hybrid vigor, and heterosis is enhanced when the two parent strains or breeds are distantly (rather than closely) related. Hybrid vigor rapidly

dissipates, with the offspring of two first-generation cross-bred animals expected to have approximately half the heterosis of their parents. As the COI increases for each parent, the magnitude of heterosis of the offspring is also expected to increase. When compared to plant species, the benefits of hybrid vigor are not as pronounced for animal species (Nicholas 2010).

The study of true heterosis in the dog has largely been limited by a lack of precise classification of mixed breed dogs, though its presence in other species implies hybrid vigor also exists in the dog. Conclusions of the available studies on true heterosis in this species have shown mixed results, with both purebred and crossbred dogs found to have a higher prevalence of the studied trait (Nicholas et al. 2016).

Outbreeding depression describes the loss of fitness experienced by interpopulation hybrids (especially second generation, or F2 hybrids), and may occur due to the disruption of interactions between the genome and the environment or disruption of interactions between the genes themselves (Edmands et al. 2007). For example, F2 hybrids resulting from interbreeding of two geographically and genetically distinct populations of largemouth bass were found to have a mortality rate 3.6 times greater than either F1 hybrids or native fish when experimentally infected with a novel virus (Goldberg et al. 2005). This phenomenon has not been well-studied in mammals, and most studies have evaluated the effects of crossbreeding between species (Adavoudi et al. 2022). To the author's knowledge, the presence and effect of outbreeding depression have not been studied in the dog.

Genetic Counseling

Problems Faced by Purebred Dogs
Many breeds are predisposed to certain proven or presumed inherited health conditions. Examples include lymphoma in the Golden retriever and BOAS in the Bulldog. These breed predilections are likely because of both the loss of heterogeneity (increase in COI and therefore the accumulation of deleterious genetic mutations) as well as the exaggeration of anatomical features, which may be considered desirable by the breed's standard (Hedhammar et al. 2011).

General Breeding Recommendations
An ideal breeding candidate is historically healthy, passes all breed-required health testing, has a nice temperament, meets its breed standard, contributes positively to its breed, meets the goals of their breeder, and helps maintain the genetic diversity of the breed. With many breeds (especially those with a small gene pool or those with high rates of inherited disorders whose registration bodies disallow outcrossing to other breeds), some of these factors may need to be sacrificed to maintain a robust breeding population.

Application of Genetic Variant Testing Results to a Breeding Program
To effectively apply genetic testing results to a breeding program, the breed of dog, the variant, and the variant's mode of inheritance must all be taken into consideration.

Certain genetic variants are expected to increase risk of disease in certain breeds or populations, but not in others. For example, a variant in the *RPGRIP1* gene leads to a type of cone-rod dystrophy (PRA-crd4/cord1) in the Miniature Longhaired Dachshund. However, Labrador retrievers and French bulldogs also harbor this variant but appear to be clinically unaffected (Mellersh et al. 2006).

Other genetic variants are seen at a very high rate in certain breeds and therefore eliminating them from a population may result in a precipitous loss of genetic diversity. For example, an *FGF4* retrogene on chromosome 12 has been associated with chondrodystrophy and type I intervertebral disc disease in a variety of breeds. In the French bulldog, where intervertebral disc disease is a clinical concern, the allele frequency of this variant is 0.94 (Batcher et al. 2019). This means there are few individuals with one or no copies of this variant, and elimination of the variant must be done very slowly to avoid a genetic diversity bottleneck. This practice necessitates breeding individuals with one or two copies of this variant with careful mate selection. In instances where disease-associated variants are fixed within a population (allele frequency of 1.00), elimination of the variant is impossible without an outcrossing program to other breeds.

In cases where a variant is not fixed and is expected to result in disease, the mode of inheritance must be considered. In all cases, the allele frequency of the variant in question must be assessed prior to making breeding decisions. Additionally, the disease phenotype and its impact on the health and welfare of the dog should be considered, as well as the availability of a prevention or treatment strategy.

Generally speaking, for autosomal recessive variants in which the allele frequency is low in a given population, two carriers should not be bred to one another. Doing so will result in approximately 25% of the litter inheriting two copies of the variant allele, which means they will be at risk for the associated condition. However, carriers of autosomal recessive variants that possess other desirable traits may be bred to homozygous wild-type individuals, as this mating helps preserve genetic diversity and will not result in disease in the offspring (Bell et al. 2011; Meyers-Wallen et al. 2003).

An individual at-risk for an autosomal recessive condition may be bred to a homozygous wild-type individual, provided they are otherwise an exceptional breeding candidate and breeding will not exacerbate their condition. All puppies resulting from this pairing would be carriers of the condition. This strategy will preserve desired traits, but the affected animal should be replaced by a normal descendent that retains the desired traits when possible (Traas et al. 2006).

For autosomal dominant and codominant variants in populations where elimination of individuals from the gene pool is not expected to rapidly decrease genetic diversity, individuals with one or two copies of the variant should ideally not be bred. Instead, a first-order relative that does not possess the disease-associated allele may be utilized if available (Bell et al. 2011). If breeding these individuals is necessary to maintain genetic diversity, they should be replaced by a homozygous normal offspring when possible (Traas et al. 2006).

For X-linked variants, normal males may be bred as they cannot pass on the variant associated with these diseases. Known carrier females should not be bred, as all their male offspring would be at-risk (Traas et al. 2006).

Application of Phenotypic Health Testing Results to a Breeding Program

Studies have shown dogs judged as having phenotypically normal hips by OFA ratings may still have clinically relevant hip laxity (as shown by a PennHIP Distraction Index [DI] ≥ 0.3), suggesting OFA ratings underdiagnose canine hip dysplasia (Powers et al. 2010). Additionally, applying selection pressure to the objective measure of DI in lieu of or in addition to a subjective score has been shown to improve hip joint conformation in a population of purpose-bred dogs (Haney et al. 2020). However, selecting breeding candidates based solely on a DI of ≤ 0.3 may

result in a precipitous decrease in genetic diversity (Keller et al. 2011). The OFA database is also public, which offers better transparency and the ability to evaluate hip scores for all tested relatives (including distantly related relatives). This information can be utilized to calculate **Estimated Breeding Values** (EBVs) to better select for phenotypically normal offspring (Keller et al. 2011).

Although Grade I Dysplastic elbows typically do not produce clinical disease, progeny from a parent with this radiographic diagnosis (when bred to any other elbow grade) have been shown to have an increased frequency of elbow dysplasia (Keller et al. 2011). Therefore, treating Grade I Dysplastic elbows as “normal” (a strategy employed by some breed groups) is not recommended.

OFA eye examinations currently offer two categories of breeding advice. If substantial evidence exists of the heritability of a condition and/or the condition is expected to result in a loss of vision or other ocular function, the recommendation is not to breed that individual. As of 2021, there are eleven conditions that fall under this category: keratoconjunctivitis sicca, glaucoma, certain types of persistent pupillary membranes, cataracts, lens luxation or subluxation, persistent hyperplastic primary vitreous/tunica vasculosa lentis, retinal detachment, retinal atrophy, retinal dysplasia, optic nerve hypoplasia, and optic nerve coloboma.

The second category of breeding advice offered by OFA eye examinations is “breeder option”. This category is utilized when caution should be exercised in breeding that individual. This category may be modified to recommend not breeding or it may be removed as more evidence is accumulated regarding the heritability of the condition.

The Respiratory Function Grading Scheme for BOAS recommends removal of Grade 3 individuals from the breeding program. Dogs testing in Grades 0 and 1 are considered good breeding candidates, and dogs testing in Grade 2 may be used with caution (though should not be bred to other Grade 2 individuals). Because the inheritance of BOAS is not fully understood, it is still possible to produce affected puppies even if the RFGS is used responsibly.

Estimated Breeding Values (EBVs)

EBVs are a statistical tool that can be used in canine breeding to predict an animal's genetic merit for a specific trait, like hip or elbow dysplasia. These values provide an estimate of the likelihood of a particular animal having a desirable genetic makeup for the trait in question compared to the other animals in the dataset. These measures are typically used for polygenic traits that are not easily genetically tested.

Estimated breeding values are calculated using phenotypic data for a specific animal (if available) as well as all relatives (weighted according to relationship). The data used to calculate the EBV typically includes the trait of interest (for example, hip dysplasia) as well as any other measures that may impact the expression of the trait (sex, size, age, etc.) and that trait’s estimated heritability.

To obtain an accurate EBV, data on most or all dogs within the population is required. At least five generations of a pedigree are typically utilized, and not only breeding dogs are considered. Consistent measures also increase accuracy, which can be challenging for more subjective data such as qualitative hip scores. The accuracy of a particular EBV increases with more data, especially as progeny or sibling data become available.

The Finnish Spitz successfully utilized EBVs to decrease the rate of epilepsy in their breed. This required a concerted effort between several kennel clubs to maintain complete pedigree records as well as transparency from breeders. Pedigree data was used to calculate an “Epi Index”, which was the EBV of epilepsy in this breed. A specific breeding strategy was utilized that prohibited the use of epileptic dogs (or their siblings or progeny) or dogs that had produced epileptic offspring. However, dogs with epileptic relatives could be used and were paired smartly based on their Epi Index. Since beginning this strategy, epilepsy frequency has fallen from 5-6% to less than 1% (a smaller risk than that found in most other breeds). This is an excellent example of using EBVs to decrease the incidence of a polygenic trait.

Breed Standards and Type

Elimination of certain conditions may not be possible without changing breed standards, as some disease processes are directly related to conformation (such as BOAS in brachycephalic breeds or entropion in the Chinese Shar-Pei) (Asher et al. 2009; Traas et al. 2006). In these cases, breeding individuals with less extreme phenotypes is desirable to slowly change the morphology of the breed over time.

Maintaining Genetic Diversity

Average COI will vary greatly depending on breed, and there is no one-size-fits-all approach to maximize genetic diversity within a population. Many canine registries maintain closed stud books, meaning individuals of other breeds are not permitted to be introduced into the breed population (Traas et al. 2006). Additionally, many breeds have experienced the effects of a genetic bottleneck or popular sire syndrome at some point in their histories, decreasing the available number of breeding animals (Hedhammar et al. 2011; Leroy et al. 2011). Therefore, knowledge of the breed is essential when making decisions about maintaining genetic diversity.

In some cases, the level of inbreeding of a population may be unsustainable, resulting in the need for a “**genetic rescue**”. This is the terminology used to describe the introduction of new, unrelated individuals into a closed population with the goal of decreasing inbreeding-related health concerns.

The Norwegian Lundehund is a breed with a small population currently undergoing a genetic rescue program due to high inbreeding levels. All current purebred Norwegian Lundehunds are related to four individuals, who were also related to one another. This breed has a very high coefficient of inbreeding, and suffers from small litter size, low sperm quality, and intestinal lymphangiectasia (Melis et al. 2022). An outcross program was started to cross the Lundehund with three other breeds: the Norrbottenspets, the Icelandic Sheepdog, and the Norwegian Buhund. The aims of this project are to preserve the breed’s unique traits (such as polydactyly) while increasing litter size and decreasing the prevalence of lymphangiectasia. This project is ongoing, but initial data show the F2 generation showed higher homozygosity than the F1 generation or the Norwegian Buhund, but lower homozygosity than the Norwegian Lundehund (Melis et al. 2022). This indicates some of the acquired diversity was lost on the initial backcross to the purebred Lundehund (Melis et al. 2022).

Conclusion

Breeding healthy dogs is an imperfect science. Choosing which individuals are acceptable breeding candidates is a balancing act, and decisions should be made by the breeder and the attending veterinarian together.

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Its Been 4 Hours Now What? Priapism and Paraphimosis

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Priapism is a persistent penile erection lasting longer than 4 hours, without sexual stimulation. Priapism can be confused with paraphimosis. Paraphimosis occurs when the non erect penis cannot be ensheathed in (returned to) the prepuce. Although the penis is not erect, it may be edematous from extrusion. Paraphimosis may result from too small of a preputial orifice, inward rolling of the preputial orifice, inadequate length of the prepuce, weakened preputial muscles or trauma. Phimosis occurs when the penis cannot be extruded from the prepuce, and can occur in conjunction with priapism. Priapism is infrequently reported in dogs and cats. Idiopathic, neurological, and traumatic injuries have been reported causes in dogs and cats. Priapism in people, is categorized as either non-ischemic (arterial, high flow) or ischemic (veno-occlusive, low flow). Non-ischemic priapism, caused by increased arterial inflow through the corpus cavernosa is often caused by trauma, but may also be the result of vasoactive drugs and neurological conditions. Ischemic priapism, caused by venous congestion to the penis and enhanced blood viscosity, is often associated with sickle cell disease, hematological dyscrasias, hemodialysis, parental nutrition, heparin therapy, vasoactive drugs, neoplasia, neurological conditions such as spinal cord injury and anesthesia. Ischemic priapism is considered an emergency in people. Stuttering priapism is a subset of ischemic priapism and has been described as a pattern of recurrent events with intermittent periods of tumescence. Stuttering priapism typically lasts less than three hours. Clinically ischemic priapism is often painful, where as non–ischemic priapism is not.

The canine erection is mediated through the pelvic nerve, which arises primarily from the first and second sacral nerves (S1-S2) and is composed of parasympathetic nerve fibers. The pudendal nerve, which arises from the S1-S3 is involved by stimulating contraction of the extrinsic penile muscles. The hypogastric nerve, a sympathetic nerve originating from the L1-L4 spinal cord segments, may also have a regulatory role in the canine erection. Hypogastric nerve stimulation in the dog causes an increased in blood flow into the cavernous space secondary to vasodilation of the inflow blood vessels. However, an inhibitory effect may also occur due to relaxation of the outflow blood vessels, causing increased blood outflow from the cavernous space. Sympathetic chain fibers inhibit erection. Sympathetic chain fiber stimulation increases arterial resistance, decreases corpus cavernosal pressure and decreases venous resistance. The sympathetic inhibition of the erectile process is mediated by the alpha 1 adrenergic system.

Neurological diseases in dogs associated with priapism include spinal injury, canine distemper virus, acute disc herniation post T12-T13 hemilaminectomy and was seen in a dog with a meningomyelocoele in the cauda equina and syringohydromyelia in the lumbar spine. A dysregulatory hypothesis for the pathophysiology of priapism has been suspected. Priapism could result from dysregulation of the pelvic, pudendal, hypogastric nerves or the sympathetic chain. Dysnergic neurostimulations of inflow and outflow penile blood vessels cause prolonged vascular or smooth muscle spasms. Idiopathic priapism has been reported in dogs and is considered when other causes are not identified.

Phenothiazine drugs, α- adrenergic antagonists, have been reported to cause priapism in people and in horses. In horses, it occurs because the retractor penis muscle is solely under

control of α- adrenergic fibers. Perioperative medications such as acepromazine or anesthesia is unlikely to result in priapism in dogs. One canine study demonstrated consistent erections following intracorporeal injection of chlorpromazine. However, when administered intravenously, chlorpromazine did not cause or facilitate erection.

Non-ischemic priapism, often secondary to trauma in people, results from fistula formation within cavernous tissue. Blood bypasses the typically high resistance helicine arteriolar bed. No detrimental homeostatic changes or ultrastructural tissue damage occur even after years of non-ischemic priapism in people. Thus, non-ischemic priapism is initially treated conservatively. If the condition persists arterial embolization is often successful.

Ischemic priapism has been reported in cats. A traumatic cause is common with priapism often developing during mating or after castration. Ischemic priapism occurred in a cat with FIP and an idiopathic cause has been suspected in other cats. Thus far, Siamese cats have been over represented. Penile damage and infection have led to most cats being treated via penile amputation and perineal urethrostomy. Successful surgical treatment has been reported in a cat via small incisions made bilaterally in the tunica albuginea of the corpora cavernosa penis and in some parts of the corpora cavernosa itself. Heparinized saline was used to irrigate the corpora cavernosa. Skin sutures were placed, but the tunica albuginea was not sutured. Ischemic priapism in a dog was reported after being hit in the back during copulation. Successful surgical treatment was achieved via bilateral incisions to the longa glandis and the tunica albuginea. Blood was then pressed out. Irrigation with heparinized saline was done until red blood flow returned and the tunica albuginea was sutured.

Clinical signs and history may help differentiate between and ischemic and non-ischemic priapism. People with ischemic priapism typically have a painful, rigid penile shaft with a soft glans, whereas in non-ischemic priapism the entire penis is often partially rigid and non painful. Color flow doppler ultrasonography can help evaluate for an arterial to cavernosum fistula or may detect high systolic flow into the cavernosal artery. Ultrasonographic evaluation may also detect anatomic abnormalities and is done in the perineum and then the entire penile shaft, as perineal portions of the corpora cavernosa may be abnormal in trauma. Ultrasound may also rule out etiologic factors such as neoplasia, emboli, and other obstructive causes. Retrograde urethrogram may also be used to rule out urethral obstruction.

Aspiration of blood from the corpus cavernosum may be both therapeutic and diagnostic. Cavernous blood gas evaluation may differentiate ischemic and non-ischemic priapism. Ischemic priapism typically results in a pH < 7.25 a PO_2 < 30 mm Hg and a PCO_2 >60 mm Hg whereas non-ischemic priapism typically results in a pH of 7.4 a PO_2 >90 mm Hg and a PCO_2 <40 mm Hg.

Very little controlled data exists regarding the use of systemic drugs in people. Human studies with terbutaline suggested a trend of possible benefit, but were not statistically significant. Given the limited data and a consensus panel review, systemic therapies are not recommended in treatment of ischemic priapism in people. The management goal in stuttering priapism is prevention of future episodes. Therefore, systemic therapies such as GnRH androgens, estrogen, anti-androgens, baclofen, gabapentin, terbutaline, hydroxyurea, and PDE-5 inhibitors have been used for prevention, but are not recommended for individual episodes of stuttering priapism.

Guidelines for therapy in people have been established. Initial aspiration to obtain blood gas analysis and to provide pain relief is done. If priapism is determined to be ischemic irrigation may be done following aspiration. Aspiration +/- irrigation is about 30 % successful in people. Intracavernous injection of an alpha-adrenergic sympathomimetic agent is recommended in ischemic priapism either initially or if initial aspiration +/- irrigation has failed. Phenylephrine is the preferred drug in people due to its limited cardiovascular risks compared to other sympathomimetic drugs with greater β- adrenergic activity. The phenylephrine is diluted with saline to 100 to 500 mcg/ml and 100-200 mcg every 5-10 minutes is injected until detumescence is achieved with a maximal dose of 1000 mcg in adults. Smaller doses are used in children and patients with higher risk profiles. Resolution rates with aspiration and use of sympathomimetic agents range from 43 % to 81%. Patients are monitored closely for cardiovascular adverse effects during and after sympathomimetic injections. Concurrent treatment of any underlying causes for priapism is recommended. If intracavernous therapy is not successful within 48 to 72 hours surgical shunting is recommended.

The infrequent reports of priapism in dogs and cats make evaluation of treatment options in dogs and cats difficult. Given the similar pathophysiology and histopathological findings, a similar algorithm as that in people likely makes sense for dogs and cats. Distinguishing ischemic vs. non-ischemic priapism, identifying and treating the underlying cause is important. If determined to be ischemic then aspiration under sedation or anesthesia with or without irrigation should be done. Intracavernosal injections of phenylephrine should be considered. However, this may carry some risk as appropriate dosages in dogs and cats have not been determined. Starting with low dosages (1-3 mcg/kg) and cardiovascular monitoring therefore is important. Veterinary patients may have a longer duration of priapism before initial presentation compared to people. This prolonged exposure of the penis makes lubrication important to limit tissue damage secondary to exposure and excoriation. An Elizabethan collar may be indicated. If intracavernosal drainage and injections are not successful or significant tissue damage has occurred then penile amputation and perineal urethrostomy may become necessary.

The smaller size of dogs and cats compared to people and the paucity of experience with embolization procedures makes treatment of non-ischemic priapism more difficult. Thus, conservative treatment and protecting penile tissue integrity with lubrication and prevention of excoriation are recommended. If not resolving and a fistula can be identified then embolization, ligation or cauterization may be a consideration.

The success of systemic therapy for priapism is anecdotal at this time. Systemic therapy should be considered if the priapism is not considered an emergency or if intracavernous injections or surgical treatment are declined. Gabapentin therapy has been of benefit in some dogs and cats we have treated. Pseudoephedrine, an alpha-adrenergic drug was apparently beneficial in another dog.

Paraphimosis is treated with saline irrigation as the penis is placed back into the prepuce. Penile lubrication can help protect and place the penis. Sedation or anesthesia can be helpful. Diuretics do not significantly decrease swelling of the penis. Topical osmotic agents can sometimes be helpful. At times temporary purse string sutures can be helpful. It is important to make sure the sutures do not interfere with urination. Surgery may be needed if paraphimosis

cannot be resolved, recurs or becomes chronic. Surgery often involves a preputial opening revision or the correction of any underlying anatomic abnormality. Pain management, anti-inflammatory therapy and appropriate antibiotic usage should be considered as indicated.

Phimosis may be noted in young dogs, causing partial urinary tract outflow obstruction. Urine may dribble and accumulate in the preputial cavity. Phimosis may result in the inability to copulate or have semen collected manually as the preputial opening is too small. Entangled preputial hair in long coated cats may entrap the penis in the prepuce mimicking signs of phimosis. Ultrasound and endoscopy of the prepuce can be done to evaluate for hematoma, mass lesions or a ruptured tunica albicans which can also cause penile entrapment.

Surgical enlargement of the preputial orifice is done to treat phimosis. However, if the surgical correction leads to too large of an opening, the penile mucosa can be exposed.

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THE PEDIATRIC NEUROLOGICAL EVALUATION

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The neurological examination of puppies and kittens can be challenging. Pediatric patients can be uncooperative and their various stages of development lead to different expectations of normalcy compared to adults. Understanding the normal neurologic development of puppies and kittens enhances our ability to identify neurological abnormalities, correctly localize the abnormalities and generate an appropriate list of differential diagnoses.

Signalment and a thorough history are the first part of any examination. Information regarding problematic parturition, affected littermates, health of the parents, feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) status, nutrition, and vaccination history should be obtained. Time of onset of signs as well as their progression may help to rule in or rule out inherited, developmental, and acquired disorders. Identifying where the patient was obtained may be helpful, because certain animal shelters or regions may have a high incidence of distemper or panleukopenia. Catteries may have a high incidence of feline infectious peritonitis (FIP).[1]

Puppies and kittens mostly either sleep or nurse during the first two weeks of life. Kittens transition directly from waking to REM sleep[2] and may have considerable motor activity during sleep during their first week.[3] Until 3 weeks of age EEG patterns in puppies are similar during periods of sleep and waking. EEG patterns become adult like by 8 weeks of age.[4]

Vestibular function is present at birth and is important during positioning with nursing.[5] The head can be raised at birth and may be used to right them self. Initially movement is swim-like with coordination improving to reach the ability to maintain an upright posture at about 10-14 days of age.[4] At about 5-6 days of age stepping movements in the thoracic limbs may be made if weight is supported. Pelvic limb steps may be made at 7-10 days with support.[6] At 18-21 days of age an uncoordinated gait begins.[5] Adult posture and balance develops between 6 and 8 weeks of age. Breed variation does exist.[4] Histologically the spinal cord is mature by 6 weeks of age.[17] The cerebellum develops until 10 weeks of age.[8]

Tactile placing reactions begin as early as early as 2 days in the thoracic limb and 5 days in the pelvic limbs,[9] however these are not consistent until about 5 weeks of age. Hopping reactions may be detected from 6-8 weeks of age with the thoracic limbs developing first.[4,6] Extensor postural thrust can be seen by 12-14 days in puppies[10] and 14-16 days in kittens.[3]

Tonic neck reflexes, the magnus reflex, evaluate cervical tension receptors and can typically be identified by 5-6 days of age. Cervical extension produces thoracic limb extension and pelvic limb flexion. Cervical flexion yields pelvic limb extension. Lateral cervical flexion results in extension of the ipsilateral limb and flexion of the contralateral limb.[6] Kittens inhibit tonic neck reflexes. If present in kittens greater than 3 weeks of age an upper motor neuron lesion should be suspected.[3]

The “seal” reflex is seen when suspended under the chest. It consists of extension of the head, pelvic limb extension and variable posturing of the thoracic limbs.[6] The “diving” reflex occurs when held under the chest and rocked backward or forward and consists of raising the head, extending the thoracic limbs and arching the back.[4] Both the diving and seal reflexes can be seen at 3 weeks of age.[4,6] A normal response to being dropped consists of twisting into an upright position with limb in extension and are seen at 3-4 weeks of age.[3,4]

Reflexes to protect the eyes develop prior to the eyes actually opening. A dazzle reflex occurs before the retina has developed. The eyes open between 10 and 16 days in the puppy[6] and 5 to 14 days in the kitten.[3] The menace response can be seen shortly after the eyes open, however may take up to a month to appear.[3,5] Kittens may have a divergent strabismus until 8 weeks of age.[3] The time of appearance of cranial nerve tests are summarized in table 1.

Table 1: Time to onset of positive cranial nerve tests[1]

Reflex	Puppy	Kitten
PLR	10–16 d	5–14 d
Dazzle	1–2 d	1–2 d
Palpebral	2–4 d	1–3 d
Menace response	10 d–4 w	1–4 w
Corneal	10–16 d	5–14 d
Vibrissopalpebral	1–2 d	
Suckling	1–2 d	1–2 d

Abbreviations: d, days; PLR, pupillary light reflex; w, weeks.

Olfaction (CN I) is present at birth, but is likely poor.[5] The suckle response (CN V, VII and XII) is seen within 1-2 days, but can disappear by day 20 in kittens.[3] The external ear canals open between 12to 14 days of age in puppies[6,9] and 6 to 14 days in kittens.[3] Once open a startle response (CN VIII) can be produces with auditory stimulation.[9]

Spinal and myotactic reflexes such as the patellar, triceps, gastrocnemius, withdrawal and panniculus reflexes are all present shortly after birth. However these reflexes may be difficult to elicit.[4,6] If a crossed extensor reflex persists past 17 days in kittens and 3 weeks in puppies an upper motor neuron lesion is indicated.[3,6,9] Stimulation of the perineal reflex may cause urination or defecation typically up to 3 weeks of age.[4,7] Examining patients with them as relaxed as possible and positioning them in lateral recumbency can help maximize success in obtaining spinal and myotactic reflexes.

As the neurologic examination is completed localizing to a specific segment (Brain, spinal cord, neuromuscular system) of the nervous system is essential. Both cerebral and brainstem lesions may have mentation changes and circling. The circling tends to be wider with cerebral lesions and tighter with brainstem/vestibular lesions. Placing deficits are contralateral with cerebral lesions and ipsilateral with brainstem lesions. Some patients with brain lesions may vocalize and which can be confused with pain. Head tilts can be seen with brainstem and cerebellar lesions. Ataxia can be seen with intracranial lesions, particularly when the vestibular system is involved. Profound paresis without mentation changes typically suggests a myelopathy. Some painful myelopathic patients can be quieter than normal due to their pain.

When assessing a myelopathy, I first ask myself are all 4 limbs affected or just the pelvic limbs? If all 4 limbs are affected, I must decide between a localization of either C1-C5 or C6-T2. Reflex deficits, a thoracic limb lameness (not of orthopedic origin) or a short strided thoracic limb gait indicate a localization of C6-T2. If the thoracic limb gait is normal and only the pelvic limbs are affected then one must decide between a localization of T3-L3 or L4-Cd. Reflex deficits or a pelvic limb lameness suggest a L4-Cd lesion.

Generalized neuromuscular disease may present with a history or signs of weakness or exercise intolerance. Reflexes may be decreased in multiple limbs. Muscle atrophy, hypertrophy or decreased muscle tone may be seen. Megaesophagus may be present, particularly with myasthenia gravis. On occasion a brainstem lesion can cause a megaesophagus. Likewise, a change in voice or dysphagia can be seen with neuromuscular conditions and less commonly brainstem lesions.

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Diagnosis and treatment of inflammatory endometrial disorders in the bitch

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Abstract

Endometritis is inflammation of the lining of the uterus without extension into the deeper muscular layers of the uterus. It may be due to bacteria ascending via the cervix, hematogenous infection, or translocation via the abdominal cavity. It can also occur due to chronic lymphoplasmacytic, eosinophilic, or post-mating induced inflammation. Endometritis may be seen as an individual entity or associated with other endometrial or uterine pathologies. Diagnosis of endometritis is made via history, physical exam, vaginal or endometrial cytology, endometrial culture and/or biopsy, ultrasonography, hysteroscopy and lab work. Treatment may include antibiotics, non-steroidal anti-inflammatories, uterine lavage, mucolytics, ecbolics and/or stem-cell therapies. This paper will review endometritis and its diagnosis and treatment. It will also discuss the importance of the reproductive tract microbiome and our consideration of such during therapy.

Keywords: Endometritis, canine, inflammation

Introduction

Infertility in the bitch can be due to a single cause or may be multifactorial. When a bitch fails to become pregnant, resorbs all or part of her litter, or has a high number of stillborn, mummified or fading puppies, a work-up, to determine the cause, is indicated if she is to be bred again and a similar outcome is not desired[1, 2] The work-up should start with a detailed breeding history, including previous litters (length of interestrous interval, when each breeding occurred; what type of breeding management was employed, if any; what type of insemination was performed; semen quality; and the outcome of the litter). Then the clinician should start to rule out potential causes of the problem(s), until a reasonable list of differential diagnoses is formed. Starting with the most likely etiologies, diagnostic tests should be performed to rule in or out potential differential diagnoses, until a management plan for the subsequent breeding can be formulated.

This paper will review one of the most common causes of pregnancy failure or resorption – endometritis – from a clinical perspective. Endometritis is defined as inflammation of the lining of the uterus that does not extend below the stratum spongiosum[3-7]. Endometritis is a common finding in subfertile bitches, reportedly being involved in 30 – 50% of cases[4]. It is usually subclinical[4]. In addition to inflammatory infiltrates, it can also be associated with vascular congestion, edema and disruption of the endometrial luminal epithelium[8]. Inflammation may be subacute, active, or chronic[3]. It may be neutrophilic, lympho-plasmocytic, or eosinophilic, or any combination of the three[3]. The cause of this inflammation is likely either: 1) post-mating in response to sperm, bacteria and debris that enters the uterus during the breeding, regardless of

type; 2) as a result of translocation of bacteria from the vaginal canal to the uterus during the period of cervical relaxation during the estrous cycle; or 3) hematogenously[3].

These etiologies are very similar to those in the mare and cow[9]. The more we study the bitch and subfertility, the more similar the bitch appears to be to the mare in the development of endometritis. In the mare, efficient uterine clearance is critical to the resolution of PMIE. If there is deficient myometrial function, then uterine clearance post-mating is delayed infection may take hold, resulting in a persistent endometritis[9, 10]. The endometrium is also much more sensitive to the development of endometritis during the luteal phase of the cycle[3]. The endometrium is more permeable to bacteria and the glandular secretions enhance the growth of microorganisms, at the same time as being supportive of pregnancy[3].

This inflammation may affect the fertility of the bitch during sperm transport or storage in the sperm reservoir, which is at the tip of the uterine horns. It may affect fertilization, embryo transport and movement, implantation and trophoblast invasion through the endometrium[8, 11, 12]. If the bitch becomes pregnant, and the endometritis has not resolved, it may affect placental function, disturbing placental blood flow or resulting in placentitis. With chronicity and time, over one or multiple cycles, it may lead to more widespread, deeper and more severe inflammation, causing a variety of pathological fluid accumulations, including hydrometra, mucometra, hematometra, or pyometra[8, 12, 13]. It can also lead to changes in and around the glands, including cystic endometrial hyperplasia (CEH), fibrosis, gland atrophy, or pyometra, all of which are beyond the scope of this paper[5, 6, 11].

While in the mare, the cow and other domestic livestock, endometritis has been well studied due to the relative ease of acquiring samples, in the bitch, it has been more elusive because of the difficulty of sampling the endometrium[3, 4, 6, 14, 15]. Over the last 2 decades, the use of ultrasound and endoscopic procedures has led to a much better understanding of how to assess uterine health and acquire samples directly from the endometrium[4, 6, 14-21].

In addition to learning more about endometritis, we have also been learning more about the importance of microbiome health and have become more cautious in terms of judicious use of antibiotics, antifungals, antiprotozoals and antiviral medications to ensure we are treating a known disease and not just prophylactically treating with antimicrobials, as this may adversely affect the individuals microbiome (or others in their home/kennel of like or different species, including their humans) and this in turn could have negative outcomes in the reproductive tract or any one of the other body systems[22].

Reproductive Microbiome

Normal uterine and vaginal microflora

For the discussion in this paper, it will be important to understand not only how endometritis develops, its significance for sub-or infertility, but also our approach to treatment. Over the last 2 decades, a mountain of research on the microbiome of all species has been accumulating. The importance of having a healthy microbiome (including bacteria, archaea [eukaryotic and prokaryotic bacteria], viruses, fungi and

protozoa) cannot be underestimated both in terms of general health and reproductive health[22]. The microbiome is derived from contact with oral, internal and inhaled sources[23]. Colonization of the reproductive tract microbiome has been suggested to contribute to a basal, healthy immune state[23]. The presence of a healthy microbiome may also serve to alter or limit other more pathological components of the microbiome[23].

The canine vaginal microbiome is higher in richness, while the endometrial microbiome is higher in its diversity[22-24]. The canine vaginal microbiome is very similar during all stages of the estrous cycle except during estrus, while the uterine microbiome is the same during all stages of the estrous cycle[24]. The placenta also has its own unique microbiome – it is not sterile as once was believed[22, 23]. The placental microbiome may be colonized via the oral cavity, GI tract or vaginal ascension[23]. Having a healthy microbiome versus an unhealthy one may be the reason for a bitch to fail to conceive (due to cervicitis or endometritis), for conception failure to occur (due to endometritis) or for resorption or abortion to ensue (due to endometritis, placentitis or cervicitis)[23].

The vaginal and cervical microbiome tends to more dynamic, being affected by things like stage of the estrous cycle, hormonal concentration and sexual activity[23]. The vaginal and cervical microbiomes are likely affected by the gastrointestinal tract microbiome, especially in species where anatomy causes fecal secretions to drip down the perineum towards the vulvar lips[23]. There are 4 proposed routes of inoculation of commensal or pathologic bacteria into the uterus: 1) ascent from the vaginal canal; 2) retrograde via the oviductal fimbria from the abdominal cavity; 3) via invasive procedures; or 4) hematogenously[23].

Inflammatory bacteria in humans are hypothesized to be due to dysbiosis rather than pathogenic invasion[23]. The vaginal microbiome changes in response to hormone concentrations and pH, and thus is diverse from the pre-breeding state through the time of delivery[23]. In the bitch, the vaginal microbiome was similar in animals with normal fertility and with subfertility[25]. There were no lactic acid producing bacteria found in the bitches vaginal canal, unlike in humans where it is the predominant bacterial type[25].

Subfertile women are often diagnosed with vaginal dysbiosis[23]. Reduced diversity of organisms has been associated with endometritis, stillbirths and pre-term delivery in humans, horses and cows[23]. Currently, there is much less information in veterinary species than human, but already associations are being made between bitches with pyometra and certain phyla of bacteria and dysbiosis of the reproductive tract[23]. Certainly, there is much more to come in terms of research, diagnostics (metagenetic testing) and treatments for these dysbioses.

Normal vaginal and uterine flora based on traditional cultures

There are higher numbers of bacteria in the vaginal canal during proestrus and estrus[26]. This may be due to the presence of blood from the uterus providing an ideal medium for bacterial growth, or it may be due to decreased immune function during these stages of the estrous cycle[26].

Bacteria are frequently present in the uterus of healthy animals during proestrus and estrus and rarely at any other time[26]. This may be due to the open cervix, allowing translocation of bacteria from the vaginal canal, across the cervix and into the uterus or

it may be due to alterations in immune function during this stage of the cycle[26]. By day 10 of diestrus, the presence of bacteria present in the uterus has returned to zero in healthy bitches[26]. The normal uterus is also free of bacteria during anestrus[26].

Normal flora in healthy vaginal and uterine culture samples include bacteria of the following species: β-hemolytic streptococci, *Escherichia coli*, *Staphlyococcus* spp, *Streptococcus* spp., *Bacillus* spp., *Proteus mirabilis*, *Corynebacterium* spp, *Hemophilus haemoglobinophilus, Enterobacter spp.* and *Klebsiella pneumoniae*[26-29]. Mycoplasma spp can be isolated from more than 60% of clinically normal bitch's vaginal canals and higher numbers are isolated from those with genital tract infections[30]. Ureaplasma can be isolated from 16-38% of normal vaginal cultures, with higher percentages in bitches with infertility[30].

Types of endometritis

Endometritis, regardless of cause, is one of the leading causes of infertility across all species[8, 9]. Endometritis decreases fertility due to a multitude of factors, including decreased sperm transport and storage, decreased fertilization rates, lower oocyte and zygote maturation and its negative impact on embryonic and fetal development[8].

Endometritis was found in 42.6% of uterine biopsy specimens of infertile bitches[6]. In these cases, 52.4% were classified as chronic lymphoplasmacytic, 30.0% were mixed inflammatory reactions (acute and chronic changes) and 17.6% were acute inflammatory changes with either neutrophils or eosinophils or both. In clinically healthy bitches, 54% of diestrual biopsies revealed endometritis[27]. In other studies endometritis was found in between 29 – 50% of bitches with infertility[12, 14]. In most cases, bacteria are not associated with endometritis, even early in the luteal phase when neutrophilic endometritis is most common, but when bacteria are isolated, it is usually associated with early diestrus or pyometra[27]

It has been recommended that a classification scheme, similar to what is done in the equine species, be created for bitch but it is much more complicated in the canine, and as such, a scheme has not yet been established for the prognosticating about future fertility[5, 7, 31, 32]. Uterine tissues frequently had multiple lesions present (2.7 lesions/case on average)[6]. There did not appear to be an association with age and the presence of lesions.

The presence of endometritis has been suggested to be a better prognostic indicator of subfertility than other morphologic changes[3, 5, 6, 12], although mild inflammatory changes may be present with normal fertility.

Numerous factors (i.e. lactoferrin, Mucin-1) have been investigated as causative for endometritis, but thus far none has been identified as definitive suspects either by their presence or by the lack of their presence in the bitch's uterus[33].

Neutrophilic endometritis

Acute inflammation is more common in the early portion of the luteal phase[6, 7, 34]. This type of inflammation is due to the contamination that occurs with breeding and from the inflammatory response to spermatozoa (post-mating induced endometritis)[11, 35]. It

also may occur once infection has evolved to a point that there are secretions accumulating in the uterus and bacterial growth begins to proliferate[6, 7, 32].

Lymphoplasmacytic endometritis

Chronic inflammation is more common the late luteal phase both in subfertile and clinically normal bitches[6, 7]. Chronic endometritis was more common than acute endometritis in subfertile bitches[5, 6, 14]. Lymphoplasmacytic inflammation may extend beyond the stratum spongiosum and into the myometrium in bitches with subfertility[5]. Chronic endometritis may persist through diestrus and into anestrus and may be diagnosed by the presence of lumenal fluid during these stages of the cycle[8, 36].

Eosinophilic endometritis

Eosinophilic endometritis has been closely associated with fetal loss during the most recent cycle[5, 6]. It is not clear if eosinophilic endometritis is a cause of fetal loss or a response to it[5, 6]. Further research is needed to clarify this[5, 6].

Disease processes that may predispose to endometritis

Cystic endometrial hyperplasia and fibrosis

While this is not within the scope of this paper, it would be wrong not to mention these commonly identified conditions and discuss them briefly.

CEH has been identified as the most common morphological abnormality or of equal incidence to endometritis in subfertile bitches in numerous studies[5, 6, 12, 14, 20, 31, 37]. It has been noted that the presence of CEH by itself is not always a cause of subfertility. It has been suggested that microscopic CEH is less prognostic in terms of future fertility than macroscopic CEH, meaning what can be seen on ultrasonographic examination. Ultrasonographic evidence of CEH was first noted between 2.5 – 7.3 years of age[27, 37]. CEH was found in 6.8% of 2-year-olds, 60% 6-7-year-olds and 100% of 7-8+ year-olds, but this may or may not be associated with subfertility or endometritis[27, 37]. So, while the presence of CEH should always be considered a potential cause of infertility, there are many bitches with significant CEH on both biopsy and on ultrasonography, that have normal fertility, so this should be considered more significant when it is seen with other changes that are more consistently seen with subfertililty, most notably endometritis[5, 6, 12, 14, 31].

Two theories associate CEH with endometritis. The first is that endometrial hyperplasia develops first and leads initially to a chronic inflammatory reaction (L/P endometritis). Eventually, the secretions from the hyperplastic endometrium allow for growth of pathologic bacteria resulting in an influx of acute inflammatory cells (neutrophilic endometritis) and eventually pyometra develops[6, 32, 33, 38]. The second theory is that subacute and chronic endometritis lead to proliferative changes in the endometrium that then allow for growth and proliferation of pathologic bacteria under the influence of progesterone[6, 32, 33, 38]. Both theories may be correct. It has also been found that CEH can occur without any evidence of endometritis[12, 32, 38].

Fibrosis has been noted in 6.7-54% of bitches with subfertility[12, 31], but was also seen in 14% bitches with normal fertility, especially in those that had previously been pregnant[5]. It has been hypothesized that because of the placentation type of the bitch, that reorganization of the glands during pregnancy results in some degree of fibrosis that is not correlated with subfertility but rather with normal pregnancy[5]. Thus, for fibrosis to be considered a factor in subfertility, the amount present may need to be much more than what is seen in other species (i.e. mare), where any presence of fibrosis is seen as a marked negative prognostic indicator[39].

Post-mating induced endometritis (PMIE) and delayed uterine clearance

In some species (i.e., bovine, ovine, caprine), there is a thick layer of mucus on the cervix that acts as a barrier to pathogens while allowing sperm transport to occur[8]. In other species (equine, canine, porcine, camelid), there is no such barrier, and a large volume of ejaculate enters the uterus during breeding[8, 11]. In a normal, fertile animal, uterine motility and the host immune response combine to evacuate the uterus of bacteria, debris, dead sperm and other cellular factors[8, 11]. Then PMNs enter to finish the clean-up, allowing the microbiome of the uterus to return to normal[8, 11]. This transient, physiologic, inflammatory response to breeding, due to the presence of seminal plasma, spermatozoa, bacteria and debris, is called post-mating induced endometritis[8, 11]. In the mare and the bitch, bacteria also translocate between the vaginal canal and the uterus due to the relaxed cervix and may result in endometritis[3, 8].

In the bitch, PMIE is often associated with failure to conceive or small litter size[3, 8, 12, 40, 41]. In some cases, there will also be CEH present[6, 14, 37]. Delayed uterine clearance and evidence of increased numbers of PMNs on cytology may also be found [11, 40].

In normal animals, uterine motility is highest during estrus and is moderate during proestrus[11, 40, 42]. There is little to no motility during diestrus. Uterine motility increased in response to oxytocin during proestrus and estrus. Thus, oxytocin may be effective in treating bitches with delayed uterine clearance during proestrus and estrus, while the effect of estrogen on oxytocin receptors is upregulated[11, 42].

Resolution of PMIE requires normal immune function, uterine motility and uterine and vaginal microbiome[8]. In bitches with CEH, uterine motility may be affected due to physical impedance due to the hyperplastic endometrium[8, 11, 40]. Bitches with CEH also had a muted uterine contractile response to breeding that may be related to reduced prostaglandin production or diminished response to prostaglandin produced as a result of breeding[40] This results in greater PMN influx and a hostile environment for embryos when they enter the uterus[8, 11, 40]. This PMIE may be compounded by the normal translocation of bacteria during proestrus and estrus[8, 27, 43].

Bitches with CEH and endometritis also have reduced uterine perfusion on Doppler ultrasound and a blunted vasodilatory response following mating, which likely play a role in their delayed uterine clearance[11, 40]. Bitches with CEH may also have some degree of fibrosis which may affect their vasodilatory response[40]. It has been hypothesized that bitches with endometrial disease present prior to breeding, will have a either a more severe or a more persistent PMIE and that this extraordinary inflammatory response is what is responsible for their reduced fertility[11, 40]. In one study,

uterine tissue samples with CEH or endometritis (increased # of PMNs), incubated with spermatozoa, had reduced sperm binding compared to normal uteri[11, 40].

Uterine luminal fluid may be present in small amounts before breeding, with no effect on fertility[41]. This fluid is likely physiological and due to edema in the endometrium, noted under the influence of estrogen, as is noted in the mare[41]. However, detection of fluid in the uterus on days +5 or +14 post-mating (when mating occurred 2 and 4 days after progesterone attained 5 ng/ml concentrations) was associated with reduced pregnancy rates[41]. No bitches with fluid on +14 from mating became pregnant[41], similar to what is known in mares.

Treatment of PMIE in the mare involves uterine lavage and ecbolic therapy[8, 44]. However, in the bitch, lavage is not possible post mating, because the sperm reservoir is predominantly within the lumen of the uterus in the glandular crypts and at the tip of the uterine horns and not in the oviducts, as with most other species[11, 40]. Thus, other less traditional treatments must be applied to resolve a persistent PMIE.

Diagnostic tools for endometritis[1, 2]

Ultrasonography

An 8.5 -10 MHz transducer is recommended for optimal visualization of subtle lesions in the uterus[37, 41]. Endometrial edema, endometrial cysts and luminal fluid may be visualized with a high-quality ultrasound machine during the estrous cycle, particularly around the time of breeding, as well as in early–mid diestrus associated with the early ultrasound for pregnancy. Ultrasonography is the preferred method to differentiate between endometritis alone and the different fluid accumulations in the uterus (mucometra, hematometra, hydrometra or pyometra)[13]. Ultrasound can be used to evaluate the thickness and character of the endometrium, to assess for macroscopic cysts and to assess for fluid accumulation within the uterus[13]. Ultrasound cannot differentiate the type of fluid present[13]. Ultrasound of the endometrium can also be utilized to assess the degree of inflammation present, with increasing amounts of inflammatory changes causing an increase in heterogeneity and echogenicity[45]. Larger breeds are more difficult to assess because of increased abdominal depth and presence of soft tissue structures between the probe and the uterus[45].

An ultrasound examination done between 5 and 14 days post breeding may be recommended in bitches with a previous pregnancy failure or small litter size, to determine if delayed uterine clearance is evident, and thus allow for a better management plan on a subsequent breeding attempt[41].

Vaginal cytology

Vaginal cytology is useful to assess the stage of the cycle and should always be correlated to vaginal speculum examination, because in the case of early proestrus and early diestrus, the cytologic interpretation will be very similar but the appearance of the vaginal mucosa will be completely different (pink and edematous in early proestrus vs. blotchy pink and flat in diestrus), allowing for differentiation of the stage of the cycle. An increase in the number of PMNs or other inflammatory cells at any time when they are

not expected should instigate an investigation as to the cause. This investigation might include ultrasonography of the uterus and endometrial culture, cytology, or biopsy[1, 2, 29].

Vaginal culture

While anterior vaginal culture with a double guarded swab is a well-known and accepted technique, care should taken when interpreting results. It is normal for there to be bacteria present at all stages of the cycle, with numbers increasing significantly in proestrus and estrus[1, 2, 29]. Confirmation of the presence of inflammatory cells or uterine pathology should be made before making any assumptions about the validity of culture results. Certainly, in cases where the uterus cannot be breached (i.e. pregnancy) and there is vaginal discharge, anterior vaginal culture will be the only option for diagnosis beyond laparotomy[29]

Endometrial culture and cytology

Endometrial culture and cytology are most commonly performed using endoscopy[4, 15-18]. Uterine lavage with centrifugation of the efflux to concentrate the cells is the most common method of collection, but the cytobrush can also be used[4, 15, 17]. In one study, 40% of bitches diagnosed with endometritis on culture and cytology, also had visible discharge emanating from the cervix before uterine catheterization[3]. Vaginal or cervical hyperemia and lymphoid hyperplasia in the anterior vaginal vault may also be indicators of endometritis[3].

At present, the use of the ureterorenoscope for these procedures is much more successful than the cystoscope and this has made the use of uterine endoscopic procedures more commonplace and less problematic[16, 17]. Difficulties noted with the cystoscope that are not typically seen with the smaller, narrower angle of view ureterorenoscopes, include difficulty passing the scope into the paracervical area in small or maiden bitches, vaginal puncture, difficulty cannulating the cervix and adequate insufflation of the vaginal canal[18]. Vaginal tearing with the cystoscope is more common in diestrus and anestrus when the paracervical area is much narrower[18].

Repeated catheterization can cause endometritis or pyometra[18]. The cytobrush is more traumatic to the endometrial lining and should not be used during diestrus, due to the increased risk of precipitating pyometra[15, 17, 18]. Collection of samples during diestrus will also increase the risk for pyometra. If a transcervical procedure is deemed necessary during the progesterone phase of the cycle, some type of preventative action should be performed to induce luteolysis and reduce this risk (i.e., aglepristone or PGF2α treatment following the procedure)[4]

Cell types found on endometrial cytology include normal or degenerate endometrial cells, red blood cells, white blood cells (neutrophils, lymphocytes, plasma cells, eosinophils, macrophages) and bacteria, as well as foamy debris and proteinaceous material in the background[15, 19]. Certain cell types are normal during specific stages of the cycle[15, 19]. Normal endometrial cells are commonly noted in proestrus, early diestrus and late anestrus[15, 19]. During estrus, mid–late diestrus and early–mid proestrus, there are more degenerate endometrial cells visible[15, 19]. Degenerate endometrial cells are also seen with endometritis and CEH[15]. Normally,

endometrial cells are clumped in small to large groups, but in early diestrus and late anestrus, single cells are more frequently noted[15, 19]. Naked nuclei were most common in proestrus and early diestrus[15, 19].

Neutrophils are the most common type of white blood cell seen on cytology in both normal bitches and those with pathologic conditions[15]. PMNs are most common in proestrus, estrus and with endometritis or pyometra[15]. Lymphocytes are more common in late anestrus, with endometritis or benign fluid accumulation without CEH[15]. Eosinophils are most commonly seen during proestrus, estrus, during uterine involution and in cases of subinvolution of the placental sites (SIPS)[15]. Plasma cells were noted during late anestrus, pyometra, uterine stump inflammation and SIPS[15]. Macrophages were seen in late diestrus, early anestrus, SIPS, pyometra and with uterine stump inflammation[15].

Bacteria are commonly seen in proestrus, estrus and late anestrus. Foamy debris was noted in early diestrus and early- mid anestrus; proteinaceous debris was seen during early diestrus and SIPS; amorphous debris was seen with CEH; and necrotic debris with pyometra[15]. Cultures performed during diestrus are commonly negative for bacterial infection, and if positive and they were obtained using a transcervical technique, one should consider vaginal contamination as a possible source[4].

The presence of increased numbers of leukocytes on endometrial cytology, purulent vaginal discharge or a peripheral leukocytosis are necessary to ascertain if bacteria isolated from endometrial cultures are significant or not[26]. The presence of intracellular bacteria is associated with endometritis or more severe infectious disease[15]. Cytologic assessment correlated well with histologic assessment[15].

Endometrial biopsy

The stage of the cycle when biopsy is performed is important and should always be noted when sending samples to the pathologist[6, 12, 14, 34]. Samples obtained mid–late diestrus will have more pathology present than in early diestrus, and this pathology tends to resolve during early anestrus. Therefore, it is recommended that samples be obtained either during mid–late diestrus if performing surgical biopsies or in very early anestrus if obtaining transcervical biopsies. If transcervical biopsies are obtained during diestrus, the risk of inducing pyometra is high[3, 14, 20, 21]. The stage of the cycle will impact the successful completion of the procedure, especially if using the cystoscope because there may be inadequate room to pass the scope through the paracervical areas without causing vaginal rupture[16-18, 21]

Transcervically collected biopsy samples provide diagnostic sample size and similar results to full-thickness biopsy, and since this is a less invasive approach than laparotomy, its usefulness in the diagnosis of uterine pathology is tremendous[21]. A previous study did not yield very successful results, with only 31% of the samples obtained of diagnostic quality due to crush artifact or poor sample size[21]. While another study yielded much better results[14]. Differences in biopsy technique were present between the 2 studies, the most notable being the type of endoscope being used. A cystoscope was in the original study with a 4Fr biopsy instrument vs ureterorenoscope in the latter study with a 5 Fr biopsy instrument[14, 21]. It has been this author's experience

that we obtain diagnostic samples in more than 80% of our endometrial biopsy specimens using a similar biopsy instrument (5 Fr) and the ureterorenoscope. Crush artifact is present in a small percentage of samples obtained using the ureterorenoscope[14]. While sample size is considerably smaller with the transcervical technique and a small percentage will have crush artifact, multiple biopsy specimens are generally taken from multiple sites along the uterine body and distal horns, allowing more opportunities to diagnose focal or multifocal lesions[16, 17, 46].

Hysteroscopy

Hysteroscopy is more easily performed with the cystoscope due to the wider angle of view (30°) vs the narrower view of the ureterorenoscopes (5°)[16-18]. In some bitches it is not possible to pass the larger scope through the external os of the cervix. Hysteroscopy may be useful to collect biopsy samples from focal lesions noted on ultrasound examination, or to evaluate the endometrial surface in bitches previously diagnosed with endometritis but failing to respond to therapy. Hysteroscopy should be performed under anesthesia due to increased risk of perforating the uterus with any sudden movement in a conscious bitch[16-18].

Labwork

In cases where there is neutrophilic or eosinophilic inflammation on vaginal cytology or there is uterine luminal fluid or endometrial edema on ultrasonography, especially during the luteal phase of the estrous cycle, a minimal database of a complete blood count (CBC) and chemistry panel, including proteins and acute phase proteins (where available) is recommended to determine if there is evidence of acute bacterial endometritis that may require additional therapies (PGF2α, aglepristone, antibiotics, misoprostol, etc.). C-reactive protein is higher in bitches with endometritis, with or without clinical signs of disease, so it is a not a good lab marker for subfertility[27]

Treatments

Mucolytics

For bacteria that produce biofilm, the use of mucolytics before antibiotic therapy can be useful to increase antibiotic contact and decrease the amount of time needed for treatment and bactericidal activity. Biofilms, typically produced by gram-negative bacteria, are a layer of extracellular polymeric substances, secreted by the bacteria to provide protection, under which the bacteria can proliferate without being attacked by immune cells or antibiotics. *E. coli*, the most common bacteria to cause pyometra, along with *Pseudomonas* and *Klebsiella* spp, all produce biofilms[47].

The author uses acetylcysteine in a 1:10 dilution with saline following all endometrial cultures, to remove any potential biofilm that may be present (unpublished results). Ideally, it would be best to wait for the culture's results to be completed, but often this is very close to the time of breeding or sometimes even after breeding, at which time it would be too late to perform such a therapy.

Antibiotic therapy

When evidence of inflammation is present on endometrial cytology or vaginal cytology at the time of ovulation or between ovulation and diestrus d1, focused antibiotic therapy should be applied based on culture and sensitivity whenever possible. If culture and sensitivity are not available and unexpected inflammation is noted, a culture should be obtained that day. Antibiotics can be started based on an educated decision after evaluating for the type of bacteria present (cocci vs rods) using the most focused antibiotic that is reasonable. Care should be taken not to disrupt the microbiome of the bitch whenever possible[48]. Overuse of antibiotics when there is no clear indication of infection may result in resistant bacteria[49, 50].

There is some evidence that short courses of antibiotics (i.e. 4 days of amoxicillin/clavulanic acid TWICE DAILY, starting on the last day of breeding) in selected cases of bitches with documented CEH or PMIE, may be beneficial and may increase pregnancy rates[11, 35, 40].

NSAIDs

In addition to their anti-inflammatory action, they are also analgesic, anti-pyretic and antimicrobial in action. Their antimicrobial mode of action is to inhibit cyclooxygenase, which in turn decreases prostaglandin synthesis[51]. Salicylic acid can reduce *E. coli* and *P. mirabilis* flagellin production, which reduces binding to uroepithelial cells[51]. Diclofenac inhibits the growth of *E. coli* [51]. NSAIDs can also alter gene expression of *E. coli* and *P. aeruginosa*, and they can reduce capsule production in these gram-negative bacteria.

NSAIDs have been used successfully in bitches believed to have potential concerns with PMIE. There is increased expression of COX-2 enzymes found in the luminal and glandular epithelium and stromal cells in the uterus in bitches with endometritis[43]. Thus, protocols using NSAIDs to treat endometritis have been introduced.

Therapy with NSAIDs for endometritis has been successfully used in 2 different studies. In the first, 33 bitches with a history of at least 3 failures to conceive and evidence of CEH or endometritis, were treated with meloxicam starting on the day after the first insemination (3-5 days post LH) for 3 days and then again at the time of expected implantation days 15-17 post LH[52]. In this study 43% of bitches who had a history of subfertility, conceived with a mean litter size of 6.8 +/- 4.5 puppies[52]. In another study, bitches with a history of at least 2 failures to conceive or resorptions of their pregnancies, were administered carprofen twice daily or meloxicam once daily for 5 days starting on the day after breeding, and they achieved a pregnancy rate of 80% with a mean litter size of 7.7 puppies (range 4-14)[53].

Care should be taken not to administer NSAIDs before 2-3 days after the onset of ovulation so as not to affect ovulation via COX-2 inhibition during luteal cell transformation[54].

In mares, the use of glucocorticoids for the treatment of PMIE is commonplace[55]. Their use in the bitch is more concerning because of the pre-ovulatory rise in

progesterone and subsequent immune suppressing effects of the uterine environment. Coupling this rise in progesterone with additional immune-suppressing effects of glucocorticoids may reduce PMN phagocytosis and increase the risk of bacterial presence in the uterus, and thus may increase the risk of endometritis. Therefore, NSAIDs have been more studied to treat PMIE in the bitch to combat endometritis associated with breeding. There have been studies in mares showing that NSAIDs are effective in modulating inflammation associated with mating[56, 57].

Ecbolics

Oxytocin can be used to facilitate uterine clearance if care is not taken to disrupt sperm transport and storage[36]. England, et al theorize that oxytocin, post -breeding, will improve uterine clearance in bitches with CEH[11]. This author uses a treatment protocol for bitches diagnosed with delayed uterine clearance during endometrial cultures or during a post-ovulation ultrasound examination. If delayed uterine clearance is diagnosed at the time of endometrial cultures, then oxytocin is administered starting early in proestrus, continuing daily to every other day until the day of breeding. The day of breeding is skipped and then the bitch is treated TWICE DAILY for 3 days post breeding. This has resulted in pregnancies in some bitches affected by delayed uterine clearance (unpublished results).

Immunomodulators

Pentoxifylline is used routinely in mare with placentitis both as an anti-inflammatory and anti-cytokine[55]. It inhibits the NFkβ pathway and acts against tumor necrosis factor and interleukin-6, which are pro-inflammatory cytokines[55]. Pentoxifylline increases erythrocyte flexibility and reduces blood viscosity, making it useful to treat many ischemic conditions or conditions where improving capillary microcirculation is beneficial, as in endometritis or placentitis. The author routinely uses pentoxifylline during pregnancy when endometritis or placentitis is diagnosed based on ultrasound evaluation, although no controlled studies have yet to be performed in the bitch.

There are numerous other immunomodulators that have been used in the mare that remain untested in the bitch (bacterial cell wall extracts, fresh-frozen plasma, lactoferrin)[55].

Stem cell therapy

In mares with PMIE, there is an excessive accumulation of luminal fluid and delayed uterine clearance due to diminished immune response to the bacteria and dead spermatozoa that remain in the uterine lumen following mating[9, 10]. Recently, embryo-derived mesenchymal stromal cells (EDMSC) and their extracellular vesicles (EV) have been used to treat PMIE to mitigate the inflammation associated with spermatozoa and breeding[58, 59]. The intrauterine administration of EDMSC 24 hours before breeding resulted in an immediate reduction in the excessive and inappropriate inflammatory response that is seen in mares with PMIE[58] and improved per-cycle pregnancy rates.

Currently, a similar study is being performed in dogs with successful results (Hollingshead, unpublished data). In this study, 25 bitches with a history of 1-4 failed breedings (non-pregnancy or resorption) were treated with canine-derived mesenchymal stem cell extracellular vesicles (CDMSC-EV) 24 hours before breeding. Seventy – Four percent of these bitches had CEH visible on ultrasound exam 24 hours prior breeding which was when the EV administration via transcervical catheterization occurred. Twenty-two percent of the bitches had luminal fluid present just before breeding and 26% had luminal fluid 24 hours post breeding. Pregnancy rates were 76%. Thirty-six percent of these bitches had 1 or more absorbing sites noted at their pregnancy ultrasounds. The number of inseminations was 84.5% had 1 TCI; 11% had 2 TCI; 0.5% had 3 TCI. The type of semen used varied with 38.4% of the bitches being bred with fresh semen, 50% with chilled semen and 19.2% with frozen semen. Considering this cohort of bitches all had 0% pregnancy rates on their last breedings (often for multiple breedings), it appears that use of CDMSC-EV may be useful to treat PMIE in bitches.

There may be other uses for stem cells that are yet to be explored - for example, during the treatment of chronic endometritis or the medical management of pyometra.

Future research

Other medications like antihistamines, statins, opioids and SSRI antidepressants may also have antimicrobial properties and will likely be researched soon in veterinary medicine, following reports in humans[51]. Additionally, treatment protocols with Tris-EDTA, acetylcysteine, lactoferrin, platelet-rich plasma and regenerative stem cell therapies might be studied to assess their effectiveness in the bitch, since they have been successful in the mare - the species that appears most like the bitch in their expression of endometritis.

Conclusions

Endometritis is one of the most common causes of infertility in the bitch. A careful diagnostic workup is needed to determine the type of endometritis present and if any other concurrent uterine pathologies exist. Transcervical uterine procedures and ultrasonography have made diagnosis of endometrial disease readily available without significant risks to the bitch. There are many treatment modalities available. Since bacterial endometritis is less common and a healthy reproductive microbiome is critical for uterine health, indiscriminate use of antibiotics is contraindicated for the treatment of endometritis in bitches.

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Cardiology Essentials for The Theriologist- Part I: Murmurs in Puppies and Kittens

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SURPRISE: A new murmur!

- New murmur in puppy or kitten
- What does this mean?
- Differentiate innocent from pathologic murmurs
- Workup of murmurs in pediatrics
- Discuss most important congenital heart diseases
  - Diagnosis, medical management, interventional or surgical treatment

What is an Incidental Murmur?

- Detected unexpectedly when evaluating a patient for another reason
  - Wellness examination
  - Preanesthetic evaluation
  - Evaluated for other systemic problems
- No clinical signs detected referable to the cardiovascular system
- Very common in practice
- Can be stressful for both the client & veterinarian

Incidental Murmurs

Non-pathologic murmur

Pathologic murmur

- Not caused by a structural or functional abnormality of the heart
- Innocent- no underlying physiologic cause
- Functional- systemic cause of the murmur
  - Anemia, hyperthyroid

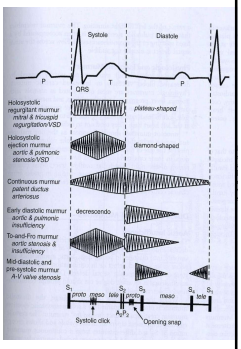
- Caused by a structural or functional cardiac abnormality
- Does not imply it is causing clinical signs or imply prognosis
- Many causes- Congenital or acquired heart disease

Non-pathologic Murmurs in Dogs

- Young dogs- typically pediatric - 2 yr old
- Soft I-II/VI left systolic murmur
  - Usually basilar; may be early systolic
  - Does not radiate
  - No other cardiovascular abnormalities present
    - Femoral pulses
    - Jugular veins
    - Arrhythmia
    - Pulmonary assessment
- +/- Further workup with echo
  - Breeding status, client concern, breed, persistent murmur > 6 months age

Pathologic Murmurs in Dogs

- Murmur characteristics that *increase* suspicion of pathologic heart disease
  - ≥III/VI systolic murmurs,
    - Radiating from PMI
  - Right sided systolic murmurs: VSD (basilar) tricuspid valve disease (apical)
  - Left apical systolic murmurs: Mitral regurgitation
  - Continuous murmurs: Patent ductus arteriosus
  - Diastolic murmurs: Aortic insufficiency



Ettinger Textbook of Small Animal Medicine

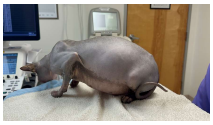
Cardiovascular Examination

Auscultation
- Location (left, right, basilar, apical)
- Timing (systolic, diastolic, continuous, to & fro)
- Intensity

Femoral pulses
- Fair to weak (SAS)
- Bounding (PDA)

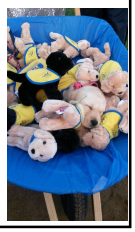
Mucous membrane color
- Cyanosis (generalized versus differential)
- Right to left shunting defects

Right heart clinical signs
- Jugular venous distension, hepatomegaly +/- ascites



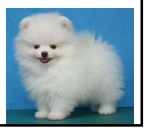
Congenital Heart Disease

- A morphological defect of the heart or great vessels that is present at birth
- Not synonymous with heritable, although many defects are heritable
- Spontaneous mutations may be passed down to progeny
- Diagnosis important to the individual, and for breeding purposes



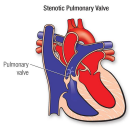
Congenital Heart Disease

- General practitioners are the <u>front line</u> for dx
- Prevalence 0.46- 1.6% dogs; 0.13-0.2% cats
- Echo required for dx & management
- Radiographs inaccurate
  - TXR correct dx in only 37-40% of puppies
  - Prioritize if respiratory signs or echo declined



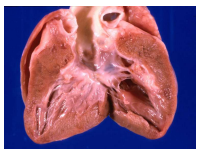
Classification of Congenital Heart Defects

- Defects Causing Pressure Overload
  - **Subaortic stenosis (dog)**
  - **Pulmonic stenosis (dog)**
- Defects Causing Volume Overload
  - Tricuspid valve dysplasia **(cat)**
  - Mitral valve dysplasia **(cat)**
  - Left to right shunting defects-
    - Atrial septal defect (ASD)
    - Ventricular septal defect (VSD) **cat**
    - **Patent ductus arteriosus (PDA) dog**
- Defects Causing Cyanosis
  - Right to left shunts
    - Tetralogy of Fallot, right to left PDA, Eisenmenger's syndrome

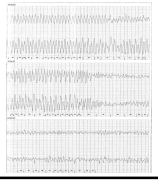


Subaortic Stenosis

- Obstruction of the left ventricular outflow tract (LVOT) by a fibrotic ring or ridge
- Most common in large breeds
  - Newfoundlands- auto dom
  - Golden retrievers, Rottie, Dogue de Bordeaux, Bull mastiff- auto rec
  - Boxers
  - German Shepherd dogs
  - Bull Terrier – valvular AS
- Worsens over first few months to 12 months of life



LV pressure overload ⇨ LV hypertrophy, myocardial hypoxia, myocardial fibrosis Ventricular arrhythmia & Sudden death



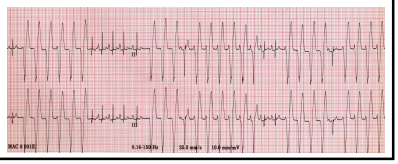
Subaortic Stenosis

Cardiovascular exam

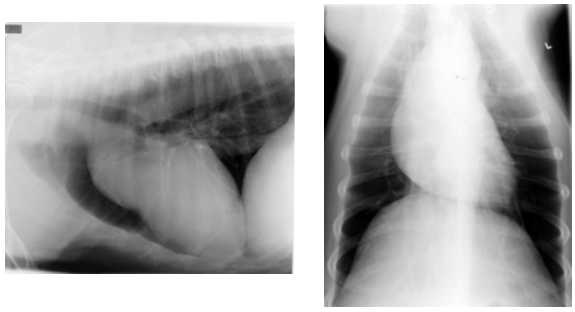
- Left basilar holosystolic murmur
  - May radiate to right, up the carotids
- Hypokinetic (dampened) femoral pulses

Electrocardiogram

- Ventricular arrhythmia
- ST segment depression or elevation > 0.2 mv regional myocardial hypoxia
- ↑ QRS amplitude- LV hypertrophy

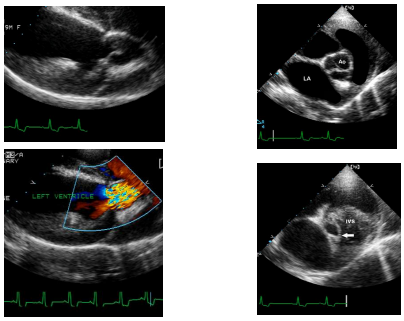


Subaortic Stenosis- Thoracic Radiographs



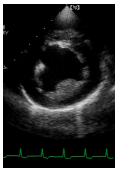
Subaortic Stenosis- Echocardiography

Fibrotic band, ridge, or tunnel just beneath the aorta

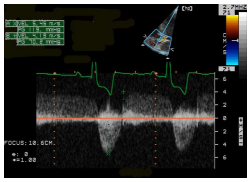


Subaortic Stenosis- Echocardiography

- Concentric LVH secondary to pressure overload
- Subendocardial hyperechogenicity (fibrosis)

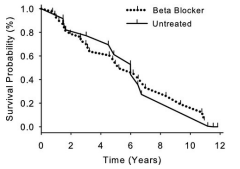


- Continuous Wave Doppler- LVOT & aortic blood flow velocity
  - Normal < 2 m/s
- Mild < 50 mmHg
- Moderate 50-80 mmHg
- Severe >80 mmHg

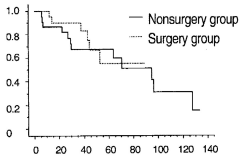


Subaortic Stenosis Treatment

- Atenolol?
  - Ventricular arrhythmias
  - Atenolol 0.5- 1.5 mg/kg PO BID
  - No effect on survival (PG >80)[1]
- Interventional (balloon valvuloplasty) or open heart surgery[2] - No effect on survival
- ↑ risk of infective endocarditis, perioperative antibiotics



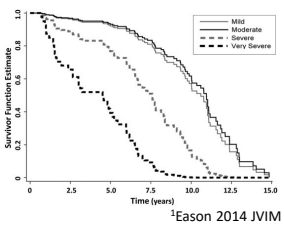
[1]Eason 2014 JVIM



[2]Orton 2000 JAVMA

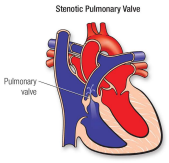
Subaortic Stenosis- Prognosis

- Severe SAS (PG >80) 16x risk of sudden death than mild or moderate
- Mild (<50 PG) MST 10.6 yr
- Moderate (50-79 PG) MST 9.9 yr
- Severe (80-129 PG) 7.3 yr
- Very Severe (130 PG) 3 yr

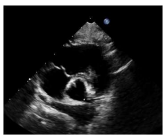


[1]Eason 2014 JVIM

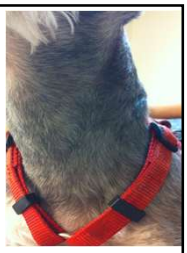
Pulmonic Stenosis



- 20-34% of CHD in dogs
- Valvular most common
  - Valve thickening, fused cusps, +/- annulus hypoplasia
  - Subvalvular, supravalvular uncommon
  - Anomalous left coronary artery in Bull dogs & Boxers, causing subvalvular extramural compression
- Breeds
  - French bull dogs, Pit bull, terriers, English bull dog (autosomal recessive), boxer



Pulmonic Stenosis

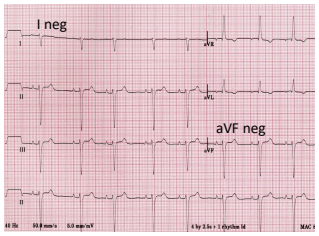


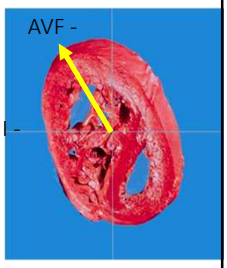
Presenting complaints
- Asymptomatic with murmur
- Syncope, fatigue, exercise intolerance, ascites

Cardiovascular exam
- Left basilar holosystolic murmur
- Normal femoral pulses
- Possibly right sided CHF
  - Hepatomegaly, ascites, jugular venous distension

Pulmonic Stenosis- ECG

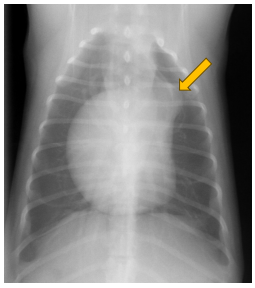
Right ventricular hypertrophy pattern
Right axis deviation (MEA 120 to -90)
Deep S waves





Pulmonic Stenosis- Thoracic Radiographs

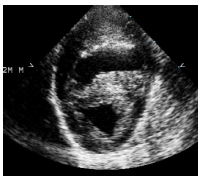
- Right ventricular enlargement
- Main pulmonary artery dilation (2 o'clock)
- Right atrial dilation
- Possible dilation of caudal vena cava + hepatomegaly

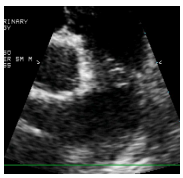


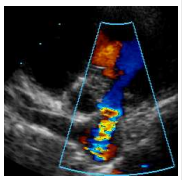
Pulmonic Stenosis- Echocardiography

- Right ventricular concentric hypertrophy
- Hyperechogenic subendocardium (fibrosis)
- Septal flattening

- Pulmonic valve thickened, dome during systole vs immobile
- Turbulent color flow at valve
- Post-stenotic PA dilation
- Severity: RVOT:PA PG same as SAS



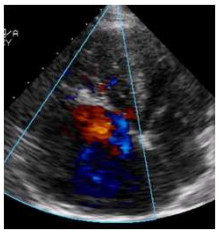




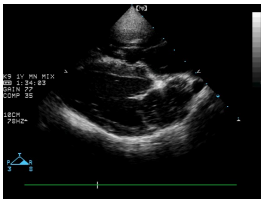
Pulmonic Stenosis- Echocardiography

Concurrent defects

- Tricuspid valve dysplasia
  - Neg impact of TR with RVOT obstruction

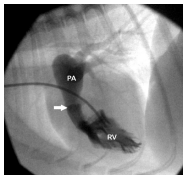


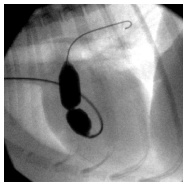
- Patent foramen ovale
  - Bubblegram
  - Rt to left shunting blood flow
  - Hypoxemia



Pulmonic Stenosis Treatment

- Nothing if mild, good prognosis
- 30% of dogs with severe PS die suddenly
  - 25x risk of death compared to mild or moderate
- Atenolol for ≥ moderate PS
- Percutaneous balloon valvuloplasty
  - ☑ Severe PS
  - ☑ Moderate PS with significant TVD, rt CHF, PFO
  - 53% ↓ risk of sudden death & improved QOL
- Percutaneous pulmonary artery stenting
  - Dysplastic valves or lack of improvement with ballooning
- Anomalous left coronary artery- atenolol, possible stenting





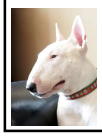
Breeding Screening Programs for AS & PS

- Italian Boxer breed screening program for AS & PS over 6 years
  - Auscultation & Echo:
  - Baseline PS + AS (isolated or combined) 17.8% incidence, ↓ to 12.68%
- Another Boxer screening program reported similar improvement in PS from 1997- 2017
  - ↓ Incidence from 35% PS to 23.8%
- French Boxer Club mandatory 17-year program for Boxers
  - Reduction in the proportion of Boxers compared to other breeds
  - 3,126 dogs screened for AS & PS
  - 2005-2009 Boxers 1st most common breed dx SAS (42.9%); PS 3rd most common (7%)
  - 2017-2021 Boxers 3rd common SAS (10%); PS 6th most common (2.4%)

Dysplasia Of The Atrioventricular Valves

Mitral valve dysplasia

- Bull terriers, Great Danes, large breed dogs
- Most common feline CHF
- Clinical Abnormalities
  - Left apical holosystolic murmur
  - Left sided CHF: cough, dyspnea, adventitious lung sounds



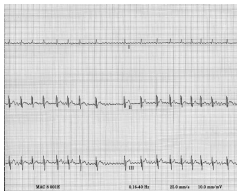
Tricuspid valve dysplasia

- #1 defect in Labrador retrievers
  - Auto dominant trait with incomplete penetrance (carriers) Localized to chromosome 9
- 40% of dogs with TVD also have MVD
- Most common feline CHD
- CV abnormalities
  - Right apical holosystolic murmur
  - No murmur in most mild TVD
- Right sided CHF

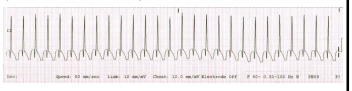
TV Dysplasia- ECG

- Arrhythmia- 16-22% of dogs
- Atrial premature complexes, supraventricular tachycardia, atrial fibrillation
- +/- Right axis deviation
- +/- Accessory pathway with orthodromic AV reciprocating tachycardia

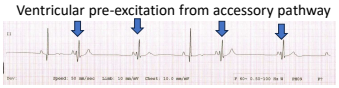
Splintered QRS complexes- 60%



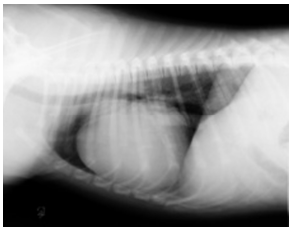
Supraventricular tachycardia in Lab with OAVRT

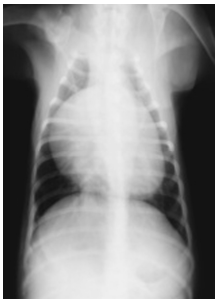


Ventricular pre-excitation from accessory pathway

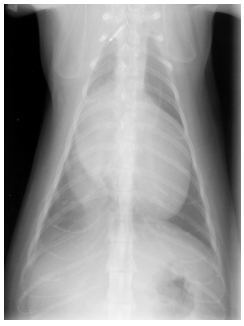


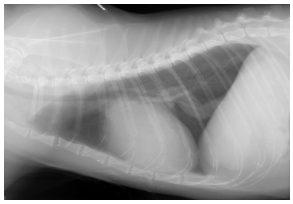
Thoracic Radiographs in TVD



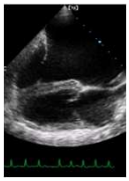


Thoracic Radiographs- Feline

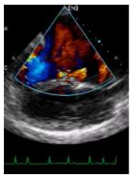


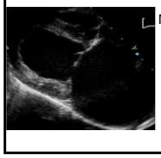


AV Valve Dysplasia- Echocardiography

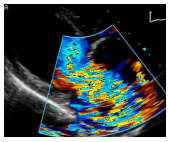


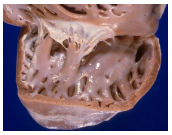
TVD





MVD





TVD

Valve abnormalities

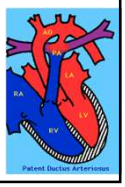
- Short, thickened chordae tendinae
- Absent chordae with fused papillary to the valve
- Thickened, clubbed leaflets
- Abnormal papillary position
- AV insufficiency
- Atrial dilation

AV Valve Dysplasia: Clinical Management

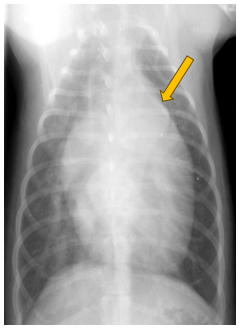
- No treatment necessary if mild to moderate
- Standard treatment of CHF
  - Quad therapy: pimobendan, Furosemide, ACE inhibitor, & spironolactone
  - Rate control if atrial fibrillation or SVT present
    - Diltiazem, sotalol
- Open heart surgery for TVD
  - Annuloplasty, +/- valve repair/ replacement
  - Mature dogs usually > 10 kg
  - Uncertain of current availabilities

Patent Ductus Arteriosus Clinical Abnormalities

- 8.5- 32% of Congenital heart disease
- Female predisposition
- Breeds: Poodle, Dachshund, Maltese, GSD, Dobie
- Auscultation
  Pathognomonic left basilar <u>continuous</u> murmur (No break in the murmur)
  - Emphasize LISTEN in arm-pit for continuous murmur
- Bounding femoral pulses (75% of dogs)
  - Marked ↓ diastolic pressure, mild ↑ systolic pressure
- Possible tachypnea, dyspnea, adventitious lung sounds if CHF



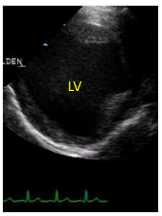
Patent Ductus Arteriosus Thoracic Radiographs

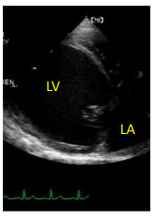


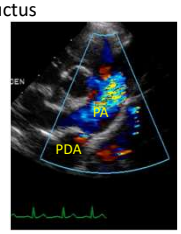
- Left ventricular enlargement
- Aortic- ductal aneurysm (ductal bump)
- Pulmonary overcirculation (a + v)
- Possibly pulmonary edema due to left sided CHF

Patent Ductus Arteriosus- Echocardiography

- LV and LA volume overload
- Continuous turbulent flow in the PA, retrograde from ductus

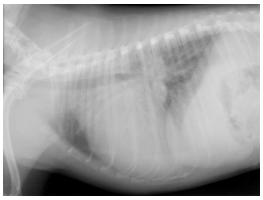


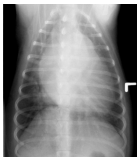




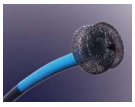
Patent Ductus Arteriosus Clinical Sequelae

- Congestive heart failure
  - 64% of puppies die before 1 year of age if PDA is not closed
  - RX: furosemide, pimobendane, & timely closure of the PDA
- Pulmonary hypertension and shunt reversal
  - Rare
  - Cannot close a reversed PDA

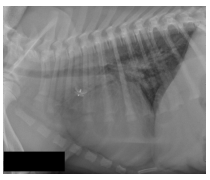


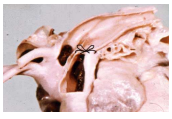


Patent Ductus Arteriosus Treatment



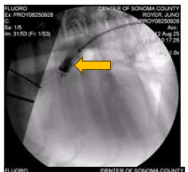
- Minimally invasive catheter-based closure
  - ACDO, coils
  - ACDO requires >3-3.5 kg dog size
  - ACDO 98-100% success rate, least device migration & lowest residual flow
  - Femoral arteriotomy & return home next day
  - Prognosis is excellent with successful closure
- Surgical ligation
  - Left lateral thoracotomy
  - Low mortality (<5%) in highly experienced surgeons
  - Mild morbidity associated with thoracotomy

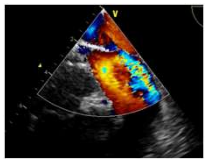




Patent Ductus Arteriosus- ACDO

Pre





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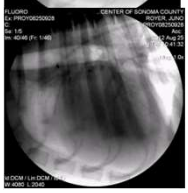
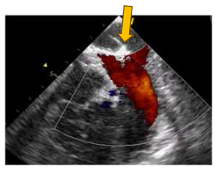


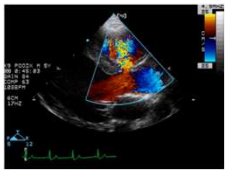


Ventricular Septal Defect

- VSD 4.8- 14% of CHD
- 75% subclinical at 9.6 years of age
- MST 12 yrs, individual variability
- #1 CHD in cats, occasional in dogs
  - English springers (auto dom), Keeshonds (polygenic), Fox terriers, Beagle (auto rec)
- Moderate to large VSD causes L- CHF
- Large VSD may lead to Eisenmenger's syndrome with right to left shunt reversal
- 45% have concurrent PS

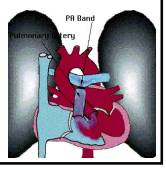
Characteristic murmur:

- Right basilar holosystolic murmur
- Murmur intensity is INVERSE to severity of shunt
  - Loud murmurs with smaller defects



Treatment of Ventricular Septal Defects

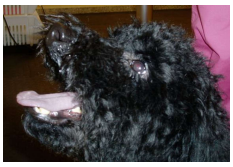
- Rx if left heart enlargement & CHF
- Surgical options
  - Pulmonary artery band
    - Animals > 6 months old
    - Thoracotomy, ↓ PA diameter by 60% to ↓ left to right shunting; TEE assessment of flow
- Medical management
  - Arterial vasodilator
    - Amlodipine 0.2-0.3 mg/kg q12-24 hr
  - CHF rx: quad therapy

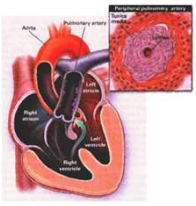


Cyanotic Congenital Heart Diseases

Pulmonic to systemic shunts (Right to left)

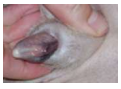
- Tetralogy of Fallot
  - Most common cyanotic CHD
- Right to left patent ductus arteriosus
- Eisenmenger's Complex from large VSD
- Tip off: RV Hypertrophy



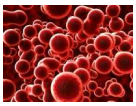


Cyanotic Congenital Heart Diseases

- Complex dx requiring cardiologist
- Hypoxia and polycythemia are the main clinical problems
  - Cyanosis occurs with PaO2 of 40 mmHg, SaO2 70%
- Clinical signs: weakness, exercise intolerance, syncope, dyspnea
- Generalized vs. differential cyanosis
- Treatment aimed at control of polycythemia
- Correction of defect (rt L PDA, Eisenmenger's VSD) not indicated



Treatment of Polycythemia



- Target PCV 60-65%
- Hyperviscosity syndrome if ≥70%
  - Poor perfusion, tissue ischemia, platelet aggregation, thrombosis

Phlebotomy

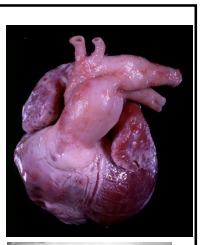
- Removal of blood (according to equation) and simultaneous replacement of 1-2 x volume with IV fluids

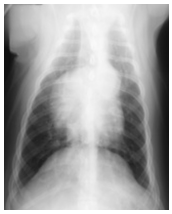
Hydroxyurea

- Reversible BM suppression
- 30 mg/kg/day x 7 d then 15 mg/kg/day taper dose as needed

Right to Left Patent Ductus Arteriosus

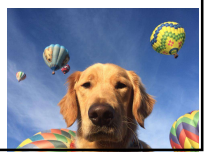
- Large, unresistive PDA
- No murmur
  - Possible split S2
- Differential cyanosis of caudal body
- Hind limb weakness
- Polycythemia
- Irreversible pulmonary hypertension
- MST 627 days, if right CHF 58 days
- Sildenafil independent association with improved survival (1839 vs. 302 days)





Conclusion

- Differentiation of innocent from pathologic murmurs in pediatrics is first step
  - Pathologic murmur characteristics, concurrent cardiovascular abnormalities
- Congenital heart disease requires comprehensive, detailed echo by cardiologist to determine management
- Interventional procedures and medical management depending on patient specifics
- Many CHD patients can live long lives



Cardiology Essentials for The Theriologist- Part II: Acquired Heart Disease in Small Animals

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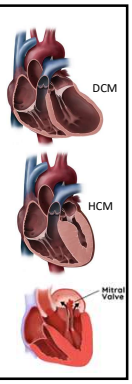
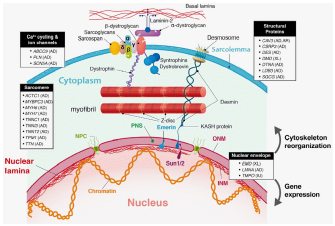
Acquired Heart Disease in Small Animals

- Genetic etiology & testing
  - DCM, HCM
- Preclinical diagnosis
  - Diagnostic testing
  - Management and clinical outcomes
- Dilated cardiomyopathy (dogs)
- Mitral valve degeneration (dogs)
- Hypertrophic cardiomyopathy (cats)



Etiology- Dilated Cardiomyopathy

- 1^0 cardiomyopathy
- In people, mutations in > 50 genes
- Doberman- auto dom.
  - DCM1- Pyruvate dehydrogenase kinase (PDK4)
  - DCM2- Titin
- Boxer- auto dom.
  - ARVC1 gene: Striatin- intercalated disc
  - ARVC2 gene: regulatory gene
- Great Dane- X linked
- IWH- auto recessive



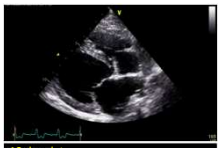
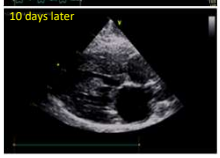
- Welsh Springer Spaniel- auto dom- phospholamban, high penetrance all develop DCM
- Standard schnauzer- recessive; RNA binding motif

DCM Phenotype: 2^0 myocardial failure Don't miss these!

- Tachycardiomyopathy
- Nutritional

  Taurine deficiency
  - Golden retriever
    Cocker spaniel
    Newfoundland
  - Whole blood taurine <150;
    Goldens <200 (200-250 equivocal)
    Plasma <40 nmol/l;
    Goldens <60 (60-70 equivocal)

  Carnitine deficiency unclear
  - 50 mg/kg PO TID?

Diet Associated DCM

- Non-traditional diets = Boutique, exotic, grain free
  - Legumes
  - AAFCO label of nutritional adequacy; WSAVA standards
- Most have normal taurine levels
- Diet △ greater improvement in systolic function, LA size [1]
- Diet △ 30% alive vs. *none* in no diet △ or normal diet DCM over 9 months

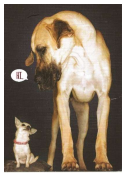
- FDA- No direct causality proven
- GF DCM younger dogs
- Adin et al: n=36 GFD vs. 12 grain based + DCM[2]
  - None taurine deficient
- GF larger weaker hearts, more advanced disease
- Diet enhanced pathophysiology
- Improvements 3- 9 months after taurine, Δ diet , cardiac meds

[1] Freeman L, ACVIM abstract 2020; [2] Adin, J Vet Cardio 2019

Signalment

MV degeneration

- 75% patients are small breed (<20#)
- Large breed dogs may have MVD + 2^o myocardial failure
- Geriatric age 10 +
  - CVCS middle age 6 yrs
  - 30% of small breed dogs >10 yrs old have MVD
- Male > female



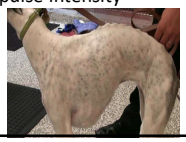
Dilated Cardiomyopathy

- Medium to large breed dogs
  - 90% Doberman, GD, IWH, Newfie, St. Bernard, Boxer, Dalmation, Cocker
    - Portugese water dog- juvenile form, auto recessive
  - Smaller dogs:
    - Standard Schnauzer
    - Irish Terriers
- Middle age to older
- Male > female

Cardiovascular Examination

MV Degeneration

- Left apical systolic murmur
  Small dogs: I-II typically mild
  V-VI severe
  III-IV anything goes!
- ↑ Precordial impulse intensity
- +/- Arrhythmia



Dilated Cardiomyopathy

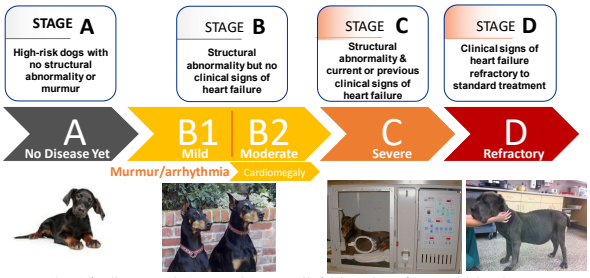
- Soft left apical systolic murmur
- S3 gallop
- Arrhythmia
- Weak pulses

Breeding Screening Programs in MVD- Do they work?

- MMVD screening
- Danish Kennel Club mandatory program- auscultation & echo from 2002-2011[1]
  - 2002-2003 vs. 2010-2011 groups compared
    - Dogs product of breeding program 2010-11: 73% reduced risk of MR murmur; non-product of breeding program not different than 2002-3
- UK voluntary program did not reduce prevalence of MR murmur
  - 21% of puppies from Cavalier Club partipating in program
  - Only 4% of dogs in program followed breeding guidelines

[1]Birkegard 2015 JVIM

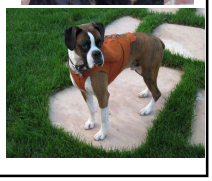
ACVIM Classification



Material summarized from publication: Keene, BW, Atkins, CE, Bonagura, JD, et al. ACVIM consensus guidelines for the diagnosis and treatment of myxomatous mitral valve disease in dogs. J Vet Intern Med. 2019; 33: 1127– 1140.

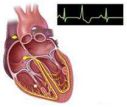
Stage A DCM- Genetic Screening Tests

- NC State Cardiac Genetics Laboratory
- DCM in Dobermans- 2 mutations
  - DCM1- Pyruvate dehydrogenase kinase; 68% penetrance
  - DCM2- Titin, 47% penetrance
  - 2 concurrent mutations worse clinical course
  - 15% have DCM & no mutation
- Arrhythmogenic right ventricular cardiomyopathy in Boxers
  - ARVC1 Striatin- desmosomal protein
    82% penetrance in heterogygous; 100% in homozygous
  - ARVC2- regulatory protein

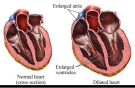


The silent threat- Preclinical DCM

- Asymptomatic DCM patients may have moderate to severe disease
- Breeding considerations
- 30% risk of sudden death in Stage B DCM
- ↑ Anesthetic risk
- May have unremarkable CV exam
- Abnormal CV auscultation
  - Murmur in 46% of preclinical DCM[1]
    - Soft murmur cannot be equated as mild disease
  - VPCs in 48% of preclinical DCM[1]
    - 26% complex ventricular arrhythmia
  - +/- S3 gallop



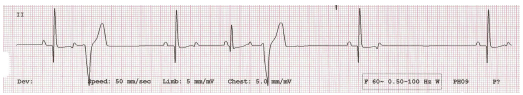
- Stage B1 DCM
  - Arrhythmia
    - Ventricular arrhythmia
    - Atrial fibrillation
- Stage B2 DCM
  - Myocardial failure
  - Arrhythmias common



[1]Gordon et al 2022, J Vet Cardio

VPC's: The Tip of the Iceberg

- VPC's in a Boxer, Doberman, or other predisposed breed may be an indication of occult ARVC or DCM & cannot be dismissed!
  - 1 VPC/5 min is 97% specific for occult DCM in Dobermans
- Run 5 min ECG as a preanesthetic evaluation in at-risk breeds
- If VPC or atrial fibrillation present, an echocardiogram & ideally holter are needed for further evaluation



Screening Stage B DCM

Best practice screening tests

- Holter monitor
  - <50 VPC/24 hr normal
  - Doberman: >50 VPC/24 hr or complexity
  - Boxer: >300 VPC/24 hr
    - 100-300 VPC equivocal
    - Couplets, complexity abnormal
- Echocardiogram
- Annually in dogs ≥ 3 yrs

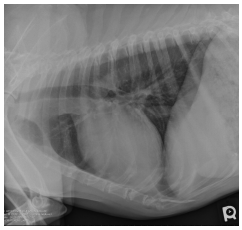
GP screening if best practice not possible

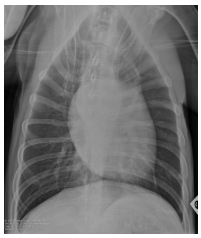
- ECG- 5 minute
  - Recommend echo if
    - 1 VPC/5 min
    - Atrial fibrillation
- Nt- ProBNP
  - Detects myocardial disease
  - >500 pmol/L Doberman (sensitivity 78.6%, specificity 90.4%)
  - >900 pmol/L other breeds

GP Screening for Stage B2 DCM

Thoracic Radiographs

- TXR insensitive to screen for preclinical DCM

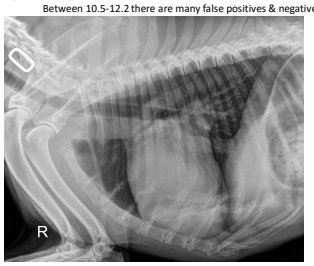


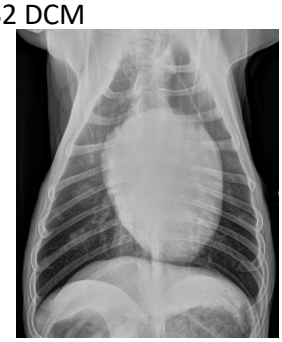


Thoracic Radiographs in B2 DCM

Dogs >20 kg, VHS >12.3: 73% had B2 DCM [1]

Between 10.5-12.2 there are many false positives & negatives



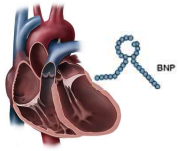


[1] Gordon et al JVC 2022

GP Screening for Stage B2 DCM

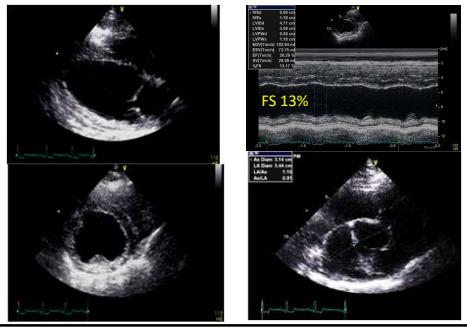
Nt-ProBNP

- 77- 90% specificity, 79- 100% sensitivity for myocardial failure (B2)
- 76% sensitivity, 77% specificity (n= 324) if VPCs (B1) *or* myocardial failure (B2)[2]
- Combination Nt-proBNP + Holter
  - 94.5% sensitivity, 87.8% specificity

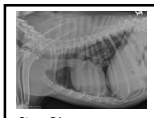


Wess G, J Vet Intern Med 2009; 23:686.

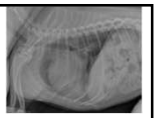
Gold Standard Test: Echocardiogram Stage B2 DCM



Nt-Pro BNP in Stage B MV disease

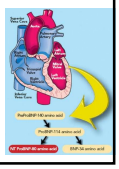


Stage B1



Stage B2

- Less accurate for differentiating B1 from B2, better at differentiating heart failure from preclinical or normal
- Differentiating B1 from B2 MVD
  - Pro-BNP > 1100: sensitivity 53%, specificity 85%, PPV 57%[1]
- >1500 6x higher risk for CHF within 3- 6 months [2]
  - Closer & more frequent monitoring
  - Impact rx decisions?
- Elevated Nt-ProBNP predicted onset of CHF in 12 months[3]
  - 80% sensitivity
    76% specificity



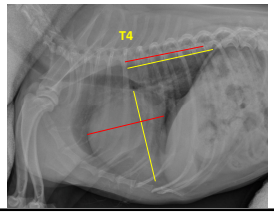
[1]Wilshaw JVIM 2021, 23:686. [2] Reynolds C, J Vet Cardiol 2012. [3] Chetboul JVIM

TXR for prediction of Stage B2 MV disease

Prediction of VHS >10.5 in dogs with MVD [1]

- 33% of dogs with radiographic cardiomegaly had normal LA:Ao and LVIDd (false positive)
- 21% of dogs with mild to moderate LA dilation and LVIDDN >1.7 had normal VHS (false negative)
- 14% of dogs with severe LAE on echo and LVIDDN >1.7 had normal VHS (false negative)

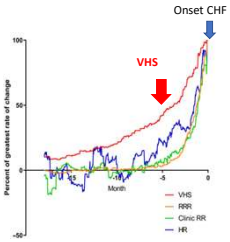
- VHS >11.5 most predictive for echo EPIC criteria to start pimobendan



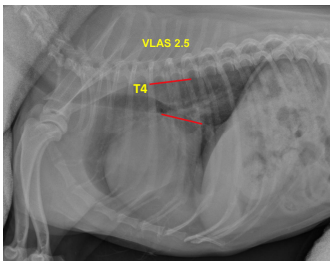
[1] Franchini et al, 2021, J Vet Cardio

GP Management of Stage B MVD Dogs

- Thoracic radiographs
  - VHS helpful (>11.5 EPIC criteria)
  - Submit for radiologist review
  - Baseline for comparison over time



Vertebral LA size- VLAS

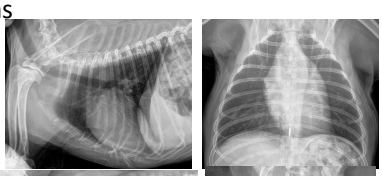


- Carina- center & ventral to LA caudal border jxn with dorsum of cd vena cava
- T4 vertebra- measure in 0.1 increments
- > 2.3- 2.5 predict LA dilation EPIC criteria [a,b]
- > 2.8- 3 high specificity for LA dilation EPIC criteria [a,b]

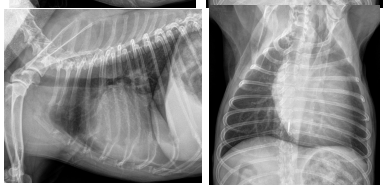
[a] Visser L, ACVIM abstract 2020; [b] Stepien R, JAVMA 2020 p 1129

Serial Radiographs

Baseline
Early Stage B2

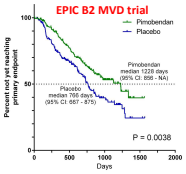


12 months later
Progressive B2

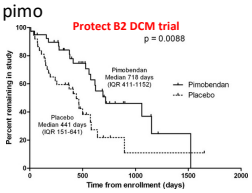


What's the motivation to treat Stage B2 dogs with pimobendan?

- 15 more symptom-free months for B2 MVD dogs on pimo
- Median time to heart failure: 3.5 yrs on pimo



- 9 months more symptom-free months in B2 DCM dogs on pimo
- Median time to heart failure: 2 yrs on pimo



What is the motivation to Prolong Stage B preclinical disease

Stage C is harder on dogs & clients

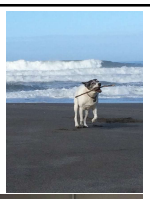
PU/PD

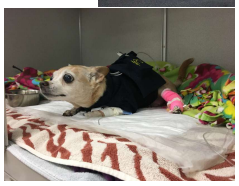
Coughing, Dyspnea

Fatigue

More rechecks with more tests

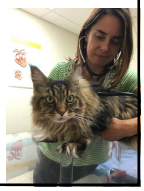
More medications for optimal therapy





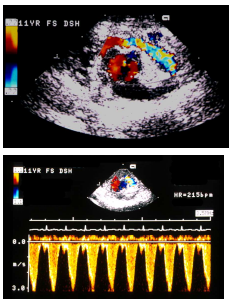
Incidental Murmurs in Cats

- Murmurs are common in apparently healthy cats
  - 16-44% of adult cats in hospital or shelter
- Heart disease caused the murmur in 16- 77% of cats
  - 62% of cats with incidental murmurs had HCM
- Impossible to assess if pathologic or non-pathologic by auscultation alone
- Loud murmurs >3/6 usually congenital or HCM
- Echocardiogram is the test of choice



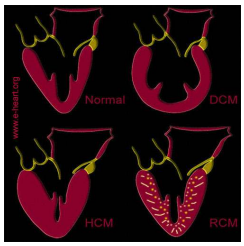
Non-pathologic Murmurs

- Dynamic RVOT stenosis
  - Most common cause (8%) of non-pathologic murmur
- Systemic diseases
  - High output states
    - Anemia
    - Hyperthyroidism
  - Diseases causing systemic hypertension
- Innocent murmur of unknown cause



Pathologic Murmurs in Cats

- 2^0 to cardiomyopathy
  - 80% of cats with HCM
  - 20% in DCM
  - 36% in RCM
- Increased suspicion of cardiac disease if:
  - Gallop present
    - S3 or S4 heart sound
    - 80% in DCM
    - 33% in HCM
  - Arrhythmia present



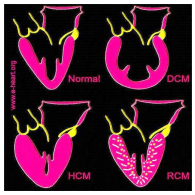
Comprehensive Echocardiogram

- Diagnoses the etiology of heart disease
  - Specific cardiomyopathies
  - Congenital heart disease
    - Most common: MV/TV Dysplasia, VSD
  - Assess systolic and diastolic dysfunction
- ID Risk factors for adverse events and impacts treatment recommendations
  - Left atrial dilation, smoke
- Determines prognosis depending on disease & severity
- Breeding screening annually for HCM
- Focused echo by trained nonspecialist- accuracy for dx mod severe dz 93-100%, low for mild dz 45.6%[1]

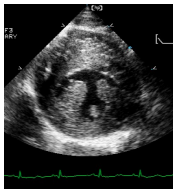
[1]Loughran 2019 JVIM

Classification of Cardiomyopathies

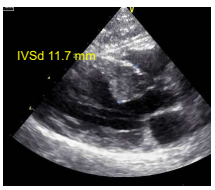
- Pathophysiologic abnormalities
  - Echocardiographic assessment
    - LV hypertrophy
    - Myocardial failure
    - Diastolic filling abnormalities
  - Various etiologies with common distinct hemodynamic features
  - Limitations
    - Overlap in functional groups
    - Subsequent shift from one functional group to another due to cardiac remodeling



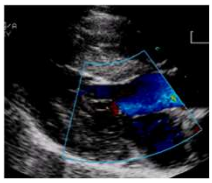
Hypertrophic Cardiomyopathy



LV wall ≥ 6 mm



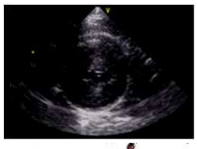
Basilar septal hypertrophy



LVOT obstruction- SAM HOCM

Hypertrophic Cardiomyopathy

- Concentric LV hypertrophy without other systemic causes
- Diagnosis of exclusion- rule out other causes
- Secondary causes of LV hypertrophy
  - Systemic hypertension
  - Hyperthyroidism
  - Acromegaly
- Prevalence of HCM
  - Overall 14.7-16% [1-2]
  - Sphynx 20% [3]
  - Maine Coon + Ragdoll 26%[4]



[1] Paige 2009, [2] Payne JVC 2015, [3] Chetboul J Vet Cardiol 2015, [4] Gundler 2008

HCM: Patient Characteristics

- Dominant heritability
  - Maine Coon cat
  - American shorthair
  - Ragdoll
  - Sphynx
- Other predisposed breeds:
  - Scottish Fold, Rex, Siberian, Egyptian Mau, Bengal
- DSH most common breed
- Male > female 3:1
- Age: young to old (<1- >15) Mean age 4.8 - 7 yr

Auscultation

- Murmur common (80- 90%)
  - 81.7% sensitivity; 66.3% specificity for HCM[1]
- Gallop (7- 30%)[2, 3] > Symptomatic
- Arrhythmia (6- 13%)[2, 4] > Symptomatic
- 7- 20% Normal auscultation[2,3]

[1] Payne JVC 2015, [2] Trehiou JVIM 2012, [3] Rush JAVMA 2002, [4] Fox JVIM 2018

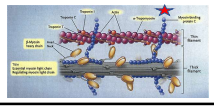
Stage A HCM- Genetic Mutations

Maine Coons & Ragdolls

Myosin binding protein C

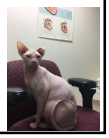
- 2 different mutations
  - A31P mutation in Maine Coons- 34-41% prevalence (10% homo; 90% hetero)
  - R820 mutation in Ragdolls- 17-27% (1.4% homo)
- Carrier heterogygous cats may not develop HCM phenotype
  - Incomplete penetrance
  - Time-dependent onset
- Homozygous affected more severe
- HCM wild type also occurs

Testing submitted to NCSU Cardiac Genetics Laboratory



Sphynx

- ALMS1 gene associated with HCM in 60% of affected Sphynx cats (NCSU)
  - Other mutations likely
- Alstrom syndrome in humans causes multisystemic disease including cardiomyopathy
- ALMS1 regulates cell cycle proliferation in perinatal cardiomyocytes



HCM Natural History

- 2 retrospective studies- 10 yrs duration [1-2]
- Asymptomatic HCM cats shorter MST 10.9 y than normal cats[1]
- Cats with cardiac death were younger (7.9 y) than non-cardiac deaths (11.2y)[2]
- 12- 30% risk for developing CHF or ATE over 10 years [1-2]
  - Sudden death 8%[1]
  - 1st Cardiac event: CHF 67%; ATE 16%[2]
- Once cardiac event occurs, regardless of age, survival is same 1.3 +/- 1.7 yrs[1]
- The longer a cat lives with HCM, the greater risk of dieing from it- progressive disease
  - CV death risk:
    1 in 15 cats after 1 yr
    1 in 4.4 after 5 yrs
    1 in 3.5 after 10 yrs
- Maine Coons first event at 2.5 yrs old compared to 7 yrs old all other breeds[2]
  - 50% MC died

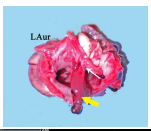
[1] Fox et al JVIM 2018, n=1000 HCM n= 728 normal cats; [2]Trehiou JVIM 2012- 10 yr, 169 asymptomatic, 48 symptomatic

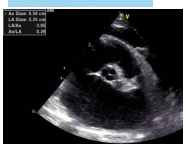
Natural History of HCM

Clinical – Symptomatic HCM

- End-stage of disease
- CHF: MST 15 months[1]
- ATE: MST 4- 8 months[2-3]
- Sudden death

- Predictors for cardiac mortality
  - Left atrial size- most important
  - Severity of LVH
    - > 9 mm wall thickness = independent predictor
  - Others: myocardial failure, LA function

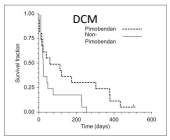




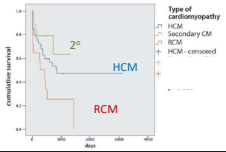
[1]Fox PR, JVIM 2018,[2]Hogan D, J Vet Cardiol 2015, [3]Borgeat JVIM 2014

Outcomes in Feline Cardiomyopathies

- **DCM**
  - Nearly all have CHF or ATE at presentation
  - Non-taurine: pimo 49 days vs. 12 days no pimo[1]
  - Taurine deficiency measure WB +/- plasma taurine for any myocardial failure or supplement
    - 35% die within 2 weeks
    - If survive > 2 weeks, 96% chance of long term survival with excellent prognosis



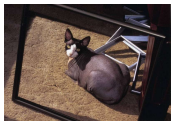
- **RCM**
  - MST 273 days (9 months), 86% died[2]
  - Earlier studies pre- pimo: 100-132 days
  - Dx criteria include LA dilation
    - Almost all had HF at presentation or ATE
- **2º Cardiomyopathy** - 80% lived 10 years[2]
- **HCM**- MST 865 days; 44% died[2]



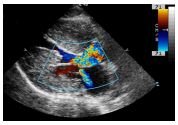
[1]Hambrook JFMS 2012, [2]Spalla JFMS 20

Treatment of HCM

- Asymptomatic & compensated: controversial whether to treat
  - No data to indicate optimal treatment that actually impacts clinical outcome
- Severe HCM no CHF:
  - Mod- severe LAE- antiplatelet, ACE inhibitor
  - Beta blocker?
  - Rapamycin?
- Severe HCM & CHF:
  - Furosemide, ACE inhibitor, & Antiplatelet



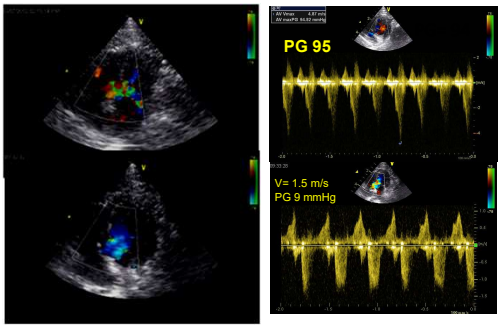
Treatment of HCM
Beta blockers



- Best treatment to reduce SAM
- Reduce LV hypertrophy
  - Anecdotal
  - Mild ↓ in septal hypertrophy[a]
- Rx of tachyarrhythmias (supraventricular or ventricular)
- No benefit in prolonging survival in symptomatic cats
  - Worsened outcome? Avoid starting if CHF unless tachyarrhythmia; ↓ dose or wean off if develop CHF
- Atenolol 6.25 – 12.5 mg PO q24 hr- BID

[a]Schober, 2007 ACVIM abstract

Effect of Atenolol on SAM

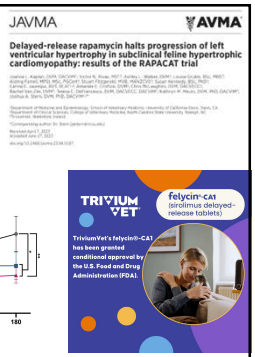


Treatment of Secondary Causes of LV Hypertrophy

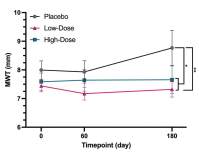
- Hyperthyroidism:
  - Methimazole, I – 131
    - Remember to measure BP post- treatment (30% new onset hypertension)
- Systemic hypertension
  - Amlodipine 0.625 mg – 1.25 mg PO q24 hr - BID
  - Telmisartan- angiotensin receptor II blocker
    - PLN + HTN
  - Target systolic BP < 150- 160 mmHg
- Regression of LV hypertrophy occurs over a couple of months
  - If LVH remains, concurrent HCM likely

Rapamycin

- Rapamycin- mTor inhibitor
  - mTor- anabolic,↑ protein & lipid synthesis; ↓ catabolic effect ↓ autophagy
  - Rapacat study: ↓ Maximal wall thickness day 180 low-dose rapamycin group (n= 14; placebo n= 12)
- Felycin-CA1
  - While awaiting HALT clinical trial results
- HALT trial
  - Preclinical HCM
  - Double-blinded, placebo controlled, duration 1 yr
  - Enrolling since 2024


JAVMA AVMA

Delayed-release rapamycin halts progression of left ventricular hypertrophy in subclinical feline hypertrophic cardiomyopathy: results of the RAPACAT trial



ProBNP tests

- SNAP ProBNP bedside test
- Visual comparison of test well to reference well
  - Positive = sample darker than reference well; >100-150 pmol/ml
- ELISA SnapShot Dx scanner not significantly superior to visual detection

- Quantitative Cardiocare ProBNP
  - Results not immediate
  - Can use as part of a senior panel in at risk cats
  - Different levels of elevation
    - <100 pmol/l normal
    - 100-270 pmol/l abnormal- may have heart disease
    - >270 pmol/l suggestive of heart failure in dyspneic cats
- V-check quantitative ProBNP bedside test

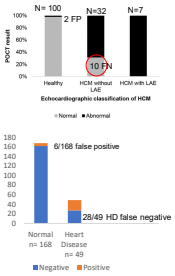
Nt-ProBNP in asymptomatic cats

- Screening asymptomatic cats is less accurate
  - Detects moderate to severe disease with high specificity
  - May miss mild disease
  - Not appropriate for breeding screening
- Performance of test depends on disease prevalence
  - Performs better with higher prevalence of disease
    - Auscultation abnormalities or clinical signs

Machen 2014 JVC

ProBNP in Asymptomatic Heart Disease

- POC SNAP for asymptomatic HCM in referral patients
  - Sensitivity: 65-88%
  - Specificity: 81-100%
  - Accuracy improves for severe heart disease
    - SE 100%, SP 98%
  - Lower sensitivity in milder disease (false negatives)
    - SE 69%, SP 98%
- Screening for (any) preclinical heart disease in GP practice[2]
  - Lower prevalence & severity of heart disease than referral patients
  - POC SNAP in 221 healthy cats
    - SE 43%, SP 96%
    - If screened only murmur cats, SE 71%, SP 92%
    - Positive likely true positive, but many false negatives- missed dx



ProBNP in cats with systemic diseases

- Elevated ProBNP in systemic diseases that affect the circulatory system
  - Hyperthyroidism- 70% elevated (1/3 were >270 pmol/l)[1]
    - The same magnitude of elevation as HCM cats- cannot differentiate based on ProBNP[2]
  - Systemic hypertension with CKD
  - Severe chronic kidney disease- possible mild elevation

ProBNP levels decrease with euthyroid or normotensive state

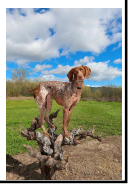
May still be elevated- 40% of euthyroid cats 3 months after rx

Underlying cardiac disease possible

[1] Sangster JVIM 2014; [2] Sangster JVIM 2013

Conclusion

- Detecting cardiac disease in preclinical animals is challenging but important
- Dx clinically relevant disease which enables treatment prior to end stage, hopefully improving outcome
- Breeding recommendations based on screening
  - Annual exams for acquired diseases necessary
- Preanesthetic screening for predisposed breeds



Delay of puberty with deslorelin in dogs and cats

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Gonadotropin-releasing hormone (GnRH) is secreted by the hypothalamus in a pulsatile pattern with species-specific differences. In adult dogs, slow GnRH-agonist-releasing implants lead to desensitization and down-regulation of GnRH receptors in the pituitary gland, leading to inhibition of gonadotropin release after an initial stimulation defined as flare-up. The only currently available GnRH-agonist implant is the deslorelin implant Suprelorin® (Virbac, F), available as 4.7 mg and 9.4 mg implants. The 4.7 mg implant is among others licensed for the use in prepubertal female dogs.

Delay of puberty is sometimes desired by the owner to postpone the first oestrus and to prevent unwanted pregnancies. In previous studies, the use of one deslorelin implant before puberty was shown to not affect ovarian function and body development in male and female dogs (Trigg et al. 2006, Sirivaydjapong et al 2012, Kaya et al 2013, 2015, 2017, Schäfer-Somi et al 2018). In prepubertal bitches of medium body size, both the 4.7 mg and the 9.4 mg deslorelin implant were found to delay puberty for >8 to 25 months; duration of efficacy was independent from dosage (for review: Schäfer-Somi et al 2022). In male dogs, onset of puberty after delay with a 4.7 mg or 9.4 mg deslorelin implant, inserted at the age of 4 months, was on average 34 months; in most cases even longer in the 9.4 mg group (Sirivaidyapong et al 2012). Interestingly, when two 9.4 mg deslorelin implants were administered s.c. in neonatal male and female dogs immediately after birth, puberty occurred significantly later in comparison to control dogs who received a placebo (72.7 vs 35.8 weeks) (Faya et al 2018). In female cats, postponement of puberty was possible until the age of 9-36 months, when a 4.7 mg implant was administered at the age of 3-9 months (Risso et al 2012, Cecchetto et al 2017). In two tomcats, puberty was delayed until at least 16 and 20 months, resp., when the study ended (Romagnoli et al 2010). And similar to the experiment in postnatal dogs, female and male cats were administered a 1.6 mg deslorelin implant 24 h after birth. Puberty occurred at the age of 58 weeks in males and at 67 weeks in females. Body development was not disturbed (Carranza et al 2014).

An interesting effect is that the serum concentration of gonadotrophins will not increase as observed after gonadectomy. In dogs, the long-term effect of increased LH-serum-

concentrations is currently under investigation as it may predispose to several diseases (Kutzler 2023).

In dogs, the use of one implant does not seem to completely suppress ovarian activity as the development of the secondary organs was undisturbed, whereas epiphyseal closure was delayed (Kaya et al 2015). In another study, the vulva remained juvenile, when three 4.7 mg deslorelin implants were inserted subcutaneously at the age of 4.5, 9, and 13.5 months (Marino et al 2014). This may indicate a more effective suppression of *GnRH* and/or *Kiss* gene activity within the hypothalamus or on GnRH hormone release and/or a more effective receptor downregulation, when the application is more frequently done; however, the effect of the GnRH-agonist implant on the central nervous system during the prepubertal period remains to be investigated.

The short- and long-term effects of deslorelin implants administered at different time points of the prepubertal period differ from those of adult dogs. The age of the prepubertal bitch at the time of implant insertion appears to be the main determinant of the response to deslorelin. Most studies to postpone puberty in bitches where started at the age of 3-7 months, to prevent a flare-up; however, flare-up symptoms with visible effects of estrogens were sporadically described in several studies in prepubertal dogs and comprised vulva swelling and/or increase in superficial cell index and sometimes bloody vaginal discharge; in some cases, increased E2 concentrations were the only sign of ovarian activity (Trigg et al. 2001,2006, Marino et al 2014, Kaya et al 2013, 2015, Gontier et al 2022). In recent studies (Karadag et al 2023, 2024), 4.7 mg implants were inserted in 16 prepubertal bitches at the age of 7-8 months. The late application caused a flare-up in all dogs; however, the effect was variable and different from the flare-up in adult dogs implanted in anoestrus. Similar studies in cats are not available.

In conclusion, prepubertal administration of a 4.7 mg or 9.4. mg implant safely postpones puberty in male and female dogs and cats; however, a flare-up may occur in any case. The probability of an induced proestrus increases with age at implantation. In dogs, the deslorelin-mediated long-term delay of puberty so far did not have negative effects on subsequent ovarian functionality, serum steroid hormone concentrations, uterine health and fertility. However, more studies are needed concerning the best time of administration. Similarly, the long-term effects on the development of orthopedic problems and tumor diseases remain to be investigated in both dogs and cats.

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Melatonin treatments in cats

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Manipulation of the oestrus cycle in female cats and contraception in both male and female cats is a prominent issue. Especially the short-term delay of oestrus in female breeding cats is a challenge; some treatments may cause severe side effects and decrease fertility. For short-term suppression of oestrus, most breeders in many countries use progestin pills (Schaper et al. 2025), since it can be administered once weekly, the effect is fully reversible, and fertility usually recurs within one month. Administration of a GnRH-agonist implant (4.7 or 9.4 mg deslorelin; Suprelorin®, Virbac, F) can be used off-label in female cats; duration of efficacy is variable but may be more than a year (Göricke-Pesch et al 2013, Baldan et al 2025). To reduce the time of duration, it can be removed; cats will return to oestrus 3 to 7 weeks after removal (quicker during increasing photoperiod; Ferré-Dolcet et al 2022), irrespective of treatment length and queen's age or body weight. Melatonin, a neuromodulatory substance, is produced and secreted in the pineal gland during autumn and winter, while daylight intensity decreases. When duration of daylight decreases to < 8 hours, the increase in serum melatonin concentration becomes measurable. Melatonin affects on the hypothalamus by decreasing GnRH secretion; consecutively, FSH and LH secretion decrease, and ovarian function will be blocked. Therefore, melatonin was considered a contraceptive decades ago. Early studies from the 1980s used frequent injections or tablets. The clinical effects of these schemes varied, and the studies are difficult to compare. Different preparations, concentrations, experimental periods, and light protocols were used. Leyva et al (1989) applied 5 mg/cat i.v. every other day and observed oestrus suppression for 60 days. Faya et al (2011) administered 4 mg/cat once daily and duration of suppression was 50 days on average. Graham et al (2004) used 30 mg/cat once daily and observed oestrus suppression for an average of 33 day. Time of duration were additionally influenced by season as well as intensity and duration of day light. During the time of application, ovulations may occur, this happened in >30% of cases (Faya et al 2011). No side effects were observed in any study.

During the last two decades, the interest in melatonin implants increased. Most studies found a significant increase in the interestrus interval in comparison to controls receiving no melatonin

or a sham implant, when 12-60 mg melatonin where applied. Interestrus interval was 63.8 to 113 days (Faya et al 2011, Gimenez et al 2009), and duration of suppression from application to next oestrus was 21 to 277 days (Faya et al 2011, Schäfer-Somi 1013, Furthner et al 2020), when an 18 mg melatonin implant was used. As with tablets and injections, ovulations occurred in approximately one third of cases. The lack of relation between melatonin dosage, plasma concentration of melatonin and duration of oestrus suppression is independent from application. However, duration of efficacy can be increased by reducing intensity and duration of light; serum melatonin concentrations in the domestic cat were 15-fold higher during the dark phase than the light phase. Furthermore, Melovine® 18 mg implants (Ceva Santé Animale, F) should be administered during the interestrus phase, shortly after oestrus. When administered brief time before the next oestrus, a fertile cycle may occur shortly after the application (Schäfer-Somi 2017). In a cat, mated by chance during Melovine® treatment, normal brooding care was described after delivery (Schäfer-Somi 2017); this authors thereafter repeatedly used Melovine® in puerperal cats and observed particularly good brooding care (Schäfer-Somi unpublished). In ferrets, an increase in prolactin was measured after oral melatonin application (Ramer et al 2006); the use in puerperal cats should therefore be more investigated.

One problem with the use of any melatonin preparation is that repeated application is difficult. This is supposed to be due to the so-called photo refractoriness. This phenomenon describes a desensitization of the hypothalamo-pituitary axis after prolonged melatonin treatment (Leyva et al 1989). Graham et al (2004) described that after application of melatonin orally for 30 days, more than 50 days of exposure to long days were necessary to restore responsiveness of the gonads to melatonin. Another problem is the highly individual response to melatonin as well as the possibility of failure, which may happen with any implant (Schäfer-Somi 2017).

In contrary to adult cats, prepubertal cats did not respond with a delay in oestrus occurrence, when an 18 mg implant was used; there was no difference in puberty occurrence in comparison to a non-treated control group (Faya et al 2011). In tomcats, application of melatonin significantly decreased sperm-quality; however, as spermatogenesis was not completely suppressed, melatonin is not a contraceptive in male cats (Nunez Favre et al 2014).

Many questions remain to be answered. In one study, uterine pathologies were observed, when 12-60 mg melatonin implants were used in female cats. Authors describe occurrence of endometrial thickening, and endometrial cysts; however, in their study, cats were not examined before the beginning of the study (Griffin et al 2001). In a recent study (Tugce et al 2025), a group of 10 cats received 18 mg melatonin implants and cats were ovariohysterectomized during the consecutive oestrus, while 10 non-treated control cats were ovariohysterectomized

during the interestrus stage. Uterine tissue was examined for histopathological changes, for protein expression of interleukin (IL)-1ß and for gene expression of inflammatory cytokines as well as progesterone receptors (PR)-A, and PR-B. Oestrus was delayed for on average 96.5 days and no side effects were observed; however, histopathologically, authors found mild inflammation in uterine tissues from 2/10 control cats and in the Melovine® group, they found mild inflammation in 7/10 animals, moderate inflammation in 1/10 cats and severe inflammation in 1/10 cats. Expression of IL-1ß matched these findings, with higher expression levels in the melatonin group. Furthermore, gene expression of inflammatory cytokines was upregulated in uterine tissues of Melovine® treated cats. Thus, authors concluded that Melovine® implants may cause a uterine inflammatory response. Duration of this inflammation, clinical effects and the effect on consecutive fertility remain to be observed; especially when higher or lower concentrations are used. During one study, a cat pretreated with Melovine® was mated during the next oestrus and became pregnant; she gave birth to a healthy litter (Schäfer-Somi 2017). In another study, twelve cats treated with a Melovine® implant, were allowed to mate after sexual recovery and all gave birth to healthy litters of 2-6 kittens (Furthner et al 2020). Negative effects of Melovine® on embryo or foetal development are therefore unlikely; however, in melatonin pretreated cats, delayed oviductal embryo transport and a lower embryo recovery rate after embryo flushing were reported, while embryo quality was not changed (Graham et al 2000, 2004).

Concerning the practical use of Melovine® implants, limiting factors like off-label use, highly individual duration of efficacy, and the problem to purchase packages with 2x25 pieces and outside the USA, were published (Schäfer-Somi 2017). Meanwhile, melatonin implants produced in the USA and licensed for cats like Melawin® are available in different concentrations; for cats, the 12 mg implant is recommended by the manufacturer. Advantagous is that the implant is provided with a reusable applicator syringe and hypodermic needles. Another preparation is dermatonin (Melatek LLC, USA), available as 8 mg, 12 mg and 18 mg melatonin preloaded implanting device. The manufacturer recommends for cats one 18 mg implant when needed, irrespective of body weight; however, it is licensed for treatment of hair loss and no scientific study is available.

To summarize, melatonin tablets and implants can be useful for short-term suppression of oestrus in female cats; however, the owner should be informed about the individual duration of effectiveness. The use in puerperal cats should be further investigated, while melatonin at a dosage of 18 mg was not reliable for postponement of puberty in female cats or contraception in male cats.

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Mycoplasmosis in dogs

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Mycoplasmas (M.) and ureaplasmas (U.) are aerobe, facultative anaerobe microorganisms, which characteristically do not have a cell wall. They are very small (0.3-0.8 µm) and have a small genome. They can be found in intracellular as well as extracellular compartments. The pathogenicity of some species is apparently caused by the complex interplay of specific adhesion structures and metabolism products of the antigens (Razin et al. 1998): the secretion of sialidase, a neuraminidase, can promote the colonization and tissue invasion. For some M., intracellular colonization is possible. *M cynos* is able to increase cytoadherence by producing the hemagglutinin HapA (Kastelic et al. 2015). These ubiquitous microorganisms are commensals or parasites of mucous membranes, especially of the respiratory and urogenital tract, and only a few of them cause infections. They are mostly facultative pathogens in the female and male genital tract and can be isolated in healthy and diseased dogs (Bjurström und Linde-Forsberg 1992, Jagodka et al 2024, Schäfer-Somi et al 2024). An infection is supposed, when exclusively mycoplasmas are detectable, and inflammation is evident; however, not even in these cases, the isolated bacteria may be the cause of the disease.

In dogs, mostly *M. canis* and *Ureaplasma (U.) canigenitalium* were isolated in case of inflammatory diseases of the urogenital tract of male and female dogs (Jagodka et al 2024). *M. cynos,* which is host specific and considered to be the most pathogenic among canine mycoplasma spp, was sporadically detected in seminal fluid and prostate secretions of healthy male dogs and of dogs with testicular degeneration, benign prostate gland hyperplasia (BPH) and prostatitis (personal communication).

Mycoplasmas and ureaplasmas mostly cause mild, chronic infections due to host immune reactions and inflammation (Razin et al. 1998). Dogs get infected after direct contact with other infected dogs, also during mating. Mycoplasmas survive semen freezing. Infection of dogs via licking cannot be excluded.

In the male dog, the whole urogenital tract can be infected (Mimouni 1997). Inflammation of the prepuce, penile mucous membranes, urethra, scrotum, testes, epididymes and the prostate gland as well as infertility are described. The course of infection is slow and there must not necessarily be an infection; mycoplasmas are able to directly infect spermatozoa and to damage

them (Laber und Holzmann 1977). In bovine semen, primary defects of the nucleoplasma, tail defects, regressive changes of the plasmalemma and the acrosome as well as vesiculo-lamellar bodies in the periacrosomal region were described (Hrudka 1984). In some dogs with a history of infertility and bad semen quality, *M. canis*, *Ureaplasma (U.) canigenitalium or M. cynos* can be isolated without any clinical symptoms. In these cases, treatment may be rectified to prevent chronical diseases of the urogenital tract and irreversible infertility.

The infected bitch can show recurrent inflammation of the vagina, infertility, resorption, abortion or birth of premature or weak puppies; puppies get infected either in the uterus or during parturition (Mimouni 1997; Chalker 2005; Pretzer 2008). However, even in cases, where monocultures of M. canis are isolated from a diseased dog or a weak or dead puppy, it will be difficult to proof that it was the causative agent. In a recent study, in case of abortion and neonatal mortality, the term Mycoplasma-positive cases was used when the real cause could not be determined; authors found a prevalence of 7% (8/114) (Marenzoni et al 2024). In the same study, a relation between isolation of M. canis in the investigated kennels and the incidence of puppy deaths and low litter sizes was found. However, incomplete samplings and analyses for other bacteria/viruses or other causes make correct interpretations difficult.

The identification and quantification of mycoplasma/ureaplasma spp. is necessary for decision finding whether treatment is recommendable or not. In cases, where clinical symptoms are present or a history of infertility, treatment may be indicated. Cultivation of these bacteria is time consuming, and the running costs are relatively high. PCR is a quick and precise method, when a certain species is sought. MALDI TOF mass spectrometry is the preferred method for the identification and differentiation of mycoplasmas/ureaplasmas, since the method is quick, precise and running costs are low (Spergser et al. 2019). When a swab is taken, care must be taken that suitable media are used, such as Amies medium. Furthermore, the laboratory must be able to perform species identification and quantification. Only then, interpretation of the findings is possible.

Mycoplasmas are resistant against ß-lactam antibiotics; however, resistances against other antibiotics are emerging. Therefore, a resistance test to detect the minimum inhibitory concentration (MIC) of an antibiotic, should be performed in case of chronical infections and when long-term treatment is required; specialized laboratories offer these tests. A treatment is not necessary in case of a low to medium grade positive culture without clinical symptoms. Only in case of severe inflammation combined with high-grade monoculture of a pathogenic mycoplasma species, antibiotic treatment can be necessary.

To avoid infections with mycoplasmas, the breeding history of the breeding partner should be scrutinized. In case of a previous inflammatory disease in the urogenital tract or infertility, a gynaecological/andrological examination before mating, inclusive a swab for bacteriological examination should be performed. Stress factors like crowding, bad hygiene and feeding must be avoided. If a therapy is necessary, a control should be performed one week after the end of the antibiotic therapy. More investigations of the pathogenic mechanisms of certain mycoplasmas will contribute to better interpretation of bacteriological findings and to decision making as to treat or not.

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DO YOU WANT THIS BULL OR A BULL? DIAGNOSIS, MANAGEMENT, AND PROGNOSIS OF COMMON PREPUTIAL, PENILE, AND SCROTAL INJURIES

J. Koziol DVM, MS, DACT

1

AGENDA

- Anatomy and physiology review
- Penile hematoma
- Retropreputial abscesses
- Preputial lacerations
- Inability to make intromission
- Scrotum abnormalities

2

ANATOMY

- Sheath is considered a double invagination of skin
- Length and diameter of prepuce varies from bull-to-bull
- Interdigitating layers of elastic tissue between tunica albuginea of the penis and the epithelium of the prepuce

3

PHYSIOLOGY OF ERECTION

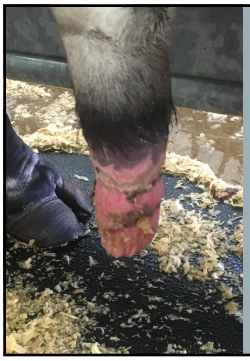
- Fibroelastic penis
- Erectile tissues of the CCP contained within relatively inelastic tunica albuginea
- Following sexual stimulation
  - Increase blood flow through deep artery of the penis into the crura and then CCP
  - Retractor penis muscles relax
  - Ischiocavernosis muscle contract pushing crura against the ischium preventing blood flow out of CCP
  - Creates closed hydraulic tube

4

EVERSION OF THE PREPUCE

- Occurs in bulls carrying the polled gene
  - Retractor prepuce muscles
- Bos indicus type bulls with pendulous sheaths
  - Length of internal lamina of the prepuce
  - Size of external os of the prepuce
- Bulls with high degree of preputial eversion are more subject to preputial laceration, and other traumas

5



EVERSION OF THE PREPUCE

6

PREPUTIAL LACERATIONS

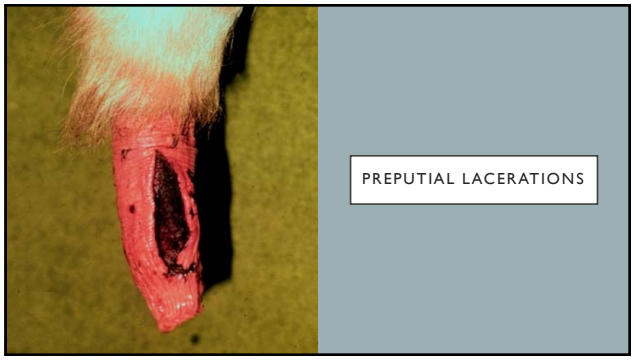
- Pendulous sheaths and excessive preputial skin
- Most preputial lacerations occur at the time of the ejaculatory lunge
- Preputial skins slides caudally up shaft of the penis and redundant skin gather
- Redundant preputial skin then gets entrapped between the bull's abdomen and the bony pelvis of the female
- Bursting of the epithelium and damage to the underlying elastic tissue

7

PREPUTIAL LACERATIONS

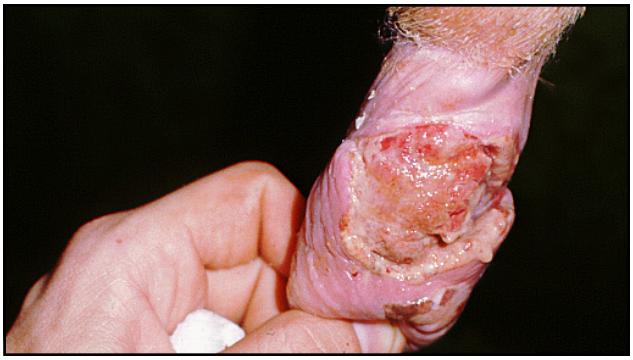
- Laceration with subsequent preputial prolapse are more common in *Bos indicus* breeds and their crosses
- Even serious preputial injuries in *Bos taurus* bull rarely result in preputial prolapse

8



PREPUTIAL LACERATIONS

9



10

PREPUTIAL LACERATION – POST INJURY

- Bos taurus bull retract damaged prepuce into preputial cavity
- Injury goes unnoticed until
  - Cellulitis, abscessation, or stenosis occur
  - Retropreputial abscess

11

PREPUTIAL LACERATION – POST INJURY

- Bos Indicus bulls will usually prolapse the prepuce
  - Edema in the traumatized tissue
  - Dependent edema increases size and weight

12

Category	Description
I	Simple preputial prolapse with slight-to-moderate edema without laceration, necrosis, or fibrosis. Respond well to medical or surgical treatment. Good prognosis for return to service
II	Prolapsed prepuce has moderate-to-severe edema, may have superficial lacerations or slight necrosis, but no evidence of fibrosis. Surgery is usual course of therapy. Good to guarded prognosis
III	Severe edema of the prolapsed prepuce with deep lacerations, moderate necrosis, and slight fibrosis. Surgery is indicated with guarded prognosis
IV	Prolapsed prepuce has been exposed for an extended interval and there is severe edema, deep lacerations, deep necrosis, fibrosis, and often abscessation. Culling of bull usual recommendation. Guarded to poor prognosis follows surgery.

13

PREPUTIAL LACERATION – MEDICAL MANAGEMENT

- Control sepsis
- Reduce edema
- Eventually return damaged tissue to the preputial cavity

14

CONTROL SEPSIS

- Clean and flush wound with dilute antiseptic
- Gentle debridement
- Emollients and topical antibiotics
- Topical Application of Petermycin/Petercillin
  - Mixture of Lanolin, Scarlet oil and Oxytetracycline

Scarlet oil is not FDA approved

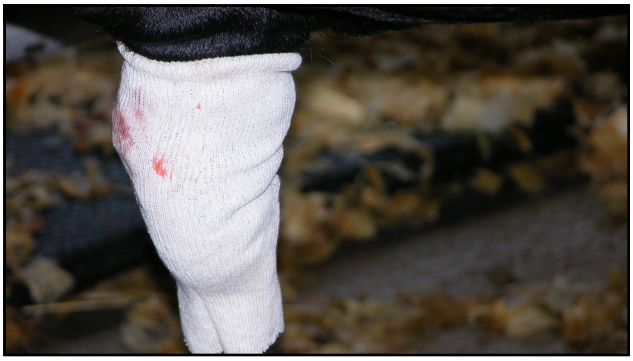
15

REDUCE EDEMA

- Hydrotherapy
- Compression
- Support prepuce

16

17



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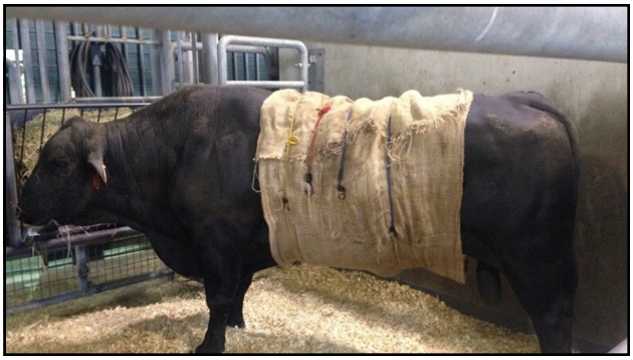

22

SURGICAL REPAIR OF PREPUTIAL LACERATIONS

- Resection and anastomosis of the preputial scar can improve outcome
- Warranted when bull's value warrants the investment
- Surgical outcomes are improved by preoperative wound management

23

RETROPREPUTIAL ABSCESS

- Most commonly occurs in *Bos taurus* bulls
- *Bos taurus* bulls retract the penis into the sheath and prolapse of the penis
- Injury goes unnoticed until laceration becomes contaminated
  - Cellulitis
  - Abscessation
- Phlegmon may extend from the preputial orifice to the scrotum
- Confined to a small well-defined area along the sheath

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RETROPREPUTIAL ABSCESS

- Differentiate from other conditions
  - Retropreputial abscesses are usually asymmetrical and located closer to preputial orifice
  - Urolithiasis/Water belly
  - Careful physical examination +/- ultrasound to differentiate from an abdominal abscess

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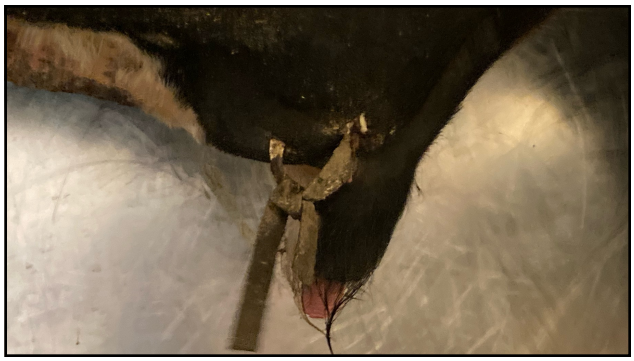

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TREATMENT OF RETROPREPUTIAL ABSCESSES

- Limited to systemic antibiotic therapy and local treatment with cold water hydrotherapy
- Needles should never be introduced
- Never drain through skin of the sheath
  - Infection extend through the peripenile layers and increase subsequent adhesion formation

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PROGNOSIS FOR RETROPREPUTIAL ABSCESS

- Always guarded to grave
- Adhesions within elastic tissue or between elastic layers and the skin can interfere with penile extension
- Only small % of bulls will heal sufficiently or without adhesions of the elastic tissues

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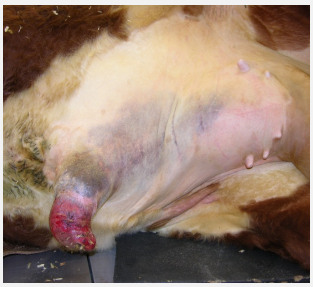
PENILE HEMATOMA

- Rupture of the tunica albuginea of the penis
- Broken of fractured penis
- Most common site of rupture is the dorsal surface of the distal sigmoid flexure
  - Just above the point of insertion of the paired retractor muscles



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PENILE HEMATOMA – HOW OCCUR

- During normal erection pressure within the penis rises to ~14,200 mmHg /275 psi
- During coitus the bull mounts and makes 1-2 searching motions followed by forceful lunge
- If penis is outside the vulva during ejaculatory lunge or the female collapses severe angulation of the penile shaft can occur
  - drastically increasing the intracorporeal pressure

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PENILE HEMATOMA

- The erect penis only contains about 250 mL of blood at the time of rupture of the tunica albuginea
- Blood forcefully enters peripenile tissue
- Creates symmetrical swelling in sheath immediately cranial to the base of the scrotum
- Hematoma may grow if bull continues to breed
- Occasionally swelling may extend caudally along the retractor penis muscles

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PENILE HEMATOMA – DIAGNOSIS

- Diagnosed by physical examination
  - Subcutaneous hemorrhage/bruising may be evident on light colored bulls
- Mild to moderate prolapse of the prepuce may occur as well
  - May be first sign owner's notice
- Palpation of the symmetrical swelling on the dorsum of the distal bend of the sigmoid flexure confirms rupture
- Penis is unlikely to extend due to swelling and should not attempt to extend due to possibility of causing more damage

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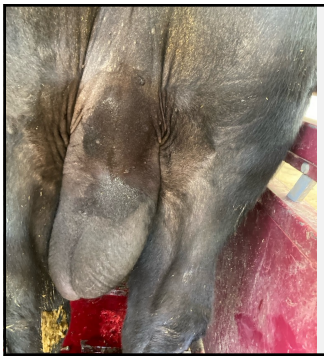

PENILE HEMATOMA

- Blood can travel caudally along retractor penile muscles in some cases

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PENILE HEMATOMA - COMPLICATIONS

- Rupture of the tunica albuginea may result in loss of reproductive function
  - Abscess formation at the site of hematoma
  - Adhesions between the penis and peri-penile tissues
  - Development of vascular shunts between CCP and surrounding vasculature
  - Injury to prolapsed prepuce
  - Damage or destruction of the dorsal nerves of the penis

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PENILE HEMATOMA – MANAGEMENT

~ 50% of bulls with penile hematoma resume breeding following conservative treatment

- Minimum of 60 days sexual rest
- Prophylactic systemic antibiotics to prevent abscess formation following hematoma
- Hydrotherapy of the sheath
- Conservative treatment may be indicated when
  - Diagnosis is delayed
  - Bull does not justify the expense of surgery

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PENILE HEMATOMA – SURGICAL

- Surgical intervention is aimed at removing blood clot and closing rent in tunica albuginea
- **Perform 3-7 days after injury**
- Surgery can result in ~80% of bulls returning to breeding soundness

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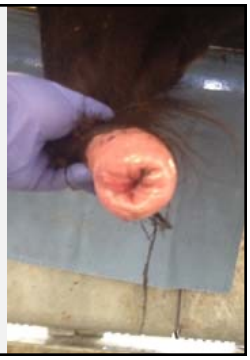
PHIMOSIS



- Inability to extend the penis
- Most common secondary to preputial stenosis secondary to scar tissue
- Other reasons for phimosis
  - Short penis
  - Adhesions within elastic layers of the prepuce
  - Large penile fibropapillomas

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PHIMOSIS

- Extension is dependent on gliding of elastic tissues and preputial epithelium
  - Trauma
  - Adhesions
  - Abscesses
- Site of adhesions may be felt by palpation or may notice puckering or wrinkling of the skin during attempted erection

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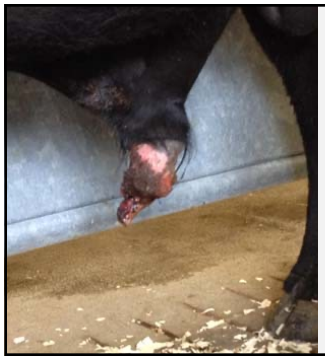

PARAPHIMOSIS

- Inability to retract the penis
  - Most common following preputial laceration or preputial trauma
  - Rare bulls with balanoposthitis
- Regardless of the reason exposed skin of the penis and prepuce will desiccate

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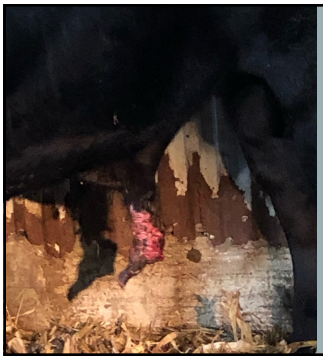

PARAPHIMOSIS

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PARAPHIMOSIS - MANAGEMENT

- Emollient ointment
- Hydrotherapy and daily bandage changes
- Bandage in same manner as preputial laceration
- Paraphimosis secondary to preputial lacerations have only a fair-guarded prognosis
- Severe devitalization of tissue has guarded prognosis

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ERECTION FAILURE

- Penile engorgement begins prior to mounting in the normal bull
- Full erection should occur as the bull mounts to make intromission
- Determination of cause begins with history, physical exam and observed test mating
- Breeding history important for distinguishing between congenital or acquired conditions
- Test mating more reliable to diagnosis erection failure than electroejaculation

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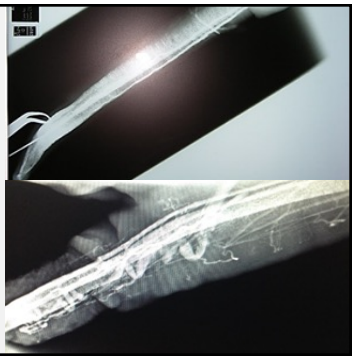
ERECTION FAILURE

- When erection failure is present
- Contrast cavernosography is useful to determine if failure is caused by vascular abnormalities
  - Vascular shunts
  - Thrombosis of the CCP



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SPIRAL DEVIATION OF THE PENIS

- Most common of the penile deviation
- Deficiencies in function of the dorsal apical ligament or denervation
- Dorsal apical slips to the left side of the free portion of penis prior to intromission
- Affected bulls often have 1 or more seasons of successful breeding before being diagnosed
- Diagnosis of spiral deviation can only be made after observation of the breeding act or during a test mating
- Spiraling during electroejaculation is frequently observed and is not predictive of premature spiraling during natural mating

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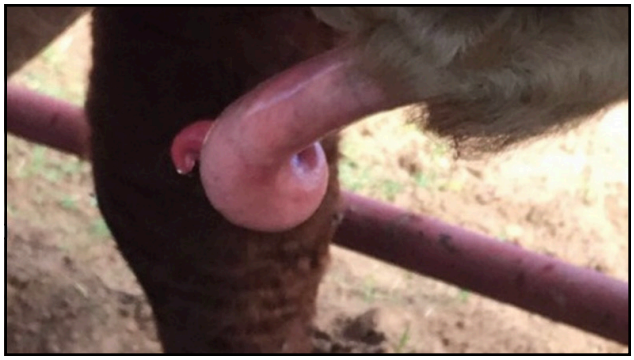

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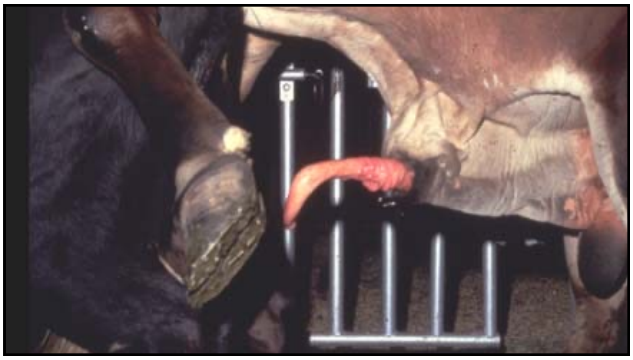

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VENTRAL DEVIATION OF THE PENIS

- Ventral deviation occurs less commonly than spiral deviations
- Exact etiology is unknow
  - Altered architecture of the tunica albuginea of the penis
  - Altered blood flow through the ventral portion of the CCP

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S-SHAPED DEVIATIONS

- S- shaped curvature is the least common of the reported penile deviations
- Typically occurs in bulls 4-years of-age or older
- Thought to be due to inadequate apical ligament length
- No successful therapy for correction has been described

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CONDITIONS OF THE SCROTUM

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FROSTBITE

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FROSTBITE

- Results in scrotal inflammation that impedes normal thermoregulatory function
- Hydrotherapy to aid in reduction of inflammation
- 2-4 weeks to recover to normal function
- In general bulls that develop adhesions or skin lesions that extend more than half the length of the scrotum have a poor prognosis

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INGUINAL HERNIAS

Inguinal hernias are usually classified as direct or indirect

Indirect are result of abdominal viscera passing through the inguinal ring into the vaginal cavity

Majority of cases are left sided although they can be seen on either side

Unilateral castration is treatment of choice

Can also perform standing surgery

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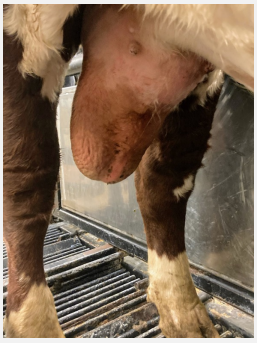

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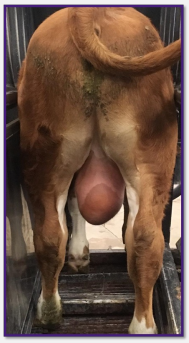

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HYDROCELE/HEMATOCELE

- Most common cause of scrotal enlargement is fluid accumulation in the tunica vaginalis cavity
- Hydroceles may occur unilateral or bilateral
  - Insulation
  - Usually temporary
- Hematocele usually unilateral
  - Trauma to scrotum, testicle, or testicular vascular cone
  - Marked distention of the scrotum
  - Poor prognosis for return to fertility of that testicle

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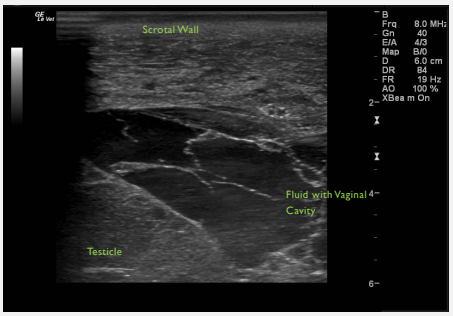

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CONDITIONS OF THE TESTES

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TESTICULAR CONDITIONS

- Evaluation of testicular tone
- Testes should by symmetrical
  - No more than 10-15% disparity

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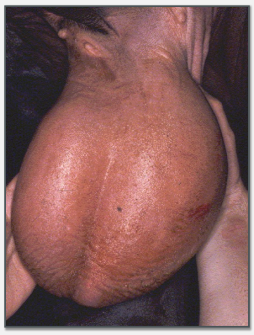

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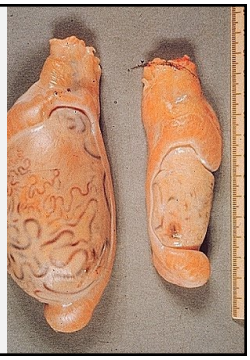
TESTICULAR HYPOPLASIA

- May be unilateral or bilateral
- Differentiate hypoplasia from atrophy/degeneration
  - Hypoplasia is congenital
  - Degeneration is acquired



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TESTICULAR FIBROSIS

- Damage to seminiferous tubules may be followed by invasion of fibrous tissue
- May range from a few small areas to large areas of fibrosis
- Presence of large number of fibrotic lesions does not preclude production of ejaculates with normal morphology

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ORCHITIS

- Range from subclinical to severe
- Occurs following hematogenous dissemination of infectious organism or result of extension of infection of the lower genitourinary tract
- Pain on palpation

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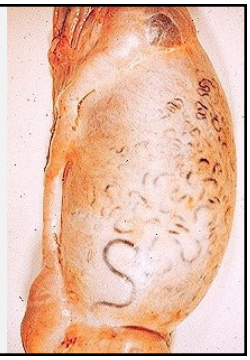
EPIDIDYMITIS

- Present as swollen painful cauda epididymis
- Effect on semen quality may or may not be evident
- Eventual fibrosis of epididymis can occur if left unresolved



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QUESTIONS

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Key Elements for Successful Bull Breeding Soundness Evaluations

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Abstract

Conducting bull breeding soundness evaluations (BBSEs) is a critical service in bovine veterinary practice. These assessments are aimed at identifying bulls with reduced or absent fertility. Using such bulls for breeding can significantly impact producers' bottom lines due to fewer calves born, extended calving intervals, and ultimately reduced weaning weights across the herd.

Introduction

BBSEs are multifaceted procedures involving a combination of assessments—physical health, scrotal measurements, sperm motility, and morphology. This presentation highlights challenges commonly encountered by new veterinarians and outlines strategies that contribute to effective evaluations.

Challenges in Semen Collection: Handling Difficult Bulls

Achieving erection, penile protrusion, and ejaculation during semen collection depends largely on the method and consistency of stimulation—either manual, electroejaculation, or a combination of both.

Importance of Quality Equipment

Proper equipment plays a crucial role in the success of a BBSE. A reliable electroejaculation system—which includes the control unit, probe, and cord—is essential. Various models offer different functionalities: some allow manual adjustment of voltage via a rheostat, while others operate using preset, automated patterns. Certain units provide both options.

The probe, inserted rectally to stimulate pelvic reproductive organs, typically has ventrally positioned electrodes to target specific tissues while avoiding others. Probes generally come in 60, 75, or 90 mm diameters. Larger probes offer better contact and more effective stimulation. Selection should be based on what the bull can comfortably accommodate—60 or 75 mm probes are usually suitable for bulls weighing 1200–2000 lbs, while 90 mm probes may be necessary for larger or Bos indicus-type bulls.

Weighted probes improve electrode contact by pressing more firmly against the rectal wall. Many probes are equipped with a yoke—either horizontal or upward-facing—to secure the tail and stabilize the device during collection. The upward-facing yoke reduces the risk of injury if the bull moves against a collection barrier. Cleaning the electrodes with a scrub pad prior to use ensures optimal conductivity.

If the machine fails to deliver consistent stimulation, inspect and potentially replace the cord, as this is often a common issue.

Stimulation and Semen Collection Technique

Before inserting the probe, it's important to perform a thorough transrectal exam of the internal genital structures. Begin by removing feces to allow clear palpation, then massage the prostate, ampullae, and pelvic urethra with the fingers for about 30 seconds. This encourages relaxation of the prepuce and may initiate penile protrusion or emission of pre-seminal fluid.

After this, the lubricated probe is inserted and stimulation begins. The process should start at the lowest voltage, with gradual increases in intensity. Bulls should respond with controlled, rhythmic movements—avoid abrupt or jerky adjustments. Manual settings on non-automated machines should be applied smoothly using the rheostat, with rest intervals between pulses to allow relaxation of the retractor penis muscles.

Effective stimulation leads the bull through a predictable sequence: erection, protrusion, and ejaculation. If the process stalls, a gentle increase in intensity can help resume progress. For bulls struggling to protrude the penis, an assistant may provide support by applying pressure to the sigmoid flexure behind the scrotum.

Evaluating Sperm Motility and Morphology

Given time constraints, this topic is broad, but foundational preparation improves accuracy. Warm all slides, coverslips, and pipettes to avoid cold shock, which can hinder motility and cause defects like bent midpieces. Be mindful of media limitations—eosin-nigrosin stains can degrade, and solutions like buffered formal saline or PBS may become hypo-osmotic, causing clumping or artifacts.

Always examine morphology under oil immersion at 1000x magnification. If field analysis isn't feasible, label slides and evaluate them later at the clinic. Research shows more bulls fail due to poor morphology than motility, making careful analysis critical.

Managing Bulls Near Morphology Cutoffs (69–71%)

When a bull's sperm morphology falls into this gray zone, it's essential to re-evaluate carefully. Count 300–500 cells to improve diagnostic accuracy, and examine all quadrants of the slide to ensure an even assessment. Bulls near the cutoff are typically deferred and scheduled for a follow-up exam. If no improvement is noted, the discussion shifts to classification as subfertile.

Owners often appreciate visual evidence of abnormalities and are usually responsive to culling or alternative breeding recommendations.

Deferral vs. Unsatisfactory Classification

Most bulls that don't meet satisfactory criteria are deferred for re-evaluation. However, some are designated as unsatisfactory without a recheck:

- Bulls with irreversible musculoskeletal injuries (e.g., hock or stifle damage).
- Animals with non-repairable reproductive injuries (e.g., scrotal, penile, or preputial damage).
- Bulls producing semen with specific morphological issues (e.g., pyriform heads, knobbed acrosomes) suggesting chronic testicular dysfunction.
- Mature bulls (≥2 years) that fail minimum scrotal circumference thresholds.
- Aged bulls with a declining trend in sperm morphology over successive evaluations.

All other bulls are usually recommended for a recheck after 4–6 weeks.

Navigating Dystocias and Fetotomies

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Causes of Bovine Dystocia

Dystocia in cattle can arise from various maternal and fetal factors:

Maternal Causes

- *Primary Uterine Inertia*: Inadequate myometrial contractions due to overdistention (e.g., hydrops), nutritional deficiencies, or infectious conditions.
- *Secondary Uterine Inertia*: Exhaustion or pain resulting from prolonged labor.
- *Physical Abnormalities*: Twinning, inguinal hernia, uterine didelphys, vaginal hypoplasia, and breed-specific characteristics.
- *Nutritional Factors*: Insufficient or excessive nutrition leading to fetopelvic disproportion or uterine inertia.
- *Other Factors*: Insufficient age at breeding, restricted exercise, infections, uterine torsion, and pelvic fractures.

Fetal Causes

- *Malpresentation*: Incorrect alignment of the fetus's spine relative to the dam's spine.
- *Malposition*: Incorrect positioning of the fetus within the maternal pelvis.
- *Malposture*: Abnormal positioning of the fetus's limbs and head.
- *Fetal Anomalies*: Conditions such as hydrops amnion, achondroplasia, prolonged gestation, and fetal monsters.

Stages of Parturition

Understanding the stages of parturition is crucial for timely intervention:

Stage I

- Duration: 1-4 hours
- Signs: Restlessness, anorexia, abdominal discomfort, increased pulse and respiration, cervical dilation, myometrial contractions, availability of colostrum, and rupture of the chorioallantois.

Stage II

- Duration: 30 minutes to 4 hours.
- Signs: Rupture of the amnion, active straining, and expulsion of the fetus.

Stage III

- Duration: 8-12 hours in bovines.
- Signs: Placental expulsion and uterine involution.

Obstetric Management

Effective management of dystocia involves several key steps:

Restraint and Preparation

- Proper restraint of the dam is essential to ensure safety and facilitate examination.
- Cleanliness and lubrication are critical to prevent infections and ease manipulations.

Examination

- Assess the position, posture, and presentation of the fetus.
- Determine if there is cervical dilation, uterine torsion, or fetopelvic disproportion.

Fetotomies

Fetotomies are performed to resolve dystocia when the fetus cannot be delivered naturally. The procedure involves the dissection of the fetus to facilitate its removal.

Common Fetotomy Cuts

- **Head/Neck**: Amputation of the head or neck to reduce the size of the fetus.
- **Forelimb**: Removal of one or both forelimbs to aid in delivery.
- **Trunk**: Dissection of the trunk to manage large fetuses.
- **Pelvis**: Removal of the pelvis to facilitate extraction.

Rules for Fetotomies

- Limit the number of cuts to avoid excessive trauma (more than four cuts may necessitate a cesarean section).
- Always leave one appendage for traction (head counts as an appendage).
- Ensure complete removal of limbs, including the shoulder or pelvis, to prevent complications.

Conclusion

Managing bovine dystocia requires a thorough understanding of the causes, stages of parturition, and appropriate intervention techniques. Fetotomies, when performed correctly, can be a lifesaving procedure for both the dam and the fetus. Emphasizing gentleness, cleanliness, and adaptability in techniques is crucial for successful outcomes.

Review of Semen Evaluation in the Bull

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Abstract:

Assessing sperm morphology is a pivotal element of comprehensive breeding soundness evaluations across species. Spermiogram interpretation provides valuable information about disruptions in spermatogenesis and allows for predictions regarding potential recovery. This facilitates more informed discussions between veterinarians and producers about a bull's prospective breeding capability, enriching standard classifications such as satisfactory, deferred, or unsatisfactory potential breeders.

Introduction:

Analyzing sperm provides an indirect but informative evaluation of testicular and epididymal function, akin to a testicular biopsy. A spermiogram—a detailed analysis of sperm morphology—can shed light on disruptions in sperm production. When combined with clinical history and a physical exam, it can help identify the underlying cause and determine prognosis or therapeutic strategies.

Common contributors to impaired spermatogenesis include thermoregulatory failure, hormonal imbalances (particularly those induced by stress), exposure to toxins, and genetic anomalies. Stress, for instance, elevates cortisol levels, suppressing luteinizing hormone (LH) and testosterone, both vital for normal spermatogenesis. These hormonal changes significantly affect the primary spermatocytes, often manifesting as early signs of testicular dysfunction.

Sperm Immaturity

Signs of immaturity in sperm samples include an abundance of round cells (spheroids) and a high percentage of sperm with proximal and distal cytoplasmic droplets. Young, peripubertal bulls often show these features, particularly proximal droplets, which typically decline as maturity progresses.

Immature sperm vary in size depending on their developmental stage. Differentiating these from white blood cells in semen is crucial and can be done by staining air-dried smears with Diff-Quik®, new methylene blue, or Wright's Giemsa. Accurate identification guides the diagnosis and determines the need for follow-up, often recommended after 4–6 weeks.

Testicular Degeneration and Recovery

This condition typically arises due to compromised scrotal thermoregulation. Diagnosis is based on physical findings—often soft testes—and a spermiogram showing low sperm concentration

and increased morphological defects. Additional signs may include immature cells and "medusa" formations in semen.

Degeneration may result from systemic illness, extreme temperatures, scrotal injury, or structural anomalies like hydroceles or hernias. Chronic cases, especially in older bulls, are often associated with testicular fibrosis caused by vascular damage.

The spectrum of morphological abnormalities varies depending on the time elapsed since the initial insult. For example, proximal droplets may appear within 9 days post-injury, while acrosomal defects might not be evident until 30 days later. Given the 61-day spermatogenic cycle and roughly 9–11 days of epididymal transit, repeated spermiogram analysis may be needed to assess the stage of degeneration or recovery.

Effects of Stress

Stress—whether environmental, social, or illness-related—can profoundly affect hormone levels by increasing cortisol and suppressing FSH, LH, and testosterone. This alters both testicular and epididymal function. A hallmark of stress-induced sperm defects is the distal midpiece reflex (DMR), which stems from dysfunction in the distal cauda epididymis.

While DMRs are strongly associated with stress, other anomalies may include proximal droplets, detached heads, mitochondrial defects, coiled tails, and nuclear vacuoles. Differentiating stress-related changes from thermoregulatory issues often requires detailed clinical history and physical evaluation.

Genetic Factors

Although environmental causes are most common, some sperm abnormalities have a genetic basis. When a specific morphological defect predominates across a high percentage of sperm, with few other abnormalities present, a hereditary condition should be considered. A list of such genetically linked defects is provided in Table 1 (not included here).

Toxic and Nutritional Influences

Various toxins can impair sperm development, though naturally occurring cases are rare. One well-documented toxin is gossypol, found in cottonseed products. Even low daily intake can induce significant morphological defects—such as segmental aplasia of the mitochondrial sheath, proximal droplets, coiled tails, and detached heads—which are usually reversible within 28 days of discontinuing exposure.

Veterinarians and producers often question the effects of medications on semen quality. While corticosteroids like dexamethasone can disrupt hormonal function, most antibiotics and anti-inflammatory drugs appear to have minimal impact.

Proper nutrition is also essential for maintaining testicular mass and sperm production. A balanced intake of protein, energy, and micronutrients supports hormonal function and spermatogenesis. Severe deficiencies, particularly in Vitamin A, can severely damage the germinal epithelium, leaving only Sertoli cells and spermatogonia. Other micronutrient deficiencies may indirectly affect reproduction by impairing overall health.

Iatrogenic Effects

Errors in sample handling and slide preparation can cause artifacts that mimic true morphological defects. Hypo-osmotic damage—due to improper staining techniques, temperature extremes, or delayed drying—can lead to characteristic bent midpieces. These defects can be distinguished from genuine DMRs by the absence of retained droplets in the bend. Cold shock is another artifact that can impair sperm motility, often noted as sluggish, erratic, or circling movements.

Equine In Vitro Embryo Production: Navigating the Absence of Consensus

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[b]Veterinary Reproduction Innovations, San Luis Obispo, CA

Over the past decade, in vitro embryo production (IVP) in horses has significantly reshaped the breeding industry and theriogenology practices across the US. Despite its growing influence, comprehensive data on the outcomes of IVP remain elusive. The most recent report from the International Embryo Technology Society (IETS) in 2023 recorded over 3,000 IVP embryos[1] . However, the actual numbers are likely much higher, as these figures do not capture the output from most commercial intracytoplasmic sperm injection (ICSI) laboratories.

In parallel, the demand for oocyte retrievals has markedly increased in our region (Central Coast of California), a trend that likely reflects a nationwide surge, evidenced by the emergence of more than 20 new laboratories according to a recent web search.

Our experience with equine ICSI began in 2006[2,], after which time IVP efficiency has advanced to approximately 1.8 embryos per oocyte retrieval session Nonetheless, overall success remains modest, with fewer than 20% of retrieved oocytes developing into blastocysts. Pregnancy rates following embryo transfer vary widely among laboratories, ranging from 50% to 80%, and early embryonic loss remains relatively high (10%–25%) compared to in vivo-produced embryos. Moreover, logistical challenges associated with the interstate shipment of oocytes and embryos further complicate the process.

Several factors contribute to the variability and slow progress in equine IVP, including the limited supply of abattoir-derived oocytes, a degree of technological protectionism, and—most notably in the authors' view—a lack of consensus within the field.

In contrast, human assisted reproductive medicine operates within a highly regulated framework overseen by federal and state agencies such as the FDA, CDC, and governed by standards like the Clinical Laboratory Improvement Amendments (CLIA), with professional oversight from organizations including the American Board of Obstetrics and Gynecology and the American Society for Reproductive Medicine. Human IVF is among the most heavily regulated medical procedures in the United States[3]. Furthermore, standardization on laboratory practices has been achieved through initiatives such as "The Cairo Consensus,"[4,5] leading to uniform procedures and improved efficiency (an average of 8 embryos per oocyte retrieval for women under 35, and 4 embryos for women over 36).

Similarly, in cattle, years of collaborative research through the IETS and the American Embryo Transfer Association have fostered a consensus that supports an overall efficiency of 5 embryos per oocyte retrieval session[1], with pregnancy rates about 10% lower than those achieved with in vivo-produced embryos.

The authors propose that leadership in equine assisted reproductive technology should come from ACT and SFT, with a focus on training, research and development for veterinarians and

scientists. As theriogenologists, it is essential not only to agree on basic scientific principles but also to foster a spirit of collaboration and shared learning to improve an inefficient yet highly demanded procedure with ethical concerns within the industry (i.e. unregulated use of semen, sale low quality embryo production, unlicensed individuals performing procedures). The goal of the proposed program is both educational and transformative: to encourage research, stimulate clinical innovation especially with the new promise of IVF and ultimately give birth to a unified framework—the Sacramento Consensus in Equine Assisted Reproductive Technology (ART).

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The Delicate Dance: Intricacies and Challenges in Equine ICSI and Embryo Cryopreservation

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In vitro embryo production (IVP) is a multifaceted process that involves fundamental steps such as oocyte in vitro maturation, fertilization, embryo culture and cryopreservation. Since 2006 we have been on the journey to optimize the equine IVP system in clinical settings. Even though embryo production rate via IVP is more efficient than in vivo embryo recovery, this avenue remains inefficient when compared to cattle and humans IVP. In respect to the oocyte, there has been some progress in understanding the basic physiology of the mare follicular and oviductal environment and oocyte metabolism[1–4], however, the developmental competence of oocytes remains at a 50-70% maturation, 50-70% fertilization and 15-25% blastocyst rate per injected oocyte with an overall yield of approximately 1-2.2 embryos per oocyte retrieval. Furthermore, pregnancy rates are 10-20% lower and embryo loss is 10-25% higher after transfer when compared to in vivo produced embryos[5–14] .

The first component is the spermatozoa, in which DNA integrity, chromosomal normalcy, morphology and motility play a fundamental role[15]. Prior to ICSI or IVF, sperm are typically selected via swim-up, swim-up through a membrane, density gradient centrifugation (DGC) and sperm wash or combinations of these. As a result, one obtains a more normal sperm population [16]; but linear motility and normal morphology remain the most valuable evaluation at the time of ICSI[17]. One sperm sorting methodology may not be adequate for all stallions, as viability of sperm vary greatly, especially among frozen vs fresh or cooled vs refrozen samples. Our clinical data (n=680 cycles) using DGC indicated higher cleavage (57.6 and 70.1), blastocyst (13.4 and 25.8 %), number of embryos produced per cycle (1.16 and 2.24) and initial pregnancy rates (72 and 81.5) for cut-frozen straws and cooled semen respectively[8]. One pitfall of the report is that some of the frozen samples were of poor quality upon thawing and very few sperm (2 or 3 sperm cells per field of view at 200x magnification and dilution of 1:4 sperm pellet and 5% PVP (5ul) were motile after DGC; or in some samples, the motility was of short duration (<5 min) after DGC. On further investigation, time lapse morpho dynamics evaluation of a subset of embryos (n=150) up to the blastocyst stage, for zygote first cellular division , normality of cleavage and size of resulting blastocysts indicated a nearly identical normality of cleavage and development for embryos obtained by frozen or cooled semen[18]. Therefore, we recently evaluated a subset of cycles (n=270) wherein cut-frozen semen straws (n=113) were thawed in 1.5 ml of IVF medium and centrifuged once and the semen for ICSI was taken from the resulting pellet. The cooled semen samples (n=57) were processed by DGC. With the single centrifugation of the frozen samples, the number of spermatozoa per field of view in the PVP drop increased and the motility lasted > 10 min. Cleavage (59.8 and 68.5%), blastocyst (15.8 and 26.3%), and number of embryos produced per cycle (1.4 and 2.3) were lower for frozen vs cooled semen respectively. This clinical data indicates that in our IVP system cooled semen samples increase

the overall efficiency. Interestingly, the use of cryopreserved or fresh semen in humans for ICSI does not make a difference[19]. Can one speculate that cryopreservation may affect sperm decondensation parameters, PLCz content and/or sperm Zn signatures, leading to lower fertilization rate, mitotic aneuploidy and blastomere multinucleation?

The next step is ICSI: the utilization of the piezo driven injection system dates to the early 2000's and at the time, produced the most consistent fertilization rates. The piezo device delivers rapid, precise mechanical pulses to penetrate the zona pellucida and the oolemma minimizing damage to the oocyte and potentially, has positive effects on sperm decondensation and oocyte activation. Piezo-ICSI in cattle and humans seems to improve IVP outcomes, [20,21] and a direct comparison with conventional equine ICSI indicated faster sperm remodeling and resumption of meiosis, but only one stallion of high IVP fertility was evaluated.[22] Is the piezo injection system needed? Clinical data indicates doubt, as some laboratories utilize conventional ICSI with outcomes similar to the published piezo ICSI results. Furthermore, the incidence of abnormal cleavage (direct, explosive and indirect) was 27 % in a laboratory using conventional ICSI [23] compared to 20% with our time lapse embryo annotated observations (n=150) using piezo-ICSI[24]. To note upon conventional ICSI, the oocyte shape is deformed drastically, and cytoplasm is aspirated to break the ooplasm infrastructure.

Following is the crucial criteria of embryo in vitro culture (IVC). In humans the culture system is based on the composition of human tubal and uterine fluid analysis[25]. The chemical composition of commercially available IVC media varies, but the components are nearly similar among brands as disclosed for FDA approval. Furthermore, independent chemical analysis of human IVC media vary slightly in glucose, pyruvate and lactate concentrations and the ratio of pyruvate/lactate[26]. There is evidence that the embryo adapts to different culture conditions, but the blastocyst rates in humans is robust and consistent among practices [27]. Furthermore, the IVC target pH is the same for all medium and it is based on the knowledge of the internal pH of the human embryo. Consequently, the morphology of in vivo and in vitro produced human embryos is very similar. In cattle, the consensus is the use of synthetic oviductal fluid (SOF) that was formulated from biochemical analysis of ewe oviducts in 1972 [28]. Today, most bovine IVF laboratories use SOF IVC medium with slight modifications with consistent results; however, the IVC conditions bring about blastomere lipid accumulation and metabolic aberrations that decrease pregnancy rates (10% lower than in vivo derived) and compromise cryopreservation of low-quality embryos. [29] In horses, there is very little knowledge on the oviductal and uterine biochemical composition, and only one study has been conducted looking at the metabolism of in vivo derived equine embryos.[30] It is troublesome to formulate an equine IVC medium; we do not even know what the internal pH of the equine embryo in the reproductive tract is. The first medium (DMEM/F12 supplemented with 10% FCS) was described by Woods et al. [31] when studying the effects of calcium on cell culture and subsequent cloning of the mule. For many years, the DMEM/F12 (17 mM glucose plus 51 more ingredients) was used as primary medium and nowadays is mostly used after day 5 of IVC or in combination with another low glucose medium (60:40%) from day 1 of IVC. Others use for the first days of IVC, human medium (Global, GTL, Sage, etc.) or cattle medium (SOF, KSOM). In addition, the commercially available equine IVC medium is problematic as its components are proprietary and lack major studies to support its use. The bottom line is no consensus and little evidence exists on the appropriate equine IVC medium. Furthermore, there is evidence that the current culture practices

induce aberrant embryonic gene expression patterns. [32] Morphologically, IVP equine embryos differ vastly to the in vivo produced ones: darker (increased lipid content?), more dispersed ICM, lack of development of a capsule, higher incidence of monozygotic twins, lower pregnancy rate and most importantly, higher embryo loss rate after 14 days of gestation. Indeed, there is an immense opportunity for comprehensive and holistic research in this area.

Currently, most IVP equine embryos are cryopreserved by vitrification. Vitrification is the process of transforming a biological sample into a glass-like, solid state without forming ice **crystals** by ultra-rapid cooling in the presence of high concentrations of cryoprotectants and a very fast cooling rate (>2000°C/min). We were the first to document vitrification of equine IVP embryos utilizing a methodology first described in humans using Open Pulled Straws (OPS) and using bovine IVP embryos (n=400) as experimental models. The technique was further modified by Choi et al. utilizing Cryolocks [33]. Currently, there are several commercially available containers for vitrification mostly for the human market: Cryolocks, Cryotops, Cryotec, OPS to name a few. Note that all devices used for humans were designed to work with commercially available vitrification media containing DMSO and ethylene glycol (EG) as permeable and sucrose or trehalose as non-penetrating cryoprotectants based on the pioneer work of Ishimore et al. in 1992.[34] Also, most protocols call for step-wise warming media supplemented with sucrose. Such protocols appear to work for equine IVP embryos but have not been optimized. Furthermore, commercially available kits for equine embryo vitrification are proprietary; however, one can speculate similarity in composition to the formulation described by Caracciolo di Brienza et al. [35] This protocol uses EG and glycerol as cryoprotectants and a long equilibration time (10 min) prior to vitrification, additionally, it was tested with a relatively large vitrification drop (30 ul) and for in-straw rehydration. Currently, in most clinical settings isosmotic warming of equine embryos is used with high survival rates[36] . Surprisingly, the embryos can survive an extreme osmotic shock from a vitrified state of 6500-7000 mOsm to 290 mOsm. For this reason, performing in silico vitrification and warming studies, looking at the interactions of cryoprotectant concentration, temperature and time of exposure including repeated re-vitrification of embryos is warranted to discern small changes in survival rates.

Equine IVP is currently the most efficient method for achieving pregnancies. Nonetheless, in the authors' view, the system remains suboptimal and presents significant potential for improvement. Drawing on two decades of experience with the implementation of this technology, we addressed key challenges and propose strategies for collaborative efforts to advance the field.

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The Secret Life of Equine Oocytes: Maturation Dynamics

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In assisted reproductive technologies (ART), the oocyte is widely considered the cornerstone of success. Its quality determines the ability to undergo proper maturation, be fertilized, and support early embryo development. While sperm quality, laboratory conditions, and technical expertise are also critical, they cannot compensate for a poor-quality oocyte. Successful oocyte maturation is a highly orchestrated process that encompasses three interdependent components: nuclear, cytoplasmic, and epigenetic maturation. Each of these domains contributes to the oocyte's developmental competence—the ability to resume meiosis, be fertilized, and support early embryogenesis.[1,2] The synchronization of these maturation processes is essential; disruption in one domain can impair the entire developmental trajectory.

Nuclear maturation refers to the progression of the oocyte through meiosis. In vivo, this process is triggered by the preovulatory luteinizing hormone (LH) surge, which initiates signaling cascades within the follicular granulosa cells. These cells release epidermal growth factor (EGF)-like ligands—amphiregulin (AREG), epiregulin, and betacellulin—that act on EGF receptors in surrounding cumulus cells. The resulting downregulation of cAMP, cGMP, and NPPC via gap junctions with the oocyte leads to the activation of maturation-promoting factor (MPF) and the resumption of meiosis, evidenced by germinal vesicle breakdown (GVBD). The maintenance of meiotic arrest prior to LH stimulation is mediated by the oocyte GPR3-driven cAMP accumulation and PDE3A inhibition, which together prevent premature MPF activation.[3]

Cytoplasmic maturation involves complex biochemical and ultrastructural changes that prepare the oocyte for fertilization and post-fertilization development. Organelle redistribution is a key factor: 1) Mitochondria: relocate from a perinuclear or scattered distribution to a more uniform, often cortical pattern. This repositioning enhances localized ATP production, which is essential for fertilization and embryo development.[4] 2) Endoplasmic Reticulum (ER): aggregates into cortical clusters that serve as calcium reservoirs, facilitating Ca^{2+} release upon sperm entry—crucial for oocyte activation.[5] 3) Golgi Apparatus: transitions from a single, centralized organelle into multiple vesicular structures, dispersed throughout the cytoplasm during maturation[6]. 4) Cortical Granule Migration: cortical granules move to the subplasmalemmal region, where they play a vital role in blocking polyspermy through exocytosis upon fertilization.[7] 5) Cytoskeletal Reorganization: microtubules and microfilaments assist in organelle positioning, spindle formation, and the asymmetrical division of cytoplasm during meiosis.[3]6) mRNA and Protein Storage: the oocyte accumulates and stabilizes maternal mRNAs and proteins necessary for early embryonic development, as the embryo remains transcriptionally silent until the maternal-to-zygotic transition.[8] 7) Metabolic Shifts: enhanced metabolic activity supports increased demands for energy, biosynthesis, and redox regulation during fertilization and cleavage stages.[4]

Epigenetic maturation refers to a series of molecular modifications that regulate gene expression patterns essential for meiotic progression**,** genomic imprinting, and early embryonic

development**,** all without changes to the underlying DNA sequence.[9] The main components of epigenetic regulation include: 1) DNA methylation**,** primarily mediated by DNMT3A and DNMT3L**,** which establish de novo methylation patterns during oocyte growth. These are especially important for the regulation of imprinted genes**,** which exhibit parent-specific monoallelic expression crucial for fetal growth and placental development.[10] 2) Histone modifications, such as H3K4me3 (associated with active chromatin) and H3K9me3/H3K27me3 (associated with transcriptional repression), are dynamically regulated during oocyte maturation.[10] These modifications contribute to chromatin remodeling and gene silencing necessary for meiotic competence and transcriptional regulation. 3) Non-coding RNAs, including miRNAs and long non-coding RNAs, also play a role in chromatin structure modulation and mRNA stability in oocytes, although their specific roles remain less defined.[10] To note that transcriptional activity is low during the fully grown GV stage and undergoes marked changes post-GVBD. Although detailed transcriptional kinetics have not been fully characterized in the horse, studies in bovine and porcine models suggest that transcriptional reactivation occurs shortly after GVBD and supports the synthesis of key transcripts for oocyte activation and early embryo development.[11]

In current clinical practice, equine oocytes are typically collected via ovum pick-up (OPU) at unsynchronized stages of the estrous cycle, resulting in a heterogeneous population of oocytes then held overnight in some sort of embryo holding medium. These oocytes vary in terms of growth stage, degree of atresia and cytoplasmic and epigenetic maturity. The majority are immature and arrested in prophase I of meiosis, corresponding to the germinal vesicle (GV) stage, which is further subclassified into GV1, GV2, and GV3, based on chromatin condensation and nuclear organization[12]. A major limitation of in vitro maturation (IVM) lies in the frequent asynchrony between nuclear, cytoplasmic and epigenetic maturation, which contributes to suboptimal fertilization rates, aberrant spindle formation, early embryonic arrest, and poor blastocyst development. One of the key barriers is the absence of granulosa cells during IVM and the fact that LH receptors have not been definitively identified on cumulus cells or oocytes of most species including the horse[13,14]. As a result, the canonical signaling pathways triggered by the preovulatory LH surge in vivo cannot be faithfully replicated in vitro. In addition, within the follicular environment, the oocyte expresses GPR3, which sustains high intracellular cAMP levels, maintaining meiotic arrest[15]. When the oocyte is removed from this environment during OPU, cAMP levels decline, triggering spontaneous resumption of meiosis.

Most IVM systems rely on supraphysiological concentrations of FSH to induce maturation.[16] FSH promotes cumulus cell expansion and the closure of gap junctions within the cumulus–oocyte complex (COC), disrupting intercellular communication. Moreover, FSH activates protein kinase C (PKC) in cumulus cells, which elevates intracellular calcium and reduces the activity of natriuretic peptide receptor 2 (NPR2). This leads to decreased cGMP levels, thereby releasing the inhibition on PDE3A. Activated PDE3A hydrolyzes cAMP within the oocyte, resulting in the activation of maturation-promoting factor (MPF) and progression through meiosis.[17] While this artificial stimulation initiates nuclear maturation, it does not guarantee the synchrony of cytoplasmic and epigenetic maturation, ultimately limiting the success of current IVM protocols.

In cattle, protocols for IVM are largely standardized[18]. Synchronization of the follicular wave, combined with low-dose FSH stimulation followed by a 48-hour coasting period prior to ovum pick-up (OPU), yields a relatively homogeneous cohort of oocytes, predominantly at the GV2 chromatin configuration stage. This population is associated with enhanced developmental competence, resulting in approximately five embryos per donor, or a ~50% blastocyst rate per oocyte placed in IVM. It should be noted, however, that in this context, the first polar body status—a typical marker of nuclear maturation—is often not assessed. In humans, the clinical application of IVM has only recently gained broader acceptance. A novel approach known as diphasic or capacitation IVM (CAPA-IVM) has emerged to improve maturation outcomes[19,20]. During the pre-IVM phase (24 hours), C-type natriuretic peptide (CNP) is used to maintain oocytes in meiotic arrest. CNP binds to its receptor NPR2 on cumulus cells, sustaining elevated intracellular cGMP levels within the oocyte via gap junctions. This, in turn, inhibits PDE3A activity, thereby preserving high levels of cAMP and maintaining meiotic arrest. Following this pre-maturation period, a second IVM phase is initiated and extended for an additional **30 hours** with AREG supplementation. This two-step protocol significantly improves oocyte quality: the proportion of oocytes exhibiting the GV2 configuration increases to approximately 70%, and both blastocyst formation and live birth rates improve markedly—from 5% to 20%, and 25% to 50%, respectively, when compared to conventional IVM methods.[19] It is important to note that the human IVM medium used in CAPA-IVM is partially defined and typically supplemented with human serum albumin, in contrast to undefined serum-containing media often used in equine systems.

Clinical Perspective

Significant inter-laboratory variation persists in equine IVP primarily due to the absence of standardized protocols, which sharply contrasts with practices observed in human and even bovine ART. For example, reported concentrations of FSH range widely—from 5 to 100 mIU/mL, utilizing ovine, porcine, or recombinant human glycoprotein sources[21,22] while some protocols describe 0.5 μg/mL of commercially available porcine FSH[23]. In addition, in most equine IVM systems, 10% (v/v) fetal calf serum (FCS) is added to base media such as TCM-199, DMEM/F12, DMEM/F12/Global IVC, or synthetic oviductal fluid (SOF)[21–25]. However, FCS is an undefined and highly variable supplement, containing hundreds to thousands of components whose composition differs between batches and suppliers. In addition, there is inconsistent oxygen culture conditions (e.g., atmospheric air vs. hypoxic environments) which contributes to unpredictable outcomes and may negatively affect cumulus–oocyte complex (COC) metabolism[26,27]. Several modifications have been explored to improve maturation efficiency, including the addition of EGF, LH, follicular fluid, and extracellular vesicles to the IVM medium to name a few, as well as alterations in IVM duration (24–36 hours)[28–34]. However, these adjustments have yielded limited or inconsistent improvements. Furthermore, there remains a lack of consensus regarding the optimal phase of the estrous cycle for oocyte retrieval in mares[35]. In our clinical program, synchronization of the follicular wave via follicular ablation did not reliably improve outcomes. While some mares produced up to 12 embryos, approximately 23% failed to yield an embryo from the first retrieval, and in certain cases, multiple retrieval attempts were required to achieve a viable pregnancy. Currently, maturation rates hover around 50–70%, as estimated by the presence of a first polar body. However, there is limited understanding of the synchrony between nuclear, cytoplasmic, and epigenetic maturation, which may compromise

oocyte competence. Additionally, an estimated 10-20% of retrieved oocytes are intrinsically non-viable. Of the oocytes that reach MII and are injected, approximately 20% develop into transferable embryos, which show a reduced pregnancy rate (50–80%) and a higher embryonic loss rate (10–25%) compared to in vivo-derived embryos. In the author's opinion, the current IVP system for equine reproduction remains costly and inefficient**,** necessitating a reevaluation of protocols starting with the oocyte and the implementation of evidence-based, standardized approaches to improve consistency and reproductive success.

Pilot Clinical Trial

Sibling oocytes (n=3) were randomly picked from mares (n=25) with at least 12 oocytes retrieved and which owners only wanted one pregnancy and injected with 15 different stallions (frozen and cooled semen). Treatments and outcomes were as follows:

Treatment	Oocytes (n)	Maturation rate (n, %)	Cleavage Rate (n, %)	Blastocyst Rate (n, %)
CAPA 6 h - IVM 24h	30	20, 67	15, 75	3, 15
CAPA 12 h - IVM 24 h	32	23, 72	17, 74	6, 26
CAPA 24 h - IVM 24 h	15	6, 40	3, 50	0, 0
IVM-sibling control	260	176, 68	123, 70	36, 20

CAPA 24 h was detrimental as the maturation and cleavage rates were clearly reduced, an no embryos were produced. Furthermore, the results are inconclusive for the 6h and 12h CAPA due to the few numbers of oocytes and the lack of evaluation of the chromatin configuration after the CAPA treatment. In a recent study using slaughterhouse derived equine oocytes, CAPA 6 h increased developmental competence of oocytes including cleavage and blastocyst rates; however, number of oocytes was also low and only one stallion was used in the experiment[36].

The Need for a Standardized Consensus in Equine In Vitro Maturation (IVM)

To improve the consistency and developmental outcomes of equine IVP, there is a critical need to establish a consensus framework for IVM protocols. Key areas requiring standardization and further investigation include:

- The implementation of a defined, serum-free IVM medium, avoiding FCS and a definedoocyte culture formulation mimicking equine follicular environment and COC metabolic needs
- Precise titration of FSH concentrations to optimize cumulus expansion and meiotic resumption without overstimulation.
- The incorporation of AREG instead of FSH into IVM systems using a more physiologically relevant approach, possibly mimicking the biphasic structure of CAPA-IVM.
- Determination of the optimal stage of the estrous cycle for retrieval of oocytes enriched in the GV2 chromatin configuration, which is associated with higher developmental competence.

- Assessment of GV2 status following CAPA-IVM treatment, to verify chromatin progression and synchrony of nuclear and cytoplasmic maturation.
- Exploration of the use of exogenous equine FSH, if available, particularly following follicular wave ablation, to promote a more synchronized cohort of competent oocytes.
- Overall, optimizing IVM protocols and integrating them more effectively with recently described IVF protocols is expected to significantly enhance the efficiency and reliability of equine IVP**.**

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What's Good for Sperm Isn't Always Good for Pregnancy: The Seminal Plasma Paradox

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Abstract:

The survival of the semi-allogeneic fetus from the attacking maternal immune response has intrigued researchers for centuries. In the human, it has been theorized that paternal antigen within the seminal plasma fraction of the ejaculate is presented to the maternal immune system during intercourse. The paternal antigen is then proposed to stimulate naïve T lymphocytes to mature into regulatory T cells that detect the developing semi-allogeneic fetus as self and encourage an immunotolerant environment. A variety of assisted reproductive techniques (ART) are commonly used in humans in addition to numerous animal species, including the horse. This includes artificial insemination (AI), oocyte retrieval/aspiration, embryo transfer (ET), intracytoplasmic sperm injection (ICSI), and *in vitro* fertilization (IVF). The majority of these techniques are performed with reduced or eliminated seminal plasma, and although pregnancies are established, the success rates is generally lower in comparison to natural breeding practices in the presence of seminal plasma. Approximately 2% of all infants born in the US are conceived utilizing ART, and massive strides have been taken to increase the use of ART in the equine breeding industry in order to increase the genetic pool available, heighten biosecurity practices, and increase ease of use due to immense research performed to optimize breeding success. While research utilizes the production of a viable neonate as the primary endpoint for ART success, quality and health aspects of the conceptus and offspring have not been studied and minimal work has gone into the potential for negative consequences of ART usage, and possible mitigations.

Seminal plasma:

The deposition of semen into the uterus activates numerous aspects of maternal immunity, including both innate and adaptive. The fluid portion of the ejaculate, seminal plasma is believed to impact pregnancy outcomes through a variety of avenues, including a) the innate immune response to breeding that selectively secures sperm transport of viable sperm and clearance of the uterus of excess sperm and contaminating bacteria [1-3], b) expose the maternal immune system to paternal-derived antigens and thereby activate the adaptive immune response to tolerate the semi-allogeneic fetoplacental unit [4-6], and c) increase the expression of various embryokines (LIF, IL-6, CSF2, TRAIL) and transcripts relating to embryo health (IGF-β) to enhance the development of the early embryo [7, 8]. The impact of seminal plasma on the immune response to breeding includes an activation of cytokines and chemokines from the epithelial cells, a recruitment of immune cells into the uterine lumen, and eventual activation of dendritic cells

by paternal antigens [9-14]. The effect of seminal plasma on development of the fetus has been most well studied in invertebrates, where it has been shown to enhance inflammation post-coitus, increase egg release, and alter the behavior of females to reduce receptivity to competing males [15-17]. The impact of seminal plasma is less understood in mammals, where the majority of research on this topic has been performed in the mouse. In the murine uterus, seminal plasma induces the production of chemotactic factors by epithelial cells, and this induces the chemotaxis of various immune cells, including neutrophils, macrophages and dendritic cells. The dendritic cells reside in the endometrial stroma for 24-48 hours before migrating to the lymph nodes via the afferent lymphatics, where they present paternal antigen to naïve T lymphocytes [18]. In the murine model, the T lymphocyte response must be dominated by Tregs, and these are recruited to the uterus 3 days after breeding [6]. Tregs are believed to be essential for pregnancy maintenance, and depletion of this population leads to implantation failure, impaired uterine vascular development, and fetal loss in late term gestation [19]. A specific abortion-prone murine model does exist, wherein CBA/J females are mated with DBA/2J males. The females fail to produce an adequate Treg response, and this leads to poor pregnancy outcomes including intrauterine growth restriction, decreased placental size, and spontaneous fetal loss [20]. Additionally, numerous studies in the mouse have found the mating of seminal vesicle-excised males to normal females to decrease placental volume, litter size, and impact the health of resulting offspring [21]. Recent work indicates that seminal vesicle-derived CD38 is imperative in inducing the tolerogenic dendritic cells and CD4+FoxP3+ Tregs. In a study conducted by Kim *et al*. (2015), BALB/c females mated with CD38(-/-) males had higher abortion rates, and this could be negated by direct intravaginal injection of CD38 to CBA/J pregnant mice at the time of implantation [22]. It is unknown if the same occurs in other mammalian species, but with the increasing utilizing of IVF procedures, this topic deserves attention.

Assisted reproductive techniques:

The role of seminal plasma on pregnancy acceptance and tolerance has been elegantly studied in the murine model, but little is understood regarding the role of seminal plasma in domestic species, where artificial insemination (AI) and embryo transfer (ET) in the absence (reduced or significantly diluted) presence of seminal plasma still results in a pregnancy. In the horse, this includes artificial insemination (AI), embryo transfer (ET), and intracytoplasmic sperm injection (ICSI), and more recently, traditional *in vitro* fertilization (IVF). Seminal plasma is generally reduced to 5-20% the original volume in preparation for the cryopreservation involved in artificial insemination with frozen sperm. While this has been shown to enhance sperm viability *in vitro*, the removal of seminal plasma has also been shown to increase the duration of inflammatory cells within the uterine lumen *in vivo* [23]. Recently, studies have emerged which indicate that human offspring born to IVF practices experience altered health outcomes [24, 25], and this has been linked to the reduced or eliminated seminal plasma volume [21, 26]. The issue can be remedied through infusion of seminal plasma at the time of mating, or through the additive of seminal plasma to incubation media exposed to the embryo [21]. In the pig, farrowing rates improved from 70% to 81% when seminal plasma was administered before live cover, while both farrowing rates and litter size improved when seminal plasma was added during artificial insemination [27]. In jennies, a trend towards an increase in fertility rates was observed when

seminal plasma was added to the insemination dose [28]. Additionally, cattle have noted a dramatic decrease in the production of live offspring following *in vitro* production of embryos [29], but it is unknown if this is due to eliminated seminal plasma. While pregnancy can be achieved utilizing ART with eliminated or reduced seminal plasma concentrations, there is evidence to support that seminal plasma and its components can improve fertility, increase fecundability, alter endometrial environment, and boost offspring health [4, 6, 18, 21, 26, 27, 30]. However, it is unknown if breeding with reduced exposure to seminal plasma when ART is implemented in the horse leads to a compromised pregnancy, abnormal fetal development, or impaired offspring health.

Impact on breeding-induced endometritis:

In the horse the majority of spermatozoa are eliminated from the uterus shortly after breeding, with only a minor subset reaching the oviductal sperm reservoir. The excess sperm need to be eliminated from the uterus in a timely fashion to provide a compatible endometrial environment for embryo descent into the uterine lumen at approximately 6 days after fertilization [31]. This is accomplished through uterine contractions and sperm-induced inflammation, characterized by a balance of endometrial pro-and anti-inflammatory cytokine expression leading to a rapid influx of polymorphonuclear neutrophils (PMNs) into the uterine lumen [9]. This inflammatory response is referred to as breeding-induced endometritis, or inflammation of the endometrium. Endometritis results in prostaglandin release [32], which causes additional myometrial contractions 2-6 hours after the initial contractions [33]. These contractions physically clear the uterus from excess spermatozoa, contaminating bacteria, and residual inflammatory fluid/products within 24-36 hours. Impairment of any aspect of this response to breeding has been associated with infertility due to the persistent inflammation being incompatible with the survival of an embryo [34].

There is accumulating evidence that seminal plasma plays an important role in breeding-induced endometritis. This is specifically noted in the protection of viable spermatozoa for safe transport in the presence of an inflammatory uterine environment, while allowing dead spermatozoa and bacteria to be eliminated through PMN phagocytosis [2, 3, 35, 36]. Additionally, sperm viability was found to alter the innate immune response to breeding, as insemination with dead sperm significantly increased the expression of IL-6 [37]. In one study, only 5% of the mares became pregnant when inseminated into an inflamed environment in the absence of seminal plasma, while a normal pregnancy rate of 77% was achieved in the presence of seminal plasma [23]. Additionally, specific seminal plasma proteins have been identified that may play a role in this sperm selection. In one study, the seminal plasma protein complex of lactoferrin and SOD-3 (LF/SOD3) was found to interact specifically with dead spermatozoa, and increase the binding to PMNs [36]. Seminal plasma derived CRISP-3 was later identified as a protein responsible for protecting spermatozoa from PMN-binding and phagocytosis, and this protective effect was subsequently shown to be selective for live spermatozoa and has no effect on dead sperm or bacteria [2, 35]. Additional work is needed to elucidate the function of additional seminal plasma proteins and their impact on the immune response to breeding.

Impact on pregnancy outcome:

Establishment of pregnancy involves a fine-tuned balance between protection and tolerance within the maternal immune system, as the female needs to accept a foreign antigen (the semi-allogenic fetus) while still being able to combat pathogens from the uterus. In the horse, the first uterine exposure to paternal antigens is during mating when sperm is introduced to the tissue and draining lymphatics of the uterus. Spermatozoa itself plays an important role in preparing the female tract for a suitable immunologic environment, and this is believed to be governed by various T lymphocyte populations [26, 38]. Each T lymphocyte begins as naïve, and through a series of interactions with cytokines, contact with antigens, and access to specific transcription factors, the cells mature into either effector (Th1, Th2, Th9, Th17), regulatory (Treg), or cytotoxic populations [39]. Originally, pregnancy was believed to be a balance between the effector T cell populations, and specifically the Th1 and Th2 cell responses [40-42]. The invasive properties of placentation and implantation are governed by a primarily Th1, or pro-inflammatory, involvement [43, 44]. An increasingly Th2, or anti-inflammatory process, follows this as the fetus matures and the maternal immune system becomes tolerant to the developing and enlarging semi-allogeneic tissue [45, 46]. Finally, as parturition is initiated, the immune response switches back to a Th1 response in order to synchronize contractility and the degradation of tissue adhesion between the endometrium and chorioallantois [47, 48].

An optimal balance between the pro-inflammatory and anti-inflammatory functions of the Th1 and Th2 systems was believed to be critical for fetal survival [49, 50]. Interestingly, while an increase in Th1 cytokines in mid to late gestation was found to be embryotoxic [51, 52], Th2-deprivation was not shown to be abortogenic [53], leading to further research that deviated towards the regulatory arm of the T cell population. Tregs are involved in overall immune suppression and have been found essential for the development of the graft-host tolerance involved in benign tumor survival, which pregnancy mimics to an extent [54]. Women develop a large Treg population in both circulation and within the feto-maternal interface during pregnancy, [55], and this has also been indicated in the horse [56]. The Treg infiltration suppresses the effector functions of Th1/Th17 and is key for the regulation and recognition of the feto-placental unit as not being entirely foreign or requiring attack [57, 58]. Decreased Treg cell populations correlate with preeclampsia [59], spontaneous abortion [60], and unexplained infertility [61] in humans. In the murine model, it has been shown that the initial induction of Tregs development is initiated by seminal plasma, and this may assist with the acceptance of the paternal antigens that are found within the embryo and associated tissues [21, 38, 62, 63]. The importance of Tregs in equine pregnancy is supported by the observation that a low circulatory Treg population in estrous correlates with an increased risk of early embryonic loss [56, 64], and that a decrease in Treg-related transcripts was noted at the feto-maternal interface following the induction of ascending placentitis [65]. The number of circulating Tregs in women is found to be highest in the second trimester, and then gradually declines as parturition nears [66].

In addition to improving implantation, the addition of seminal plasma has been shown to benefit the health of the fetus throughout gestation. This is well studied in humans, where exposure to seminal plasma varies dependent on the length of cohabitation, use of barrier

contraceptives, in addition to the number of sexual partners. Reduced contact with seminal plasma has been found to correlate with an increased risk of preeclampsia [67], and this exposure is partner dependent, as multiparous women who conceive with a new partner are at an increased risk of this complication [68, 69]. The increased risk of preeclampsia is associated with the use of an oocyte donor, sperm donor, or the use of ICSI [70, 71]. Women with preeclampsia have reduced numbers of Tregs in both endometrium and within periphery, and this is believed to be the link between reduced seminal plasma volume and increase risk of disease [72]. A decreased peripheral population of Tregs has been associated with early pregnancy loss in the horse [56]. Additionally, a shift from a Treg response to a Th17 response has been noted following the induction of ascending placentitis in late term gestation [65]. It is unknown if a reduction of seminal plasma is associated with any equine pregnancy-related complications, or if reduced seminal plasma is associated with decreased Treg populations.

Impact on offspring:

Seminal plasma is a major regulator of endometrial gene transcription, thereby impacting the environment for the embryo. This can permanently alter embryo development through epigenetic effects, potentially impacting the phenotype of the resulting offspring. Seminal plasma has been shown to enhance the endometrial and oviductal production of various embryotrophic factors, including CSF1, CSF2, CSF3, IL-6, LIF, VEGF, and TRAIL in both mice and pigs [7, 73]. When females are mated with seminal vesicle-excised males, a reduced rate of zygote cleavage has been noted [21], while infusion of seminal plasma at the time of insemination in these seminal vesicle-null animals reinstates the appropriate inflammatory response, enhances embryo development, and increases the number of offspring [30]. IVF in humans has been shown to alter placental development [74], neonatal outcomes [75], and offspring health [24, 25]. In mice, seminal plasma deletion caused by seminal vesicle ablation has been shown to alter growth trajectories in resulting offspring, with elevated adipose tissue, hypertension, and reduced glucose tolerance observed, and this was primarily found in males [21]. The alteration in offspring phenotype is hypothesized to be linked to the reduced embryokine production in the female reproductive tract following insemination with seminal plasma-voided ejaculates.

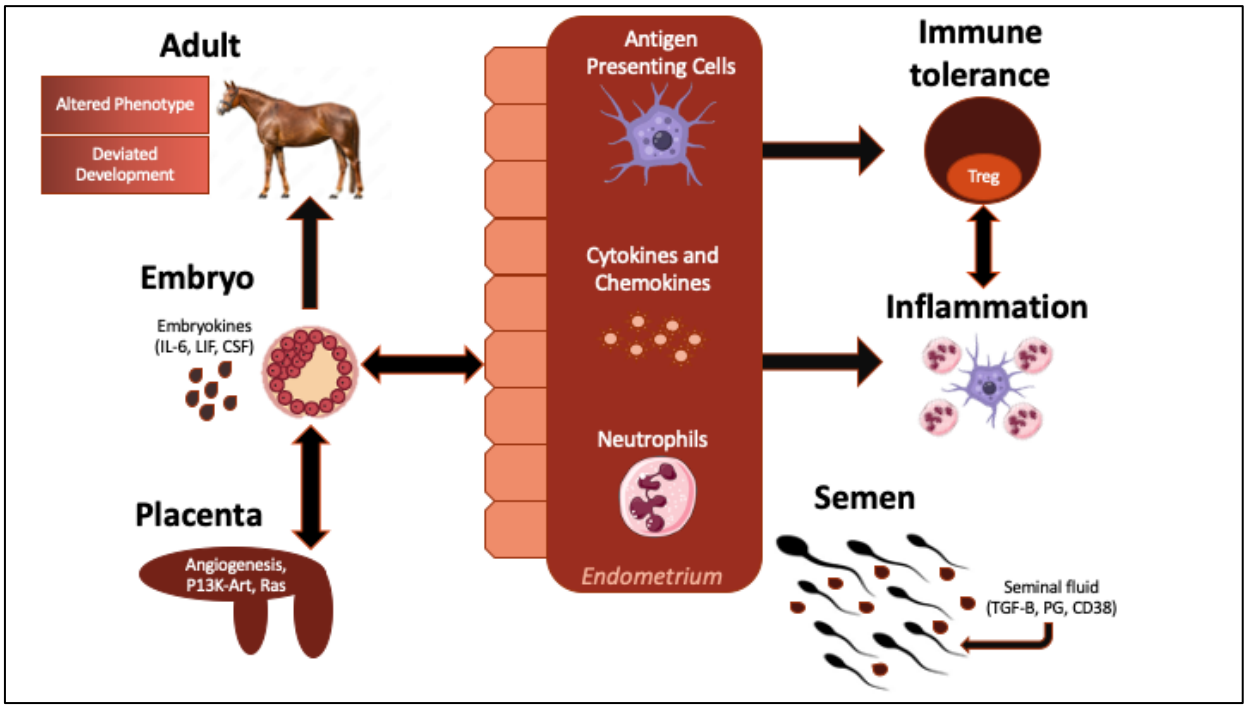

Figure 1: Schematic representation of the proposed impact of seminal plasma on the maternal immune response to breeding and pregnancy. *Seminal plasma is believed to impact the maternal immune response to breeding, embryo growth and development, in addition to immunotolerance of the developing fetus, all of which are believed to improve pregnancy outcomes and optimize the growth and development of offspring. Proteins, cytokines, and paternal antigen within seminal plasma modulates the immune response to breeding, leading to the production of various cytokines which assist with the innate immune response to breeding, in addition to the cell-mediate immune response to pregnancy. Stimulated antigen presenting cells travel through the draining lymphatics of the uterus to increase the proliferation of immunotolerant lymphocyte populations, specifically Tregs. Seminal plasma is also believed to increase the production of various cytokines, deemed embryokines (IL-6, LIF, CSF), which enhance embryo growth and development. Breeding techniques in which seminal plasma is reduced or eliminated are believed to alter the endometrial and placental transcriptome, in addition to altering cell-mediated immunity both peripherally in addition to the reproductive tract. (Modified from Bromfield 2016 [26]).*

Recent research:

Recent research from our laboratory characterized the endometrial transcriptome following exposure to seminal plasma. Following this, the placental transcriptome of pregnancies produced *in vivo* (fresh semen; carry own) or *in vitro* (ICSI; embryo transfer) was evaluated. These two datasets allowed us to investigate the impact of ART/seminal plasma exposure on both the maternal and placental transcriptome in the equine. The data from these studies that are directly relevant to the proposed study are summarized below (Figure 2 and 3) and have been recently published [76, 77].

To determine the effect of seminal plasma on the uterine environment that the early embryo would be exposed to, we collected endometrial tissue from 6 mares following insemination utilizing an insemination dose that either had full or reduced seminal plasma fraction. Mares were bred during estrous when a pre-ovulatory follicle was noted, and endometrial biopsies were collected 7 days after insemination. Tissue was placed in either RNALater for RNA isolation/sequencing, or formalin for future immunohistochemistry. For RNASeq, RNA was extracted from endometrial tissue, and RNA sequencing performed for transcriptomic analysis. Seminal plasma appeared to have an effect on the endometrial transcriptome at seven days after breeding (roughly six days after ovulation), which is when the embryo migrates to the uterine lumen from the oviduct. When comparing groups (insemination including seminal plasma in comparison to insemination alongside reduced seminal plasma), 241 genes were found to be differentially expressed with a false discovery rate (FDR) cut off of $P<0.05$. Differentially expressed genes (DEG) were associated with a variety of pathways relating to embryo development and health, immunotolerance, and metabolism. This included genes associated with antigen presentation such as HLA class II histocompatibility antigen DRB1, HLA class II histocompatibility antigen DM Beta Chain, and RAS guanyl-releasing protein 1 (RASGRP1) alongside a trend towards an increase in HLA class II histocompatibility antigen DQA, all of which are essential in antigen processing and the stimulation of naïve T lymphocytes. Additional targets related to immune cell signaling were altered following insemination with seminal plasma, including an increase in chemokines and defense peptides such as B-defensin. Numerous targets that have been previously associated with proper embryo health and development increased following insemination with seminal plasma. Additionally, numerous targets relating to embryo metabolism and cholesterol regulation increased following insemination with seminal plasma, and this included the insulin receptor (INSR) as well as binding proteins for the insulin-like growth factor (IGFBP2/IGFBP3) alongside low-density lipoprotein (LDL), and a component of high-density lipoprotein (APOA1). This data is described in Figure 2 [76].

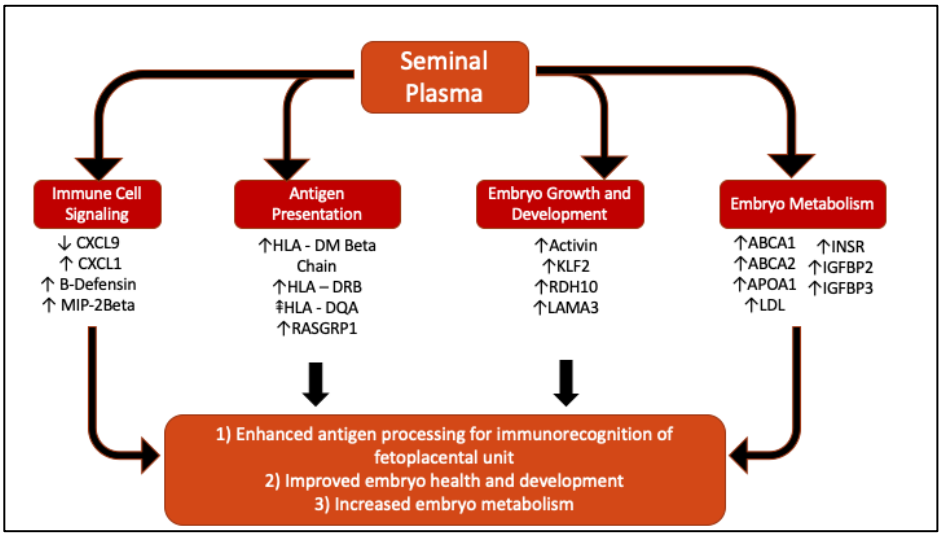

Figure 2: Select DEG transcripts following insemination with either full seminal plasma fraction or reduced seminal plasma fraction. *Seminal plasma appears to have an impact on immune cell signaling and antigen procession, alongside embryo growth, development, and metabolism (↓/↑ indicates $P<0.05$, indicates ⇞/↟ $P<0.1$)*

In addition to the endometrial transcriptome, the *in vitro* maturation of embryos (and therefore complete elimination of seminal plasma) was found to impact the placental transcriptome. To evaluate this, 17 warmblood mares were bred either *in vivo* (n=9; fresh semen, full seminal plasma), or *in vitro* (n=8; ICSI, no seminal plasma). Mares bred *in vivo* carried their own pregnancy, while *in vitro*-produced embryos were transferred to recipient mares with no seminal plasma priming. Chorioallantois was collected immediately postpartum, and RNA was extracted from tissue. RNA sequencing was performed for transcriptomic analysis where 1580 genes were found to be differentially expressed with a false discovery rate (FDR) cut off of P<0.05. Further pathway analysis was performed, indicating multiple pathways to alter in the *in vitro*-produced placenta. This included multiple pathways associated with hypoxia, including decreased metabolism and increased ribosome biogenesis. Additionally, a decrease in many members of innate immunity was noted, including interleukins and ligands (IL-1β, CCL2) in addition to various chemokines (CXCL8, CXCL5, CXCL6, CXCL2), and this was alongside an increase in members of the adaptive system, specifically noted in IL-17 signaling (CCL17, IL-17RB). Key members of the angiogenesis pipeline altered, including both the EGF pathway (VEGFR2, HRAS, BRAF, ERK) in addition to the EGF bypass pathway (PDGF, FGF, IL-6), all of which may lead to altered translation of various targets. This data has been recently published, and is summarized in Figure 3 [77].

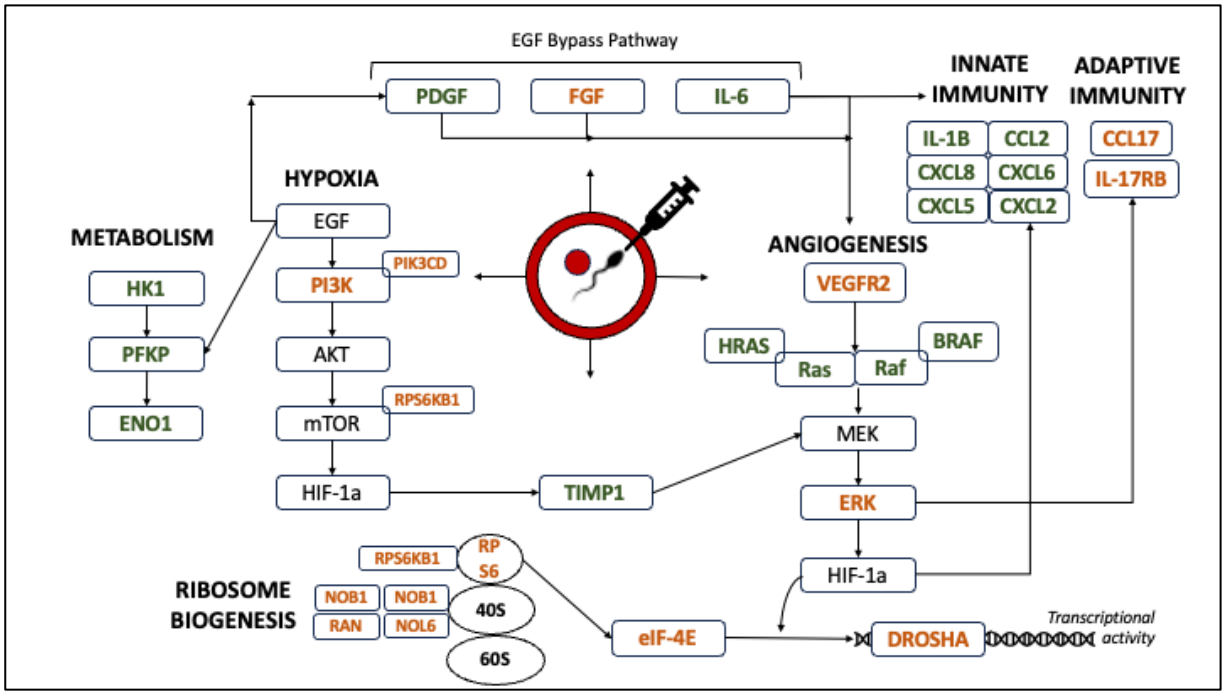

Figure 3: Biological pathways altered in the placenta of in vitro-produced pregnancies. *Differential expression of approximately 1650 genes were found associated with the in vitro production of equine pregnancies (ICSI). This included key transcripts in key cellular signaling pathways such as decreased metabolism, increased ribosome biogenesis, decreased innate immunity, increased IL-17 signaling, and altered hypoxia and angiogenesis. Orange indicates an increase in expression, while green indicates a decrease in expression.*

Conclusions:
There is accumulating evidence that seminal plasma has a distinct biological function associated with breeding in horses and other species. While seminal plasma may not be necessary for the establishment of pregnancy, it appears to facilitate the immune response of the uterus, as well as ensuring a suitable uterine environment for optimal development of the fetus. The importance of the uterine environment extends beyond gestation and can potentially affect the health of offspring later in life. The use of transcriptomics allows researchers to generate hypothesis that may better test the impact of seminal plasma on maternal immunity, placental function, and offspring health. With this information, the role of seminal plasma exposure at the time of breeding should be investigated further regarding its effect on quality aspects of pregnancy and offspring, with a specific focus on assisted reproductive techniques.

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Embryo Transfer Recipient Management in the Era of ART; What have we learned?

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Introduction

Embryo transfer has been established as an important tool in commercial horse production over the last 40 years and recipient mare management has been recognized as an important component of the procedure. While the transfer of embryos and management of recipients has become relatively standardized over these decades, the increasing number of in vitro-produced (IVP) embryos has led to some adjustments. A relative shortage of mares available as recipients, the effectiveness of cryopreserved embryos, and an increase in client-provided recipients have also changed the recipient management landscape.

The best embryo transfer programs arrived at that level of proficiency only by optimizing each aspect of the entire program. The journey from mediocre to excellent can only be realized by the aggregation of incremental gains. Each small gain not only adds to the improvement of the program but often magnifies the results of previous and future improvements. The program could be enhanced by improving selection of recipients, improving the transfer technique to decrease damage to the embryo or stimulation to the reproductive tract, improving the recipient cycle evaluation to prioritize transfers, improving transfer drug regime to improve embryo survival, improving recipient housing and holding to decrease stress, culling to improve overall recipient fertility, and improving progesterone management to minimize pregnancy loss. Improving any one of these areas may help a little, but maximizing all of them can enhance the whole program.

Selection

Selection of potential recipients has changed somewhat over time, as the frequent use of cryopreserved embryos simplifies the synchronization process, so it is much simpler for owners to provide their own recipients. A normal reproductive tract is the most important factor in selecting a recipient, but appropriate size, age, and behavior are also important. Evaluation of the reproductive tract will vary with the program and the individual mare. A young maiden mare will probably not require much more than a thorough ultrasound exam, while more mature mare with an uncertain history brought to you by an owner may require a thorough workup including a uterine biopsy. Horse owners tend to be much more critical of recipient quality if they are provided by a program and more lenient if they are to provide them themselves. In that case it behooves the veterinarian to be discerning and educate the owner on the suitability of each individual mare to provide the best possible outcome.

Identification

Positive identification of each individual recipient mare in an embryo transfer program to prevent mistakes or confusion in estrous cycle management, medication administration, transfer of the appropriate embryo, even sending the correct mare home with an owner. This identification can take the form of freeze branding or microchips. Neck collars, leg bands, and halter tags can be used, but can be lost and result in identification problems. In our program recipients receive a freeze brand and are identified on paper and verbally using both their number and the name to make it

clear which mare one is referring to. Owner recipients that are brought to the clinic are photographed and a halter tag applied. While this is not ideal, consistent monitoring of the tags and the short duration of the mare's stay allow it to work satisfactorily.

Housing

Reduction of stress and biosecurity are the two major concerns regarding housing of recipient mares. Stress in the form of environmental, nutritional, or social stress can negatively affect a program in many ways. Anecdotally we have observed an increase in numbers of recipients with low serum progesterone during periods of high environmental stress such as cold or mud. Recipients in a large program should be gathered late in the year before use, not only for biosecurity, but also so social hierarchies can be established and adapted to. These groups should be kept together and moved together if possible, preventing the stress of reforming the pecking order. Access to hay and grain in a group feeding situation should have spots for at least 10% more horses than are actually in the group, allowing more submissive animals to find a spot clear from the dominant mares.

Paddock lighting to encourage early cyclicity works well in group situations, but due to the stress of being in a group and perhaps weather and mud stress, it doesn't work quite as well as it would for individual mares in stalls. We have our mares come up for feeding late in the afternoon, starting December first. They remain in the lot until 10 PM when the lights turn off and the gate to the pasture opens. We expect most mares to have their first ovulation in 60 days, during the first two weeks of February, although there will be some stragglers in the group that will be delayed.

Biosecurity

Larger embryo transfer programs require the collection of relatively large numbers of mares that can come from many different backgrounds and potentially are exposed to infectious disease at sales and collection points. This requires the herd be assembled several months before the season so that any health concerns can be addressed. Viral respiratory diseases are quite common in mares that have been through public auctions, and as the herd is assembled together these often have to run their course. Strep equi infection can be more of a persistent problem in mares especially those assembled from auctions or dealers, resulting in an occasional carrier mare. These can be identified through pharyngeal washings and PCR or guttural pouch endoscopy which some embryo transfer programs employ.

Some form of isolation or quarantine is important for recipients entering a program or even for owner-provided recipients coming into a clinic. Owner's will unwittingly pick up mares from a dealer or a sale and bring them directly to a clinic without much thought to their suitability or the possibility of infectious disease. Serum amyloid A testing added to a good history and examination of an owner-presented recipient mare can help decrease the possibility of the introduction of respiratory disease in your clinic or herd, especially when combined with an isolation period before entering the general population.

Reproductive Management

Recipient mares should be examined by ultrasound each day of estrus to not only identify the day of ovulation, but to characterize the overall quality of the cycle. Some practices only examine mares

every other day, and while one can get by with this protocol, if you really want to optimize your program, especially with IVP embryos, this is not a good short-cut to take. Criteria for passing a mare to receive an embryo include the establishment of good uterine edema, lack of uterine fluid, an obvious ovulation with the formation of a corpus luteum, and prompt resolution of endometrial edema following ovulation. Many programs follow mares until ovulation and then check them for corpus luteum formation, resolution of edema and uterine fluid on the day of transfer. In our practice we follow the mares through ovulation continuing until a good corpus luteum is noted, endometrial edema has resolved and no uterine fluid is present. This helps us rank mares as we feel a mare that ovulated and quickly resolved edema is a superior candidate to a mare that only resolved edema and fluid by the day of embryo transfer.

Synchrony

The term synchrony is used to describe the temporal relationship between an embryo's developmental stage and a recipient mare's estrous cycle or more particularly stage of diestrus. For in vivo produced embryos the relative timing of the donor and recipient mare's ovulations were conveniently used to describe this relationship with the donor mare's ovulation being the frame of reference. This allowed for the description of synchrony numerically as "zero" if the donor and recipient ovulations were on the same day, and +1 or -1 if the recipient mare ovulated one day before or 1 day after the donor, respectively. This system works well with the occasional exception of an embryo that would be more delayed or advanced in development than would be typical, but that is relatively uncommon and the variation is usually minimal. The highest pregnancy rates with the transfer of in vivo-produced embryos are generally attributed to a synchrony of -2 with synchronies of -3 to +1 producing acceptable rates as well.[1]

The description of synchrony for the transfer of IVP embryos requires different nomenclature, as there is no donor mare ovulation and the rate of development of IVP embryos varies greatly with transferrable early blastocysts developing anywhere from day 6 to day 10 following fertilization. These embryos, despite the variation in time of development, are all transferred at nearly the same stage of development, so synchrony is described simply using the day of diestrus, the period of time following ovulation, of the recipient mare. Since IVP embryos are transferred at a stage of development roughly equivalent to day 6 in an in vivo-produced embryos, a synchrony similar to -3 to +1 used for in vivo produced embryos should be day 3 to day 7 recipients for IVP embryos to produce optimum results, and that theoretical relationship seems to hold true. (Table 1)

Some factors can shift the most effective synchrony one way or another and one of these is double ovulation in the recipient mare. Claes et al showed that the highest pregnancy rates for mares with double ovulations was shifted one day earlier, apparently the more rapid rise in progesterone initiates uterine changes sooner than with a slower rise.[2] The same appears true for non-cyclic recipients in our practice as we see the best pregnancy rates on day 3 following initiation of progesterone treatments.[3] Cryopreservation and subsequent thawing can shift timing as well. While most cryopreserved embryos re-expand shortly after thawing, many appear to not resume a normal growth rate for as much as a day or two following thawing. This leads to in best pregnancy rates, as reported by Claes, produced from thawed embryos transferred into day 3 recipient mares. Our results reflect a similar shift (table 2).[4]

Embryo Transfer

The actual transfer of embryos into recipient mares varies from practice to practice with embryos loaded in 0.25 ml or 0.5 ml straws using end-delivery or side-delivery guns, and some with the Wilsher technique.[5,6] Regardless of the specifics the goal is still the same, to place the embryo in a sterile manner into the uterus with minimal stimulation or effect on the reproductive tract and no damage to the embryo. All techniques have a take some practice to an operator to become proficient. The Wilsher technique has been shown to have a lower learning curve but may not be adaptable to all situations such as larger programs with multiple transfer locations such as ours. Transfer of IVP embryos appears to be somewhat more demanding of operator technique than the transfer of flushed embryos. We have seen a trend in operators that are able to produce good pregnancy rates transferring in vivo-produced embryos but struggle with IVP embryos until they gain further experience.

Recipient mares are commonly medicated at the time of embryo transfer, and the type of medication varies widely from program to program. Some programs administer only minimal medications, usually tranquilizer, while others use antibiotics, anti-inflammatories, and progestins in an attempt to improve pregnancy rates. Because of the expense and logistics of equine embryo transfer it is difficult to perform a blinded trial to ascertain the efficacy of any treatment. Blanco et al utilized random distribution of embryos between treatments on 300 transfers and showed that the addition of antibiotics and anti-inflammatories to the transfer regime significantly improved port-transfer pregnancy rates.[7] Our practice includes ampicillin, gentamicin, dexamethasone, flunixin meglumine, xylazine, and butylscopolamine bromide in the standard embryo transfer regime.

Progesterone Management

Progesterone and progestin supplementation has been controversial in equine reproduction for many years with debate over whether the condition of luteal insufficiency exists in the horse. It has been shown that embryo transfer or even sham embryo transfer can result in luteolysis and a decrease in serum progesterone, and recipients have been shown to have progesterone lower than 4 ng/ml at the time of the first pregnancy exam.[8,9] Many embryo transfer programs use some sort of progestin supplementation in an effort to avoid any pregnancy loss from insufficient progesterone, and they do it in many forms. Whether supplementation is administered from the time of the transfer throughout pregnancy, it is avoided altogether, or some form of testing is combined with targeted supplementation, each program seems to be different. It is apparent though that some recipients will be more likely to carry a pregnancy to term with supplementation. The serum progesterone concentration generally accepted in the industry for pregnancy maintenance is 4 ng/ml or greater, although pregnancy can be maintained with concentrations as low as 2 ng/ml (ref). One study showed 31% of embryo transfer recipients had serum progesterone values less than 4 ng/ml one week after transfer, and serum progesterone concentrations at the time of transfer were not predictive of the concentration 1 week later.[10] Administration of altrenogest administered for pregnancy maintenance can also reduce the production of endogenous progesterone. (McCue), and administration starting at the time of transfer does not influence the result of the first pregnancy check or pregnancy maintenance as long as mares with low concentrations are supplemented at that time.[11]

Non-cyclic mares supplemented with estradiol and then progesterone or progestins can be successful embryo transfer recipients and have been used in many programs. The use of non-cyclic recipients can be very convenient and as successful or even mores successful than cyclic mares

(Table 1 & 2). The only drawback is their reliance on exogenous progestins for pregnancy maintenance. While the placenta largely takes over pregnancy maintenance by producing pregnanes after 75- 100 days of pregnancy, in our experience mares that have had supplemental progesterone will often not have sufficient support from the placenta until closer to 150 days of gestation. Radioimmunoassay (RIA) for progesterone, unlike other progesterone assays, cross reacts with pregnanes, allowing testing of mares beyond 100 days of gestation. Our practice is to evaluate serum progesterone (and pregnanes) via RIA every 30 days starting at 120 days of gestation for mares that are on supplemental progestins such as non-cyclic recipients. Once concentrations reach 4 ng/ml, supplementation is stopped.

Culling

Perhaps the single most important step in maintaining a high-producing embryo transfer program is culling of the recipient mare herd. Culling will keep the herd at a relatively high level of fertility by eliminating and replacing mares of questionable fertility. Mares should be culled for obvious reproductive abnormalities such as endometritis, persistent intrauterine fluid, cervical abnormalities, and failure to become pregnant after embryo transfer. We usually consider culling a mare after her second failed transfer with an exception if an embryo was of poor quality. Mares should also be either culled as they get older, or at least scrutinized more completely to be maintained in the herd. Since overall mare fertility starts to decline around age 14 we routinely cull all mares by age 14, as well as perform a uterine biopsy in the fall on all open and returning recipients 8 years of age and older before the next season. Behavior issues can also result in culling, particularly for mares that are difficult to handle, mares that are not good mothers, and mares that don't adapt well to living in a herd situation.

Table 1: 14-day pregnancy rate and maintenance to 30-day pregnancy for **fresh** ICSI embryos by recipient day of ovulation.

	14d Pregnancy	Maintenance to 30d Pregnancy
Noncyclic recipients	41/47 (87.2%)	32/36 (88.9%)
Day 7 post-ovulation	9/14 (64.3%)	9/9 (100%)
Day 6 post-ovulation	65/90 (72.2%)	49/61 (80.3%)
Day 5 post-ovulation	168/220 (76.4%)	136/168 (81.0%)
Day 4 post-ovulation	293/376 (77.9%)	249/287 (86.8%)
Day 3 post-ovulation	112/164 (68.3%)	87/111 (78.4%)
Day 2 post-ovulation	2/2 (100%)	1/2 (50%)

Table 2: 14-day pregnancy rate and maintenance to 30-day pregnancy for **vitrified** ICSI embryos by recipient day of ovulation.

	14d Pregnancy	Maintenance to 30d Pregnancy
Noncyclic recipients	49/64 (76.6%)	18/21 (85.7%)
Day 7 post-ovulation	2/4 (50%)	2/2 (100%)
Day 6 post-ovulation	10/16 (62.5%)	7/9 (77.8%)
Day 5 post-ovulation	76/100 (76.0%)	61/71 (85.9%)
Day 4 post-ovulation	349/478 (73.0%)	243/282 (86.2%)
Day 3 post-ovulation	86/124 (69.4%)	62/74 (83.8%)

Day 2 post-ovulation	0	0

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Sperm's Vital Role: A Functional Perspective on Fertilization

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The ultimate goal of the sperm is to survive long enough to reach and fertilize the oocyte. The sperm goes through a dedicated and well-orchestrated journey, undergoing amazing molecular and structural changes to acquire the fertilizing ability. Major events such as capacitation and acrosome reaction are synchronized changes that prepare the sperm for zona pellucida recognition, membrane fusion, and oocyte activation. Oocyte activation is initiated when a fertilizing sperm delivers sperm-borne oocyte activating factor into the oocyte cytoplasm. Experimental evidence indicates that phospholipase C zeta 1 (PLCZ1) meets the criteria of the soluble oocyte-activating factor responsible for initiating calcium (Ca^{2+}) oscillations after gamete fusion at mammalian fertilization. The fertilizing sperm releases PLCZ1 into the ooplasm, triggering Ca^{2+} oscillations via the inositol trisphosphate ($InsP_3$) signaling pathway through the hydrolysis of organelle membrane-bound substrates. The oscillatory pattern of intracellular Ca^{2+} rises activates pathways that result in the resumption and completion of meiosis II, the extrusion of the second polar body, cortical reaction, and the initiation of preimplantation development. In fertilized and successfully activated oocytes, Ca^{2+} oscillations also regulate short- and long-term embryonic developmental events, confirming the essential role of sperm-borne PLCZ1 for fertilization and embryo competence in mammals.

Most clinical investigations of PLCZ1 have been performed in humans due to the importance and implications for oocyte activation failure after in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). The importance of oocyte activation is especially notable after in vitro fertilization due to the physiologic sperm selection that occurs in the female reproductive tract is bypassed. The relationship between male infertility and PLCZ1 in men has been confirmed in several clinical and experimental studies. The absence, reduction, and abnormal localization of PLCZ1 in human sperm or *PLCZ1* mutations in men are associated with ICSI failure, low fertilization rates, and impaired embryo development. In this context, the assessment of PLCZ1 in sperm samples used for IVF and ICSI has diagnostic and predictive value for fertility.

In stallion sperm, PLCZ1 is first observed in round spermatids over the developing acrosome. In mature. PLCZ1 is in the acrosomal, equatorial, and postacrosomal regions, connecting and principal piece of the tail. The localization of PLCZ1 in the principal piece of the tail is a unique feature of stallion sperm, which is enzymatically active and capable of promoting oocyte activation. The relative content of PLCZ1 in stallion sperm has been associated with fertility in vivo and in vitro. Some stallions with low in vivo fertility also

performed less efficiently after heterologous or homologous ICSI, suggesting the implication of a male infertility factor. In equine clinical ICSI, in frozen-thawed sperm samples from stallions with proven fertility in vivo, the relative content and proportion of positively labeled sperm for PLCZ1 exhibited a wide range of values, differing from stallion to stallion. When oocytes were injected with frozen-thawed sperm from samples with low or high PLCZ1, oocytes injected with sperm from samples with low PLCZ1 consistently demonstrated lower cleavage rates after injection in heterologous and homologous ICSI. The male factor plays a critical role in the outcome, especially when sperm used for injections come from a heterogeneous population of stallions and from sperm samples frozen under varied conditions. The content and localization of equine PLCZ1 have intrinsic variations among stallions that are exacerbated by cryopreservation procedures impairing cleavage success and embryo competence.

The regulation of PLCZ1 expression in stallions and its association with fertility are largely unknown. There are a handful of reports on genes that affect stallion fertility. In stallions, single nucleotide polymorphisms (SNPs) within genes have been identified to contribute to the estimated breeding values of the paternal component of the pregnancy rate per estrus cycle, showing a significant connection with stallion fertility. Recently, an SNP was located within the PLCZ1 gene in Hanoverian stallions. The validation of polymorphisms showed that those SNPs are associated with the paternal component of pregnancy rates as a male fertility trait. However, PLCZ1 mutations that affect stallion fertility under in vivo or in vitro conditions have not yet been documented. Genetic variants and the altered protein expression of PLCZ1 may influence sperm quality traits in stallions and consequently impact fertility in vivo and in vitro. Among stallions, the PLCZ1 content of sperm samples largely varies and sperm samples with low PLCZ1 are associated with low cleavage rates after ICSI. Genetic screening and protein evaluation for PLCZ1 could be markers to predict male fertility in vivo and in vitro, and an oocyte-activating tool to improve assisted fertilization outcomes. Further research is needed to determine whether PLCZ1 as an artificial oocyte-activating treatment is a physiological, efficient, and safe method for improving assisted fertilization in the horse.

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Optimizing Equine Embryo Production *In Vitro*: Insights from the Follicular and Oviductal Environments

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Production of equine embryos *in vitro* is currently a well-established commercial technique and a reliable way of producing embryos for vitrification or uterine/oviductal transfer. To obtain these embryos, immature oocytes are retrieved from mares via ovum pick-up (OPU) or post-mortem. Following oocyte *in vitro* maturation (IVM), mature oocytes are fertilized by intracytoplasmic sperm injection (ICSI). Embryos are then cultured in specific media until day 8–10 of development. However, in the best of the scenarios roughly 10% of the oocytes used for ICSI result in successful pregnancy and foaling (Fernández-Hernández et al., 2020). Surprisingly, when *in vivo* matured equine oocytes are transferred into the oviducts of live mares, the likelihood of pregnancy rises to 75%, contrasting with the only 40% when *in vitro* matured oocytes are transferred (Hinrichs, 2018). All these data clearly reflect that the current media used for IVM and embryo culture in the horse are far from ideal and thus, more efforts are needed to better design current commercial media that accurately replicate the natural reproductive environment. Therefore, our research focuses on gaining a deeper insight into the *in vivo* condition to replicate these conditions *in vitro*. For this purpose, we are investigating the composition of preovulatory follicular fluid (FF) and oviductal fluid (OF), the latter before and after ovulation.

Hence, we initially conducted our research in OF and FF aiming to better disclose their physicochemical properties (ion content and osmolarity) and their metabolic and proteomic composition (Fernández-Hernández et al., 2020; González-Fernández et al., 2020). Ovaries were obtained from an abattoir, and OF was retrieved from oviducts ipsilateral to a preovulatory follicle (PRE) or to the ovulation site (PST). FF was obtained from follicles >35 mm and the reproductive tract showed an evident uterine oedema when opened at the abattoir; a recent ovulation was considered when a recent CL was observed after ovarian dissection. All samples were centrifuged at 16000 g for 20 min at 4 °C, and the supernatants were stored at −80 °C. Osmolarity was measured in pure FF or OF diluted 1:1 in distilled water using an Advanced™ Model 3320 Micro-Osmometer. Ion content was determined using inductively coupled plasma mass spectrometry system. Normality was assessed using the Shapiro-Wilk test and comparisons were run by one-way ANOVA; a $P < 0.05$ was considered statistically significant and values are presented as mean ± standard error of the mean (SEM). The osmolarity (mOsm/kg) of FF was 288 ± 2.4 (n = 11), 298.3 ± 9 for PRE-OF (n = 6) and 260.8 ± 11 for PST-OF (n = 6); the osmolarity of PST-OF differed significantly from both PRE-OF and FF ($P < 0.05$). The analysis revealed that the following ions were present in all the samples: Na^+, Mg^{2+}, K^+, Ca^{2+}, Fe^{2+}, P^+, Ni^{2+}, Cu^{2+} and Zn^{2+} (n = 9 for FF, n = 3 for PRE-OF, and n = 2 for PST-OF). Significant differences were observed for K^+ in FF (4.9 ± 0.1 mM) when compared with PST-OF (54.8 ± 1.6 mM; $P < 0.05$) and from PRE-OF (45.9 ± 6.5 mM). Similarly, the concentration of Zn^{2+} was significantly higher in PRE-OF (87.5 ± 25 µM) and PST-OF (89.5 ± 22.4 µM) compared to FF (5.2 ± 0.5 µM; $P < 0.05$). Our first batch of results demonstrated that significant differences exist in osmolarity, and ion concentrations present in equine FF and OF at the PRE and PST stages (Proceedings of the 24th Annual Conference of the ESDAR, 11-16 October 2021).

Then, the metabolic and proteomic composition of FF and OF (PRE and PST) were investigated. Post-mortem OF was obtained from 8 PRE and 6 PST mares, combined to provide a total of 5 samples per group (10 µl each), and analyzed by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) (González-Fernández et al., 2020). A total of 18 metabolites were identified, being the most concentrated lactate, myoinositol, creatine, alanine and carnitine. Statistically significant differences were observed only in fumarate and glycine, which were higher in PST samples ($p < 0.05$). These metabolite concentrations in OF were compared with those in commercial media used for equine *in vitro* fertilization, such as Menezo B2; Whitten's medium (MW); Tyrode's albumin lactate pyruvate (TALP). These culture media contain glucose at approximately 5 mM; lactate is present at concentrations ranging from 0.45 to 21.6 mM, and pyruvate ranges from 0-5 mM. A major difference between the concentrations of these common energy sources in commercial media and those determined in our study was that the concentration of glucose in OF was lower (0.18 ± 0.04 and 0.57 ± 0.2 mM preovulatory vs. postovulatory) and the concentration of lactate in OF was higher (54.66 ± 10.7 and 69.25 ± 7.3 mM preovulatory vs. postovulatory). Furthermore, no measurable glucose was found in 3 of 5 PRE oviductal fluids. We also took into consideration that lactate increases in post-mortem tissue, due to anoxic metabolism, and these glucose and lactate measurements might not be entirely representative of the actual content of fluid in the live mare. However, the extremely high lactate concentrations (55–70 mM) cannot be solely attributed to the metabolism of plasma glucose (~5 mM), suggesting that such levels are intrinsic to the OF. These data revealed important metabolome signatures that could reflect physiological needs of the spermatozoa, oocytes and zygotes in the oviduct and should be taken into consideration to design commercial media. Regarding FF composition, similar research was conducted using preovulatory FF samples obtained from post-mortem mares (n = 6) using ^{1}H-NMR. Results obtained were contrasted against the composition of the two commonly used media for equine oocyte IVM: TCM-199 and DMEM/F-12. Twenty-two metabolites were identified in equine FF; of these, 9 are not included in either DMEM/F-12 or TCM-199 media. These include acetylcarnitine (0.37 mM ± 0.2; mean ± SEM), carnitine (0.09 ± 0.01; mean ± SEM), citrate (0.4 ± 0.04; mean ± SEM), creatine (0.36± 0.14; mean ± SEM), creatine phosphate (0.36 ± 0.05; mean ± SEM), fumarate (0.05 ± 0.007; mean ± SEM), glucose-1-phosphate (6.9 ± 0.4; mean ± SEM), histamine (0.25 ± 0.01; mean ± SEM) and lactate (27.3 ± 2.2; mean ± SEM). Besides, the mean concentration of core metabolites such as glucose varied (4.33 mM in FF *vs.* 5.5 in TCM-199 *vs.* 17.5 in DMEM/F-12). Again, our data suggested that the currently used media for equine oocyte IVM can be further improved (Fernández-Hernández et al., 2020).

The proteome of PRE and PST-OF was also investigated, revealing significant differences among both fluids. OF samples were obtained post-mortem from oviducts ipsilateral to a pre-ovulatory follicle (n = 4) or recent ovulation (n = 4) and the samples were kept at -80 °C until analysis. Following protein extraction and isobaric tags for relative and absolute quantification (iTRAQ) labelling, the samples were analyzed by nano-liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The analysis of the spectra resulted in the identification of a total of 1173 proteins present in pre-ovulatory and post-ovulatory samples; among these, 691 were unique for *Equus caballus* (FDR < 0.01). Proteins from post-ovulatory oviductal fluid were compared with proteins from pre-ovulatory oviductal fluid and were categorized as up-regulated (positive log fold change) or down-regulated (negative log fold change). Fifteen proteins were found to be down-regulated in the post-ovulatory fluid and 156 were up-regulated in the post-ovulatory OF compared to the pre-ovulatory fluid. Among the up-regulated proteins, 87 were involved in protein metabolism pathways. Identified proteins were involved

in sperm-oviduct interaction, fertilization, and metabolism, among others (Fernández-Hernández et al., 2021).

All this data collectively underscores the importance of adapting *in vitro* conditions to more accurately reflect the natural equine reproductive environment, aiming to improve the success rates of equine embryo production. Although not all these findings may be immediately applicable to optimizing the composition of current *in vitro* maturation or embryo culture media, they significantly enrich our understanding regarding the physiological processes of oocyte maturation, fertilization, and early embryo development in the horse.

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Early antibiotic and IV fluid therapy of the critical neonatal foal

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Early Antimicrobial Therapy

Early antimicrobial administration and cardiovascular support are important components of the early sepsis bundle in humans. The pediatric guidelines for treatment of sepsis and septic shock in children and infants (Surviving Sepsis Campaign) is to start antimicrobial therapy as soon as possible, within one hour of recognition of septic shock, and within three hours of recognition of sepsis-associated organ dysfunction, but without shock (Weiss et. al, 2020). Empiric broad-spectrum therapy is recommended with one or more antimicrobials that cover all likely pathogens. Once pathogens and antimicrobial susceptibilities are available, the recommendation is to narrow antimicrobial therapy coverage. (Weiss et. al, 2020).

Foals: In foals, the aim is similar for early administration of empiric antimicrobial therapy to cover as many likely pathogens as possible. Two approaches for antimicrobial administration in foals include: 1) Prophylactic use for foals considered at high risk for sepsis, and 2) Therapeutic administration for foals with clinical and/or laboratory evidence of sepsis. For foals that are at high risk of developing sepsis but are not yet obviously ill, prophylactic antimicrobial therapy is warranted. These include foals with failure of passive transfer, neonatal maladjustment syndrome, premature foals, and those born in contaminated environments.

In a study assessing cumulative susceptibility at 'foal level' of bacteria isolated from foals with sepsis at hospital admission, the following antimicrobials or combinations had the highest % of susceptibility (Theelen et al, 2019):

Amikacin + ampicillin 91.5%
Ceftizoxime 89.7%
Ceftiofur + amikacin 89.6%
Amikacin + penicillin 88.6%
Ceftiofur 86.3%
Gentamicin + ampicillin 83.6%
Gentamicin + penicillin 82%
Chloramphenicol 81.6%
Trimethoprim/sulfamethoxazole (TMS) 59.6%

For **prophylactic use**, ceftiofur is a reasonable option in the field at extra-label doses (5-10 mg/kg Naxcel; ceftiofur sodium IM or SC, q 12 h). Ceftiofur crystalline free acid can also be used in foals, however extra-label doses (up to twice) and reduced dosing intervals (q 48-72 hours) are often needed to target the likely pathogens associated with sepsis in foals. As noted above, TMS is not an optimal choice.

For **therapeutic use** in hospital, the combination of amikacin + ampicillin is excellent when renal function is preserved. When aminoglycosides are used, the author uses these guidelines: the foals must be rehydrated first, noted to produce urine on serial ultrasound exams, and have a creatinine that is decreasing with time (ideally ≤ 2 mg/dL). Therapeutic drug monitoring is very helpful to monitor the efficacy and safety of amikacin. If renal physiology is a concern, then either ceftizoxime, ceftazidime, or cefotaxime, in combination with ampicillin, is used by the author, especially if neutropenia is present on the CBC. If the white blood cell count is normal, then ceftiofur + ampicillin is often initiated instead. Ampicillin is added for its activity against enterococci and anaerobes, which cephalosporins lack or have minimal activity against, respectively. Once blood culture results are available, treatment may be modified.

Early Hemodynamic Support

The Surviving Sepsis Campaign suggests the administration of up to 40-60 mL/kg crystalloids in boluses (10-20 mL/kg per bolus) over the first hour, titrated to clinical markers of cardiac output and discontinued if signs of fluid overload develop, for the initial fluid resuscitation of children with sepsis or septic shock (Weiss et. al, 2020). Clinical makers of cardiac output include heart rate, blood pressure, capillary refill time, mentation level, serial lactate measurement, and urine output. Advanced hemodynamics, including cardiac index, systemic vascular resistance and central venous oxygen saturation are recommended to be used whenever available.

Signs of fluid overload include clinical signs of pulmonary edema and hepatomegaly. The suggestion is to use balanced crystalloids rather than 0.9% saline. They recommend against the use of starches or gelatin in children with septic shock or sepsis (Weiss et. al, 2020).

Foals: In foals, the author utilizes a somewhat similar "fluid challenge method" for resuscitative or replacement fluid therapy of the hypovolemic or dehydrated foal. This entails administration of 10-20 mL/kg of commercial, balanced crystalloids administered over 15-30 minutes, with subsequent reassessment of the foal's hemodynamic status. Perfusion parameters (mentation, extremity temperature, heart rate, capillary refill time, and pulse quality), mean arterial blood pressure, serial lactate, and urine output are used as markers of cardiac output and perfusion. Urine output in particular, as monitored using serial ultrasound exams, is very helpful for monitoring response to fluid therapy. Once the bladder begins enlarging, the rapid boluses can stop. Cardiac output can also be used for monitoring for positive responses to fluid therapy. In foals, indirect or noninvasive estimates of cardiac output can be used. The author uses the Bullet method for monitoring serial cardiac output measurement in individual foals (Giguere et. al, 2005).

If the hemodynamic status of the foal is not stabilized after 2-3 of such fluid challenge boluses, the author begins inotrope support (dobutamine 3-10 μg/kg/min) in conjunction with fluid therapy. Following this, norepinephrine may be added to the fluid+dobutamine protocol as a vasopressor (0.1-0.5 μg/kg/min). For foals with refractory hypotensive shock, vasopressin or low-physiological doses of hydrocortisone may be added as infusions as well. Premature foals may also benefit from physiological doses of hydrocortisone as an infusion, depending on their ACTH and cortisol status.

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Evaluating Sperm Fertilizing Potential: What Can We Predict? - Preview-

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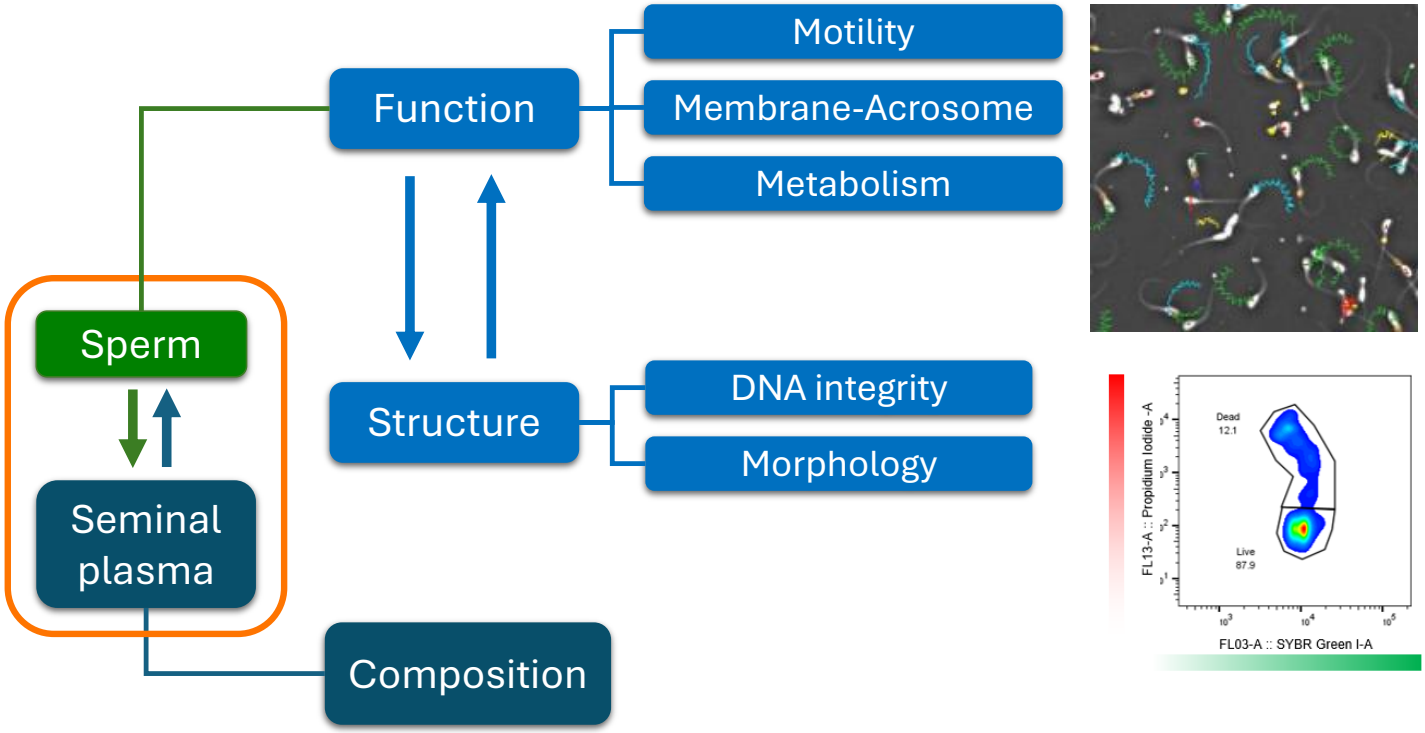
Primary approach of sperm evaluation

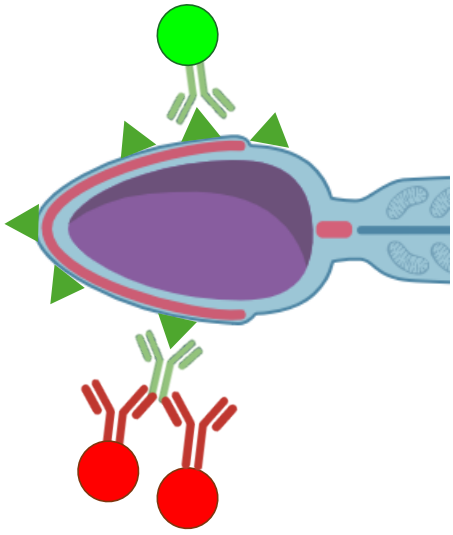


Advanced analysis

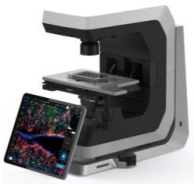
- Protein abundance/localization → fertility markers
- ROS production/accumulation → oxidative stress
- Respiration → Oxygen consumption/ ROS production → real-time sperm metabolic status
- Proteomic
- Lipidomic
- Epigenomic
- Genomic

Protein abundance/localization → Immunodetection

Primary antibody (Ab)-conjugated
(enzyme/fluorochrome)

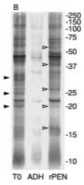


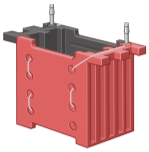
Primary antibody (Ab)
+ Secondary antibody-conjugated



Epifluorescence

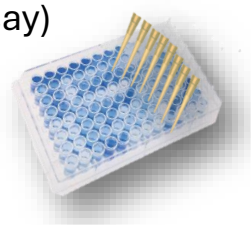
Flow cytometry



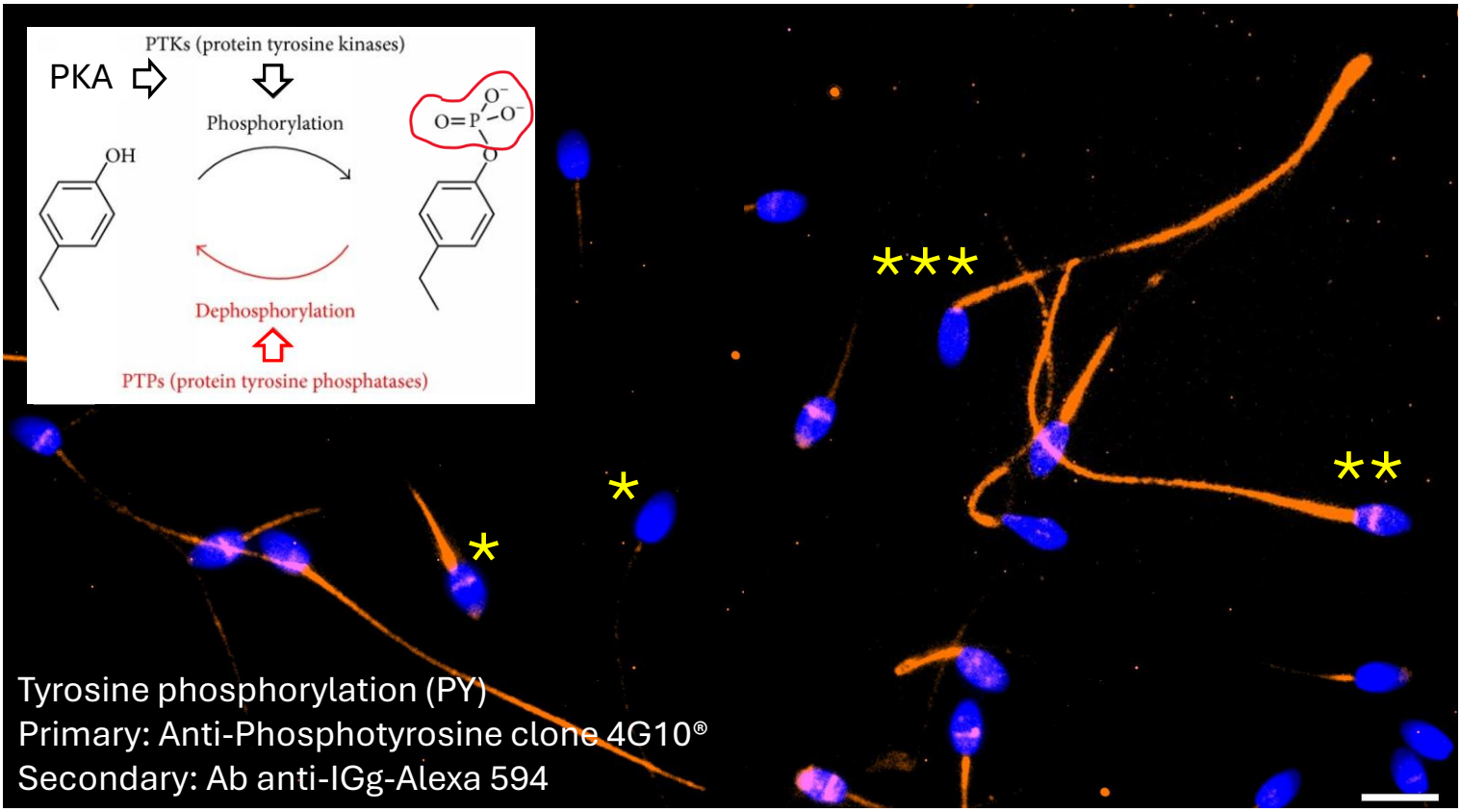


Western blotting

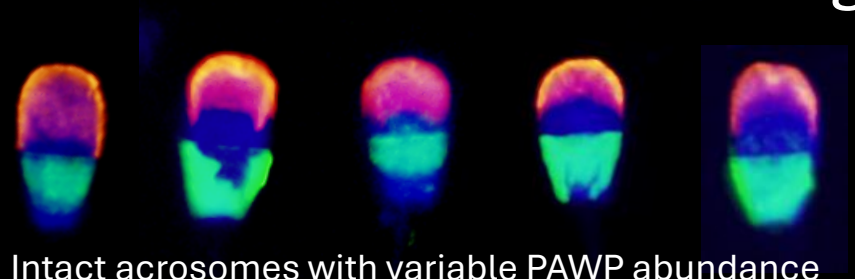
ELISA (enzyme-linked
immunosorbent assay)



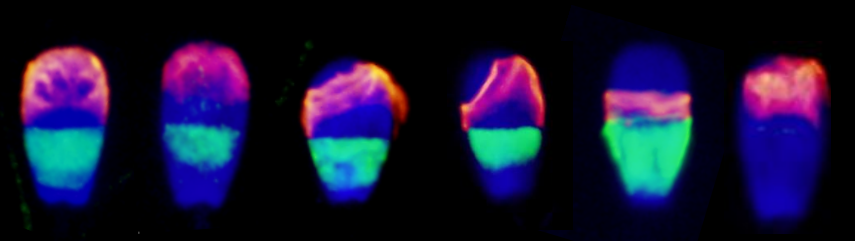
Immunofluorescence of tyrosine phosphorylation patterns



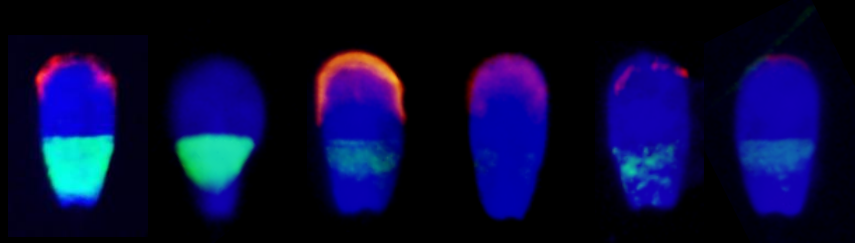
Immunofluorescence of protein marker and acrosome integrity



Intact acrosomes with variable PAWP abundance



Damage acrosomes with variable PAWP abundance



Reacted acrosomes with variable PAWP abundance

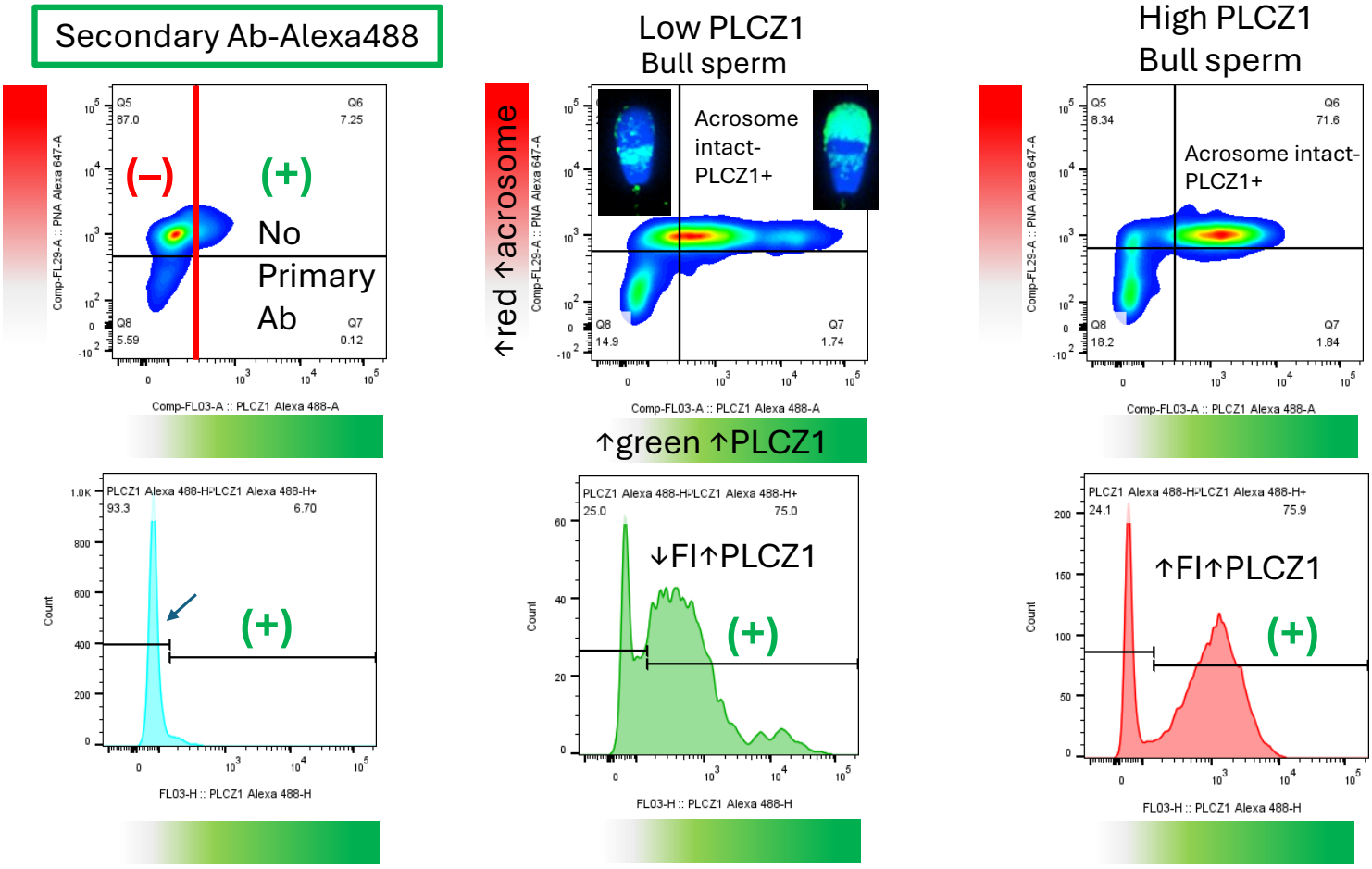
Bull sperm heads

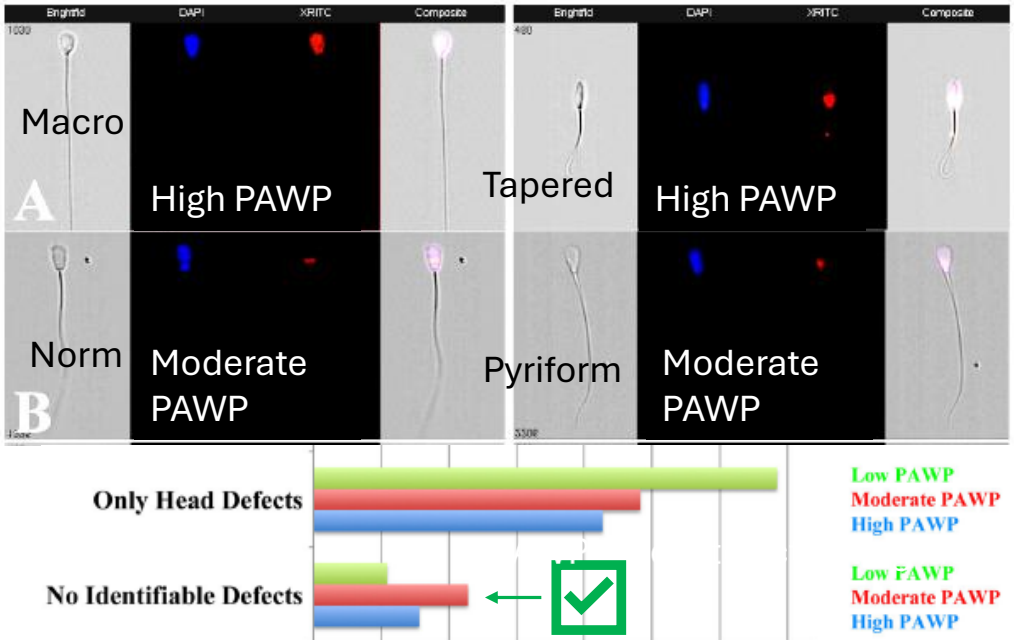
BLUE
→ DAPI
→ dsDNA → A-T rich region

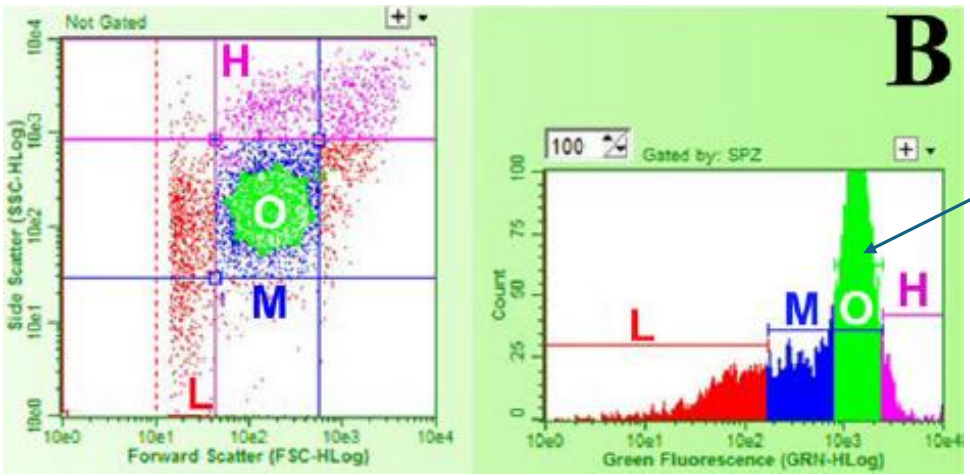
RED
→ PNA-TRITC
→ glycoconjugates in the outer acrosomal membrane (Gal-β(1-3)-GalNAc (galactosyl (β-1,3) N-acetylgalactosamine)

GREEN
→ PAWP/WBP2NL-Alexa488
→ Postacrosomal region

Flow cytometry: PLCZ1 and acrosome integrity







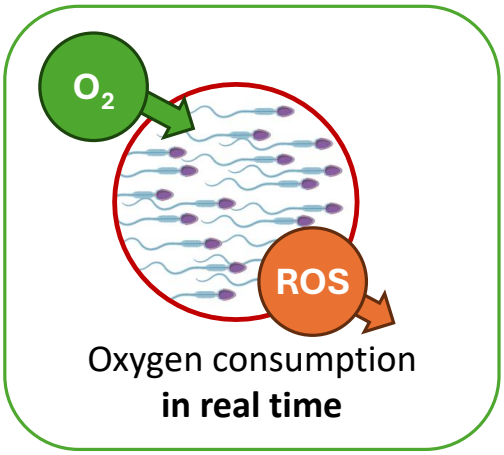
Imaging flow cytometry

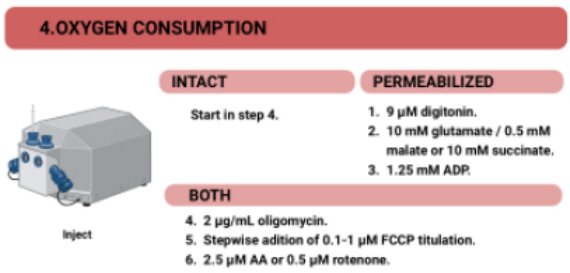
- ✓ PAWP/WBP2NL → fertility marker in bulls
- ✓ Abnormal sperm morphology alters protein content and distribution

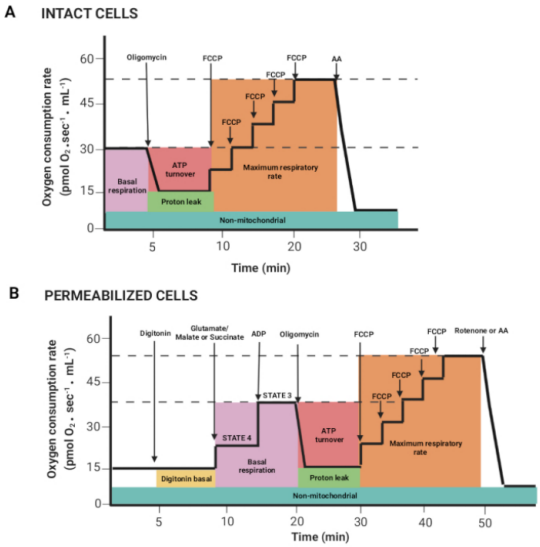
"Optimal" sperm subpopulation

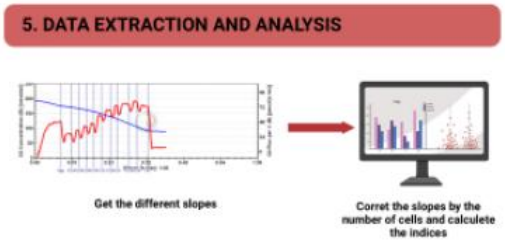
Mol. Reprod. Dev. 81:436–449 (2014)

High-Resolution Respirometry
→ Oxygen consumption









Oxygraph-2 K

Irigoyen P, Sapiro R, Cassina A. High-Resolution Respirometry to Assess Mitochondrial Function in Human Spermatozoa. J Vis Exp. 2023 Jun 23;(196). doi: 10.3791/65493. Erratum in: J Vis Exp. 2023 Sep 26;(199). doi: 10.3791/6574. PMID: 37427915

High-Resolution Respirometry

Malate/glutamate/succinate
→ mitochondrial complex I or complex II

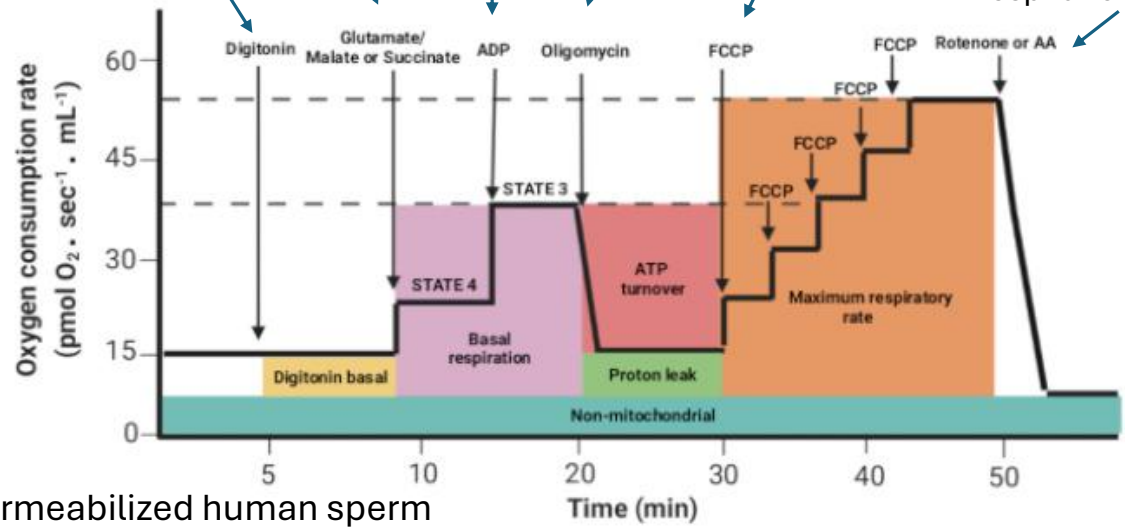
Oligomycin → ATP synthase inhibitor
(non-phosphorylating respiration → no ATP)

Digitonin
→ permeabilization

ADP
→ for ATP

FCCP → protonophore ↑ proton permeability → uncoupled respiration → no ATP production

AA → mitochondrial respiration inhibitor

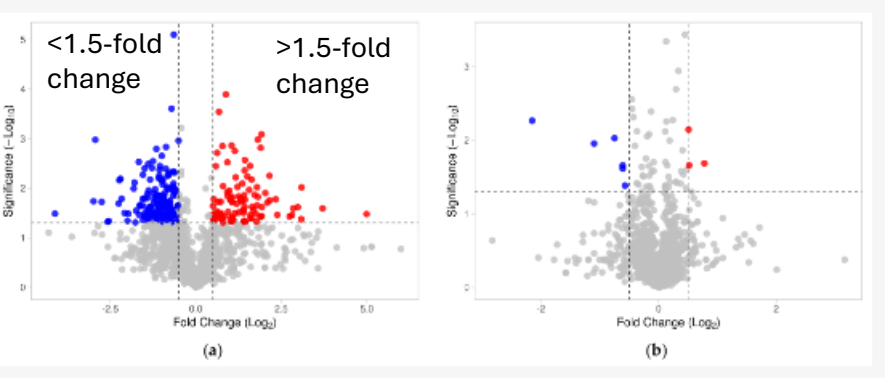


Permeabilized human sperm

Irigoyen P, Sapiro R, Cassina A. High-Resolution Respirometry to Assess Mitochondrial Function in Human Spermatozoa. J Vis Exp. 2023 Jun 23;(196). doi: 10.3791/65493. Erratum in: J Vis Exp. 2023 Sep 26;(199). doi: 10.3791/6574. PMID: 37427915

Omics .... Proteomic

Proteomic evaluation of boar sperm



Capacitated x 4h

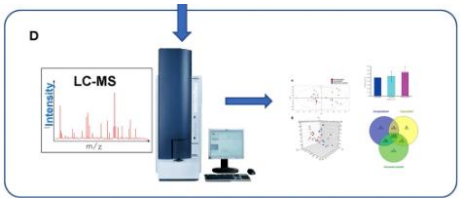
Control

Volcano plot representation of changes in **sperm surface protein abundances** before and after 4 h of in vitro capacitation

Biomolecules **2023**, *13*, 996

- ✓ Proteins
  - Capacitation
  - Hyperactivation
  - Metabolism
  - Acrosomal exocytosis
  - Fertilization
- ✓ 14 proteins different >1.5-fold
- ✓ More abundant → NIF3L1, CSE1L, NDUFB7, PGLS, PPP4C, STK39, TPRG1L
- ✓ Less abundant → BPHL, GSN, GSPT1, PFDN4, STYXL1, TIMM10, UBXN4

Omics .... Lipidomic

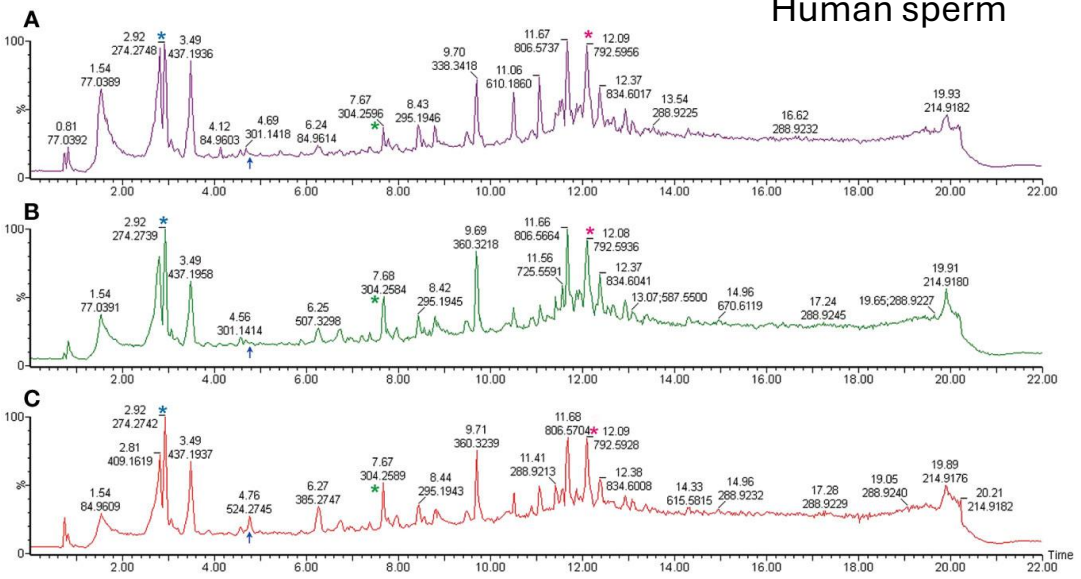


Mass Spectrometer

Mass-to-charge ratio → MW

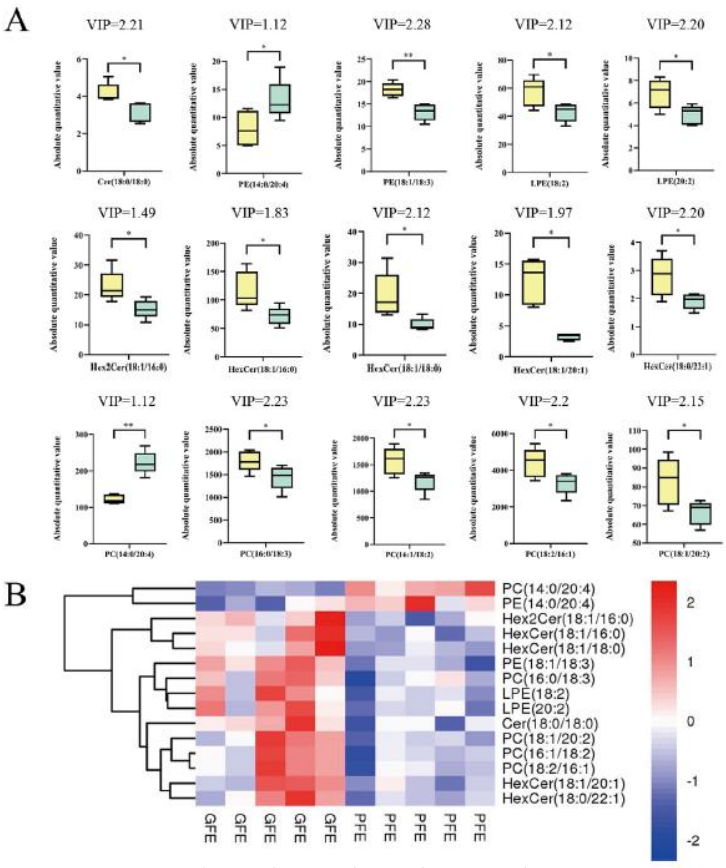
Human sperm

After lipid extraction, the results look like this.....



Front. Endocrinol. , 06 November 2023
Sec. Reproduction
Volume 14 - 2023 |
https://doi.org/10.3389/fendo.2023.1273878

Omics .... Lipidomic



Differential and hierarchical analysis

Theriogenology 206 (2023) 28–39

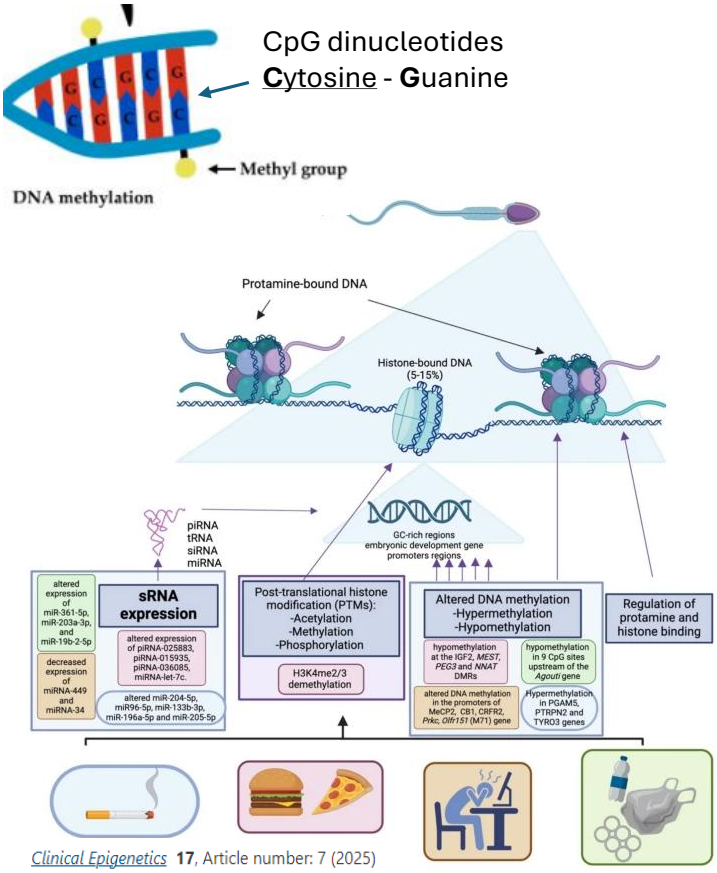
Good freezer

Poor freezer

.... but after analysis of a huge data set:

- ✓ Lipid identification
- ✓ Lipid profile changes
- ✓ Treatment effect
- ✓ E.g. Lipid composition of boar sperm between good and poor freezer are different expressed.

Omics .... Epigenomic



Clinical Epigenetics 17, Article number: 7 (2025)

DNA methylation → sperm epigenome

- ✓ Sperm quality
- ✓ Ability to fertilize
- ✓ Via MAKP81IP3 signaling pathway.

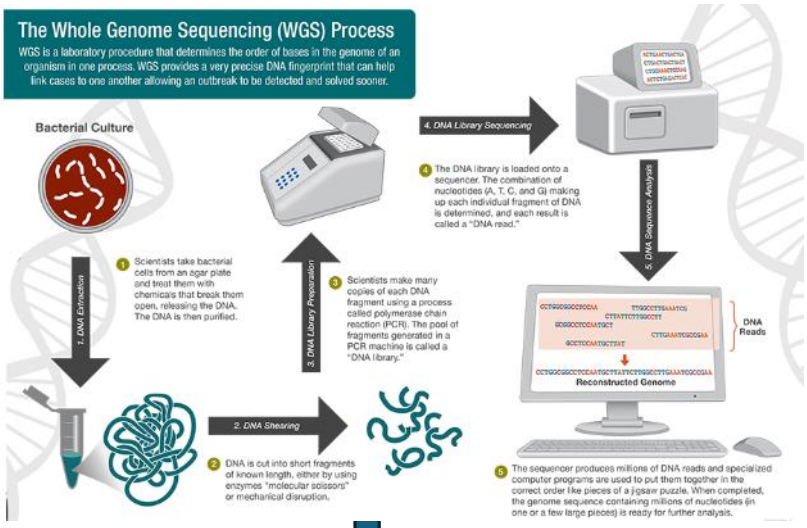
- Paternal exposure to toxic endocrine-disrupting chemicals (EDCs) → transgenerational transmission.

- Predisposition to disease, infertility, testicular disorders, obesity

- Human ART → paternal diet, BMI, and alcohol consumption, male lifestyle choices and environmental factors.

Omics .... Genomic

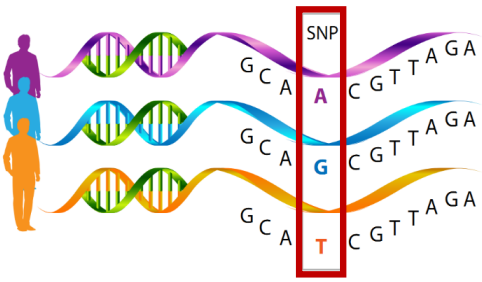
Whole genome sequencing (WGS)
→ Complete DNA sequence



https://www.cdc.gov/

Genetic variability underlying phenotypic variations in male fertility traits and semen/sperm characteristics.

Genotype



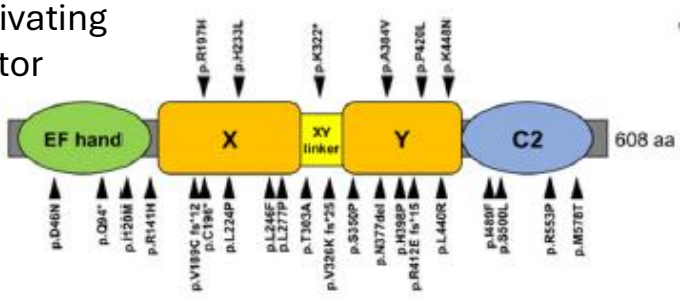
Single Nucleotide Polymorphism
SNPs

- ✓ No sperm sample
- ✓ Genetic study for individuals

- ✓ Genetic component of the herd
  → Bull prepuberal selection

Omics .... Genomic

PLCZ1 → sperm oocyte activating factor



Abnormal PLCZ1 → Oocyte activation failure

Zygote, Volume 31, Issue 4, August 2023, pp. 316 - 341

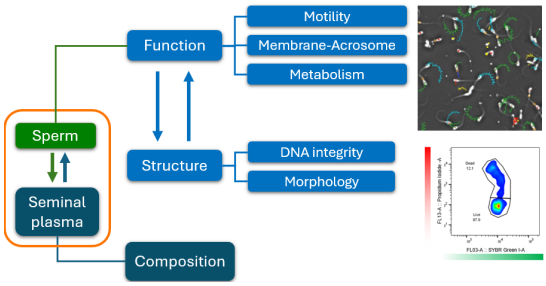
✓ Human

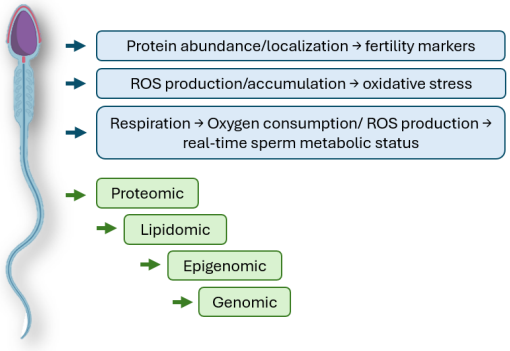
- 27 variants in PLCZ1
- 18/21 variants → deleterious for structure/function
- 1/21 → no deleterious
- 2/21 → unknown

- 63.1% variants bi-allelic variants
- Pathogenic variants for PLCZ1 also heterozygosity

Lay Summary

Primary approach of sperm evaluation





Evaluating Sperm Fertilizing Potential: What Can We Predict?

While traditional sperm analysis focuses on evaluating individual sperm attributes, a sperm sample is actually a heterogeneous collection of sperm. It contains a "super" sperm subpopulation with optimal fertilization capabilities alongside a "poor" subpopulation that is deteriorating with sublethal changes and undergoing apoptosis. Therefore, a multiparametric approach, integrating functional, structural, and molecular assays, is crucial for a thorough evaluation. This comprehensive view offers a range of diagnostic and predictive tools to assess sperm's fertilization potential across different reproductive strategies (in vivo, IVF, ICSI) and production objectives.

The secretome, a promising tool in equine assisted reproduction

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The secretome, encompassing the entire spectrum of proteins and other molecules secreted by cells, tissues, or organisms, is crucial for intercellular communication in numerous biological processes including immune responses, metastasis and tissue repair among others (Damous et al., 2018; Luis-Calero et al., 2024b). The term was first used in the context of the array of proteins secreted by *Bacillus subtilis* (Tjalsma Harold et al., 2000), and it includes all proteins released by cells, such as cytokines, growth factors, hormones, enzymes, glycoproteins, coagulation factors, and extracellular vesicles (EVs). EVs are membrane-bound structures such as exosomes and microvesicles; these EVs transport regulatory molecules including different RNA types (microRNAs, long non-coding RNAs, mRNAs), lipids, metabolites, and DNA fragments. These elements are crucial for cell-to-cell signaling (Harmati et al., 2021) and have been shown to play a vital role in oocyte maturation, meiotic progression, and developmental competence (Luis-Calero et al., 2024a). Secretome can be obtained from the supernatant of cultured cells or directly from native biological fluids, and can be conceptually divided into two main fractions: the EVs fraction, considered the "solid" fraction, consisting of exosomes, microvesicles, and apoptotic bodies (which are classified based on size, biogenesis, and release mechanism), and the EV-free fraction, which consists of bioactive molecules secreted in soluble form (Papait et al., 2022). Both the complete secretome and isolated EVs have been used as adjuvants in assisted reproduction techniques (ARTs) in domestic species. However, the isolation of EVs requires expensive equipment or time-consuming techniques and leaves behind a subset of core biologically active compounds that need further exploration. Furthermore, EVs isolation from equine preovulatory follicular fluid yields very low EVs quantities (data not shown), and thus, our research was directed towards the use of complete secretome.

In assisted reproduction, secretome supplementation has been shown to enhance embryo quality in both mice and bovine (Marinaro et al., 2019; Perrini et al., 2018). Under our point of view, applying a similar strategy in the horse could lead to better outcomes in equine embryo production *in vitro*, and thus, the use of this approach was chosen to improve equine oocyte maturation and to improve subsequent embryo production and/or quality.

Hence, we analyzed the composition of equine preovulatory follicular fluid (FF) secretome and tested its effects on meiotic competence and gene expression in oocytes subjected to *in vitro* maturation (IVM) (Luis-Calero et al., 2024a). To this aim, preovulatory FF was obtained, concentrated using ultrafiltration with *cut-off* of 10 kDa, and stored at -80 ˚C. The metabolic and proteomic composition was analyzed, and its ultrastructural composition was assessed by cryo-transmission microscopy. Oocytes obtained *post-mortem* or by ovum pick up (OPU) were subjected to IVM in the absence (control) or presence of 20 or 40 µg/ml (S20 or S40) of FF secretome. Following IVM, oocytes were also analyzed for chromatin configuration or snap frozen for subsequent gene expression analysis. Proteomic analysis detected 255 proteins in the *Equus caballus* database, mostly related to the complement cascade and cholesterol metabolism. These proteins were associated with official gene symbols and 125 unique genes were used for the enrichment and pathway analyses. For the enrichment analysis, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were used as annotation databases, revealing enriched terms as *extracellular space* (GO:0005615, 61 proteins), *innate*

immune response (GO:0045087, 13 proteins), and *serine-type endopeptidase inhibitor activity* (GO:0004867, 21 proteins). According to the KEGG and GO analysis, many proteins were involved in lipid, phospholipid and lipoprotein metabolism (ecb04979, GO:0010873, GO:0042157), activity (GO:0051006, GO:0060228), exchange (GO:0033344, GO:0043691), clearance (GO:0034382) and binding (GO:0005543, GO:0034380, GO:0034375, GO:0031210, GO:0070653, GO:0010903) (Luis-Calero et al., 2024a). Metabolomic analysis identified 14 metabolites being the most concentrated glycerol (4228.0 ± 512.0; µM ± SEM) and lactate (136.0 ± 5.6; µM ± SEM) vividly differing with the composition of native FF (Fernández-Hernández et al., 2020). Analysis of secretome by cryo-transmission electron microscopy revealed also the presence of extracellular vesicles. Additionally, cytokine profiling revealed the presence of IP-10 (173.1 ± 36.3; pg/ml ± SEM), FGF (78.8 ± 1.7 pg/ml ± SEM), eotaxin (49.6 ± 2.5; pg/ml ± SEM) and RANTES (1.1 ± 0.3; pg/ml ± SEM) which were similar to concentrations found in native FF (Luis-Calero et al., 2023). However, supplementation of the IVM medium with secretome did not result in significant differences in chromatin configuration (metaphase II, metaphase I, germinal vesicle or degenerated) regardless of secretome dosage or oocyte source (OPU or *post-mortem).* In parallel, the relative mRNA expression of quality-related genes in equine cumulus oocyte complexes (COCs), namely TNF alpha induced protein 6 (*TNFAIP6*), growth differentiation factor 9 (*GDF9*) and bone morphogenetic protein 15 (*BMP15*) was analyzed in COCs subjected to IVM in the presence or absence of secretome. *GDF9* and *BMP15* expression significantly increased in OPU-derived oocytes compared to *post-mortem* oocytes (fold increase ± SEM: 9.4 ± 0.1 *vs.* 1 ± 0.5 for *BMP15* and 9.9 ± 0.3 *vs.* 1 ± 0.5 for *GDF9*, respectively; $p < 0.05$), thus reflecting the superior quality of equine oocytes retrieved *in vivo*. Interestingly, secretome addition enhanced the expression of *TNFAIP6* in *post-mortem* group at 40 µg/ml and in OPU group at both 20 and 40 µg/ml concentrations. This gene has been demonstrated to be expressed in the extracellular matrix of equine follicles, as well as in COCs, and has been related with cumulus expansion and extrusion of the detached complex during ovulation in the mare, possibly reflecting improved cumulus expansion when secretome is added during IVM (Luis-Calero et al., 2024a). To further understand the influence that secretome addition during IVM could exert on equine oocytes obtained by OPU or *post-mortem*, the metabolomic profile of the IVM medium was analyzed pre- and post-maturation (pre-IVM and post-IVM) by Nuclear Magnetic Resonance. Addition of FF secretome during IVM resulted in a significant increase in lactic acid concentration post-IVM for the S20 and S40 groups compared to the control medium pre-IVM in OPU and *post-mortem* derived oocytes, ranging from 110.5 ± 4.9 (µM ± SEM) in control to 221-273 (µM ± SEM) when secretome was added disregarding the oocyte source ($p < 0.05$). Interestingly, glucose consumption post-IVM significantly decreased only in OPU-derived COCs supplemented with secretome at 40 µg/ml compared to the control medium pre-IVM (Control pre-IVM vs. S40 post-IVM: 117.24 ± 7.72 vs. 82.69 ± 4.24; µM ± SEM). However, this reduction did not directly parallel the observed increase in lactate production, as it would be expected (Luis-Calero et al., 2024b). Moreover, a similar effect was noted in the cytokine concentration pre- and post-IVM in the S40 group for FGF (37.4 ± 3.7 vs 17.8 ± 5.6; pg/ml ± SEM) and Eotaxin (72.7 ± 6.3 vs 41.9 ± 10.8; pg/ml ± SEM) ($p < 0.05$), but not in the control group. These findings suggest that the secretome may facilitate the uptake of cytokines and certain metabolites by equine COCs during IVM (Luis-Calero et al., 2023). Hence, secretome addition during IVM seems to be capable of modulating equine COCs' metabolism and cytokine uptake that could potentially be directed towards a more physiological IVM process. In a subsequent yet unpublished study, the developmental competence of *post-mortem* derived oocytes subjected to IVM in the presence or absence of secretome was assessed. In this study, equine oocytes matured in the presence or absence of secretome were subjected to intracytoplasmic sperm injection (ICSI). A cohort of 242 oocytes

for control, 261 for S20 and 263 oocytes for S40 were included in the study. Maturation rates significantly increased with secretome supplementation, from 35 ± 3.6 (% ± SEM) in the control group to 46.5 ± 2.7 in S40 resulting in statistically significant differences ($p < 0.05$). These results contrast with the formerly referenced works by our own laboratory (Luis-Calero et al., 2024a, 2024b), which reported non-significant differences, likely due to smaller sample sizes (maximum 66 oocytes per group versus up to 263 in the current study). However, cleavage rates (ranging from 60.9 ± 3.7 to 73.6 ± 5.5) and blastocyst rates did not significantly vary ($P > 0.05$; embryo yield was 20.4 ± 3.3 for CTR; 20.9 ± 4.7 for S20 and 22.2 ± 5.5 for S40). Interestingly, a non-significant tendency for higher successful ICSI (defined as the ICSI session that yielded at least one embryo) was observed with increasing secretome doses (% successful ICSI sessions: 75% for control, 83.3% for S20 and 91.7% for S40). Moreover, pregnancies and foaling have been achieved showing the safety of FF secretome supplementation during IVM in horses (unpublished data).

In conclusion, the comprehensive studies conducted on the use of the secretome in equine assisted reproduction highlight its potential to significantly enhance oocyte maturation but not embryo development. The observed improvements in cellular metabolism and gene expression with secretome supplementation suggest a promising avenue for optimizing reproductive techniques. Continued research into the mechanisms and effects of secretome components as well as its effect on gametes and embryos will be crucial in harnessing its full potential, aiming to keep optimizing assisted reproductive technologies in horses.

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Semen evaluation, conservation and transport

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Semen evaluation in dogs is required for breeding soundness evaluation or in cases of sub- or infertility; such an examination can be done easily with little expenses and few materials. Most important is the mindful collection, handling and preparation of the semen; furthermore, the interpretation of the results considering the history and the clinical findings of the dog. Semen examination usually comprises macroscopical evaluation, measurement of pH, sperm concentration, total number of spermatozoa, total and progressive motility and evaluation of cell morphology (Pena-Martinez 2004). During the lecture, useful methods will be discussed.
For evaluation of sperm kinematics, microscopical evaluation of a sperm sample is still worthful; good semen samples show a progressive motility of >70% (Johnston et al 2001). Estimated motility well correlates with data objectively measured by computer assisted sperm analysers (CASA). For prediction of fertilizing capability of a semen sample, commercially available kits like the pro A-kinase anchor protein 4 (proAKAP4) assay are of interest; in the canine species, a positive correlation between proAKAP4 concentration in the sperm rich fraction and total motility, progressive motility and further velocity parameters was reported (Le Couazer et al 2019). However, to recognize subfertile samples, individual analyses with CASA and evaluation of more kinematic parameters like VCL may be helpful. In one study, a VCS value of 121.8±54.8 µm/s was measured in subfertile dogs, while in the fertile group, a significantly higher value was detected (160.7±19.7 µm/s) (Rijsselaere et al 2007). A VCL value < 161 µm/s was later shown to be related to bad post-thaw values despite good fresh semen quality (Schäfer-Somi and Tichy 2019). Another useful test is the life-dead stain (eosin-nigrosin) as it is cheap and easy to do (Johnston et al 2001), whereas viability assessment by using PI/SYBR-14 requires special equipment. More insights into sperm functionality are provided by the hypoosmotic swelling test (HOST) which gives information about the adaptability to changes in osmolarity (Göricke-Pesch and Failing 2013). The zona binding assay is useful to examine the in-vitro fertilizing capability of sperm; however, it is not easy to do (Holst et al 2001). Measurement of the inner mitochondrial membrane potential (IMM) by using JC-1 requires a fluorescence microscope; however, as results correlate with kinematic parameters, it is not necessary for routine examinations (Volpe et al 2009). Morphology tests

are helpful as in most cases, good semen samples have more than 80% of morphologically normal cells; however, this may vary between breeds (Johnston et al 2001). Semen samples can be evaluated by using Hancock's solution containing formalin for immobilization of cells (Schäfer-Somi et al 2006); another useful tool is the Spermac stain (Minitube, Germany), allowing to evaluate the intactness of the acrosome, midpiece and tail separately (Göricke-Pesch and Failing 2013). However, fertility prognosis is largely dependent on the kind of morphological changes. Primary changes, developing during spermatogenesis as well as abnormalities of the midpiece and its attachment have a poorer prognosis, i.e. acrosome damages and head deformities, proximal cytoplasmic droplets, bent or coiled tails, and reflexed midpiece, may cause fertilizing problems. Cytological evaluation of a stained smear for presence and number of white blood cells (WBCs) may be helpful. Presence of 7 to >10 WBCs per High-Power-Field (light microscopy) was described to be associated with inflammation (Johnston et al 2001). In cases of infertility, analysis of DNA damages of spermatozoa may be helpful. Simple tests for the practitioner's lab are available like the toluidin blue assay. These assays are, however, very subjective as the stained samples are not easy to evaluate (Monachesi et al 2019). Finally, it is important to note that a thorough interpretation of results is not possible after collection of a single ejaculate; especially when the dog was not collected for a longer period of time, collections should be repeated which will in some cases improve the semen quality.

Semen conservation can be done by chilling or freezing. For chilling, manifold extenders were tried and variable results were obtained by using skimmed milk, non-fat dried milk, lecithin or egg-yolk containing extenders or commercially available extenders. Motility after cooled storage for 48 h varies between 41 and 99% (Igouer-ouada and Verstegen 2001, Witte et al 2009). Meanwhile, commercially available cooling extenders containing lecithin do not necessarily contain egg yolk; however, the latter may be added. As it is difficult to keep the samples cool for more than 48 hours and the cooling chain constant, antibiotics are contained in most extenders, and it is recommendable to ship within 48 hours. For semen chilling, the sperm rich fraction should be kept at room temperature and the cooling medium (room temperature) added drop wise at a ratio of 1+1 or 1+2. The diluted semen should be put into a glass with cold tap water and equilibrated in the fridge at +5°C for 1-2 hours. The semen can then be put into the transport box. Fertilizing results with cooled canine semen vary, dependant on semen quality, fertility of the bitch, insemination timing and skills of the vet; however, the results are on average comparable to those obtained with intravaginal application of fresh semen or natural breedings.

For semen freezing, abundance of protocols are available with different freezing extenders, cryoprotectants, freezing curves, one- or two-step protocols, warming rates, etc. (for review: Dorado and Ortiz 2025). From the authors point of view, the two-phase system with 1h of equilibration after addition of the cooling extender, and addition of the freezing extender briefly before freezing, is still advantageous. Freezing-thawing rates should be moderate. The method using a modified Uppsala extender (Linde-Forsberg (2001), was described by Schäfer-Somi et al (2006). Semen freezing can be done in a simple Styrofoam box; straws can be placed in the gaseous phase, at 3 cm over the surface of liquid nitrogen, for 10 minutes and then plunged into the liquid nitrogen. Pregnancy rates vary as with cooled canine semen; however, are on average 10-20% lower, in addition dependant on the timing and method of insemination. Frozen-thawed semen should be placed into the uterus at 2-3 days after ovulations (for review: Joonè 2024).

For semen transport, within the USA or Europe, semen quality can be maintained for 1-4 days with cooled semen, and in some cases for up to 2 weeks; however, this requires good semen quality and a good cooling chain. Even though egg yolk is still a valuable and frequently used cooling and freezing extender ingredient, its variable composition and some hygiene problems are concerns. Diluents without egg yolk but containing lecithin or low-density lipoproteins are valuable alternatives and commercially available (Kmenta et al 2011, Bencharif et al 2013, Dalmazzo et al 2019, Belala et al 2019). The chilled semen can easily be sent in a neopore box with ice packs keeping the temperature low for 48 hours. A styrofoam box or an ordinary thermos flask can also be used. Most important is that the temperature does not drop below 0°C (32.0°F) otherwise the semen may freeze (Linde-Forsberg et al 2001). For longer transports, use of frozen semen is recommended. Frozen semen should be transported in dry shippers. An appropriate size guaranties maintenance of semen quality f or up to 10 days. Each shipment must be accompanied by a hygiene certificate stating that both testicles were in the scrotum and the dog was healthy at the time of semen collection and free of any infectious disease; furthermore, by a proper semen quality report and a recommendation, how to thaw and inseminate the semen (Linde-Forsberg 2001). Important is to always include a hygiene certificate, a semen quality report and a thawing and insemination recommendation. The semen samples should be easy to identify; the species, the dog's name, chip number and the place and date of collection must be indicated on each straw or vial.

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Semen evaluation for technicians

Kara A. Kolster DVM DACT

Introduction

Thorough and accurate semen evaluation is vital to ensuring the best chance of successful breedings, semen shipments, and evaluating potential causes of infertility.

The main areas to consider in evaluation of canine semen quality are:

1. total motility
2. progressive motility
3. morphology
4. total sperm count

For detailed instructions on performing common procedures, such as semen collection, slide preparation for bright field microscopic motility and morphology evaluation, and use of a Neubauer hemacytometer, the reader is referred to previously published literature[1,2].

Motility

Total motility and progressive motility can be evaluated subjectively on wet mount by bright field microscopy. Individual sperm should be visible for evaluation, which may necessitate diluting the sample. The author recommends focusing on the non-motile sperm first, then expand your field of vision to include approximately 10 total sperm and assess how many are motile versus non-motile. Repeat this evaluation over multiple microscopic fields to determine the average total motility of the sample. Use a similar technique for evaluating progressive motility, focusing on sperm with forward velocity versus those that are spinning in circles, shivering in place, or being pushed around by other sperm instead of exhibiting their own motility. Progressive motility can be graded as a percentage or on a scale of 0-5, with 0 being no movement and 5 being fast forward progression[3].

Morphology

Spermatogenesis and spermiogenesis are the processes through which spermatogonial stem cells develop and mature into spermatozoa. The stages of spermatogenesis and spermiogenesis occur at very specific locations within the seminiferous tubule. Aberrations of the testicular environment at any of these stages can result in the production of abnormal spermatozoa. Accurate evaluation of sperm morphology requires appropriate stains, quality bright field, phase-contrast, or differential interference contrast (DIC) microscopy, and knowledge and experience in identifying sperm abnormalities. Canine semen morphology is commonly evaluated using an eosin-nigrosin stain or a modified Giemsa stain[8]. When using a quick-prep modified Giemsa stain (e.g. Diff-Quick), immersion at each step for 5 minutes is recommended[2]; this is significantly longer than manufacturer recommendations. Rapid drying of

the slide will minimize staining artifacts[1]. Phase-contrast microscopy uses light to produce a high contrast image of transparent cells, therefore eliminating the need for stain. A minimum of 200 sperm cells should be counted, and percentage of normal and abnormal sperm calculated[2]. There are several systems for categorizing sperm abnormalities. In the most commonly used classification system, abnormalities are classified as affecting the head, midpiece, or tail of the sperm.

Total sperm count

The total number of sperm in an ejaculate is related to the dog's body size & testicular volume, and the success of the collection procedure. Dogs will typically ejaculate more sperm in the presence of an estrus teaser. Dogs that are not trained or comfortable with the collection procedure, are nervous in the collection environment, or for various other reasons are not exhibiting normal reproductive behavior may not ejaculate their full potential number of sperm. Total sperm count must be differentiated from total volume; the total volume of the ejaculate is highly dependent on the amount of prostatic fluid, or third fraction, of ejaculate collected. The second, sperm-rich, fraction is the most important sample for evaluation. Semen concentration can be determined using a hemocytometer, a CASA system, other photometer or densimeter, or fluorescent markers. Concentration per milliliter is multiplied by ejaculate volume to obtain the total sperm count. Correct dilution in any methodology is vital to obtain an accurate result. This can be done using calibrated laboratory pipettes, a commercial leukocyte dilution system, or diluents appropriate to a specific CASA system. CASA systems exhibit lower variability than human-derived, e.g. hemocytometer, measurements of semen concentration[5,6].

CASA systems

Computer-assisted sperm analysis (CASA) systems can be used for semen evaluation in place of manual analysis by a human evaluator. They are most useful for motility and concentration analysis, and accuracy varies based on methodology of the specific system. CASA systems are automated systems combining computer and microscope hardware and software to provide objective analysis of semen parameters. Many CASA systems are either photometers or densimeters. Photometers (eg. CellSoft, Hamilton-Thorne, SpermVision) function by obtaining multiple digital images of a field of sperm cells in rapid succession, identifying individual sperm cells, and tracking those sperm across frames to assess motility and concentration[4]. Sperma-Q is a photometer that measures concentration but not motility of a semen sample. Densimeters (eg. ARS Densimeter) are instruments that measure semen concentration, but not motility, based on transmittance of light through the sample as compared to a standard buffer. All photometers and densimeters are calibrated to a certain concentration range and to the parameters by which they identify a sperm cell; therefore samples may need to be diluted for accurate measurement and the instrument must be calibrated for the specific species being examined. Certain components of semen extenders, such as egg yolk particles, other cell types in an ejaculate, such as red or white blood cells, and debris may alter the accuracy of photometer and densimeter evaluations. The Nucleocounter SP-100 identifies sperm cells by fluorescent labeling and excludes background debris from measurement[4]. For this reason, it is useful for measuring sperm concentration in semen extended with egg yolk.

Some CASA systems have the ability to evaluate morphology; however this is generally limited to analysis of the sperm head and is highly dependent on the software settings of a particular instrument[9].

Additional testing

Live/dead ratio is evaluated using bright field microscopy and a vital stain, such as eosin-nigrosin. The intact sperm membrane is not permeable to certain stains. Cells that exclude stain are considered live. Cells that take up the stain have a permeable membrane and are likely dead. The Nucleocounter SP-100 can also evaluate sperm membrane integrity through its fluorescent labeling.

Sperm membrane integrity can be evaluated by the hypo-osmotic swelling test (HOST). When exposed to a hypo-osmotic environment, sperm cells with intact membranes will allow fluid to enter the cell to balance the osmotic gradient. The influx of fluid will cause the sperm cell to swell. This is most evident as curling of the tail, which can be seen on wet mount under bright field microscopy. HOST results in fresh semen samples are positively correlated with progressive motility and normal morphology[7].

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Chilled and frozen semen handling for technicians

Kara A. Kolster DVM DACT

Canine semen is commonly chilled or frozen for transport to breed females in geographically distant locations and, in the case of frozen semen, for long-term storage and use after the male is no longer fertile or deceased.

Chilled semen shipment

1. Evaluation
    a. Semen should always be thoroughly evaluated with motility, morphology, and total count prior to chilled shipment. Include the semen evaluation form with the shipment. If the semen quality is questionable, it is advised to contact the bitch owner and/or their veterinarian before shipping the sample.
2. Centrifugation and extension
    a. Centrifuge semen sample at 400g to 900g for 5 to 10 minutes[1]. Remove the supernatant, leaving 10% of its volume. Resuspend the pellet in extender to an ideal concentration of 200 million sperm per mL[2].
    b. Sperm samples should always be handled gently to minimize damage to the cells. Add extender slowly and mix gently, avoiding creating air bubbles. Always use extender that is warmed to the same temperature as the semen sample before combining them.
    c. Label samples with a minimum of stud & owner names, breed, and date collected. Many reproductive clinics are busy and receive multiple shipments daily. Lack of proper labeling increases the chances of inseminating a bitch with the wrong semen sample.
    d. The tube should be sealed with Parafilm to prevent leakage and put in a zipper-close bag or Whirl-pac to contain any leaks that may occur. Covering the tube cap with standard tape does not prevent leakage.
3. Packaging & shipping
    a. Canine semen is shipped in a Styrofoam box with a frozen ice pack, all contained within an outer cardboard box (eg. Canine Express, Rover, MiniTube, Puppy Pak, or similar). There must be an adequate layer of insulation between the semen sample and the ice pack. Inadequate insulation can result in over-cooling or freezing of the semen, thus destroying its viability.
    b. Shipping is by overnight carrier because the semen is most viable for pregnancy when inseminated within 24 hours of collection. Make sure to note the tracking number on the shipment so its progress can be monitored, especially if delivery is delayed for any reason.
    c. Chilled shipping boxes are meant to be disposable but are commonly reused. A box that is damaged or does not close/seal completely should not be used as this compromises the cooling properties of the box and risks damaging the semen sample.

Frozen semen shipment

1. Dewar preparation
    a. A dry shipper or cryoshipper is a specialized container for shipping frozen semen samples. The shipper is “charged” with liquid nitrogen, which is absorbed into porous material within the tank. Nitrogen vapor is slowly released from to maintain freezing temperatures (-150C) inside the tank. When the tank is opened, a “puff” of nitrogen vapor is released, indicating that the tank is charged.
    b. Thermo-protective gloves and protective eyewear should always be used when working with liquid nitrogen. Make sure you are in an area with good ventilation.
    c. The dry shipper is filled with liquid nitrogen until it reaches the full weight specified by the manufacturer, or until no more liquid is being absorbed. Liquid nitrogen will initially bubble when poured into the dry shipper. When the bubbling stops, that is an indication that no more liquid is being absorbed. The author prefers to use both weight and visible liquid remaining in the canister as indications that the tank is fully charged. The tank is then allowed to sit for 12-24 hours; at that time if there is still visible liquid in the canister and weight is still appropriate, you can pour off the excess liquid and use load the tank with semen for shipping.
    d. The foam plug is always placed in the neck of the shipper to slow the rate of nitrogen release. Line up the groove on the plug with the canister handle before inserting the plug. Failure to do so can damage the plug and increase the risk of shipper failure.
    e. The dry shipper is always placed inside a hard-sided protective shipping container. Many of these outer containers are mushroom-shaped to ensure that the tank remains upright. Turning the tank on its side or upside down increases the rate of nitrogen release and risk of tank failure.
2. Semen handling
    a. When semen is removed from the storage tank and transferred to a shipping dewar, it moves from liquid nitrogen to vapor in the neck of the storage tank, room air, and back into vapor in the shipping dewar. Increased length of time at higher temperatures (eg room air) is correlated to sperm damage so this transfer must be done as quickly as possible[3]. Semen straws and vials should only be handled with pre-cooled forceps, not hands, to avoid accidental warming of the semen. If semen needs to be transferred between canes or otherwise examined, fill a secondary container such as a Styrofoam box with liquid nitrogen, transfer the cane to that box and perform any manipulations in the nitrogen to avoid warming the semen.
    b. Send identifying information, semen analysis, thaw instructions, and specific thaw media if indicated, with the shipment.
3. Shipping
    a. Frozen semen shipping is typically by overnight carrier to minimize the time that the semen is in transport and at risk of damage. Make sure to note the tracking number on the shipment so its progress can be monitored, especially if delivery is delayed for any reason. When the tank arrives at its destination, open immediately to check for the vapor puff indicating the tank is still charged, and transfer semen to a liquid nitrogen storage tank as soon as possible. When the

tank returns to its origin, the author recommends monitoring the tank daily until the nitrogen is fully evaporated. This has helped identify tanks that may have been damaged during shipment as they evaporate more quickly than normal. Any suspicious tank should be re-tested by fully charging and monitoring until evaporated; if the tank is not holding its charge for an appropriate time period it should be replaced.

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Speaker Proceedings

Title: *The Art of Client Communication for Support Staff*
Presenter: Samantha Snyder, BS

Overview:

Effective communication with clients is one of the most essential but often most overlooked skills for veterinary support staff. Nowhere is this more critical than in reproductive medicine, where timing, emotion, and expectations run high. This session will explore the foundational elements of excellent client communication, with an emphasis on empathy, professionalism, and practical tools that empower both staff and clients.

Presentation Outline:

1. Understanding the Client Point of View
Reproductive clients are often operating from a place of high emotional and financial investment. Whether they are hobby breeders or professionals, their success depends on precise timing and variables beyond anyone's control. As support staff, we are often their primary point of contact and the first to receive their stress. This talk begins with reframing client behavior through a lens of empathy and understanding.

2. Balancing Empathy with Professionalism
While empathy is crucial, it must be balanced with professionalism. Technicians must learn to validate client emotions while also communicating medical facts, timelines, and expectations clearly and confidently.

3. Mastering Your Message: Know What You're Communicating
Clear communication starts with internal clarity. Techs must feel confident in the information they're delivering: whether it's timing instructions, medication protocols, or semen storage guidelines. This portion emphasizes the importance of double-checking plans, asking clarifying questions internally, and preparing before speaking with clients.

4. Consistency Matters: Being in Sync with Your Veterinarian
Misaligned messaging between veterinarians and staff creates confusion and erodes client confidence. Here we discuss how to establish communication preferences with your vet, understand their medical reasoning and language, and mirror those approaches in client-facing conversations.

5. Communicating Clearly in Stressful Moments
Clients in reproductive programs are often overwhelmed. This section focuses on delivering clear, concise, and actionable instructions, especially around high-stakes timelines like ovulation, AI timing, or whelping. Techniques like teach-back, simplified language, and written instructions will be reviewed.

6. Timely Replies: Building Trust Through Responsiveness
Often, it's not the *content* of communication but the *timing* that matters most. Even a simple acknowledgment can make clients feel heard and reduce their anxiety. Attendees will explore low-effort, high-impact ways to maintain trust through timely follow-up, even amidst a busy clinical schedule.

7. Tools That Support Communication
Templates, handouts, and checklists can improve both clarity and efficiency. We'll look at examples of practical client tools—such as whelping checklists, hormone testing schedules, and discharge summaries—that reinforce verbal communication and reduce callback burden. These tools also ensure consistency across team members and improve the client experience.

Speaker Proceedings

Title: *The Dream Team: How to Be an Asset to Your Repro Vet*
Presenter: Samantha Snyder, BS

Overview:

Veterinary reproductive work is precise, time-sensitive, and deeply client-centered. While most focus is often placed on veterinarian performance or client education, the truth is: the technician-veterinarian dynamic is the heartbeat of any successful practice. A strong support team can streamline operations, improve client satisfaction, and allow the veterinarian to operate at their highest level.

This session explores what it truly means to be an asset to your repro vet, not just through skill and knowledge, but through communication, integrity, anticipation, and self-sufficiency. We'll cover practical strategies for building mutual trust, syncing communication styles, and becoming a professional extension of your veterinarian in both clinical work and client interactions.

Presentation Outline:

1. What Makes a "Dream Team"?
This session begins with defining what a "dream team" means in a high-pressure, emotionally charged field like veterinary reproduction. Technicians and veterinarians must function not in hierarchy, but in partnership. Building this kind of relationship requires more than just competence, it takes clear communication, emotional intelligence, and daily intention.

2. Communication with Your Vet: Clear, Concise, Consistent
Just as client communication must be clear and compassionate, internal communication between support staff and veterinarians must be efficient and precise. This section addresses how to structure updates, flag concerns, and streamline messaging throughout the day: verbally, via EMR, or through team chat systems. The goal: reduce noise and increase clarity.

3. Gaining Trust Through Communication and Honesty
Trust is the bedrock of a successful vet/tech relationship. It starts with transparency being honest about what you know, what you don't, and what needs clarification. Techs build trust not by pretending to know everything, but by showing that they know when to speak up, when to ask, and how to follow through.

4. Setting Each Other Up for Success: The Morning Matters
A productive day begins with a smooth start. This portion will offer tips for how technicians can prepare the practice, the vet, and the schedule to ensure the day flows efficiently. From pre-loading patient notes to prepping tools and anticipating supply needs, a proactive start sets the tone for teamwork.

5. Anticipating Needs: Thinking One Step Ahead
Anticipation is a learned skill. With time, vet techs can learn to recognize patterns in their veterinarian's approach and begin preparing before they are asked—whether that means grabbing the right medication, setting up for a TCI, or having consent forms ready. This section focuses on proactive behavior that reduces stress for the entire team.

6. Being an Extension of Your Vet: Earning Client Trust by Proxy
When clients feel confident talking to a tech, they feel more confident in the practice overall. Techs should strive to mirror their veterinarian's tone, messaging, and priorities so clients feel like they're hearing one unified voice. This leads to smoother interactions, fewer escalations, and more trust in the practice as a whole.

7. Self-Sufficiency and Proactive Problem Solving
A top-tier tech doesn't just wait for direction—they look ahead and manage tasks independently where appropriate. This section emphasizes judgment: knowing when to act and when to escalate. It also covers how to present potential solutions rather than just problems, saving your vet time and building your credibility.

8. Professionalism with Personality: Integrity Always Comes First
While it's natural to form close bonds with your veterinarian, professionalism must always guide your behavior—especially in front of clients. The session concludes by underscoring the importance of maintaining boundaries, modeling professionalism, and representing the practice with integrity at all times. You can have fun and enjoy your job—just never lose sight of who's watching and who you represent.

2025 Annual Therio Conference ACT and SFT Student Abstracts

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Comparing neonatal puppy growth between overweight and lean bitches

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Lactation is the final pathway for maternal metabolism to influence the neonate. In women, metabolic hormones positively correlate with maternal body mass index and these hormones pass through breastmilk. As a result, they affected infant weight gain through the first 6 months of life.[1] Although studies have examined the effect of many variables (i.e. breed, sex, etc.) on neonatal puppy growth rates,[2] body condition of the dam has never been examined. We hypothesized that puppies born and nursed by overweight bitches have increased growth rates compared to those from lean dogs. The aim was to compare growth rates of puppies of lean and overweight bitches during the neonatal period. Fourteen litters from 13 healthy medium to large breed client-owned dogs were enrolled after whelping. Dogs were classified based on initial postpartum body condition into lean (LE, body condition score (BCS): 4-5/9, n = 7) and overweight (OW, BCS: 6-7/9, n = 7) groups. A total of 106 puppies were born to LE (n = 58) and OW (n = 48) bitches. Birth weights and daily weights of pups in the litter were recorded by the owners until 21 days of age before nutritional weaning began. Puppies were solely nursed by the dams without supplemental feeding. Birth weight, daily body weights, average daily weight gain (ADG, %), and average weight gain since birth (AGB, %) of the puppies were analyzed using mixed model ANOVA with significance at $p < 0.05$. Puppy birth weights were not affected by maternal BCS group or litter size. Puppy growth curves based on daily weight changes were significantly different between the two maternal groups, despite pup weights not being different on any given day between OW and LE bitches. ADG on days 1, 2 and 4 was 2.4%, 6.3% and 3.5% higher, respectively, in pups of OW dams compared to LE, whereas pups of LE dams gained 2.2% more on day 13. AGB increased significantly over time but was not affected by maternal BCS group. Larger litter size accounted for significantly lower AGB. Pups with higher birth weights had generally higher weights throughout the neonatal period, although they were gaining (ADG, AGB) at a slightly slower rate. In conclusion, despite the similar neonatal body weights between maternal groups, puppies from OW dams grew differently in the first 21 days compared to those from LE bitches. Litter size or birth weight are also significant determinants of pup weight and growth. Milk composition may explain some of the differences observed in growth pattern between puppies from OW and LE dogs and is currently being investigated.

Keywords: Dog, growth rate, average daily gain, puppy, neonate, body condition

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Uterine transcriptome during parturition in the dog

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The molecular basis of uterine events leading up to and through the parturition cascade have not yet been fully unraveled in dogs. We hypothesized that global gene expression analysis will pinpoint crucial biological functions (e.g. inflammatory and immune response, steroid hormone-mediated and contractility-associated processes) that will elucidate how uterus prepares for and progresses through labor. Aim of this study was, therefore, to perform RNA sequencing (RNAseq) on canine uterine samples to determine differentially expressed genes and functional pathways from term prepartum through second stage labor. Full-thickness uterine biopsies were collected during cesarean surgery from female dogs divided into 3 groups based on serum progesterone concentrations (P4) and clinical presentation: planned cesarean surgery (PCS) at term pregnancy without first stage labor signs (n = 7, P4 ≥ 3.4 ng/ml); elective cesarean surgery (ECS) at term pregnancy after temperature drop and/or first stage labor signs (n = 6, P4 <1.5 ng/ml); obstructive dystocia (OD) at second stage of labor presenting with strong spontaneous abdominal contractions (n = 5). RNA isolation was performed as described,[1] followed by DNase treatment and RNA purification (RNA clean & concentrator, Zymo Research). RNA integrity (RIN) was assessed with a 2100 Bioanalyzer Instrument and RNA 6000 Pico Kit (Agilent). Samples included in the study had RIN between 7.4-9.7. Differential transcript abundance for contrasts PCS versus OD, PCS versus ECS, and ECS versus OD were determined by employing the quasi-likelihood negative binomial generalized log-linear model from the R package 'edgeR'[2] and the Wald test from the R package 'DESeq2'.[3] False Discovery Rate (FDR)[4] threshold was set < 0.05 in both tests for a gene to be differentially expressed (DEG). There were 4 and 5 genes with transcripts exclusively detected in OD and ECS samples, respectively. A total of 541 DEGs were identified for PCS versus ECS, 3443 DEGs for PCS versus OD, and 10 DEGs for ECS versus OD. After filtering gene ontology terms by FDR < 0.1 and at least 6 genes per category, preliminary analysis for PCS versus ECS contrast highlighted changes in biological and cellular processes such as angiogenesis, positive regulation of cell migration and cell population proliferation, positive regulation of phosphatidylinositol 3-kinase/protein kinase B signal transduction, cellular response to hypoxia, and negative regulation of apoptotic process. In the PCS versus OD contrast, there were DEGs involved in functions that were overlapping with the PCS versus ECS contrast, and additionally, other processes such as actin cytoskeleton organization, G protein-coupled receptor signaling, inflammatory response and immune system process, and carbohydrate transport that appeared to

be more characteristic of the actively contracting uterus during second stage labor. These preliminary results provided insights into a broad range of biological and molecular processes that regulate uterine function during the parturition process in the dog.

Keywords: Pregnancy, parturition, uterus, dog, gene expression

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Unlocking the future of equine fetal sexing: mass spectrometry analysis of maternal conjugated estrogens in serum

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Since 1976, estrogen concentrations in pregnant mares have been thought to be unrelated to fetal sex, but these studies were based on immunoassays. Despite their widespread use, these assays have limited specificity due to cross-reactivity and are rarely validated in equine, compromising their accuracy. Liquid chromatography tandem mass spectrometry (LC-MS/MS) provides precise steroid quantification, allowing deeper insights into steroid metabolism and potential fetal gender differences. The current standard for fetal sexing, transrectal ultrasonography, requires technical expertise, has a limited gestational window, and may yield inconsistent results. This study hypothesizes influence of fetal sex on maternal sulfonated estrogen levels and aims to develop a non-invasive method for fetal gender determination in mares. From 2020-2024, 68 mares from Belgian stud farms, managed under standardized conditions regarding diet, housing, and reproductive practices, were included, resulting in 115 pregnancies. Blood samples (n = 596, median per gestation: 5 (Q1–Q3: 4–6)) were collected from 4-11 months to quantify estrone-sulfate (E1S) and estradiol-sulfate (E2S) using a validated LC-MS/MS method for equine. Mares with placentitis were excluded. Statistical analyses (SAS 9.4, $p < 0.05$) used logistic regression to assess fetal sex effects, accounting for maternal age, breed, and parity, and estimate associations between fetal sex and maternal hormone concentrations. Most mares were Warmbloods (45.6%) or Spanish purebred horses (44.1%). Male foals accounted for 51.7% of the births, with a sex ratio of 1:1.07. Parity and breed did not affect the sex ratio, while age tended to be significant ($p = 0.06$). Estrone and estradiol-sulfate concentration followed a quadratic trajectory ($p = 0.0003$), peaking at 5 months for females and 6 months for males. Fetal gender influenced hormone concentrations for E2S ($p < 0.0001$) and E1S ($p = 0.012$). Males exhibited higher E2S from 169-308 days, with the most significant differences at 169-196 days ($p = 0.0003$), 197-224 days ($p = 0.0019$), and 225-252 days ($p = 0.0031$). Females had higher E2S at 113–140 days ($p = 0.032$). These results contrast with previous reports, where no fetal sex-related differences in maternal estrogen concentrations were observed. Fetal gonads secrete androgens that drive placental estrogen production, and their bioavailability influences maternal estrogen concentrations. At 5 months, female fetuses reach peak E2S concentration, exhibiting higher concentrations than males. Around six months, male fetuses surpass females in E2S concentrations as they reach their peak. This shift is due to differential steroidogenic activity. Although male fetal gonads contain fewer interstitial cells at this stage, their enzymatic machinery is more developed, leading to greater androgen production. These are aromatized in the placenta into estradiol by cytochrome P450 19A1, whose transcript

expression also peaks around 6 months, before placental sulfotransferases responsible for estrogen sulfonation increase E2S excretion in maternal circulation, particularly with male fetuses. These findings suggest maternal E2S concentrations differ by fetal sex, indicating LC-MS/MS could serve as a reliable, non-invasive alternative to ultrasound for fetal monitoring. Expanding the cohort earlier in pregnancy could improve predictive accuracy and enable fetal sex determination as early as ultrasound, optimizing breeding management.

Keywords: Mare, pregnancy, sulfonated estrogens, fetal sex, liquid chromatography tandem mass spectrometry

Comparison of the effect of motility stimulants on frozen-thawed semen in stallions

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Intracytoplasmic sperm injection (ICSI) can be used to produce equine embryos from low quality sperm. However, some sperm are of such low quality that identifying a motile sperm for injection can be difficult. Pentoxifylline (PTX) has a positive effect on motility of frozen-thawed sperm,[1] and the combination of penicillamine, hypotaurine, and epinephrine (PHE) has supported longevity of motility in sperm preincubated for IVF.[2] This study was performed to compare the effects of PTX and PHE on total and progressive motility of both good-quality semen (1 x frozen) and in a model of poor-quality semen (semen frozen and thawed 4 times, 4x frozen). Our aim was to identify a method to stimulate sperm motility for ease of selection during ICSI. Semen from each of 3 stallions was frozen in MFR5 commercial extender (E-Z Freezin – MFR5; Animal Reproduction Systems, Ontario CA, USA). A subset of the straws from each ejaculate were thawed and refrozen 3 additional times to produce 4x frozen straws. For each replicate, 1x and 4x straws were thawed and diluted with a modified Hank's balanced salt solution containing the following treatments 1. no additives (Control); 2. with 2 mg/ml PTX; 3. with 4 mg/ml PTX; 4. with 1x PHE (9 mM hypotaurine, 18 mM penicillamine, and 1.8 mM epinephrine, the concentrations used for IVF[2]); or 5. with 2x PHE (twice the concentration as for 1x PHE). After centrifugation, the pellet was resuspended in the corresponding medium to 50 x 10^6 sperm/ml. A sample was assessed by computer assisted sperm analysis to determine total motility (TMOT) and progressive motility (PMOT) immediately after resuspension (T0) and after holding at 38ºC in air for 30 minutes, 1 hour and 2 hours. Data were analyzed using the Kruskal-Wallis test with pairwise Wilcoxon tests, with a significance of $p < 0.05$. Initial TMOT and PMOT for 1 x semen were 28.0 ± 4.9 and 17.2 ± 3.3 (mean ± SEM), respectively; these values for 4x semen were 1.9 ± 0.5 and 1.1 ± 0.3, respectively. Treatment with PHE at either concentration had no significant effect on TMOT or PMOT at any time in either 1x or 4x semen. In contrast, for 1x semen, treatment with PTX2 and PTX4 significantly increased PMOT at T0 and T30. For 4x semen, treatment with PTX2 and PTX4 significantly increased both TMOT and PMOT at essentially all time points; for example, at T30, PMOT for 4x semen in the Control and PTX4 treatments were 0.18 ± 0.1 and 2.6 ± 0.5, respectively ($p < 0.0001$). We conclude that PTX at either of the tested doses increased TMOT and PMOT in poor quality frozen-thawed semen and may have application during selection of sperm from poor quality semen for ICSI. In contrast, PHE did not have an effect on sperm TMOT or PMOT under the tested conditions.

Keywords: Sperm motility, motility stimulants, low quality semen, pentoxifylline, PHE

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Effects of firocoxib on oocyte quality in mares undergoing repeated transvaginal ultrasound-guided follicular aspiration procedures

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Advanced reproductive techniques, such as transvaginal ultrasound-guided follicular aspiration (TVA) and intracytoplasmic sperm injection (ICSI), have seen remarkable growth in the equine industry. Many donor mares in TVA-ICSI programs are older and managed with nonsteroidal antiinflammatory drugs (NSAIDs) for chronic musculoskeletal conditions. A recent study identified a transient decline in oocyte quality and embryo development following TVA-ICSI of mares treated with oral phenylbutazone (4.4 mg/kg) once daily for 10 days. We hypothesized that using a COX 2 specific inhibitor (firocoxib) maintains or improves oocyte developmental competence in mares undergoing TVAs. Four mares (8.7 ± 2.1 years) underwent 5 TVAs at 14-day intervals. Oral firocoxib (0.1 mg/kg) was given once daily for 10 days before the fourth TVA. Collected cumulus oocyte complexes (COCs) were transported in a holding medium (EmCare, ICPbio), matured in vitro (38.2ºC, 6.7% CO_2, 5% O_2), fertilized by ICSI using frozen-thawed semen from a single proven stallion, and cultured (38.2ºC, 5.1% CO_2, 5% O_2) until blastocyst formation. Liquid nitrogen frozen follicular fluid and plasma were submitted for quantification of pronflammatory chemokines. Cumulus cell expansion rates, oocyte maturation, cleavage and blastocyst development were compared by chi-square tests. Comparisons for chemokine concentrations were completed using repeated-measures ANOVA or mixed effect analysis (significance $p < 0.05$). COC expansion rates were significantly higher after the fifth TVA (~ 2 weeks after firocoxib) than in previous TVAs. Although oocyte maturation, embryo cleavage and blastocyst rates did not reach statistical significance, they were numerically highest following firocoxib treatment (TVAs 4 and 5). No significant differences in chemokine concentrations in follicular fluid or plasma were observed. Ongoing analyses will evaluate gene expression of proinflammatory cytokines and enzymes in granulosa cells and firocoxib concentrations in plasma/follicular fluid. These preliminary findings suggested that firocoxib treatment did not negatively impact oocyte quality in mares undergoing TVA-ICSI.

Keywords: Mare, firocoxib, oocyte, maturation, TVA

Analytical validation of different diagnostic tests for the detection of leukocytes in canine semen

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Leukospermia in dogs is defined as an abnormal concentrations of white blood cells (WBC) in the ejaculate (> 2,000 WBC/µl)[1]. Occasionally, disorders associated with the prostate such as prostatitis are responsible for this finding.[2,3] The most frequently used tool to identify WBC in semen is cytology. However, the morphological differentiation between germ cells and WCB is often challenging. Objectives of this study were to validate 5 diagnostic tests for leukospermia (hemacytometer, leukocyte esterase dipstick test, peroxidase stain, semen cytology, and immunostaining CD45+) and to evaluate the effect of varying concentrations of WBC on sperm motility parameters. We hypothesized that all diagnostic tests have a diagnostic value and that > 2.5 x 10^6 WBCs/ml affects motility parameters. A total of 9 semen samples from 7 healthy sexually mature adult dogs were analyzed. Leukocytes were purified from autologous blood samples by density centrifugation. Leukospermia was induced in aliquots of 50 x 10^6 purified sperm in different concentrations: negative control (NEG = no WBC, only sperm), positive control (POS = no sperm, only WBC), treatment 1% (T1% = 0.5 × 10^6 WBCs/ml), treatment 5% (T5% = 2.5 × 10^6 WBCs/ml), and treatment 15% (T15% = 7.5 × 10^6 WBCs/ml). Total and progressive motility were evaluated 0, 24, and 48 hours after induction of leukospermia at 37ºC. Five diagnostic tests were used to quantify concentrations of WBC in each sample: hemacytometer, leukocyte esterase dipstick test, peroxidase test, semen cytology, and immunostaining CD45+. The median number of cells counted in the hemacytometer, peroxidase test, and cytology was significantly higher in T5 and T15% than in NEG, whereas the CD45 immunolabeling was higher in T15% than in the NEG group (Kruskal-Wallis test with a Dunn's post-hoc test; $p < 0.05$). Concentrations of WBC detected with the leukocyte esterase dipstick test was different among treatments (Chi square test; $p < 0.05$). Both 5 and 15% of WBC in the ejaculate lowered sperm total and progressive motility at time 0. By 24 hours, total and progressive motility were only different between control and T15%, but by 48 hours, all treatment groups had lower total and progressive motility than the control group ($p < 0.05$). Thus, samples in T5 and T15% were considered positive for leukospermia. Peroxidase test had the best analytical sensitivity (96.3%) followed by cytology (92.6%). Peroxidase test and leukocyte esterase dipstick had the lowest analytical specificity (88.9%). Cytology had the highest positive predictive value and positive likelihood ratio (96.2% and 16.67, respectively) and the peroxidase test had the highest negative predictive value and the lower negative likelihood ratio (94.1% and 0.04, respectively). As hypothesized samples with ≥ 2.5 x 10^6 WBCs/ml had impaired motility parameters at times 0, 24, and 48 hours after induction

of leukospermia. Both cytology and peroxidase test were considered the ideal methods to diagnose induced leukospermia in purified semen samples in the dog.

Keywords: Dog, semen, round cells, leucocytes, leukospermia

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Effect of supplementation of donor mares with altrenogest on embryo recovery and size

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Embryo transfer (ET) enhances genetic progress in sports horses and endangered breeds, yet the embryo recovery rate from donor mares remains as a substantial bottleneck. Given progesteroneìs critical role in embryonic support, this study hypothesized that altrenogest supplementation in fertile donor mares improves embryo recovery rates and size and optimizes the uterine environment. Objective was to evaluate the effect of altrenogest supplementation to donor mares on embryo recovery and size, corpus luteum size, and uterine environment. Five fertile donor mares were inseminated with fresh semen from the same fertile stallion over 2 estrous cycles using a crossover design. Control (CON) mares did not receive any exogenous hormones, whereas altrenogest (ALT) treatment mares received oral altrenogest supplementation (0.044 mg/kg daily between days 3-7 after ovulation). Embryo recovered on day 8 after ovulation via uterine lavage; embryo morphology and size, number and diameter of the corpus luteum (transrectal ultrasonography), and endometrial inflammatory cell count (endometrial cytology from the recovered fluid) were evaluated. Embryo recovery rate per cycle was 100% in ALT treatment and 83.3% in CON treatment ($p > 0.05$; Chi-square test). When calculated per ovulation, more embryos were recovered from ALT (100%) than CON (56%) treatments ($p = 0.042$; Chi-square test). Embryos from ALT treatments had larger median diameter (1135 ∂m; IQR 670-1140 ∂m) than embryos recovered from CON treatments (670 ∂m; IQR 560-840∂m) ($p = 0.134$; Kruskal Wallis test). Although not statistically different, this increase represented the recovery of ~ 2 additional embryos per every 10 donor mares enrolled in an ET program when altrenogest was used. There was no difference in corpus luteum size (CON 26.8 ≤ 1.49 mm, ALT 28.98 ≤ 2.01 mm) (mean ≤ SD). No inflammatory cells were observed in the cytology of the fluid recovered from the uterus after embryo flush. In summary, embryo recovery rate increased by 20% per flush and 40% per ovulation in ALT-treated mares compared to controls, embryos were larger in ALT treatments. This study presented a novel application of altrenogest in mares, highlighting its potential for improving embryo recovery rates and size.

Keywords: Horse, embryo transfer, altrenogest, uterine environment

Transcriptomic profile of single immature and in vitro matured equine oocytes after holding

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Previous studies have observed a progression in chromatin condensation during holding of equine cumulus-oocyte-complexes (COCs) at room temperature. However, the impact of holding on the transcriptome of these gametes has yet to be determined. This study aimed to characterize the effects of holding COCs at a controlled room temperature (22°C) on the transcriptomic profile of immature and in vitro matured (IVM) oocytes. We hypothesized that holding COCs is associated with progression into transcriptional silencing. A total of 125 compact COCs were collected from 37 mares through transvaginal aspirations of follicles ≤ 25 mm in diameter. Only COCs with a minimum of 3 layers of cumulus cells were selected and allotted into 4 groups: 1- CT (n = 20), processed at collection; 2- H (n = 30), held in commercial embryo holding media (ABT Holding, ABT 360) at 22°C for 24 hours; 3- IMM-IVM (n = 40), placed immediately into IVM for 30 hours; and 4- H-IVM (n = 35), placed into IVM for 30 hours after holding for 24 hours. COCs were denuded at various time points and stored in liquid nitrogen. Single oocyte RNA extractions, concentration, and quality assessments were performed, leading to 33 samples (CT: n = 9; H: n = 8; IMM-IVM: n = 8 metaphase II; H-IVM: n = 8 metaphase II) being selected for library preparation and sequencing (150 pb-PE). Reads were trimmed, mapped (equine reference genome, ECab 3.0), and quantified using featureCount, with similar read counts observed among oocytes. A total of 13,263 genes were analyzed. As expected, only 2 differentially expressed genes (DEGs) were observed between CT and H oocytes, being upregulated, whereas the number of transcripts was not different among groups for the reminder of genes. Moreover, there were only 24 DEGs in mature oocytes (IMM-IVM and H-IVM) and all were upregulated. Gene ontology analysis indicated an increase in protein ubiquitination during holding that has also been described as a component of chromatin condensation pathways. For mature oocytes, DEGs were associated with processes such as chromatin and chromosome organization, microtubule polymerization, regulation of transcription, translation, and protein assembly. These results suggested that a possible progression into transcriptional silencing occurs once equine COCs are removed from the follicular environment and placed in holding media, with minimal transcriptional activity accounting for chromatin changes observed in studies.

Keywords: Oocyte, holding, transcriptome, horse

Hock umbilical cord entanglement in an Angus calf

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A 6-year multiparous Black Angus cow was presented for dystocia. Vaginal examination revealed a deceased fetus in anterior longitudinal presentation, dorsosacral position with bilateral shoulder flexion. Fetid fluid was detected in the vaginal vault. A caudal epidural anesthetic was administered, and the fetal malposition was promptly corrected. However, extraction of the calf remained unsuccessful. Upon further evaluation, one of the fetus's hind limbs was entrapped within the birth canal due to the umbilical cord being tightly wrapped around the hock. A blind resection of the umbilical cord was performed, allowing the limb to be freed and the fetus delivered. The heifer calf was fully developed but showed distal limb swelling beyond the site of entrapment, along with a distinct indentation where the umbilical cord had constricted the limb. Additionally, an omphalocele was observed. Postpartum vaginal examination revealed the presence of a second fetus, which was delivered successfully and found to be a healthy heifer. Umbilical cord abnormalities are well-documented causes of abortion and stillbirth in equines. An excessive umbilical cord length (> 85 cm) can predispose to umbilical cord torsion in horses. However, reports of cord entanglement around fetal limbs in equine gestation are rare, with only one documented case.[1] In human obstetrics, umbilical cord entanglement is relatively common, often occurring around the neck (nuchal cord), and is not associated with the length of the cord.[2] A case of fetal demise due to the nuchal cord was reported in a camel, leading to abortion.[3] In cattle, the umbilical cord is relatively shorter than in equines, with an average length of approximately 28 cm, making such abnormalities very rare. To our knowledge, this is the first reported case of umbilical cord entrapment leading to dystocia in cattle. This condition should be considered a potential fetal factor contributing to malposition during dystocia management.

Keywords: Bovine, obstetrics, dystocia, umbilical cord abnormalities

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Management of unilaterally fixed twins in a multiparous American Quarter Horse mare

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A multiparous 13-year American Quarter Horse mare was presented for reproductive evaluation 13 days after ovulation and breeding via artificial insemination. Transrectal ultrasonography revealed 2 embryos, positioned in the uterine body and uterine horn, respectively. Sedated manipulation and crushing of 1 embryo with the ultrasound probe were unsuccessful. On days 14-15 after ovulation, 4 additional examinations revealed close apposition of identically sized embryos that prohibited embryo reduction. During next 90 days, unilaterally fixed twins grew at similar rates and maintained heartbeats. On day 111 of pregnancy, fetal intracardiac injection of 1 twin was successfully performed at a referral hospital. Serial transabdominal ultrasonographic examinations in the following months revealed 1 viable fetus that was born without complications on day 348 of pregnancy. Placental evaluation revealed a mummified fetus invaginated within the live twin's placenta. Natural reduction of 1 twin occurs in 89% of unilaterally fixed twins with 82% of reductions occurring by 30 days of pregnancy.[1,2] However, when both embryos persist past 40 days of pregnancy, the likelihood of complete pregnancy loss is 63%.[3] With a 93% success rate, manual reduction of 1 embryo prior to fixation is the preferred twin reduction method.[4] Following fixation, multiple reduction procedures have been described, with varying success rates and pregnancy windows of utility. Examples of reduction procedures include transvaginal ultrasound-guided twin reduction and craniocervical dislocation.[5] Transcutaneous ultrasound-guided twin reduction, as performed in this mare, involves ultrasound-guided injection of potassium chloride or procaine penicillin directly into the fetal heart through the mare's abdomen on days 66-168 of pregnancy.[5] This case highlighted the utility of postfixation twin reduction techniques when embryo dynamics render preferred methods of prefixation reduction unsuccessful, even in a well-managed broodmare under the care of an experienced veterinarian.

Keywords: Horse, twin embryos, postfixation, reduction

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Testicular neoplasm associated polyostotic hyperostosis in a male budgerigar

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A 4-year, intact male budgerigar was presented for evaluation of dyspnea. The bird had a chronic history of mild respiratory distress that progressed to increased respiratory effort, lethargy, and anorexia. Physical examination revealed a cere color change and caudal celomic mass. Oxygen therapy was initiated, and the bird was treated supportively for infectious and inflammatory etiologies with no improvement. Radiography revealed a dorsal solitary celomic mass adjacent to the left kidney, polyostotic medullary hyperostosis of the long bones, and possible increased opacity of the thoracic air sacs and lungs. The mass was prioritized as a testicular neoplasm, with seminoma or Sertoli cell tumor as top differentials.[1,2] Polyostotic medullary hyperostosis,[2] typically seen in female birds during ovulation, suggested a paraneoplastic syndrome associated with a testicular neoplasm. The bird was treated with a deslorelin implant to shrink the testicular mass,[3] but did not respond to treatment. Exploratory laparotomy confirmed a testicular mass and suspected metastasis throughout the celomic cavity. The patient died during surgery. Postmortem histology confirmed a metastatic sex cord tumor. This case highlighted the importance of reproductive neoplasms as a differential for respiratory distress in avian species, and utilization of imaging for early detection and diagnosis.

Keywords: Celom, mass, testicle, paraneoplastic, deslorelin, hyperostosis

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Clinical workup of a hyperechoic structure in the uterus of a chronically infertile mare

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Hyperechoic images in the uterus of the mare can include air, foreign objects (culture swab tips, marbles), tissue debris, medications (ampicillin, gentamicin), urine, blood, mucus, retained fetal membranes, endometrial cups, and fetal bones.[1] A 10-year Quarter Horse mare was presented in April, 2024 for a reproductive examination due to chronic infertility. The mare aborted a 5-month fetus in October 2022 and had not been rebred in 2023. Before admission in 2024, the mare was bred during 2 estrous cycles, and the referring veterinarian noticed by transrectal ultrasonography a hyperechoic image at the base of the right uterine horn that persisted following multiple uterine lavages. The lavage fluid had cultured positive for *E. coli*. Transrectal palpation/ultrasonography evaluation of the reproductive tract indicated moderate uterine edema, ~ 8 cm of intrauterine fluid, and an elongated hyperechoic structure in the lumen of the left uterine horn that was palpably hard. Purulent fluid, containing numerous cocci and neutrophils, was lavaged from the uterus. The following day, uterine endoscopy was performed, and an irregularly shaped, white-to-tan hard structure (resembling a 4 cm pelvic bone) in the lumen of the left uterine horn was identified and removed. Retained fetal bones can occur as a result of fetal death and result in maceration (which is associated with bacterial contamination [likely this case])[2] or mummification.[3] This mare aborted what appeared to be an intact single fetus 18 months before admission, so this would have been the only opportunity for the fetal bone to be retained, possibly due to an unrecognized retained twin. This case highlighted the diagnostic challenge of ultrasonographic 'hyperechoic' images and the eventual diagnosis, in this case, of fetal bone. In addition, following abortion, the uterus should be examined for the presence of a twin fetus.

Keywords: Horse, uterus, fetal maceration, retained bone

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Granulosa cell tumor in a yearling Angus donor heifer

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Granulosa cell tumors (GCT) are one of the most common ovarian tumors in cattle with a prevalence rate of 0.5%.[1] The appearance and size of these tumors are variable with some masses measuring > 30 centimeters in diameter.[1,2] Malignancy is rare.[3] Clinical signs of GCT cases can vary, but may include anestrus, masculinization, udder development, lactation, and nymphomania.[4] A 1-year Aberdeen Angus heifer was presented for evaluation of an enlarged left ovary. One week before, the heifer had presented for follicle aspiration at a commercial embryo transfer facility. It was discovered at that time that the left ovary was enlarged and unable to be aspirated. On presentation, vitals were within normal limits, and presented with a history of persistent signs of estrus. Transrectal ultrasonography of the reproductive tract revealed an enlarged, abnormal left ovary measuring 9 cm with a small, inactive right ovary. Top differentials for the abnormal ovary included granulosa cell tumor, fibroma, and teratoma. Surgical removal of the affected ovary was elected and performed via standing laparoscopy. Ovary was submitted for further diagnostic evaluation that confirmed the suspected diagnosis of granulosa-theca cell tumor. Three months after ovariectomy, the heifer was introduced to a bull and became pregnant; calved during the winter of 2024 with no complications.

Keywords: Heifer, granulosa-cell tumor, ovariectomy

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Metritis in a postpartum mare associated with a retained hippomane

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Toxic metritis is an important condition in postpartum mares, commonly associated with retention of fetal membranes.[1] A 23-year Quarter Horse mare was presented 24 hours after dystocia with a sick foal. On presentation, the mare was normal except for mild trauma on the ventral vulva associated to dystocia and obstetric manipulation. Owners reported that the mare had passed her fetal membranes. The day after admission, the mare was lethargic, uncomfortable, had slight tachycardia (52 bpm), and low-grade fever (102.3ºF). Transabdominal and transrectal ultrasonography were performed. Transabdominal ultrasonography identified minimal free fluid in the abdomen and a distended bladder. Also, a hyperechoic, elliptical structure consistent with a hippomane was floating in the uterine fluid.[2] There was no internal or external evidence of retained fetal membranes. The hippomane required manual removal since it would not pass though the lavage tube. The recovered uterine fluid, of brown-red discoloration, was examined by cytology (numerous neutrophils and rod-shaped bacteria) and culture (pure growth of *E. coli* after 24 hours of culture). Follow-up treatments included antibiotics, antiinflammatories, oxytocin, and lavage until minimal uterine free fluid and no evidence of bacteria was observed. Of clinical relevance, the hippomane was not identified via transrectal ultrasonography, due to the limitation in the scanning depth of the ultrasound but was only identified following transabdominal ultrasonography. In addition, if the hippomane had not been identified prior to lavage, it is unlikely that the hippomane, due to its size, would have passed through a lavage tube, and would have been retained in the uterus. Toxic metritis is commonly caused by retained fetal membranes and can lead to laminitis and death. This case is unusual since a retained hippomane, not retained fetal membranes, was associated with the toxic metritis.

Keywords: Horse, hippomane, metritis

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Chronic balanoposthitis and urethritis secondary to phimosis in a Tennessee Walking Horse gelding

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A 22-year Tennessee Walking Horse gelding was presented for a 16-month history of phimosis[1] and urination within the sheath. Gelding also had a 2-month history of urethritis and balanoposthitis. Initial management prior to referral included 2 smegma transplantations and urethral endoscopy that identified a hyperemic distal urethra. Gelding failed to improve and was referred for a phallectomy. Clinical evaluation revealed a white caseous, crusted discharge on the prepuce, surrounding skin, and inner hindlimbs, ulcerations on the distal penis, thick and hyperplastic glans penis, and a hyperemic, inflamed distal urethra. Preoperative bloodwork revealed a mild leukopenia (4.9×10^3 cells/µl) but no other clinically substantial abnormalities. A sheath ablation and en bloc resection with total phallectomy was performed using the Williams technique.[2] Surgery was performed under general anesthesia following a caudal epidural and pudendal nerve block, and preoperative and postoperative antibiotics were given. The dorsal aspect of the penis was tacked to the body wall and a Jackson-Pratt drain was placed. A 25 x 17 x 5 cm segment of prepuce and distal penis was submitted for histopathology. Histology findings were consistent with chronic, moderate, multifocal to coalescing, ulcerative, lymphoplasmacytic balanoposthitis with granulomatous folliculitis with furunculosis and dermal fibrosis. These results confirmed the clinical diagnosis of chronic balanoposthitis. No evidence of neoplasia or dysplasia was noted. The patient remained hospitalized for 5 days after surgery and was discharged as planned. Phimosis is classified as either congenital or acquired. Acquired phimosis is a common sequela of local neoplasm or trauma.[1] Surgical management is often the only treatment option.[3] In this case, the chronic balanoposthitis likely resulted from dermal injury by recurring urination within the sheath. Due to the chronicity of the inflammation and consequential urethral stenosis, surgical management was indicated with a positive result that increased the gelding's quality of life.

Keywords:

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Concurrent cystic endometrial hyperplasia, leiomyosarcoma, and pituitary adenoma in a goat

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A 12-year female intact Nigerian Dwarf goat was presented for aggression and mammary development of 1.5-2 years duration not responsive to GnRH and dinoprost tromethamine treatment. Goat had pale/tacky mucous membranes, rough hair coat, body condition score of 2/5, and soft bilaterally symmetrical mammary enlargement. Normal urine was passed and there was no appreciable vulvar/vaginal discharge; rest of the physical examination was within normal limits. Laboratory testing revealed a stress leukogram, mild anemia, and hypoproteinemia. Transabdominal ultrasonography revealed mixed echogenicity, disorganized structure in the area of the uterus and asymmetrical ovarian structures. Computerized tomography revealed suspect uterine mass with multiple intralesional cystic changes that compressed the bladder ventrally and to the left. Humane euthanasia was elected due to the presumptive neoplasia, chronicity of disease, and poor prognosis. Gross necropsy findings included a 2.5 cm mass at the level of the cervix that contained clear fluid and purulent exudate that extended into the uterus. There were also multiple 2-3 mm tan/pink nodules and 4-5 mm cysts grossly evident in the uterine wall. Ovarian follicles ranged from 2-5 mm. Milk-like fluid was expressed from mammary glands and the pituitary gland was grossly enlarged (2-3 times normal). Microscopic findings revealed leiomyosarcoma, cystic endometrial hyperplasia, and acidophilic corticotropic pituitary adenoma. Galactorrhea has been documented in goats with pituitary tumors[1,2] likely due to increased circulating prolactin,[3,4] but the reports are scarce despite this being a well-documented condition in humans.[4] Leiomyosarcomas with and without cystic endometrial hyperplasia have been documented in goats,[2,5-7] but Nigerian Dwarf goats are largely underrepresented and concurrent pituitary adenoma, leiomyosarcoma, and cystic endometrial hyperplasia has not been reported to the authors' knowledge. Due to increasing numbers of pet goats and thus prolonged lifespan, an understanding of neoplasia in these patients is essential and frequency of diagnosis is likely to continue increasing in coming years.

Keywords: Neoplasia, mammary, pituitary, uterine, goat

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A detailed characterization of *Streptococcus zooepidemicus* mechanism of infection during equine placentitis

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Several methods have been employed to study pathogen infections. Here, we demonstrate how the integration of multi-omics alongside strict experimental settings allows for a detailed characterization of host-pathogen interactions. Specifically, we leveraged ultra-deep sequencing in a prospective randomized controlled trial to evaluate the interaction of *Streptococcus equi* subs. *zooepidemicus,* as a primary pathogen of equine placentitis (chorioallantois) with the host. We hypothesized that *S. zooepidemicus* establishes infection through crossfeeding with other microbes that reside in the placenta. Six out of 12 healthy pregnant mares at 272 days of pregnancy were randomly assigned to receive an inoculation of a *S. zooepidemicus* isolated from equine placentitis, whereas the remaining 6 mares served as negative controls. The progress of the disease was monitored for 8 days before placental samples were collected for ultra-deep shotgun-DNA and dual-RNA sequencing, primary metabolomics, and in situ hybridization (ISH) analyses. Samples were sequenced at 200 million reads depth for shotgun-DNA and 150 million reads for dual-RNA sequencing. The inoculated isolate was also sequenced for whole-genome versus metagenome-assembled genome (MAG) comparisons before and during the in vivo establishment of the disease, respectively. The probes used for ISH were designed based on the assembled 16S gene from the genome, MAG, overall *Streptococcus* sp., and recovered bacteria. Microbial contaminants were assessed by sequencing reagent samples and a probe in ISH. A bioinformatics pipeline was calibrated for 100% accuracy in microbial species identification, using a published mock community and the genome of *S. zooepidemicus*. In this pipeline, host DNA and RNA reads were carefully separated from microbial reads using various bioinformatic approaches and the horse reference genome from the National Center for Biotechnology Information (NCBI). Genes were de novo assembled using microbial RNA reads and aligned against the nucleotide database from NCBI to further remove potential eukaryotic genes. Microbial classification in a highly strict mode using DNA and RNA reads revealed that other *Streptococcus* sp. potentially resided in the placenta,

such as *S. vestibularis* and *S. dysgalactiae*, or a potential new species of uncultured *Streptococcus*. A total of 1,213 microbial genes were recovered, of which 233 were differentially expressed in mares with placentitis. During infection, several microbial species had upregulated genes related to nutrient acquisition, metabolic adaptation, stress response and survival, growth, and virulence factors, but *Streptococcus* genes accounted for most upregulated ones (n = 186) from which also had adhesion and colonization genes upregulated. These genes, alongside the identified metabolites, point to microbial degradation of the placenta extracellular matrix (ECM) as the mechanism of infection, in which ECM regeneration was confirmed to be one of the main pathways upregulated in placentas with placentitis. Further analyses suggested that *S. zooepidemicus* undergo single-nucleotide (SNP) modifications, including their 16S gene. In situ hybridization analyses confirmed the presence of *Streptococcus* species in the placenta and further confirmed SNP modifications. Overall, these analyses revealed that *S. zooepidemicus* may not be the only *Streptococcus* in the placenta during equine placentitis and that other microbes may reside in healthy placentas.

Keywords: Metatranscriptomics, metagenomics, ultra-deep sequencing

Exploring the potential of equine endometrial organoids: tissue similarities, cycle-stage influence, and long-term stability

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The endometrium is a dynamic tissue undergoing cyclic changes in response to ovarian hormones that are believed to be essential for reproductive success. Although endometrial organoids have been established as physiologically relevant in vitro models in various species, equine endometrial organoids (EEO) remain underexplored. This study aimed to characterize the structural and molecular properties of EEO and assess their fidelity to native endometrial tissue (END) across reproductive cycle stages and extended culture. We hypothesized that: 1. EEO and END exhibit a different transcriptome while retaining key endometrial markers; 2. organoid properties vary based on the reproductive cycle stage of the donor; and 3. the majority of the transcriptome remains stable over time. EEO were generated from endometrial biopsies recovered from mares in estrus and diestrus and the morphology, protein and gene expression were compared. In addition, transcriptomic stability of EEO was assessed across early (P2) and late (P15) passages. Histological evaluation demonstrated that EEO form cystic epithelial structures with a polarized columnar epithelium and basally located nuclei. Transmission electron microscopy (TEM) revealed the presence of microvilli, tight junctions, and secretory vesicles. Immunohistochemistry (IHC) confirmed the presence of epithelial (cytokeratin$^+$) and stromal (vimentin$^+$) cells in EEO. Gene expression overlap analysis revealed that 75.7% of genes were shared between EEO-P2 and END using bulk RNAseq, including key endometrial markers (*FOXA2, MUC1, VIM, CD44, P19, ACP5,* and *SCGB1A1*), at both bulk and single-cell RNA level. Paired transcriptomic analysis (FDR < 0.01) identified 1,331 genes upregulated in EEO-P2 compared to END, related to cell cycle, proliferation, metabolism, and reproduction, whereas 1,847 downregulated genes were linked to differentiation, immune responses, and vasculature development. TEM revealed significant differences in length and number of microvilli, and number of secretory granules per cell, between estrus and diestrus derived EEO. IHC confirmed estrogen receptor expression in estrus and diestrus derived EEO, whereas progesterone receptor expression was only detected in estrus-derived EEO. Bulk RNAseq identified 656, 1,705, and 185 differentially expressed genes (DEGs) when comparing estrus and diestrus in END, EEO-P2, and EEO-P15, respectively (FDR < 0.01). Genes associated with proliferation, secretion, and metabolism were upregulated in estrus

derived EEO and pathways related to ciliary function and complement activation were upregulated in diestrus derived EEO. Transcriptomic stability analysis revealed that 65.6% of DEGs identified in EEO-P2 versus END overlapped with those in EEO-P15 versus END. These findings suggested a gradual loss of systemically regulated hormonal responses over prolonged culture, while cell-intrinsic traits persisted. To conclude, this study provided a comprehensive structural and molecular characterization of EEOs, demonstrating their potential as a physiologically relevant in vitro model for equine endometrial biology. By capturing aspects of reproductive cycle dynamics and maintaining key endometrial features over passages, EEO offer a valuable tool for investigating uterine physiology, intrauterine infections, and fetomaternal interactions, ultimately advancing equine reproductive research.

Keywords: Horse, endometrium, organoids, reproductive cycle

Ovarian remnant syndrome in a 2-year cat with inconclusive diagnostics

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A 2-year, spayed female domestic shorthair cat was presented for evaluation of estrous behavior, including lordosis, vocalization, and rolling, that occurred on a cyclical basis every 3 weeks. Clinical signs began ~ 6 months after ovariohysterectomy (OHE) performed at 19 months of age. An exploratory abdominal surgery by the referring veterinarian at 11 months after original OHE revealed no ovarian tissue. On evaluation at referral hospital, serum was submitted for antimüllerian hormone (AMH) and the concentrations were 0.14 ng/ml, consistent with a spayed female (reference range in ovariectomized cats: 0.01-0.16 ng/ml). Progesterone concentrations and were 1.3 ng/ml, consistent with no luteal tissue. Cat returned 3 weeks later for follow up examination while she was exhibiting estrous behavior. Vaginal cytology had 90% cornification of the vaginal epithelial cells. Serum was submitted for AMH and results were once again consistent with a spayed female (0.10 ng/ml). Cat was given intramuscular gonadorelin (Fertagyl, Merck Animal Health) (43 μg once) to induce ovulation. Cat returned for serum progesterone 3 weeks after gonadorelin treatment. Serum progesterone concentrations were 1.6 ng/ml, consistent with no luteal tissue. Despite the inconclusive diagnostics, an abdominal exploratory was performed based on clinical signs and confirmation of estrogen via vaginal cytology. A small, 3 mm piece of ovarian tissue was confirmed on histopathology at the location of the right ovarian pedicle. The tissue contained follicles in all stages of development and a single mature corpus luteum. This case study demonstrated that a very small amount of ovarian tissue can produce adequate estradiol to stimulate estrous behavior in the cat and produce cornification of vaginal epithelial tissue yet produce serum AMH of that seen in ovariectomized cats and a single corpus luteum can produce serum progesterone consistent with no luteal tissue generally observed in an intact cat.

Keywords: Ovarian remnant, feline, antimüllerian hormone, progesterone

Ultrastructural features and prostaglandin E secretion by equine trophoblastic vesicles

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Maternal recognition of pregnancy (MRP) in mares remains unsolved, and trophoblastic vesicles (TRVs) may provide an in vitro means of studying this process. We hypothesized that TRVs secrete prostaglandin E (PGE) in a time-dependent manner in culture. Our aim was to characterize the ultrastructural features of TRVs derived from day (D) 14 equine embryos and to determine their ability to secrete PGE over time. Mares were bred with fresh semen from a known fertile stallion. Day 14 equine embryos (n = 3) were collected transcervically, washed with supplemented DMEM/F12 Hepes buffered media, the capsule was removed, and the trophoblast was cut into 2-4 mm pieces. These pieces (n = 7 or 8) were cultured together in 500 µl DMEM/F12 media supplemented with 10% fetal bovine serum in a humidified incubator with 6% CO_2, 5% O_2 and 89% N_2 at 37.5ºC. Culture media (CM) was changed every 12 hours, pooled, filtered and stored at -80ºC. Trophoblastic vesicles were maintained in culture for 4 days, then fixed, embedded, sectioned, stained with toluidine blue, and examined using transmission electron microscopy. In CM, PGE was measured using a PGE ELISA kit (Cayman Chemical) and protein concentration determined using a Bradford assay. Prostaglandin E concentration was standardized per mg of protein and normalized by log transformation. Differences in mean CM PGE/mg protein were assessed between embryos with one-way ANOVA at $p < 0.05$. TRVs from D14 embryos were round 1-2 cell layer structures with ultrastructural features compatible with trophoblast cells including microvilli, supranuclear vesicles and mitochondria, tight junctions, lipid droplets, multivesicular bodies, and active rough endoplasmic reticulum. TRVs spontaneously formed from embryo fragments, as early as after 12 hours of culture, grew, detached and multiplied for the first 48-60 hours, became static by 72 hours of culture, with some regression or deterioration at 84-96 hours. Median PGE concentrations in the CM peaked at 188 ng/mg protein at 24 hours of culture and then ranged 33-42 ng/mg protein through 96 hours. In conclusion, D14 derived TRVs shared similar ultrastructural and functional characteristics as the trophoblast of intact equine embryos and may provide an in vitro means to study aspects of the equine maternal recognition of pregnancy.

Keywords: Horse, trophoblast, vesicles, recognition, pregnancy, prostaglandin

Associations between dismount semen evaluation, postmating antibiotics, and mare clinical parameters

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Hand mating is the only acceptable method of breeding in Thoroughbred mares. Confirmation of ejaculation and monitoring of semen quality is performed via dismount samples collected from the mare's cranial vagina or more typically, placing the glans penis in a receptacle as the stallion is coming off the mare's back after mating. Also, hand mating poses substantial challenges (e.g. transmission of venereal and infectious diseases). Strategies to minimize such issues include screening for infectious diseases and postmating uterine infusion of antibiotics such as ceftiofur or ticarcillin. This study aimed to determine the associations between dismount sample features and mare clinical parameters during hand mating. The study involved 50 matings of 25 mares and 3 fertile stallions. Ovulation was induced with a GnRH-agonist (histrelin acetate, Wickliffe), and mares were mated 24 hours later under the maiden mare sedation protocol (intravenous xylazine (100 mg), butorphanol (10 mg), acepromazine (20 mg)). Uterine cultures and cytology were performed 4 hours, and 3 and 5 days after mating. After mating, the dismount semen sample parameters were assessed for volume and concentration; semen kinetics, such as motility, were evaluated using a portable sperm analyzer (iSperm). Mares underwent daily transrectal ultrasonography for 6 days after mating to confirm ovulation and detect uterine fluid. Uterine infusions were performed 4 hours after mating and then daily for 5 days immediately after each ultrasonography. Each mare underwent a control cycle (60 ml lactated Ringers solution (LRS)/infusion, 25 cycles) followed by an antibiotic cycle (ceftiofur reconstituted in distilled water 20 ml with 40 ml of LRS, n = 15 cycles or 3.1 of ticarcillin-clavulanate reconstituted in distilled water 20 ml + 40 ml of LRS, n = 10 cycles) for 5 infusions. No other postmating therapies were applied except for uterine infusions. Pregnancy was confirmed 14 days after ovulation and then terminated with 1 intramuscular dinoprost (7.5 mg). Comparative analyses were conducted within individual antibiotic treatment groups, between each antibiotic and its respective control, and grouped comparing control versus antibiotic. Data were analyzed using generalized linear mixed models (GLMM) with significance set at $p < 0.05$. All mares ovulated 24 hours after GnRH treatment. Dismount parameters did not vary between control and ceftiofur (12.1 ± 5 ml and 11.7 ± 4.4 ml gel free volume; 71.7 ± 3.71 and 78.4 ± 3.6 total motility; 61.1 ± 8.3 and 66.7 ± 5.8 progressive motility, respectively) groups ($p < 0.05$) or between ticarcillin and control (12.3 ± 4.8 and 11.2 ± 5.5 ml gel free; 73.7 ± 5.27% and 70.1 ± 4.8% total motility; 65.5 ± 6.8% and 62.6 ± 8.1% progressive motility, respectively) groups ($p > 0.05$). Uterine infusions of ceftiofur or ticarcillin reduced ($p < 0.05$) endometrial leukocyte counts compared to their respective controls. Mares receiving antibiotics had fewer ($p < 0.05$) uterine infections (40.5%) than the control (54.2%) 72 hours after mating. Intrauterine fluid accumulation varied with time ($p < 0.05$)

but not with groups or interactions ($p > 0.05$). The group overall affected the pregnancy rate ($p < 0.05$; antibiotic 36 versus 54% control). There was an effect of the group for ceftiofur ($p < 0.05$; ceftiofur 25 versus 75% control) but no effect of the group for ticarcillin (ticarcillin 56.6 versus 43.3% control; $p > 0.05$) for pregnancy rates. In conclusion, there was no association between dismount samples and mare clinical parameters. Despite apparent satisfactory semen parameters and lower leukocyte counts postmating in antibiotic-treated cycles, fertility was low.

Keywords:

Immunological and transcriptomic insights into equine persistent breeding-induced endometritis

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Persistent breeding induced endometritis (PBIE) is a leading cause of subfertility in mares; however, the immunological and molecular consequences resulting from postbreeding inflammation resolution failure are not fully understood. We hypothesized that characterizing cytokine concentrations in low volume lavage (LVL) and analyzing the endometrial transcriptome in PBIE-susceptible versus resistant mares elucidate key immunological and molecular outcomes associated with susceptibility to persistent inflammation. Twenty-two mares were categorized based on uterine fluid clearance time into resistant (R; ≤ 48 hours; n = 8), intermediate (I; 96 hours ≥ I > 48 hours; n = 6), or susceptible (S; > 96 hours; n = 8). Low volume uterine lavages (LVL) of 60 ml and endometrial biopsies were collected from all mares 24 hours after breeding. LVL samples were analyzed for the concentrations of 15 cytokines using Milliplex Equine Magnetic Bead Panels (MilliporeSigma). The biopsies were used for RNA extraction and RNA sequencing (RNA-seq). The first cycle pregnancy rate in susceptible mares was lower compared to intermediate ($p = 0.003$) and resistant ($p = 0.03$) mares. Luminex analysis revealed elevated ($p < 0.05$) concentrations of IL-5 and IL-6 in LVL samples from susceptible mares compared to intermediate and resistant mares. Additionally, IL-8 concentrations were elevated in LVL between susceptible mares and resistant mares ($p = 0.02$). Conversely, G-CSF, fractalkine, and IL-1α concentrations were decreased ($p < 0.05$) in LVL samples from susceptible mares compared to intermediate and resistant mares. Receiver operating characteristic (ROC) analysis was performed to evaluate the potential of cytokines as biomarkers for PBIE. IL-1α and G-CSF exhibited the highest accuracy ($p < 0.05$) in distinguishing susceptible mares from intermediate and resistant mares, with area under the curve (AUC) values of 0.86 and 0.84, respectively. Additionally, IL-1α and G-CSF demonstrated the highest accuracy ($p < 0.05$) in predicting day 14 pregnancy status (pregnant versus nonpregnant) with AUC values of 0.83 and 0.84, respectively. Next generation RNA-seq was performed using NovaSeq 6000 (Illumina), and reads were mapped to EquCab3.0 (STAR-2.7.9a). Differentially expressed genes (DEGs) were identified using the DESeq2 package in R with a false discovery rate (FDR) < 0.05. RNA-seq analysis revealed 483 DEGs in the comparison of S versus R, 270 DEGs in S versus I, and 76 DEGs in I versus R mares. Gene ontology (GO) analysis of DEGs from the S versus R comparison revealed enrichment in relevant pathways, including inflammatory response (e.g. *ODAM*, *CUL3*, *TAB2*, *CHI3L1*, *MMP26*), response to oxidative stress (*PRKAA2*, *MAPK8*), nitric oxide biosynthesis (e.g. *ASS1*, *GUCY1A1*, *AQP1*), angiogenesis, and VEGF signaling pathways (e.g. *HSPB2*, *FLT4*, *ARHGAP1*, *PIK3R1*,

SHC2), and negative regulation of muscle contraction (e.g. *TPM2*, *LMOD1*, *ATP1A2*), among others. These findings suggested that the identified pathways reflect downstream immunological consequences of PBIE, potentially contributing to delayed uterine clearance and reduced fertility. This study identified consequential markers associated with susceptibility and provided insight into the molecular mechanisms sustaining unresolved postbreeding inflammation. Together, these results may inform the development of targeted diagnostics and therapeutic strategies to improve outcomes in affected mares.

Keywords: Horse, persistent breeding induced endometritis, uterine clearance, inflammation, cytokines, transcriptome

Geographical differences in prevalence and antimicrobial resistance of *Escherichia coli* isolates from dogs with pyometra

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Pyometra is a common reproductive disease that affects intact female dogs. Treatment typically includes ovariohysterectomy or conservative medical management. Antimicrobial resistance is increasing in both human and veterinary medicine. We hypothesized that there are geographical differences in the prevalence and antimicrobial resistance profile of *Escherichia coli* (*E. coli*) isolates. Our aim was to compare the prevalence and resistance of *E. coli* to 4 commonly used antibiotics (amoxicillin-clavulanic acid (AMCLA), cefpodoxime (CEF), enrofloxacin (ENRO), and trimethoprim-sulfamethoxazole (TMS)) between a veterinary teaching hospital in the United States (H1) and Switzerland (H2). Medical records of dogs diagnosed with pyometra between 2010 and 2024 (H1:2010-2023, 109 dogs; H2:2017-2024, 122 dogs) were analyzed. Chi-square or Fischer's exact test with $p < 0.05$ for significance were used. Of the dogs where a bacterial culture was submitted, *E. coli* was in 60.3% (35/58, H1) and 65.6% (80/122, H2) of the patients ($p = 0.495$). *E. coli* was in pure culture in 91.4% (32/35, H1) and 87.5% (70/80, H2) of those cases ($p = 0.751$). When comparing all *E. coli* isolates with available sensitivity testing for a given antimicrobial between H1 and H2, 82.9 and 11.2% were resistant to AMCLA ($p < 0.001$), 22.5 and 6.1% were resistant to CEF ($p = 0.012$), 9.5 and 4.1% were resistant to ENRO ($p = 0.241$), and 10.0 and 11.2% were resistant to TMS ($p = 1.00$), respectively. Although there was no complete overlap in the data selection periods between the 2 hospitals, the preliminary results revealed a similar prevalence but different antimicrobial profile of *E. coli* isolates between 2 geographical regions. Due to the retrospective nature of this study, genomic analysis was not available. Our results highlighted the need for individual, well-considered, susceptibility test based and prudent antimicrobial use under consideration of geographical aspects.

Keywords: Antibiotic resistance, *Escherichia coli,* pyometra, uterus, infection, antimicrobial

Comparative post-breeding outcomes in jennies inseminated with cryopreserved semen re-extended in seminal plasma or treated with platelet-rich plasma

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Artificial insemination (AI) with cryopreserved semen in donkeys is challenging due to poor fertility, likely caused by an exacerbated postbreeding inflammatory response. This study evaluated the effects of frozen-thawed semen reextension in seminal plasma (SP) and intrauterine infusion of platelet rich plasma (PRP) on postbreeding uterine inflammation, progesterone concentrations, and fertility in jennies. A total of 68 estrous cycles from 14 fertile jennies were randomly assigned to 1 of 5 groups: insemination with frozen-thawed semen reconstituted in 7 ml of SP (SP, n = 12); insemination with frozen-thawed semen followed by intrauterine infusion of lactated Ringer (Control, n = 14) or autologous PRP (PRP, n = 14) at 6 hours after AI; insemination with fresh semen (FS, n = 14); and a uninseminated group receiving PRP infusion at 44 hours after ovulation induction (PRP only, n = 14). Uterine lavage was performed 6 hours after AI in all cycles. Intrauterine fluid accumulation (IUF), endometrial neutrophil counts, corpus luteum (CL) volume, and plasma progesterone concentrations were assessed multiple times before and after AI. Pregnancy diagnosis was performed on day 14. FS and SP groups had lower neutrophil counts 6 hours after AI than Control and PRP groups ($p < 0.05$). PRP only cycles had the lowest neutrophil counts at 6 hours and 24 hours post AI ($p < 0.05$). Neutrophil counts were similar among all groups at 48 hours post AI ($p > 0.05$). Plasma progesterone was higher in FS cycles on days 3 and 8 compared to Control-assigned cycles ($p < 0.05$), and day 14 compared to all groups ($p < 0.05$). CL volume and IUF were similar across groups ($p > 0.05$). Pregnancy rates were higher in FS cycles (71%) compared to all other groups (Control, 0%; PRP, 14%; SP, 8%; $p < 0.05$). In conclusion, SP reduced postbreeding inflammation but did not improve fertility outcomes in jennies AI with cryopreserved semen, whereas PRP had no effect. Additionally, plasma progesterone was affected by the type of semen used for AI, but not by treatments.

Keywords: Donkey, frozen semen, PRP, endometritis, asinus

Impact of canine obesity on maternal insulin resistance and fetal metabolic profile

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Obesity affects nearly 60% of domestic dogs in the US. In humans, obesity is associated with increased pregnancy insulin resistance, leading to fetal hyperinsulinemia, disrupted growth, neural, cardiac, and pancreatic development. The impact of obesity in pregnant dogs and their offspring is poorly understood. We aimed to elucidate glucose and insulin profiles in dogs and offspring affected by obesity. We hypothesized that obese (Ob) dogs and their fetuses exhibit hyperglycemia and hyperinsulinemia compared to lean (Le) controls. Female Beagles assigned to Ob and Le groups were fed ad libitum or to meet energy requirements, respectively (n = 3-5/group). Obesity was defined as ≥ 20% weight gain. Fasting blood glucose was measured during anestrus (A), proestrus/early estrus (P/E), early, mid, and late-pregnancy using a point-of-care glucometer (Precision Xtra, Abbott). Intravenous glucose tolerance tests (IVGTT) were performed during P/E and on day 46 of pregnancy (LH surge = d0). Glucose and insulin were measured at baseline (-5 minutes), and 1, 5, 10, 20, 30, 60, and 90 minutes after intravenous dextrose treatment (0.5 g/kg bodyweight). On days 56-63, dogs underwent cesarean surgery and fetal glucose/insulin were measured (4 fetuses/litter, n = 12-16/group). Insulin was measured via radioimmunoassay (HI-14K, Millipore Corporation). ANOVAs and Tukey's tests were performed (GraphPad Prism 10.1.2). In Le and Ob dogs, fasting glucose was higher at mid and late-pregnancy than at A, P/E, and early-pregnancy ($p < 0.05$). Both Le and Ob dogs presented greater IVGTT glucose area under curve (AUC) during pregnancy than P/E. Only Ob dogs presented greater ($p < 0.05$) insulin AUC in pregnancy versus proestrus that was also higher than Le pregnancies. Glucose and insulin concentrations were not different ($p > 0.05$) between Le and Ob fetuses. In conclusion, obesity appeared to enhance insulin resistance in pregnant dogs, with unaffected late-pregnancy fetal insulin and glucose concentrations. Further research is warranted to explore maternal obesity impact in canine offspring.

Keywords: Metabolism, pregnancy, adiposity, maternal, fetal

Delineation of miRNAs as biomarkers in equine chronic endometritis during different phases of the estrous cycle

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Chronic equine endometritis is a leading cause of subfertility in mares resulting in reduced foal production and financial losses. Endometritis is a dysbiosis either as a physiologic response to breeding associated contamination or another insult to the normal microbiome. However, due to immunological or mechanical abnormalities a chronic dysbiosis can develop, hindering the mare's reproductive success. Recent advances suggest associated roles of microRNAs (miRNAs) in the pathophysiology of chronic endometritis. Aim of this study was to further define the systemic expression profiles of these transcripts in normal (n = 5) versus chronically infected (n = 5) mares in the estrus versus diestrus stages of the estrous cycle. Mare cycle status was assessed regularly via transrectal ultrasonography. When the dominant follicle size reached 33-35 mm with appropriate signs of estrus, ovulation was induced using a GnRH analog, and whole blood samples were collected. Diestrous blood samples were collected 7 days after ovulation. Whole blood was collected into EDTA tubes and centrifuged (1643xg for 10 minutes). Plasma was separated into 1 ml aliquots and snap frozen in liquid nitrogen until processing. RNA was isolated from horse serum using the miRNeasy Serum/Plasma kit (Qiagen), RNA libraries were prepared using the TruSeq Small RNA Library Preparation Kit (Illumina), and sequencing was performed using the Illumina NextSeq 2000 platform. FASTQ files generated were mapped to horse mature miRNAs from the miRbase database using Bowtie software. Bioinformatic analysis is anticipated to demonstrate that mares suffering from chronic endometritis have dysregulated circulating transcript profiles that shift their physiological state towards a chronic inflammatory response that is pernicious to the events required to establish a successful pregnancy. Delineation of transcript nuances in mares experiencing chronic endometritis provides a baseline to further explore the development of useful biomarkers in judging the efficacy of therapeutics targeting chronic endometritis.

Keywords: Endometritis, Equine, miRNA

Sperm peritonitis following transcervical insemination in healthy female dogs

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Insemination within the peritoneal cavity has been reported in humans, horses and dogs with complications leading to production of sperm antibodies, anaphylaxis, ascites, peritonitis, formation of abdominal adhesions, and systemic inflammatory response syndrome.[1-4] To the authors' knowledge, sperm peritonitis has not been reported secondary to transcervical insemination (TCI) in dogs.

Case 1: An 11-month, maiden intact female mixed breed presented for breeding management and TCI with fresh semen. Ovulation was identified via serial serum progesterone testing and inseminations were performed 24 hours prior to identified ovulation and 24 hours after ovulation (ovulation deemed progesterone concentration > 5-10 ng/ml).[5] Acutely following the second insemination, dog was noted to be lethargic, hyporexic and reactive to abdominal palpation. Dog presented to the clinic pyrexic (39.4°C), with tachycardia. Transabdominal ultrasonography demonstrated free abdominal fluid at the trigone of bladder and between intestinal loops in the caudal abdomen. Abdominocentesis yielded an effusion containing neutrophils, scant rods and detached heads of sperm on cytology.

Exploratory laparotomy was performed to decontaminate the abdomen. A 2-mm hyperemic site of perforation through the serosa was identified at the body of the uterus on mesenteric surface. The mesentery was diffusely hyperemic. Effusion fluid sampled from laparotomy was consistent with abdominocentesis fluid taken prior to surgery and cultured *Orchobactrum anthropi* sp. Dog remained in hospital receiving supportive treatments (intravenous fluid therapy, antibiotics and prokinetics); responded well and was discharged after 48 hours.

Dog was confirmed pregnant via transabdominal ultrasonography at 4 weeks after insemination with a singleton. An anatomically normal singleton fetus was delivered via timed and planned cesarean surgert on day 63 after ovulation. At surgery no adhesions or scarring was noted within the peritoneal cavity or tubular tract.

Case 2: A 6.5-year, pluriparous intact female French Bulldog presented for TCI with chilled semen. Dog had a history of 3 previous cesarean surgeries. Ovulation was identified via serial serum progesterone testing and insemination was performed 24 and 48 hours after identified ovulation. Acutely following the second insemination dog was presented with a tense abdomen and ptyalism. Transabdominal ultrasonography demonstrated free abdominal fluid at the trigone of the bladder and the broad ligament was noted to be hyperechoic. Exploratory laparotomy was performed for abdominal decontamination. During laparotomy, a small site of perforation was identified at the ventral body of the uterus at the site of a previous cesarean surgery scar. Cytology of the lavage fluid exhibited sperm.

Dog recovered well following surgery and was discharged 6 hours after procedure. Transabdominal ultrasonography at 4 weeks after ovulation revealed 4 fetuses. Dog was presented for timed and planned cesarean surgery, resulting in 4 live anatomically normal pups.

These are the first reported cases of sperm peritonitis secondary to TCI in the dog. Furthermore, both dogs became pregnant in spite of acute exploratory laparotomy after insemination. Sperm peritonitis and sequalae should be considered a complication and risk of a previously considered safe technique.[1]

Keywords: Dog, sperm peritonitis, transcervical insemination

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Postmortem ovary harvest following intrathecal lidocaine hydrochloride injection in a mare

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In the event of sudden death or euthanasia, postmortem ovary harvest and oocyte retrieval for in vitro embryo production affords owners a final opportunity to preserve a mare's genetic potential. In vitro embryo production can be affected by numerous factors including, but not limited to, season, intrinsic mare effects, pharmaceuticals, and ovary transit times. Researchers demonstrated that oocyte exposure to pentobarbital may negatively impact stages of in vitro embryo production.[1] In an emergency, many factors are beyond a practitioner's control; however, the method of euthanasia, surgical technique, and proper handling of the ovaries can be managed. We describe outcomes following euthanasia via intrathecal lidocaine hydrochloride injection as an alternative to an overdose of intravenous barbiturates. A 9-year Warmblood mare was presented for refractory septic tenosynovitis with extensive necrosis of the superficial and deep digital flexor tendons of the left hind limb. Despite heroic efforts, the owner elected to humanely euthanize the mare and requested postmortem ovarian harvest for oocyte retrieval and in vitro embryo production. Mare underwent general anesthesia; once in lateral recumbency, poll was clipped and a 4-inch spinal needle was inserted into the subarachnoid space, at the atlanto-occipital level. Intrathecally lidocaine hydrochloride 2% (dosed at 3.6 mg/kg) was given. After mare's death, a midline celiotomy was performed and ovaries were removed aseptically. Ovaries were processed, packaged, shipped, and oocytes were recovered as described.[2] A total of 22 oocytes were recovered; 11 matured oocytes were subjected to intracytoplasmic sperm injection with frozen-thawed sperm. Nine fertilized oocytes cleaved, resulting in 7 blastocysts that were vitrified. Maturation, cleavage, and blastocyst rates were 50, 81, and 64% respectively, comparable to commercial standards for in vitro laboratories in the US; however, they exceed rates published for oocytes recovered from postmortem ovary harvest.[3] In cases such as this, effective planning, procedural preparation, and transport coordination are crucial to maximize results when attempting genetic preservation. Successful execution of these elements can significantly enhance the chances of reaching the transferable blastocyst stage. In this case, the use of intrathecal lidocaine injection for euthanasia successfully produced multiple transferable blastocysts. Further research is necessary to compare this method of euthanasia to others and the effects on in vitro embryo production.

Keywords: Ovaries, oocyte, lidocaine, intrathecal, euthanasia, intracytoplasmic sperm injection

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Unilateral cryptorchidism with persistent paramesonephric duct remnants in a gelding

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A 3-year Mustang gelding (gelded before purchase) was presented for aggressive behavior and mounting mares. On palpation, there were no testes and gelding had a distinct scrotal scar. Antimüllerian hormone concentrations were > 14 ng/ml (reference value: > 0.15 ng/ml in intact horses) and serum testosterone concentrations were 526.4 pg/ml (reference value: >100 pg/ml in cryptorchid horses) suggestive of gonadal tissue in the gelding. Palpation of the inguinal area under sedation revealed no palpable testis. Transrectal and transabdominal ultrasonographic examinations revealed a discreet mass resembling testicular parenchyma surrounded by a large amount of free, anechoic fluid. Due to the presumptive presence of retained testicular tissue, surgical excision was recommended. Gelding was anesthetized and placed in dorsal recumbency in Trendelenburg position for an exploratory laparotomy. On laparoscopic imaging, a large fluid-filled structure was identified on the right side surrounded by serosa that resembled testis. Ductus deferens and epididymis were traced from the structure to the ipsilateral ampulla. Yellow, serous fluid (650 ml) was aspirated from the structure to facilitate removal. The structure was ligated and excised unremarkably. On further exploration, the left ductus deferens was traced to and passed through the inguinal ring. The scrotal scar was explored and confirmed to contain no testicular tissue, consistent with hemicastration. Routine closure was performed, and gelding recovered uneventfully from anesthesia. On gross evaluation of the excised tissue, an additional 300 ml of fluid was removed from the structure. A firm, white mass with several cystic structures on its surface was identified within the larger cystic structure. The entire mass was submitted for histopathologic evaluation. Histopathology revealed atrophied seminiferous tubules and sustentacular cells devoid of spermatogenic epithelium within the firm mass. A larger cystic structure had regions of columnar epithelium and glandular structures resembling rudimentary endometrium. A diagnosis of a cryptorchid testis due to a testicular disorder of sexual development was made. During sexual development of the male, the paramesonephric duct typically regresses. Failure to regress results in structures such as uterus masculinus and appendix testis or hydatid of Morgagni that have not been previously described in horses.[1] This case highlighted a unique disorder of sexual differentiation in the stallion.

Keywords: Cryptorchid, horse, appendix testis, hydatid of Morgagni, disorder of sexual development

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Colloidal silver effects on semen parameters in dogs

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Colloidal silver is used for therapeutic agents and drug delivery systems due to its antimicrobial and antiinflammatory properties. However, toxic effects of colloidal silver have are known on male reproductive function in mammals.[1-3] To our knowledge, this is the first report on colloidal silver effect on canine sperm parameters. A 3-year male intact Great Dane dog with previously normal semen characteristics and fertility was presented with infertility and substantial abnormal sperm motility and morphology. No significant changes on libido or ejaculation were noted. Prior to the visit, the owner reported that the dog had injured his flank and developed dermatitis. Owner also reported treatment using antibiotics followed by topical treatment with colloidal silver-based ointment for several weeks. Dog was apparently healthy on physical examination and palpation of scrotal contents was unremarkable. Semen collection was performed by digital stimulation of the bulb of the penis, protrusion to the level of the bulbus glandis, and retroflexion to simulate the copulatory tie in the presence of a teaser female. Semen analysis was performed using a phase contrast microscope for sperm motility evaluation, NucleoCounter SP-100 (ChemoMetec) for sperm concentration measurements, and eosin-nigrosin stain under 1,000 x magnification light microscopy for sperm morphology evaluation. Two semen collections, performed 4 days apart, revealed a total of 1-1.5 x 10^9 sperm within each ejaculate, 5-10% progressive sperm motility, and 4-10% morphologically normal sperm, with detached heads accounting for 70-85% of the sperm morphological abnormalities. During this visit, the dog had been on topical colloidal silver for several weeks and possibly ingesting the ointment by licking that increased suspicions of colloidal silver toxicity. Therefore, we recommended the discontinuation of the colloidal silver ointment cream and performance of another semen analysis not < 2 months after discontinuation. Semen collection 5 months after discontinuation had considerable improvement in semen characteristics represented by a total of 556 x 10^6 sperm within the ejaculate, 70% progressive sperm motility, 80% morphologically normal sperm, and only 3% of sperm with detached heads. Colloidal silver caused considerable changes in sperm characteristics in dogs but effects were reversible within 5 months after discontinuation.

Keywords: Dog, colloidal silver, semen, morphology, detached head sperm

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Association between endometrial swab bacteriology and cytology and live foal rates in Thoroughbred broodmares in the United Kingdom

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Relationships between prebreeding endometrial swab cytology and bacteriology and fertility outcomes in Thoroughbred broodmares in the United Kingdom (UK) have not been evaluated. Aims of this study were to investigate associations between cytology and bacteriology findings from the last endometrial swab taken in the breeding season and live foal rates (predicted mean probability of producing a live foal) in UK Thoroughbreds. We hypothesized that mares with a positive cytology and/or a positive bacterial culture is associated with lower live foal rates. Endometrial cytology and bacteriology findings from the last swabs taken in the breeding season (15th February-15th July) were collected from a database of all Thoroughbred endometrial swab samples submitted to Rossdales Laboratories between 2014 and 2020. Mares' status, age, and foaling outcome for each season were collected, where available, from publicly available data sources. Using a multivariable logistic regression model with mare and farm fitted as random effects, live foal rates were estimated for reported categories of cytology and bacteriology findings while adjusting for mares' age, status, number of previous swabs submitted in that season, and any interactions. Between-category rate differences within predictor variables and interaction terms were evaluated using pairwise comparisons with Bonferroni correction (statistical significance was set at $p < 0.05$). Data were available for 7,691 last swabs from 3,579 mares on 196 farms. Mares with a profuse growth of *Escherichia coli* (*E. coli*) had significantly lower live foal rates (59.1%; 95% confidence interval (CI) 43.7-74.5) compared to those with no growth (80.9%; 95% CI 79.2-82.6). Live foal rates of mares with a profuse growth of β-hemolytic *Streptococcus* were not significantly different (76.6%; 95% CI 66.5-86.6) compared to those with no growth. There was interaction between mares' age and cytology findings. In mares over 12 years, significant reductions in live foal rates ($p < 0.05$ in pairwise comparisons) were observed between mares with > 30% polymorphonuclear (PMN):endometrial cells/high power field at cytological examination and mares with ≤ 0.5% PMN. Additionally, the predicted live foal rate was significantly higher in rested (89.4%; 95% CI 83.7-94.9) and barren (85.3%; 95% CI 82.9-87.7) mares compared to foaling mares (79.0%; 95% CI 77.1-80.9). The predicted live foal rate was significantly lower in mares that had either 2 (71.0%; 95% CI 67.2-74.8) or 3-10 (53.5%; 95% CI 48.1-59.0) previous endometrial swabs during the season compared to mares having none (85.0%; 95% CI 8.2-86.7) or 1 (83.2%; 95% CI 81.1-85.4) previous endometrial swab sample submitted. Results of the study highlighted

complexities to consider when interpreting endometrial swab cytology findings and identified a subset of mares with a profuse growth of *E. coli* in which important knowledge gaps exist around etiologies underlying their poorer fertility outcomes. Careful attention regarding the reproductive management around breeding in mares over 12 years with evidence of a marked endometrial inflammatory response is warranted. Additionally, contrary to previous understanding, barren mares and rested mares were not associated with lower live foal rates in this study, suggesting that resting mares may be of benefit in terms of subsequent live foal rates.

Keywords: Horse, endometrium, cytology, bacteriology, live foal rates

Effect of N-acetyl cysteine treatment on uterine cytokine and chemokine profiles in mares with persistent breeding-induced endometritis

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Persistent breeding-induced endometritis (PBIE) is a major cause of infertility in mares; in PBIE-affected mares, treatment with intrauterine N-acetyl cysteine (NAC) 12-hours before breeding did not improve clinical signs at 12 and 60 hours after breeding.[1] The purpose of this study was to examine the effects of NAC treatment on the uterine inflammatory cytokine and chemokine profiles in PBIE-affected mares. Using a randomized, blinded, cross-over design, mares susceptible to PBIE (n = 10) were allocated to Control and Treatment cycles with at least 1 'washout' estrous cycle between the cycles. Intrauterine infusion of 180 ml of 3.3% NAC (Treatment) or sterile saline (Control) was performed 12 hours before insemination. Uterine fluid samples were collected at 12 and 60 hours after insemination to determine inflammatory cytokine and chemokine profiles. Endometrial biopsies were taken at the same time points to determine gene expression of selected inflammatory cytokines (interleukin-6, interleukin-10, interleukin-1β, and tumor necrosis factor-α). Differences between Control and Treatment cycles were analyzed for statistical significance using repeated measures ANOVA after removing data from a mare that failed 1 of the PBIE inclusion criteria. There was no difference ($p > 0.05$) in the uterine fluid inflammatory cytokines and chemokines between Control and Treatment cycles. Similarly, gene expression of the selected inflammatory cytokines did not differ between the 2 cycles. The absence of any significant effects of NAC on the uterine inflammatory cytokines and chemokines provided a potential mechanism to explain the previously reported lack of improvement in clinical signs in PBIE-affected mares treated with NAC.

Keywords: Horse, insemination, uterus, inflammation, N-acetyl cysteine

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Effect of cryoprotectant type at various steps of the cryopreservation process of stallion sperm

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Stallion sperm is cryopreserved in semen extenders formulated with penetrating cryoprotectants (CPAs), such as glycerol (G) and methyl formamide (MF). Some researchers have suggested that 'high' molecular weight CPAs such as G (92.1 g/mol) induce higher osmotic stress than 'low' molecular weight CPAs such as amides (e.g. MF 59.1 g/mol), resulting in higher oxidative stress and apoptosis in thawed sperm;[1,2] yet, experimental data demonstrating such an effect are still lacking. In this experiment, we measured the effect of adding G, MF or their combination (G + MF) to a 20% egg yolk-based (EY) freezing extender on stallion sperm quality parameters throughout the cryopreservation process. Ejaculates (n = 15) from sexually active stallions were collected and processed by cushioned centrifugation; sperm pellets were resuspended in a 20% EY freezing extender formulated with either 5% G, 5% MF, or 2% G + 3% MF (Step 1), cooled from 22 to 5°C at –1°C/minute (Step 2), cryopreserved to –196°C using an automated freezing system (–60°C/minute), and thawed at 37°C for 1 minute (Step 3). At each step, sperm motility was determined by CASA (% TMOT), whereas sperm viability (% Viab; SYBR-14/propidium iodide), apoptosis in viable sperm (% Apop-V; Yo-Pro-1/ethidium homodimer-1), and superoxide anion production in viable sperm (% SOX-V; MitoSOX red/SYBR-14/propidium iodide) were determined by flow cytometry. Data were rank-transformed for normalization and analyzed using the mixed-model ANOVA and Tukey-Kramer adjustment test (JMP Pro 17). Overall, within Steps, CPA effect ($p > 0.05$) was not observed. Mean TMOT, Viab, Apop-V, and SOX-V in fresh semen were (79, 79, 5, and 31%, respectively). In Step 1, mean TMOT, Viab, Apop-V, and SOX-V were similar among G (78, 78, 7, and 30%), MF (77, 76, 5, and 33%), and G + MF (79, 78, 6, and 32%; $p > 0.05$). In Step 2, mean TMOT, Viab, Apop-V, and SOX-V were similar among G (77, 76, 7, and 33%), MF (76, 75, 6, and 34%), and G + MF (77, 76, 6, and 35%; $p > 0.05$). In Step 3, mean TMOT, Viab, Apop-V, and SOX-V were similar among G (51, 53, 23, and 28%), MF (47, 47, 23, and 25%), and G + MF (49, 49, 23, and 28%; $p > 0.05$). When comparing the effect of each Step of the cryopreservation procedure on sperm quality, mean TMOT and Viab were similar in Steps 1 (73-76 and 75-76%), and 2 (71-73%, and 72-73%; $p > 0.05$), while higher than in Step 3 (47-51%, and 47-53%; $p < 0.05$). Mean Apop-V was similar in Steps 1 (5-7%) and 2 (6-7%; $p > 0.05$), while lower than in Step 3 (23%; $p < 0.05$). Mean SOX-V was similar across all Steps (25 – 34%; $p > 0.05$). In conclusion, cryopreservation of stallion sperm using either a 'high' (G) or 'low' (MF) molecular weight cryoprotectants, or their combination (G + MF) yielded similar sperm quality parameters throughout the sperm cryopreservation process.

Keywords: Stallion, sperm, cryopreservation, cryoprotectant, oxidative stress, apoptosis

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Rheotaxis-based sperm separation of frozen-thawed equine semen using microfluidics

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Rheotaxis is a natural behavior of sperm orienting and swimming against fluid flow. Female reproductive tract has fluid flowing from the uterine tubes to the vagina and rheotaxis may be a natural method for selecting superior sperm for fertilization. Objective was to determine if sperm selection by rheotaxis can improve the quality of frozen-thawed stallion semen. Purpose of this study is to test the hypothesis that rheotaxis-based sperm separation improves the quality of frozen-thawed stallion semen samples compared to unselected raw samples or selection by density gradient centrifugation. Frozen-thawed semen from 3 stallions of varying fertility was used for this study and the pooled sample was divided into 5 groups: control (CTL) group was not processed and left in a tube until analysis, density gradient centrifugation (DGC) group was processed with a single layer EquiPure (Nidacon) at 300 g for 30 minutes, and rheotaxis-based separation using a microfluidic chip was performed at 3 flow rates, 150 µl/hour (150 µl), 250 µl/hour (250 µl), and 350 µl/hour (350 µl). Recovered samples were evaluated for total concentration and membrane damaged sperm using a Nucleocounter (Chemomatec) and motility parameters were evaluated using a Computer Assisted Sperm Analysis system (CEROS, Hamilton Thorne). Differences between groups were determined using ANOVA and Student's t post-hoc test with the CTL group used as the reference. Progressive motility was different ($p = 0.0102$) among groups with the CTL group (32.5 ± 4.2%) having the lowest motility that was not different from the DGC (41.7 ± 16.0%) group, whereas the rheotaxis groups were higher than the CTL with 59.3 ± 12.4%, 56.25 ± 5.1%, 55.0 ± 3.1% for the 150 µl, 250 µl and 350 µl groups respectively. Motility in the 150 µl group was also higher compared with the DGC group. The proportion of membrane damaged sperm was significantly different between groups ($p = 0.0124$) and was greater in the DGC group (45.8 ± 4.3%) compared with the CTL (35.6 ± 5.9%) and rheotaxis groups 150 µl (28.4 ± 4.0%), 250 µl (26.9 ± 1.4%), and 350 µl (32.1 ± 6.4%). In this preliminary study with a small sample size, rheotaxis sperm separation appeared to improve the proportion of progressively motile sperm and potentially superior to EquiPure for this purpose. The proportion of membrane damaged sperm was higher in the EquiPure group compared with all the other groups likely due to the effect of centrifugation especially at higher forces. Rheotaxis-based sperm separation has improved bovine in vitro fertilization rates and may be able to improve fertility of equine sperm.

Keywords: Rheotaxis, sperm separation, microfluidics

Vitamin D3 as a novel treatment for equine persistent breeding-induced endometritis

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Persistent breeding-induced endometritis (PBIE) is one of the leading causes of subfertility in mares, affecting 10-15% of the population. Mares susceptible to PBIE tend to be older animals with an impaired immune response, factors that are associated with vitamin D3 (D3) deficiency in humans. Previous research from our laboratory demonstrated a robust reduction in interval to clear uterine fluid and alterations in cytokine profiles suggestive of improved inflammatory response. We hypothesized that intrauterine D3 infusion into artificial insemination doses improves pregnancy rates and helps susceptible mares to clear fluid more effectively, with cytokine and transcriptomic profiles resembling more closely to resistant mares after D3 infusion. Forty-two mares were classified based on interval to uterine clearance, resistant (R; < 48 hours; n = 21) or susceptible (S; > 96 hours; n =13). Mares falling between these 2 thresholds were classified as intermediate and not considered for the remaining analyses. Mares were bred with 1 x 10^9 progressively motile sperm containing either D3 or vehicle (Con). Low volume uterine lavage (LVL), plasma and endometrial biopsies and samples were collected from mares 24 hours after breeding. LVL and plasma samples were analyzed for concentrations of 15 cytokines/chemokines using Milliplex Equine Magnetic Bead Panels (MilliporeSigma). Biopsies were used for RNA extraction and RNA sequencing (RNA-seq). Pregnancy was diganosed on day 14 after ovulation. As in our earlier study, interval to clear fluid decreased ($p < 0.05$) in susceptible mares (Con – 7.75 ± 1.33 days; D3 2.66 ± 0.61 days;). Pregnancy rates (5/6; 83.3%) in susceptible mares improved ($p < 0.001$) with D3 treatment compared to susceptible mares treated with vehicle (0/8; 0%); although 1 D3 pregnancy was lost by 21 days of pregnancy. Pregnancy rates did not differ for resistant mares (Con = 4/8, 50%. D3 = 6/14, 43%). Cytokine profiles for LVL suggested D3 reversed trends observed in susceptible mares, with D3-treated mares having a decrease ($p < 0.10$) in IL-8, and an increase ($p < 0.01$) in IL-1α, with the susceptible D3 levels ultimately being indistinguishable from the control cycle of resistant mares. Other changes noted within susceptible D3-treated mares' plasma included an increase ($p < 0.1$) in IL-1α, decrease ($p < 0.05$) in G-CSF, and increase ($p < 0.05$) in eotaxin. These trends suggested an antiinflammatory shift towards a more eosinophil-dominant, antiinflammatory profile. Next-generation RNA-seq was performed using Illumina NovaSeq 6000. Differentially expressed genes (DEGs) were identified using JMP with a false (< 0.05) discovery rate (FDR). Our RNA-seq analysis revealed 78 genes differentially expressed between Con and D3 cycles in susceptible mares, with many of them corresponding to the genes differed between susceptible and resistant genes. Promisingly, D3 appeared to shift gene

expression to mimic that observed in resistant mares. These included genes associated with the inflammatory response (*MMP26),* response to oxidative stress *(PRKAA2),* and VEGF signaling pathways (*PIK3R1)* among others. D3 has early promise to help alleviate PBIE in susceptible mares through improved interval to uterine clearance, improved cytokine response and improved pregnancy rates in susceptible mares.

Keywords: Horse, persistent breeding-induced endometritis, vitamin D3, uterine clearance, inflammation

Estradiol cypionate-sulpiride administration to seasonally non-cycling mares: the endocrine response

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Advancing the first ovulation of the year in seasonally anovulatory mares has substantial impact on equine breeding programs. Artificial light is a reliable method to hasten transition into the breeding season but can be challenging for large groups of mares maintained outside. Combination of estradiol and the dopamine antagonist, sulpiride, in a long-acting vehicle stimulated prolactin and luteinizing hormone (LH) in deep anestrous mares, resulting in rapid follicle growth and advancement of first ovulation of the season. Original studies evaluating the ECP-sulpiride combination were conducted in the southern United States. Effects of different climates, latitudes, and elevations have not been assessed. We hypothesized that estradiol cypionate (ECP) and sulpiride stimulates prolactin and LH in deep anestrous mares in a semi-arid, continental region (i.e. Northern Colorado) similar to mares in the South. Beginning in January, 12 mares (10-19 years) housed in northern hemisphere paddocks without artificial lighting were enrolled in a crossover study. Mares were randomized to treatment (ECP (50 mg) on day -1 and sulpiride (3 g) on day 0, both intramuscularly) and vehicle (n = 6/group). Jugular blood sampling began on day of ECP treatment and continued for 11 successive days. Two weeks later, previous control mares received an identical treatment of ECP-sulpiride, and blood sampling repeated. Plasma prolactin and LH were determined via radioimmunoassay. Two-way ANOVA was used to compare prolactin and LH between treated and control mares, and between mares receiving ECP-sulpiride 2 weeks apart. A treatment by day interaction was observed for both prolactin ($p < 0.0001$) and LH ($p < 0.0001$). Prolactin was stimulated beginning 1 day after sulpiride treatment and remained elevated for 9 days. Plasma LH was stimulated beginning 5 days after sulpiride treatment and remained elevated until blood sampling concluded on day 10. Prolactin and LH responses were similar in mares treated 2 weeks apart. In this study, treatment of noncycling mares with ECP-sulpiride stimulated a robust rise in prolactin and LH that persisted for at least 9 days. The magnitude and duration of prolactin and LH secretion were similar to previous observations in a subtropical region, providing evidence that harsher winter climates and higher altitudes may not hinder the stimulatory effects of ECP-sulpiride.

Keywords: Mare, season, sulpiride, prolactin

Physiological response to estradiol cypionate-sulpiride treatment to seasonally noncycling mares

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Advancing the first ovulation of the year is a rate limiting step in equine breeding programs. Artificial light is the most reliable method but can be challenging for large groups of mares maintained outside. Typically, this requires mares being under artificial lighting ~ for 60 days. Therefore, an alternative method requiring less time to hasten ovulation in the deep anestrus (DA) mare is desirable. Sulpiride treatment, a dopamine antagonist, in estrogen-primed DA mares increased prolactin and luteinizing hormone concentrations. We hypothesized that DA mares given estradiol cypionate (ECP) and sulpiride ovulate earlier than vehicle-treated mares. Beginning in January, 12 mares (10-19 years) housed in northern hemisphere paddocks without artificial lighting were enrolled in a crossover study. At the start of the study, the largest median follicle size was 14.5 mm (range: 8-26 mm). Mares were randomized to treatment (ECP (50 mg intramuscular) on day -1 and sulpiride (3 grams intramuscular) on day 0) and vehicle (n = 6/group). Transrectal ultrasonography was performed every other day until a 30 mm follicle was detected with uterine edema, then every day until ovulation. Serum progesterone concentrations were measured on days 5 and 12-14 after ovulation. Using a two-way ANOVA, a statistically significant difference in follicle size was observed by day 7 (treatment: 24 ± 2 mm; vehicle: 17 ± 2 mm). All treated mares developed a 30-35 mm follicle with uterine edema between 7-12 days, whereas none of the vehicle-treated mares exhibited follicular development (< 30 mm). Treated mares continued to cycle and therefore were not used as controls in the crossover. Six control mares were then used for a total of 12 ECP-sulpiride treated mares from January-February. These mares developed a 30-35 mm follicle with uterine edema between 11-18 days. Ten of the 12 total treated mares developed a follicle ≥ 35 mm and uterine edema (11.4 ± 1.5 days). One mare spontaneously ovulated, whereas 71.4% of the remaining mares ovulated after an ovulation induction agent. One mare developed a hemorrhagic anovulatory follicle. On days 5 and 12-14 after ovulation, serum progesterone concentrations of ovulated mares were 7.7 ± 1.02 and 7.9 ± 0.3 ng/ml, respectively. Treatment of noncycling mares with ECP-sulpiride significantly hastened follicle development and ovulation in this population of mares. This hormone combination may be clinically useful in both individual mares and large groups of embryo recipient mares.

Keywords: Mare, season, sulpiride, prolactin

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Effect of FSH and P4 on canine cumulus-oocyte complexes metabolism

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Objective of the study was to assess the effect of supplementing the maturation medium with hormones on canine cumulus-oocyte complexes (COCs) metabolism during in vitro maturation (IVM). We hypothesized that medium supplemented with FSH or P4 stimulates canine COCs metabolism, as measured by oxygen consumption rate (OCR) and proton efflux rate (PER), compared to medium without supplementation. Ovaries from dogs (> 1.5 years) were sliced in a petri dish (60 mm) containing medium 199 with Hanks' salts supplemented with 10% fetal calf serum (FCS) and 1% v/v penicillin/streptomycin. Groups of 10 COCs were randomly allocated into the following groups: 1. Control treatment group (CON); DMEM supplemented with 10% FCS, 1% Penicillin/Streptomycin, 1 µl/ml insulin-transferrin-selenium and 2.5 mM L-glutamine; 2. FSH treatment group: CON supplemented with 5 µg/ml of FSH; and 3. P4 treatment group: CON supplemented with 40 µg/ml of progesterone. The OCR and PER of COCs during IVM were measured every 24 hours using extracellular flux analysis (Seahorse XF Real-Time ATP Rate Assay Kit, Agilent Technologies). A repeated measure analysis of variance (ANOVA) with a mixed effect model was used ($p < 0.05$). Treatment, day and their interaction were entered in the model as the fixed effects, and each replicate was entered as the random effect. At 24 hours, OCR within and between CON (10.2 ± 0.8 pmol/minute/COC), FSH (10.8 ± 0.9 pmol/minute/COC) and P4 (11.5 ± 0.9 pmol/minute/COC) treatment groups were not different but higher than at 48 and 72 hours. PER within CON at 24 hours (5.7 ± 3.2 pmol/minute/COC) was lower than at 48 hours (22.4 ± 3.2 pmol/minute/COC) but not different than at 72 hours (14.3 ± 3.2 pmol/minute/COC). PER within FSH was not different at 24 hours (18.3 ± 3.3 pmol/minute/COC) and 48 hours (29.0 ± 3.3 pmol/minute/COC) but lower at 72 hours (16.5 ± 3.3 pmol/minute/COC). Within the P4 treatment group, PER at 24 hours (15.6 ± 3.4 pmol/minute/COC), 48 hours (14.9 ± 3.4 pmol/minute/COC) and 72 hours (17.0 ± 3.4 pmol/minute/COC) were not different. Among treatment groups, PER was not different at 24 hours, but at 48 hours PER of P4 was lower. At 72 hours, there was no difference in PER among treatments. To authors' knowledge, this is the first report evaluating canine COCs metabolism during IVM using extracellular flux analysis. Supplementation of the maturation medium with either FSH or P4 did not increase the metabolism of canine COCs and the hypothesis was rejected. Real-time measurement of metabolic rate (OCR and PER) revealed that COCs produced ATP by both oxidative phosphorylation and glycolysis during the first 24 hours of IVM. However, after 48 and 72 hours of IVM, ATP production shifted

to the glycolytic pathway. In all treatments, OCR was < 4 pmol/minute/COC and < 3 pmol/minute/COC and PER was > 14 pmol/minute/COC and > 11 pmol/minute/COC, at 48 and 72 hours, respectively.

Keywords: Dog, oocyte, cumulus, metabolism, in vitro maturation

Cataloguing G-protein coupled receptors expressed in the canine placenta

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G-protein coupled receptors (GPCRs) are the largest family of mammalian cell surface receptors, more than half functioning in olfaction. The remaining nonolfactory GPCRs (endoGPCRs) modulate physiologic processes and serve as key pharmaceutical targets. Little is known about expression and function of GPCRs in the canine placenta. The dog genome contains genes for ~ 1438 GPCRs, 353 of which are classified as the endoGPCR subtype. Our project aimed to characterize the endoGPCRs present in the canine preterm and term placenta. We hypothesized that GPCR spatial gene expression is influenced by sex, placental anatomy, breed, and maternal fitness. Placental samples were collected from 12 canine litters following elective cesarean surgery. Placentas from at least 1 male and 1 female were processed per litter. Tissue was sampled from each of the 3 regions of the zonary placenta (allantochorion, transfer zone, hematogenous zone) and preserved in RNAlater (Thermo Fisher Scientific) at -80°C until RNA extraction. Demographics of sampled litters were term golden retriever (n = 3), term French bulldog (n = 3), 1-week premature fit Beagle (n = 3), and 1-week premature obese Beagle (n = 3). Extracted RNA samples (n = 72) underwent mRNA-sequencing and subsequent bioinformatic analyses, resultant data providing the repertoire of the endoGPCRs expressed in the canine placenta as influenced by sex (male versus female), placental anatomy (allantochorion versus transfer zone versus hematogenous zone), temporal change (term versus preterm), and metabolic state of the dam (fit versus obese). Data generated by this research serve as foundation for future investigation of canine placental endoGPCRs in the context of pregnancy disorders, such as dystocia, maternal health, and birth defect. Ideally, design and outcomes of future GPCR exploration will aid in development of veterinary therapies to improve pregnancy outcomes for the bitch and neonate.

Keywords: G-protein, receptor, mRNA-seq, dog, placenta

Assessing the impact of dog breeder mentorship and experiential learning on student knowledge and attitudes toward dog breeding

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The challenges of adequate knowledge and effective communication between veterinarians and dog breeder clientele are recognized,[1] yet theriogenology training is limited in veterinary curriculums,[2] requiring innovative educational tools to overcome these hurdles. Veterinary students at the Cummings School of Veterinary Medicine at Tufts University can enroll in a semester-long elective to learn about purebred dogs, breeding, and whelping directly through local breeder mentorship and experiential learning outside the classroom. A mixed-methods study was designed to determine the effectiveness of this mentorship and experiential learning on veterinary student knowledge and attitudes toward dog breeding and the dog breeder community. We hypothesized that experience improves veterinary student self-perceived knowledge on canine theriogenology topics and changes attitudes toward dog breeding and whelping. Identical pre and postcourse surveys were given at the start and end of the semester, respectively, to assess student knowledge and attitudes. The survey was comprised of 12 Likert scale questions about student perceived knowledge of canine theriogenology topics, 5 Likert scale questions about student perceived attitudes to breeder-veterinarian relationships and intentions to practice, and 4 free response questions about student perceived attitudes toward breeders and the breeding community. Survey responses were paired and Likert scale question comparisons were analyzed using Wilcoxon sign-ranked tests in Python, with significance set at $p \leq 0.05$. Free response questions were analyzed using sentiment analysis, a natural language processing method used to detect emotional polarity and explain social phenomena. An increase was observed in all 12 knowledge topic areas as well as attitudes toward breeder-veterinarian relationships, the need for purebred and purpose-bred dogs, and confidence to practice with breeder clientele after graduation. Mean differences before and after mentorship had a general increase in positive sentiment toward questions about dog breeders, purpose-bred dogs, dog shows, and topics of interest in canine theriogenology. Students initially reported a lack of knowledge or experience with breeders and the breeding community but following the elective, acknowledged a positive change in attitudes and assumptions and referenced experiential learning as a productive tool. Canine breeder mentorship had a positive impact on veterinary student knowledge and attitude toward dog breeding. Biases and assumptions toward dog breeding and the breeder community were reframed following experiential learning. Study findings may be used to support dynamic extracurricular learning and strengthen the relationship among veterinary students, early-career veterinarians, and their future dog breeder clientele.

Keywords: Dog, breeding, reproduction, education, experiential learning

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Spontaneous ovulations in cats used in a non-surgical spay study

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The underlying hypothesis of this project is that fertility and reproductive hormone production in female cats can decrease or eliminated by reducing/destroying the support cells of the ovaries (granulosa and theca cells). We developed a novel nanocomplex consisting of an antibody-guided lipid nanoparticle carrying an intracellular cytotoxin (saporin). This complex can locate the support cells via anti-Mullerian hormone 2 receptors (AMHR2), enter the cells, and deposit the cytotoxin to induce apoptosis. This study was not anticipated to impact the percentage of spontaneous ovulations (~ 30-60% can occur normally), as cats are induced ovulators. Spontaneous ovulations will produce ovarian corpora lutea (CLs). The aim of this project was to test the ability of this nanocomplex to reduce/eliminate fertility. The following parameters were evaluated: estrous behavior and blood progesterone concentrations during the study, and histology of post-spay ovarian tissue. Comparisons of spontaneous ovulations were not originally planned but were analyzed when differences between control and treated groups were observed. Approximately 1-year purpose-bred female domestic shorthair cats were purchased from a commercial breeder and divided into a control group (n = 6) and treatment group (n = 6). Cats arrived at the end of the normal nonbreeding season (December) and were housed together as a colony with access to ad lib food and water and enrichment. Cats were acclimated for 1 week, then exposed to a nonbreeding photoperiod (8 hours light/16 hours dark) in early January. After 5 weeks of low light exposure, the photoperiod was changed to a breeding season photoperiod (14 hours light/10 hours dark). This long-day light regimen continued for the remaining 4 months of the study. The nanocomplex was intramuscularly given midway through the long-day light period. Blood samples were taken from sedated cats (for progesterone (P4) concentrations and health parameters) during the short light period, then at 3 points during the long light cycle. Cats were observed daily for signs of estrous behavior (spontaneous rolling, spontaneous treading/lordosis, yowling). There were some differences between control and injected cat behaviors, but none were significant. Histologically, the striking difference was CLs in the ovaries. Five out of 6 control cats compared to 1 out of 6 injected cats exhibited CLs. Blood P4 concentrations were also increased. This result was significant at $p < 0.05$, X^2 (1, N = 12) = 5.3333, p = 0.02921. There were rare occasions of control cats mounting other cats which could explain some spontaneous ovulations, but we would expect to see this equally in both groups. In conclusion, it appeared that in a colony situation, normal cats will exhibit spontaneous ovulations, in this case 83.33%. In contrast, only 1 of the 6 injected cats produced CLs (16.67%). There is the question of whether the nanocomplex injection prevented/interfered with spontaneous ovulations. Questions remain as to whether the nanocomplex would prevent/reduce normal fertile matings.

Keywords: Cats, ovulation, nonsurgical spay

Disagreement between claimed and actual quality of shipped frozen canine semen

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Cryopreserved semen is commonly stored for long periods and shipped to facilities all over the world to preserve genetics over time and space in canine breeding programs. We compared the postthaw motility and overall sperm number in frozen/thawed ejaculates (n = 147) with what was claimed on the records accompanying the shipped ejaculate. Samples from international sources or any cryopreserved in our own laboratory were excluded. Cryopreserved semen was received at our facility from other facilities (n = 43) all over the US and promptly, quickly transferred to a liquid nitrogen storage tank where it was maintained until needed for insemination. Cryopreserved semen was thawed at 37°C in a water bath (straws) or thaw media (pellets). Any defective straws were excluded from analysis. A subsample of the thawed ejaculate was incubated in thaw media (if an adequate volume was provided by the shipping institution) or otherwise in CaniPlus AI solution (Minitube USA), for 5 minutes prior to evaluation. Sperm concentration and total motility were objectively measured using standardized protocols on a SpermVision computer system (Minitube USA), supervised by 1 of 2 investigators (BWC, n = 74; AS, n = 73). Claimed and recorded values were compared using Wilcoxon's signed-rank test. Claimed total number of sperm was included in records from 97/147 cases, of which 75% overreported by at least 16 x 10^6 sperm and 50% overreported by at least 97 x 10^6 sperm. Claimed postthaw motility was included in records from 135/147 cases, of which 3/4 overreported by at least 24% and half overreported by at least 37%. The differences between claimed and objective results were significant for both total sperm number and total motility ($p < 0.0001$, in both cases). We used a sliding scale (ranging from 50-200 x 10^6 motile sperm) based on 5 weight classes to determine a minimum, adequate breeding dose. In only 2/147 cases did the shipped dose met the minimum criteria. In some cases, multiple recommended insemination 'doses' were shipped, allowing us to thaw more than the initially recommended amount in an attempt to reach a true recommended minimum dose that we achieved in 44 additional cases. For those cases in which pregnancy data exist (n = 132), 80% of dogs that received an adequate insemination dose (n = 46) were diagnosed pregnant compared to a 69% pregnancy rate in those dogs that received a less than adequate insemination dose (n = 86). These comparisons highlighted the dire state of the canine frozen semen industry in the US. Responsible dog breeders invest much in the way of time, money, and emotion into decisions to preserve their dogs' genetics for future use. Measures taken to protect the consumer by demanding better training, transparency, and oversight in the practice of canine semen cryopreservation would serve clients better.

Keywords: Semen, cryopreservation, canine, breeding, oversight

Habitual physical activity of lean and overweight dogs throughout pregnancy measured with a triaxial accelerometer

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Active lifestyle and moderate exercise have been broadly recommended by doctors for decades to women to promote general wellbeing and a healthier pregnancy and labor. Women who regularly engage in moderate intensity physical activity during pregnancy, including overweight and obese mothers, are able to mitigate the risks of several pregnancy-associated diseases such as gestational diabetes or the risk of a cesarean surgery.[1-3] Impacts of physical activity on the reproductive success and health of the dog and her pups are unknown, and consequently, there are no recommendations on what levels of physical activity are normal during pregnancy in the dog. We hypothesized that habitual physical activity gradually decreases throughout pregnancy in dogs. We also hypothesized that dogs with an overweight body condition begins pregnancy with a lower level of physical activity. Aims of this study were to determine habitual physical activity changes during pregnancy using a triaxial accelerometer, and to compare it between lean and overweight dogs. Medium to large breed (2-5 years), client-owned breeding dogs were enrolled and classified into lean (LE, body condition score (BCS): 4-5/9, n = 7) and overweight (OW, BCS: 6-7/9, n = 3) groups. All dogs were fitted with a FitBark 2 triaxial activity tracking device (FitBark Inc.) to their personal collar from shortly before ovulation to the end of parturition during owner's care. Accelerometer activity data was recorded as total cumulative daily activity quantified as 'BarkPoints'. BarkPoints were analyzed over the 9 weeks after ovulation (weekly averages) and during the last 7 days prepartum (daily totals) using mixed models; significance was set at $p < 0.05$. The baseline activity level of the dog was a significant determinant of their activity level throughout pregnancy. Average weekly BarkPoints were not significantly different over time. OW dogs had lower average weekly BarkPoints than the LE group. In the 7 days prepartum, daily total BarkPoints were significantly higher only in a day prepartum compared to 5 days prepartum, and without group effect. Although a definite conclusion cannot yet be drawn due to the small sample size, it appeared that pregnancy did not interfere with habitual physical activity level of dogs, and that an overweight status was associated with a more sedentary lifestyle. Owner lifestyle and perception of activity likely also affected these parameters.

Keywords: Dog, physical activity, pregnancy, accelerometer, body condition

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Comparing vaginal douche, cervicovaginal mucus, and uterine lavage for diagnosis of *Tritrichomonas foetus* in naive heifers exposed to a naturally infected bull

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Trichomoniasis, caused by the obligate extracellular protozoan *Tritrichomonas foetus (T. foetus)*, is a venereal disease of cattle. *T. foetus* enters the cow through coitus and establishes infection throughout the entire reproductive tract. Inflammation of the reproductive tract secondary to *T. foetus* infection commonly results in embryonic loss resulting in prolonged interestrus intervals, low pregnancy rates, lighter weaning weights, and increased culling of open cows. Consequently, there is little known about sampling for *T. foetus* in the female. To the authors' knowledge, this is the first critical evaluation into sampling various anatomical regions in the cow using real time polymerase chain reaction (qPCR). We hypothesized that: 1. Samples taken from the uterus are more often positive for *T. foetus* compared to samples from the vagina and cervix; and 2. the prevalence of positive samples decreases over time, regardless of sample location. In naive heifers exposed to a naturally infected bull, the objective of the study was to compare samples taken from vaginal douche, cervicovaginal mucus, and low-volume uterine lavage for diagnosis of *T. foetus* (using qPCR). Eleven 14-month, crossbred virgin *Bos taurus* heifers were utilized in this study. Heifers were exposed for 30 days (September 24, 2024 to October 24, 2024) to a 5-year, bull naturally infected with *T. foetus*. Starting 30 days after the introduction of the bull, 3 sampling periods were performed, 21 days apart (T1: October 24, 2024, T2: November 13, 2024, and T3: December 5, 2024). At each sampling period, samples were collected in sequence of ascending anatomy: vaginal douche, cervical mucus aspirate, and low-volume uterine lavage. At the conclusion of each sampling day, all samples were transported to the Texas A&M Veterinary Diagnostic Laboratory Canyon, Texas location. Samples were submitted for individual qPCR. A total of 82 samples taken from 11 heifers over 3 sampling time points (T1, T2, and T3) and 3 sampling locations (vaginal douche, cervical mucus aspirate, and low volume uterine lavage) were collected. Twelve out of the 82 samples (14.6%) were positive on qPCR. Time significantly impacted the PCR results ($p = 0.005$), but location did not ($p = 0.87$). There was no significant difference among the timepoints. The positive samples were from 2 heifers, at T1 and T2. For the 2 positive heifers, all 3 specimens were positive at each respective time point. The 2 positive heifers were subsequently negative for specimens collected at T3. A uterine sample was not obtained for 1 heifer at T3. All other heifers were negative at all time points. In summary, there was no significant difference in sampling location, but there was in time. Although a small study, data suggested that the location of sampling is not important, but that sampling should occur within 51 days after exposure for best outcomes.

Keywords: *Tritrichomonas foetus*, qPCR, diagnostics, heifer, protozoan

Cooling rate effect on postwarming outcomes from bovine embryo vitrification

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Vitrification is a crucial technology to expand the use of embryo transfer but it reduces embryo viability. Cryodamage from vitrification is primarily caused by intracellular ice forming at cooling and growth during warming, which is influenced by cooling rate. We hypothesized that increasing cooling rate from the industry standard cooling (SC) rate (~ 23,000°C/minute) to ultra-fast cooling (UFC) rate (~ 600,000°C/minute) eliminates intracellular ice and improves postwarming outcomes. Aim of the study was to compare intracellular ice from cooling and ice growth during warming, using time-resolved synchrotron x-ray diffraction and to compare postwarming outcomes including reexpansion, hatching, apoptosis, and gene expression changes. Abattoir-derived bovine embryos were randomly assigned to 1 of 3 treatment groups: SC, UFC, and unvitrified control (CTL). Intracellular ice was detected and quantified using a custom synchrotron x-ray diffraction data analysis pipeline. Both SC and UFC vitrified embryos without detectable intracellular ice from cooling, but only SC had ice growth at warming detected using time-resolved synchrotron x-ray diffraction. Postwarming outcome was analyzed using time-lapse videography and differences among groups evaluated using ANOVA or Cox's Proportional Hazard models in JMP Pro v.18. CTL embryos (n = 100) had the fastest median re-expansion time 181 minutes (95% CI 142-211 minutes) compared to UFC embryos (n = 71) (226 minutes, 95% CI 191-260 minutes), which were faster than SC embryos (n = 76) (491 minutes, 95% CI 441-561 minutes). Embryo hatching rate was similar between control (92.0%) and UFC (87.3%) but higher than SC (77.6%) and the median time to hatching was faster for CTL (1,215 minutes, 95% CI 1,073-1,353 minutes) and UFC (1,153 minutes, 95% CI 1,014-1,394 minutes) compared to SC embryos (1,808 minutes, 95% CI 1,615-2,055 minutes). Apoptosis was evaluated using TUNEL staining and the proportion of apoptotic cells was higher for SC (7.3 ± 0.6%) compared to UFC (3.5 ± 0.3%) and CTL (4.1 ± 0.7%). Transcriptomic analysis was performed on 3 replicates of pooled embryos and cluster analysis showed CTL having some overlap with UFC, whereas SC had a more distinct profile compared to CTL. We identified 110 differentially expressed genes (DEGs) in SC versus CTL with downregulation of genes associated with adhesion and cell-junction assembly, whereas DNA-damage repair pathway was upregulated. UFC and CTL comparisons identified 72 DEGs with upregulation in lipid metabolism and carboxylic acid biosynthesis and downregulation of secondary-messenger mediated signaling and cell migration. SC versus UFC comparison

identified 100 DEGs with upregulation of signal transduction in response to DNA damage, lipid transport and localization and upregulation of system process, neuron differentiation and guidance. Increasing cooling rate to UFC eliminated ice formation and improved postwarming embryo outcomes as well as minimized gene expression changes related to cryodamage.

Keywords: Vitrification, cattle, embryo

Effect of parturition induction methods on delivery of cloned lambs

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Somatic cell nuclear transfer (SCNT) is a valuable tool in both medical and agricultural research, used to produce animal models for human diseases and domestic animals with high genetic value for breeding purposes. However, the low efficiency of SCNT hampers its application, with only 5-15% of transferred embryos resulting in live births in domestic animals. One of the reasons is pre and postnatal abnormalities, such as prolonged pregnancy, increased birth weight, and reduced neonatal survival. Aim of this study was to investigate different induction protocols to improve the birth and survival rates of cloned lambs. Sheep SCNT embryos were generated using our standard protocol, and recipient synchronization and embryo transfers were conducted as described. On average, 14.9 ± 2.7 one-cell stage embryos were transferred into the oviduct of each synchronized recipient that were in estrus within 12 hours of the transfer time. The status of dominant follicle size or ovulation was evaluated before embryos were transferred into a recipient. Initial pregnancies were confirmed on day 40 ± 5 of pregnancy via transabdominal ultrasonography. Two protocols were used for parturition induction. Intramuscular dexamethasone (15 mg) was given once 24 hours before cesarean surgery on day 148 of pregnancy (short protocol). Intramuscular triamcinolone (2 mg) and tulathromycin (2.5 mg/kg) was given once on day 142 of pregnancy followed by dexamethasone (15 mg) and intramuscular cloprostenol (250 μg) given once 48 hours before cesarean surgery on day 148 of pregnancy (long protocol). After delivery, the offspring remained with their dams and nursed freely until weaning at 2.5-3 months of age. Delivery methods and survival rates were analyzed using a generalized mixed model, and birth weight and days of pregnancy were analyzed using a mixed model, with oocyte source, donor cell sex, recipient surgical history, and twin or singleton status as random effects (Jamovi software, version 2.6.44). $P < 0.05$ was considered statistically significant. In total, 3109 SCNT embryos were transferred into 210 recipients, with an initial pregnancy rate of 39.5% (83 out of 210). Four pregnancies were terminated for sample collection. Of the remaining 206 recipients, 52 (25.2%) went to term, with 14 animals following the short protocol and 38 animals following the long protocol. Compared to the short protocol, the long protocol resulted in higher rate of natural delivery and a lower rate of cesarean surgery births (7.1 and 92.9% versus 71.1 and 28.9%, respectively; $p = 0.004$). Duration of pregnancy was shorter for the long protocol than that for the short protocol (148 ± 1.9 versus 150 ± 1.5 days, $p = 0.010$), but birth weights did not differ between protocols (short: 7.79 ± 2.15 kg; long 6.63 ± 1.80 kg; $p = 0.588$). Moreover, survival rate of lambs at birth and at 1 month did not differ between the 2 protocols (short: 94.1% and 25%; long: 89.1% and 58.5%; $p = 0.928$ and $p = 0.718$, respectively). In conclusion, the long induction protocol improved the birth of cloned lambs through natural delivery, shortened pregnancy, and potentially benefitted their long-term survival.

Keywords: Parturition induction, cloned offspring, sheep

Granulosa cell tumor in a caprine ovotestis

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A 3-year Nubian goat with female external genitalia was presented for evaluation of clitoral hyperplasia. Owners reported that the goat was born with a female twin, and displayed buck-like behavior, namely, mounting other goats associated with frequent Flehmen responses, and aggression as evidenced by excessive head butting and rearing around handlers. Additionally, the goat had the odor of a buck, attempted self-enurination, and possessed a beard and wattles. The goat appeared systemically healthy at presentation, and the only external abnormality noted was a hyperplastic and hyperpigmented clitoris that protruded from the vulva ~ 1 cm. Digital vaginal examination revealed a blind pouch with no external cervical os after vaginal examination with a speculum. Suspected gonads were identified by transabdominal ultrasonographic examination. The left gonad measured ~ 4 x 5 cm in diameter, and was associated with 2 cystic structures ~ 1 cm in diameter. The right gonad measured ~ 2 cm in diameter. The goat was sedated with intravenous ketamine (1 mg/kg), xylazine (0.05 mg/kg), and butorphanol (0.025 mg/kg), then placed in Trendelenburg position. Laparoscopic examination of the abdomen confirmed the abnormalities noted on ultrasonographic examination. Following intubation and maintenance of general anesthesia with isoflurane, the gonads were removed via a ventral midline laparotomy. The goat recovered uneventfully from anesthesia and received subcutaneous florfenicol (40 mg/kg), and intravenous flunixin meglumine (1.1 mg/kg). Histopathological examination of the gonads revealed tissue consistent with ovotestis and a granulosa cell tumor of the left ovotestis. Results from blood karyotyping were consistent with a disorder of sexual development, in that the goat was 80% genetically male 60, XY cells and 20% genetically female 60, XX cells. Molecular analysis revealed the goat was positive for the Y-linked *SRY* gene and positive for the X-linked androgen receptor gene. A recent search of the literature yielded no reports of a granulosa cell tumor in a caprine ovotestis.

Keywords: Ovotestis, granulosa cell tumor, os clitoris

Changes in body condition score, trace minerals by parity, physiological state, and their influence on postpartum resumption of estrous cyclicity in beef cattle

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Trace minerals (TM) are essential for immune function, reproductive health, and metabolic processes in beef cattle during the peripartum period, supporting the health of cow and calf. However, studies on the temporal variations of TM during late pregnancy and the postpartum period in beef cows are limited. This study aimed to investigate changes in peripheral and hepatic TM concentrations and examine the effects of body condition score (BCS) and parity on the resumption of estrous cyclicity in postpartum beef cows. We hypothesized that TM concentrations fluctuate to meet metabolic demands, vary by parity, and are associated with the resumption of estrous cyclicity in postpartum cows. Pregnant beef heifers (n = 22, 2-years) and cows (n = 26, 4- to 7-years, parity: 3-6) in their last trimester were enrolled. Blood and liver biopsy samples were collected during the prepartum (76 ± 20 days) and postpartum (42 ± 8.4 days) periods. BCS was assessed at each sampling time using a 9-point scale (1 = emaciated to 9 = obese). Samples were analyzed for magnesium (Mg), manganese (Mn), cobalt (Co), copper (Cu), zinc (Zn), selenium (Se), and molybdenum (Mo) using mass spectrometry. Additionally, all animals were evaluated for the resumption of estrous cyclicity 40 ± 3 days postpartum using transrectal ultrasonography. Changes (Δ) in BCS and TM concentrations from prepartum to postpartum stage were analyzed using the Wilcoxon signed-rank test. Differences in ΔTM concentrations between primiparous and multiparous cows were assessed using the Mann-Whitney U test. Estrous cyclicity data were analyzed using Fisher's exact test and logistic regression. The primiparous cows exhibited a higher reduction in median BCS than in multiparous cows (1 versus 0.5; $p < 0.001$). Overall, peripheral Co and Se concentrations decreased ($p < 0.001$) in postpartum compared to prepartum by 68 and 39%, respectively. Conversely, hepatic concentrations of Mg, Co, Se, and Mo decreased ($p < 0.01$) by 20, 42, 24, and 8%, respectively whereas Mn and Zn increased ($p < 0.01$) by 14 and 13%, respectively. Postpartum hepatic Mn and Cu concentrations increased ($p < 0.05$) in multiparous cows but decreased in primiparous cows. In contrast, hepatic Zn concentration was higher ($p < 0.001$) in primiparous cows than in multiparous cows. Postpartum multiparous cows exhibited a smaller reduction ($p < 0.001$) in hepatic Co (25.8 versus 33.7 ppm) and peripheral Co (0.7 versus 0.3 ppm) compared to primiparous cows. The ΔMo in liver was higher ($p < 0.001$) in multiparous cows than in primiparous cows (0.11 versus 0.02 ppm). Postpartum blood Cu concentrations decreased in multiparous cows but increased in primiparous cows ($p < 0.001$). Twenty-two out of 48 cows resumed estrous cyclicity at 40 ± 3 days postpartum. The proportion of cyclic cows was higher ($p < 0.001$) in multiparous cows than in primiparous cows (19/26 versus 3/22). A unit increase in ΔBCS decreased the odds of estrous cyclicity resumption by 82%. These findings

supported our hypothesis that TM concentrations vary with physiological stage and parity, with liver TM concentrations having more pronounced changes than peripheral blood. These findings suggested the importance of TM in metabolic adaptations during the postpartum period in beef cows.

Keywords: Hepatic minerals, last trimester, liver biopsy, postpartum

Effects of feeding rumen-protected choline from 21 days prepartum to 100 days postpartum on health and reproduction of Holstein dairy cows

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Choline is an essential nutrient involved in lipid metabolism, methyl group donation, and cellular membrane integrity. Supplementation with rumen-protected choline (RPC) improved liver function, energy metabolism, metabolic disorders, and lactation performance. However, the impact of RPC on health and reproduction has been inconsistent. These inconsistentcies have been attributed to restricted supplementation during the transition period (21 days pre and postpartum), a time-point that precedes reproductive events in dairy cows, and the nature of most nutritional studies feeding cows individually with small sample size that lacked power to identify differences in pregnancy and health disorders. Thus, we designed a study to investigate the hypothesis that feeding RPC from day 21 prepartum and extending supplementation until 100 days postpartum improves health and reproductive performance. Objective of the study was to compare the incidence of peripartum health disorders and the reproductive performance of cows fed with RPC prepartum and for an extended period of 100 days postpartum. Holstein cows were blocked by parity and randomly assigned to the control (CON; n = 389) or RPC (n = 385) group. The RPC cows were given orally 15 grams/day prepartum (-21 to 0) and 30 grams/day postpartum (0-100 days) of RPC (CholiGEM, Kemin Industries Inc.). Data were analyzed using logistic regression and Cox proportional Hazard models on JMP. Incidence of metritis did not differ ($p = 0.22$) between groups (CON = 4.9 ± 2.8% versus RPC = 3.1 ± 2.7%;). Similarly, there were no differences in milk fever (CON = 1.4 ± 1.8% versus RPC = 1.2 ± 1.8%; $p = 0.91$) and mastitis (CON = 38.0 ± 7.5% versus RPC = 37.4 ± 7.0%; $p = 0.88$). Nonetheless, the incidence of stillbirth tended to be lower in RPC than in CON cows (CON = 8.2 ± 3.5% versus RPC = 4.2 ± 3.4%; $p = 0.10$), and RPC cows had higher culling rates than CON cows (CON = 36.5 ± 6.2% versus RPC = 27.1 ± 6.1%; $p < 0.001$). The overall incidence of heel disorders tended to be lower in RPC cows (CON = 54.2 ± 3.6% versus RPC = 50.3 ± 3.3%; $p = 0.07$). For reproductive outcomes, RPC-fed cows had a delayed first estrus (CON = 34.0 ± 3.8 days versus RPC = 41.3 ± 3.7 days; $p < 0.01$) but had no difference in time to first artificial insemination (CON = 76.7 ± 2.2 days versus RPC = 77.2 ± 2.1 days; $p = 0.85$) or first service pregnancy per AI (CON = 31.5 ± 7.0% versus RPC = 35.9 ± 6.6%; $p = 0.29$). However, more RPC-fed cows were pregnant by 150 days in milk (DIM) than CON herdmates (CON = 56.8 ± 7.0% versus RPC = 65.7 ± 6.8%; $p = 0.03$). The results of the current study underscored that extended feeding of RPF for 100 DIM led to improved tendencies in health, reduced culling, and improved reproductive outcome in Holstein dairy cows, suggesting that besides its benefits for milk yield, RPC can be a strategic supplement to support health, reproduction, and productive life.

Keywords: Rumen protected choline, pregnancy rate, stillbirth

Granulosa cell tumor in a pregnant dog

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A 6-year, multiparous Boston terrier dog bred via transcervical insemination (based on progesterone timing) was presented for transabdominal ultrasonography pregnancy evaluation. Dog was on day 32 after LH surge at ultrasonography and breeders reported no obvious changes in behavior. There were several resorption sites in both uterine horns and there was only 1 embryo with a heartbeat and development appropriate for day 32 after LH surge in the right uterine horn. Left ovary was noted to be enlarged (5.9 x 4.7 cm) and had cystic cavitations. The margin of the spleen was clearly identified, confirming the suspicion the mass was ovarian in origin. Serum from multiple days during estrus and pregnancy were submitted for antimüllerian hormone (AMH) concentrations, which were elevated, with some higher than the upper limits of the assay (> 12 ng/ml). Concern for a possible granulosa cell tumor (GCT) was high on the differential list and unilateral ovariectomy was performed. Histopathology confirmed the diagnosis of GCT with clean margins achieved. Pregnancy was carefully monitored via transabdominal ultrasonography, tocodynamometry, and serial progesterone concentrations throughout the pregnancy. Terbutaline dosed at roughly 0.05 mg/kg (1/4 of 2.5 mg tablet) was given as a tocolytic agent by mouth every 12 hours. On day 64 after LH surge, the dog was presented for elective cesarian surgery; a viable, healthy male neonate was delivered. Subsequent AMH concentrations were substantially lower; at 72 hours the concentrations were < 3 ng/ml). To the authors' knowledge, this is a unique case of a confirmed GCT during mid-pregnancy with removal of the ovary resulting in a viable neonate at the end of pregnancy. Based on this case, use of AMH as a diagnostic tool for canine GCT may prove useful and warrants further study.

Keywords: Granulosa cell tumor, ovarian tumor, ovariectomy, antimüllerian hormone

Rectovaginal fistula in an adult English bulldog

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A 2-year, spayed female English bulldog was referred to our clinic for vaginitis and recurrent presence of fecal material within the vestibule after urination. After ovariohysterectomy surgery, weekly episodes of painful fecal eliminations with straining to urinate were noted. Accumulation of gritty material in and around the vestibule and vulva immediately after urination were observed. Physical examination was unremarkable except for slight erythema around a moderately recessed vulva. Digital rectal palpation revealed firm fecal material and a 2 cm diameter sparsely covered diverticulum on the ventral rectal wall about 3 cm cranial from a normal anus. Vaginal palpation revealed a 360° fibrous vestibulovaginal stricture and a midline dorsoventral band. No communication between the rectum and the vagina was evident when rectal and vaginal palpation was performed simultaneously. Urinary tract infection was ruled out by urinalysis and culture. Vaginitis was diagnosed via cytology containing scant bacteria and a moderate number of neutrophils. Vaginoscopy using a rigid endoscope and air insufflation confirmed a midline dorsoventral band just cranial to the urethral papilla, severely erythematous mucosa of the vestibule and vagina and numerous multifocal lymphoid follicles. There were 2 fibrous tissue bands on both lateral vaginal walls. The fibrous tissue band on the left surrounded a small diverticulum with thin mucosa, and within the diverticulum there was a small round-to-oval area of very thin mucosa or fibrous tissue. No grossly visible communication between the fistula and the rectum was seen; however, air was observed escaping out of the rectum. Based on history, physical examination, and vaginoscopy findings, we diagnosed this dog with rectovaginal fistula, although its etiology, i.e. congenital or acquired, could not be reliably determined. The owner opted for nonsurgical treatment with oral lactulose (1 ml) every 12 hours and nonsteroidal antiinflammatory therapy with oral carprofen (2.2 mg/kg) every 12 hours for 3-5 days. At the time of this report, the dog is still on lactulose, she is comfortable defecating, and the episodes of fecal material within the vestibule reduced greatly, but still occur every couple of weeks. Rectovaginal or rectovestibular fistula is an uncommon congenital or acquired defect in the ventral wall of the rectum and the dorsal wall of the vagina or vestibule that allow the passage of feces through the fistula. The condition has been reported as a single abnormality[1] or in conjunction with atresia ani and other developmental abnormalities.[2,3] Clinical signs usually become evident within the first few weeks after weaning when diet changes lead to firmer fecal consistency.[4] Surgical correction is curative and is the treatment of choice.[1,4] However, complications such as wound dehiscence, surgical reintervention, perineal swelling, fecal incontinence and recurrent constipation have been reported.[1,5] Surgical correction will be considered as the next step for this patient if her clinical signs worsen. In conclusion, nonsurgical management of the rectovaginal fistula decreased the episodes of fecal passage into the vestibule and improved the quality of life of the dog.

Keywords: Rectovestibular fistula, rectovaginal fistula, dog, vaginitis, vaginoscopy

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Severe uterine torsion and moderate anemia in a late-term pregnant Maine Coon

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An 18-month female intact Maine Coon cat of ~ 55 days pregnant presented to a referral emergency service with signs of lethargy. Pale mucous membranes were noted at time of admission. Initial bloodwork revealed moderate, normocytic hypochromic regenerative anemia (HCT 18%, MCHC 31 g/dl, MCV 49 fl, absolute reticulocyte 185 thousand/µl), thrombocytopenia (88,000/µl), stress leukogram (segmented neutrophils 13,000/µl, lymphocytes 800/µl, eosinophil 0/µl), total hypocalcemia (8.1 mg/dl), and elevated AST (123 U/l). Patient was Triple SNAP (Idexx) negative, and no hemoparasites were identified on blood smear evaluation. Ultrasonography revealed 2 deceased fetuses, 2 live fetuses with heart rates between 220-240 bpm, and suspected placental separation. Given the poor prognosis for litter survival, the owners elected for pregnancy termination and ovariohysterectomy. At surgery, a 720-degree uterine torsion at the level of bifurcation and 2 jejunal intussusceptions were identified. En bloc hysterectomy and intussusception reduction were performed without incident. Histopathology confirmed uterine torsion with transmural hemorrhage and necrosis in the affected horn. Amniotic fluid and meconium aspiration were documented in the fetuses, indicative of fetal hypoxia. Aerobic and anaerobic cultures of uterine fluid were negative, and hemotropic *Mycoplasma* PCR was undetected. Postoperative management included 1 blood transfusion, antibiotic therapy, antiemetics and pain management. The queen recovered uneventfully and was discharged 2 days after presentation. This case demonstrated the vague clinical presentation of severe uterine torsion in cats. Although uterine torsion is a rare complication during feline pregnancy, blood sequestration within the torsional uterine horn should be included when considering differentials for anemia in late-pregnant cats.

Keywords: Cat, uterine torsion, anemia, intussusception

Ovine fetal deformities due to Cache Valley virus in the Southeast

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Six multiparous Katahdin ewes were pasture bred in a controlled breeding season. Ewes were confirmed pregnant via ultrasonography at ~ 40 days after ram exposure; however, 60 days later, 3 of the ewes were diagnosed not pregnant. Three ewes underwent elective cesarean surgery. Prior to surgery, transabdominal ultrasonography confirmed that fetal pregnancy age was > 145 days. Two lambs were delivered from each ewe. First ewe had 1 normal and 1 nonviable lamb with severe limb and spinal deformities. Second ewe had 2 normal lambs; however, noteworthy fibrin was observed on the placenta. Third ewe had 2 nonviable deformed lambs with fibrin on the placenta. Abnormal fetoplacental units were submitted for necropsy that revealed systemic denervation atrophy of the muscle resulting in limb and spinal deformities, internal hydrocephaly, severe cerebellar hypoplasia, and spinal cord atrophy. Blood samples from ewes were submitted to Texas A & M diagnostic laboratory for Cache Valley virus neutralization. Virus neutralization revealed antibody titers ranging 1:32-1:512, indicating tested animals were positive. Cache Valley virus causes abortion in small ruminants infected ~ on day 30 of pregnancy. The congenital abnormalities identified in this case indicated infection ~ on days 32-37 of pregnancy. The 3 ewes that were nonpregnant were likely infected prior to day 30. Ewes infected after 37 days of pregnancy can give birth to normal offspring. This herd had no history of new additions to the herd in the last 9 months. The affected ewes were born and raised on farm, with no history of travel. The only known potential biosecurity breach is that this herd belonged to a teaching institution and is housed on the same property as a veterinary hospital. As an arthropod transmitted disease, animals adjacent to infected patients are at risk of infection. Cache Valley virus is known to cause these abnormalities; however, this is a virus typically observed in the Western US. To the authors' knowledge, the first reported case in the southeastern US, with the closest reported cases in Texas, Kentucky, and Virginia.[1,2]

Keywords: Cache Valley Fever, congenital abnormalities, vector

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Identification and management of an atypical granulosa cell tumor in a pregnant mare

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Granulosa cell tumors (GCT) are typically diagnosed when transrectal palpation and ultrasonography reveals a unilaterally enlarged ovary with the contralateral ovary being inactive and small.[1] Diagnosis of a GCT is based on history, transrectal palpation, ultrasonography, and hormone evaluation; however, diagnosis of early or atypical GCTs can be challenging.[2] A 15-year Thoroughbred mare foaled in early 2023 and was later treated for bacterial endometritis. Following treatment, the mare was bred, but no pregnancy achieved. Three weeks later, transrectal palpation and ultrasonography revealed a firm right ovary with a cystic structure as the only notable abnormality. Serum was submitted for antimüllerian hormone (AMH), inhibin B, and testosterone concentrations. Results were within normal limits for a nonpregnant, cycling mare; although, inhibin B was approaching the upper limit of the reference range. A subsequent evaluation 2.5 months later revealed that the cystic structure on the right ovary has regressed completely. Both ovulation fossae were palpable, and the ovaries were small and firm, with a corpus luteum on each ovary. GCT panel was repeated that was within normal limits. In 2024, the mare was bred, ovulated from both ovaries, and was diagnosed with twins 13 days later. One of the vesicles was manually reduced and a single remaining embryonic vesicle was identified. Pregnancy was monitored via transrectal ultrasonography until day 60 of pregnancy when the mare had a markedly enlarged left ovary. Third GCT panel was submitted and testosterone and AMH concentrations were both elevated, suggestive of a GCT. The elevation in testosterone was consistent with a theca cell component; however, laboratory reference ranges are provided only for nonpregnant mares. Pregnancy monitoring occurred monthly throughout pregnancy and the ovaries were assessed via transrectal and transabdominal ultrasonography. Ovary fluctuated in size and ranged from 6.9-15 cm. Another GCT panel was submitted at ~ 190 days pregnancy and only testosterone concentrations were elevated. Mare foaled in 2025. Six days after foaling, the left ovary was firm and enlarged with no palpable ovulation fossa. Fifth GCT panel was submitted, and testosterone concentrations were elevated, supporting the diagnosis of a granulosa theca cell tumor (GTCT). Transrectal ultrasonography 13 days after foaling revealed ovulation on the left ovary with an appreciable tumor-like structure and a small, inactive right ovary. Treatment (ovariectomy) of a GTCT is performed by colpotomy (flank, ventral midline, or paramedian surgical approaches) or by laparoscopic approaches.[1] In this case, surgery was considered when the ovary was first noted to be enlarged, but there were concerns regarding pregnancy loss. Ovarian size was monitored throughout pregnancy to determine if the ovary was approaching a diameter that would have been too large to remove via flank approach. These efforts were made in hopes to avoid surgery via ventral midline incision due to the complications that can arise.[3] This case described an atypical presentation of a GTCT and highlighted the difficulty in diagnosing these cases, along with the need to consider the pros and cons of surgical and laparoscopic approaches for ovariectomy.

Keywords: Granulosa cell tumor, mare, surgery, pregnant

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Schistosomus reflexus as a cause of cesarean surgery in a dog

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Schistosomus reflexus (SR) is an infrequent but lethal congenital anomaly most common in cattle (0.01-1.3% of dystocias) but also reported in sheep goats, pigs, donkeys, horses, dogs, cats, and several exotic species.[1] The condition is characterized by ventral curvature of thoracic vertebrae (reflexus) and exposed abdominal and even thoracic viscera (schistosomus). A 4-year Labrador Retriever dog was presented for dystocia after successfully whelping 5 live pups. On reproductive ultrasonography 3 fetuses were observed with the caudal most fetus in the birth canal displaying signs of fetal distress with a heart rate of 130 beats per minute (bpm) while the other 2 had normal heart rates above 180 bpm. No fetus or fetal membranes were palpable in the vestibule. It was elected to proceed to emergency cesarean surgery. Two live pups were delivered from the uterine horns. The last pup was obstructed in the uterine body, identified in dorsal transverse presentation, and noted to have severe congenital abnormalities consistent with SR. Gross abnormalities of the pup included: a severe ventral abdominal wall defect with intestinal, hepatic, gastric, and splenic herniation, severe vertebral kyphosis and left ventricular cardiomyopathy. To date the etiology and embryological mechanism by which SR develops is unknown, although genetic abnormalities are suspected as the primary cause. Several genes associated with midline defects have been reported in mice[2] and in humans with thoracoabdominal syndrome, a syndrome similar to SR, an X linked gene has been reported.[3] Further studies are needed to better elucidate the etiology of SR.

Keywords: Dog, schistosomus refluxus, dystocia, cesarean surgery

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Microbiota-immune interactions in the vaginal and uterine environments from late pregnancy to early postpartum in dairy cows with endometritis

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Transitional period in dairy cows is characterized by substantial microbial and immunological changes within the reproductive tract. This study aimed to investigate the association between the reproductive tract microbiota and the local innate immune response during the transitional period in dairy cows diagnosed with endometritis. We hypothesized that shifts in the vaginal microbiota before calving influence postpartum uterine microbial composition and immune response, leading to an increased risk of endometritis. A retrospective cohort study was conducted on cows categorized as clinical endometritis (n = 11) and healthy (n = 11). Blood, vaginal fornix mucus and uterine mucus samples were collected at 4-time points: 1-week prepartum (−1w) and at 1 (+1w), 3 (+3w), and 5 (+5w) weeks postpartum. Microbiota composition was analyzed via 16S rRNA sequencing, whereas cytokine concentrations of interleukin 1α (IL-1α), interleukin 8 (IL-8), and α1-acid glycoprotein (AGP) were quantified using ELISA assays. At +3w, cows with endometritis had higher concentrations of IL-1α (1.05 ± 0.01 pg/ml), IL-8 (0.73 ± 0.14 pg/ml) and AGP (2.76 ng/ml) in the vaginal fornix mucus compared to healthy cows ($p < 0.05$). The microbiota analysis revealed a postpartum shift in bacterial composition, with an increased prevalence of potentially pathogenic species like *Trueperella pyogenes* ($p < 0.05$) in cows diagnosed with endometritis. White blood cell counts peaked at +1w postpartum, coinciding with vaginal innate immune changes. The present findings suggested that inflammatory cytokines and acute phase proteins may have a pivotal role in postpartum reproductive tract immunity. Elevated AGP concentrations indicated a regulatory mechanism that balances inflammation and tissue repair. The +3w postpartum period represents a critical window for evaluating immune responses in dairy cows. Understanding the immunological and microbial interactions could enhance diagnostic and therapeutic strategies for postpartum reproductive disorders in dairy cows. These findings suggested that monitoring vaginal microbiota and inflammatory cytokines prepartum could help identify high-risk cows before clinical signs appear, enabling earlier intervention strategies such as targeted probiotic supplementation or immune modulation therapies to improve reproductive outcomes.

Keywords: Microbiota, innate immunity, cytokines, endometritis, dairy cows, postpartum

Equine dystocia managed with assisted and controlled vaginal delivery

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A 14-year multiparous Quarter Horse mare was presented for a dystocia. Mare had previously aborted twin foals and was unintentionally bred by a neighboring stallion on an unknown date. On presentation, the foal's forelimbs were observed protruding from the mare's vulva. Foal was in anterior longitudinal presentation, left dorsoileal position, with lateral deviation of the head to the left with torticollis posture. No signs of fetal viability were detected at this time. Abnormal orientation of the fetus in the birth canal is the most common cause of dystocia in the equid.[1] Mare was sedated, and an assisted vaginal delivery was attempted for 20 minutes. This was unsuccessful; mare was transported to a surgical suite, placed under general anesthesia, and positioned in the Trendelenburg position for a controlled vaginal delivery. Foal was delivered, vigorously stimulated, and a heartbeat and corneal reflex were detected. It is estimated that the mare was in stage 2 labor for ~ 6 hours; acceptable time for delivery is between 20-30 minutes.[1] A retrospective study indicated that every 10-minute increment past the normal range that a foal is in stage 2 labor, the chance of survival significantly decreased.[1] If the controlled vaginal delivery was unsuccessful, a cesarean surgery would have been indicated.[1,2] Fetotomy is indicated if the foal is declared deceased to protect the mare and her reproductive potential.[1,2] Partial fetotomies did not influence a mare's reproductive future.[3] Mare and foal were transferred to a recovery; anesthetic recovery was uneventful. Mare's fetal membranes were manually removed. Common dystocia complications include retained fetal membranes, uterine prolapse, invagination of the uterine horn, uterine rupture, or rupture of the uterine artery.[2] Mare and foal were later discharged from the hospital.

Keywords: Mare, dystocia, controlled vaginal delivery

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Endometrial polyp identified as a potential cause of infertility in a mare

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A 12-year, multiparous, barren Thoroughbred mare was presented to Hagyard Equine Medical Institute's McGee Fertility Unit for evaluation of an intrauterine mass visible on transrectal ultrasonography. The mare had been bred once, with no pregnancy identified on transrectal ultrasonography on day 14 after ovulation; however, an intrauterine mass was visualized, prompting referral. On transrectal palpation, the mare had poor uterine tone and a freely movable uterus in the abdomen. On transrectal ultrasonography the left ovary had a corpus luteum. The endometrial folds had an increased hyperechogenic surface and an ~ 2 x 2 cm solid mass in the middle of the left horn. The uterus was distended with air and hysteroscopy was performed. Both uterine horns were traversed and the oviductal papillae visualized and within normal limits. A scant volume of translucent mucus was present at the base of the right uterine horn. A sample of this fluid was aspirated for culture and cytology. The mass identified on transrectal ultrasonography was visualized near the base of the left horn. The mass was yellow and attached to the endometrium by a thin pedunculated stalk. The mass was bluntly dissected from its stalk using transcervical manual disruption. Histopathology revealed the mass to consist of a polypoid structure that extended to all cut margins consisting of endometrium. The polyp was lined by ciliated, mid height to tall, columnar epithelium. Regions of the epithelium were ulcerated, replaced by thin to moderately sized streams of fibrous connective tissue, and variably covered by necrotic debris and laminated bands of fibrin, sloughed cells, and acidophilic proteinaceous material. The polyp parenchyma consisted of numerous glands with fibrovascular support. The stroma was diffusely infiltrated with moderate to large numbers of lymphocytes and plasma cells and low to moderate numbers of eosinophils. Rare stromal vessels were thrombosed with fibrin. A large peripheral region of the polyp was necrotic. Endometrial cytology revealed several white blood cells, mostly degenerative cells, light mucus and light debris with few gram-positive cocci in pairs intra- and extracellular. Culture of the fluid grew heavy β-*Streptococcus* sp. and light *Mannheimia haemolytica*. The mare was treated for endometritis post hysteroscopy using uterine lavage with sterile saline, infusion of 30% DMSO in saline, and nitrofurantoin. Endometrial polyps are protruding growths from mucous membranes and have been classified as inflammatory or neoplastic lesions.[1-3] They can be pedunculated or sessile, frequently solitary tumors, generally small but may attain a generous size, with their shape mold to the uterine lumen. They can occur in the uterine horn or body and may protrude through the cervix. Smaller polyps are usually asymptomatic, whereas larger masses may degenerate becoming necrotic, ulcerate, and cause clinical bleeding and infection.[4] Endometrial polyps are very rare in mares, with only two cases of uterine polyps documented.[4,5] This polyp may have affected uterine clearance, embryonic mobility,

or provided a nidus for infection. After removal of the polyp and treatment for endometritis, the mare became pregnant producing a live foal.

Keywords: Hysteroscopy, endometrial polyps, uterine mass

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Effects of sinigrin in combination with a low iodine diet on equine fetal development and urine iodine concentration

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Sinigrin, a glucosinolate in *Brassica* family plants, breaks down into antithyroid compounds that cross the placenta in some species, but the effects on equine fetal development are unknown. We hypothesized that feeding mares sinigrin in late pregnancy combined with a low iodine diet interferes with fetal thyroid function and skeletal development. Mares with a mean ± SD age of 9.2 ± 4.4 years (range 3 – 18 years) were individually fed, from mid-pregnancy to term, an isocaloric and isonitrogenous diet at 2% body weight in kilograms (BW_{kg}) dry matter. Mare groups were: National Research Council (NRC) iodine (Control n = 6), no supplemental iodine (No Iodine n = 5), and no iodine plus sinigrin (Mustard n = 2). Forage contained 0.222 ppm iodine. Concentrate was fed at 2 g BW_{kg}: Control oat pellets contained 4.54 ppm iodine, No iodine contained 0.34 ppm iodine, and Mustard oats with sinigrin contained < 0.003 ppm iodine. At birth, physical examination findings, ultrasonography corrected thyroid volume BW_{kg}, carpal/tarsal skeletal ossification index (SOI) and free catch urinary iodine concentrations using inductively coupled plasma mass spectrometry were measured. Data were analyzed using Shapiro-Wilk, Chi Square and Kruskal Wallis tests at $p < 0.05$. Control and No Iodine foals had physical examination, radiographic, and thyroid findings within normal limits. Mustard foals had the following clinical findings: a 43 kg colt born on day 343 of pregnancy, hypothermia (36.5°C), SOI 1, severe mandibular prognathism, severe forelimb contracture, an enlarged umbilicus, and goiter; and a 32 kg foal born on day 320 days of pregnancy, SOI 3, mild mandibular prognathism, mild forelimb contracture, and thyroid enlargement. Median (quartiles) foal urine iodine concentrations (µg/l) on day 1 were as follows: Control 290 (160, 455), No Iodine 69.6 (23,107.8) and Mustard 7.5 (6.8, 8.3) and were different $p = 0.04$. The data indicated that sinigrin crossed the equine placenta, interfered with iodine uptake and disrupted fetal thyroid function. The lack of any clinical signs in the No Iodine foals indicated that low maternal iodine intake was insufficient to cause clinical disease, and although numbers are small, the addition of sinigrin produced signs compatible with mild to severe congenital hypothyroidism dysmaturity syndrome.

Keywords: Sinigrin, foal, thyroid, iodine

Uterine environment reduces the pluripotency of the in vitro-produced blastocyst

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With the increasing production of equine embryos in vitro, understanding how in vitro culture affects early cell lineage segregation, compared to uterine signaling in vivo, has become critical for optimizing outcomes in the laboratory and subsequent pregnancy. During early embryo development, the first lineage differentiation occurs as 1 population of cells differentiates into the trophectoderm (TE) and another group of cells into the inner cell mass (ICM), losing their totipotency and becoming pluripotent cells. The timing of these differentiation events, as well as the dispersal and intermingling of cells, may be linked to a higher incidence of monozygotic twinning observed from in vitro-derived embryos when compared to their in vivo counterparts. In this study, we sought to identify specific gene markers differing between 2 sets of embryos produced in vitro (IVP). The first group corresponds to blastocyst stage embryos vitrified, thawed, and then in vitro cultured for an additional 48 hours (TC). The second group corresponds to blastocyst stage embryos vitrified, thawed, transferred into recipients, and flushed 48 hours later (ET). Both groups of embryos underwent microscopic evaluation, followed by RNA extraction. The extracted total RNA was sequenced, and the resulting reads were trimmed (Trim Galore) and aligned (STAR) to the current equine reference genome. Mapped reads were then quantified (featureCounts) and analyzed with DESeq2 (FDR cutoff: 0.1; minimum $|\log_2 \text{fold change}| \geq 2.0$) to identify differentially expressed genes (DEGs). Blastocysts flushed 48 hours after transfer had a distinct ICM, a confluent capsule, and a marked increase in size. TC embryos did not have any distinct changes during the 48 hours of culture. Transcriptomic results revealed a large number of DEGs (n = 1,561) between the 2 groups. Notably, there was a downregulation of pluripotency-associated genes (*PODXL*, *PODXL2*, *NANOG*, *SOX2, DNMT3B*) in transferred embryos. In addition, genes involved in embryo–maternal communication—such as *ESR1, CYP19A1,* and *PTGES2*—were upregulated in the transferred group. These findings indicated that the uterine environment provides a distinct set of signals that promote differentiation of in vitro-produced embryos and diminish pluripotency in the developing embryo as the ICM initiates the lineage of cells that will transform into the embryo proper.

Decoding hormonal effects in bovine oviductal organoids: a new era in reproductive research

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Oviduct has a crucial role in gamete maturation, transport, fertilization, and early cleavage of embryos in the bovine female reproductive system. However, its anatomical positioning complicates physiological study. We aimed to develop an in vitro three-dimensional (3D) cell culture system replicating oviductal physiology to advance reproductive research. We established a 3D bovine oviductal organoid model, exposed to estradiol and progesterone at physiological concentrations to simulate estrus (2 days) and diestrus (4 days), creating a natural oviductal cellular microenvironment. Using single-cell transcriptomic profiling, we analyzed molecular responses of key cell types (mesothelial cells, secretory and ciliated epithelial cells) to hormonal stimulation. Cluster analysis revealed significant changes in the relative abundance of cell populations, with progenitor cells differentiating into more specialized epithelial secretory and ciliated cells. Single-cell RNA sequencing identified 3,106 differentially expressed genes (DEGs) between hormone-treated and vehicle-treated control groups. Among these DEGs, 3,074 genes were downregulated, whereas 27 were upregulated in hormone-treated organoids. Pathway enrichment analysis indicated significant suppression of metabolic and translational activities, particularly involving oxidative phosphorylation and ribosome-related processes in hormone-treated organoids compared to vehicle controls. These results provided valuable insights into bovine oviductal organoids that maintain a diverse array of cell types like those in vivo. Furthermore, the cell type-specific DEGs identified in the 2 treatment groups provided a comprehensive list of potential genes and pathways linked to the cell type-specific response to hormones and the physiological functions of different cell types in oviduct-embryo interactions. Bovine oviductal organoids serve as a promising model for studying oviductal biology and offer applications for enhancing reproductive technologies. Future studies will explore coculturing hormone-treated organoids with gametes or embryos or supplementing culture media with organoid-derived secretions, such as extracellular vesicles, to improve embryo viability and pregnancy outcomes.

Keywords: Cattle, oviduct, organoids, single-cell sequencing

Impact of pituitary pars intermedia disorder on the equine endometrium

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Pituitary pars intermedia dysfunction (PPID) is an age-related endocrinopathy associated with elevated adrenocorticotropin hormone (ACTH) and cortisol. Animals experiencing PPID have had elevated systemic inflammation and specifically an upregulation of the proinflammatory chemokine interleukin-8 (IL-8). It is unknown if this chronic inflammation, or the presumed fibrosis related to it, is noted within the reproductive tract. Therefore, the objective of this study was to evaluate the impact of PPID on the endometrium of the mare. We hypothesized that elevated ACTH leads to altered cytokine expression and increased fibrosis in the mare, predisposing them to endometritis. Aged mares (n = 11) were screened for PPID using a thyrotropin-releasing hormone (TRH) stimulation test in late summer. In brief, ACTH concentrations were measured before and 1-hour after intravenous treatment of 1.0 mg TRH. Of these, 7 mares were PPID positive (n = 7; ACTH > 110 pg/ml after stimulation), and 4 were PPID negative (n = 4; ACTH < 30 pg/ml after stimulation). When in diestrus (presence of a functional corpus luteum, increased uterine tone), 2 endometrial biopsies were obtained from all mares for qPCR analysis of select targets associated with inflammation (*IL-8*, *IL-6, IL-1β, IFNγ*), fibrosis (*MMP2, MMP9, TIMP-2,* and *TNF*) in addition to histology for fibrotic changes. Data were analyzed using SAS 9.4, and were assessed for normality and equal variances utilizing a Bartlett's and Shapiro-Wilk test. The impact of PPID on expression of transcripts relating to inflammation and fibrosis were evaluated using an unequal variances Student's t-test. Correlations between concentrations of ACTH and expression of transcripts were assessed using a Pearson correlation. Significance was set to $p < 0.05$. Of the targets evaluated, only *IL-8* increased in expression in the PPID population ($p = 0.02$). There was a positive correlation between ACTH after TRH stimulation and the endometrial expression of *IL-8* ($r = 0.80$; $p < 0.001$). A weak but significant correlation was also noted between ACTH and endometrial expression of *IL-6* ($r = 0.41$; $p < 0.04$) and *IFNγ* ($r = 0.63$; $p < 0.01$). There was no significant correlation between concentrations of ACTH and expression of fibrotic markers. Additionally, no significant differences were noted when assessing fibrosis based on histopathology, as fibrotic changes were noted in 4 out of 7 PPID mares and 2 out of 4 control mares ($p = 0.82$). In conclusion, the systemic inflammation previously indicated in the PPID animal was also observed within the endometrium, but this was not associated with increased fibrosis. Future research is warranted to determine if this increase in *IL-8* is associated with cytologic inflammation or active infection within the uterine lumen that would be detrimental to the fertility of PPID mares.

Keywords: PPID, equine, endometrium, inflammation, endometritis

Delayed embryonic development or long sperm survival in an embryo donor mare

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An embryo collection procedure is usually performed 7-8 days after ovulation in mares. Embryos are subsequently transferred into a synchronized recipient mare that is expected to carry the pregnancy to term. Submission of a blood or hair root sample to an approved genetics laboratory for DNA parentage verification is a prerequisite for foal registration for most horse breed organizations. The goal of this report is to document a clinical case in which the DNA parentage test of the foal produced by embryo transfer excluded the stallion whose semen was used on the estrous cycle yielding the embryo and identified as the genetic sire the stallion whose semen was utilized on the previous estrous cycle. A 21-year Arabian mare was inseminated with frozen-thawed semen from a deceased stallion (Stallion A) immediately after detection of a single ovulation. No embryo was recovered following uterine lavage after 8 days. Cooled-transported semen from a different stallion (Stallion B) was used on the subsequent cycle. An exceptionally large (2,466 μm in diameter) expanded blastocyst stage embryo was recovered 8 days after ovulation and transferred into a recipient mare. The recipient mare carried the pregnancy to term. Genetic testing of the foal excluded Stallion B as the sire and confirmed Stallion A as the genetic sire of the foal. The potential explanations for how an equine embryo could be recovered following an insemination 26 days earlier include marked delayed fertilization or marked delayed embryonic development. Equine DNA parentage testing relies on the principle of exclusion, with the inheritance of a series of short tandem repeat (STR or microsatellite) markers evaluated in the foal, sire, and embryo donor mare. Parentage assignment based solely on breeding records would have been incorrect.

Keywords: Horse, fertilization, embryonic development.

Bilateral uterine horn segmental aplasia in a female goat

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In female embryos, the Müllerian, or paramesonephric, ducts will develop into uterus, uterine horns, cervix, and the cranial third of the vagina. Failure in the development of the paramesonephric ducts during embryogenesis results in the absence of 1 or several segments of the uterus, known as segmental aplasia, and it has been described in multiple species, including goats and other ruminants. This report documents a case of bilateral segmental aplasia with secondary hydrometra and hydrosalpinx in a 2-year, nulliparous, Nigerian dwarf doe that was evaluated for failure to conceive. The doe had a normal vaginal and external cervical exam. Transabdominal ultrasonography revealed dilated anechoic fluid-filled, thin-walled segments of both uterine horns with a narrower tubular, fluid-filled structure that could be followed to the ovary; more caudally, there appeared to be normal sections of the uterus. The doe was given intramuscular cloprostenol (250 µg Estrumate®, Merck) twice, 10 days apart. After treatment there was no change in ultrasonography. The doe was euthanized due to a poor prognosis for reproductive success. Postmortem findings confirmed severe multifocal bilateral segmental uterine horn aplasia with hydrometra and hydrosalpinx. Bilaterally, several segments of the uterine horns were absent (uterine segmental aplasia). In place of the missing segments of the uterine horns, there was a thin, tan, firm fascial tissue. The distal uterine horns were markedly distended with clear, watery fluid (hydrometra). The true prevalence of uterine segmental aplasia in goats is unknown. Still, it should be included as a differential diagnosis for female infertility and as a cause of hydrometra in goats. Further research is warranted to better understand the prevalence and epidemiology associated with the etiology as well as possible genetic components of this condition in the goat.

Keywords: Segmental aplasia, goat, transabdominal ultrasonography, infertility.

Effect of intrauterine ozone therapy on postbreeding inflammatory response in mares

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Endometrial inflammation after breeding that lasts beyond 48 hours in the mare is classified as persistent breeding induced endometritis (PBIE). Proinflammatory cytokines (IL-1, IL-6, IL-8, IFNγ, and TNF-α) recruit white blood cells (WBC) to the uterus. Antiinflammatory cytokines (IL-4, IL10) work oppositely to modulate the immune response. Mares that are susceptible to PBIE have an increase in proinflammatory cytokines and a decrease in antiinflammatory cytokines compared to normal mares within 6-24 hours after insemination. PBIE more commonly presents when breeding with frozen semen, due to the absence of seminal plasma that acts to dampen the immune response. Ozone is a gas molecule made up of 3 oxygen atoms in a cyclical structure, and was able to decrease inflammatory cells and microorganisms in the postbreeding uterine environment with fresh semen. As antimicrobial stewardship becomes more important while considering the development of new therapies, ozone may have an important role as an alternative to antibiotic therapy. Our specific aims were to determine: 1. differences in the relative presence of WBC in the endometrium postbreeding in mares treated with intrauterine ozone or oxygen (control) and 2. differences in the endometrial mRNA expression of proinflammatory (*IL-1, IL-6, IL-8, IFNγ*, *TNF-α*) and anti-inflammatory (*IL-4, IL-10*) cytokines postbreeding. We hypothesized that treatment with intrauterine ozone 6 hours after breeding with frozen semen modulates the endometrial inflammatory response and reduces the rates of PBIE. Two healthy light-bred mares with negative endometrial culture and no signs of inflammation on endometrial cytology were used; mares were inseminated with frozen-thawed semen during estrus and treated with intrauterine oxygen (1.5 liter for 5 minutes) at 6 hours after breeding. An endometrial biopsy was collected at 24 hours after breeding and immediately frozen under liquid nitrogen. In addition, endometrial cytologies were collected at 24, 48, and 72 hours after breeding. These mares then received a 21-day washout period, and the experiment was repeated with intrauterine ozone (50 μg/ml, 1.5 liter for 5 minutes). Percentage of WBCs in the cytology was compared by Fisher's exact test and data for gene expression were analyzed with a linear mixed effects model with significance set to $p < 0.05$. Endometrial cytology revealed only a slight decrease ($p > 0.05$) in the percentage of inflammatory cells present in the endometrium after ozone therapy (25.6%) compared to oxygen therapy (29.4%). In this preliminary experiment, endometrial biopsies were analyzed by RT-qPCR only for *TNF-α*. The relative changes in cytokine mRNA expression of *TNF-α* decreased ($p < 0.05$) in samples from mares that had been treated with ozone after breeding compared to oxygen.

Keywords: Horse, breeding, endometritis, ozone

Factors affecting lactate-induced acrosomal exocytosis in viable frozen/thawed stallion sperm

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Stallion sperm incubated under presumed capacitating conditions (presence of calcium, bicarbonate, and albumin) in a medium with lactate as the only energy substrate (Lac-MW) undergo protein tyrosine phosphorylation and spontaneous acrosomal exocytosis (AE) while retaining viability (AE/Viable).[1,2] In addition, by 4 and 6 hours of incubation in Lac-MW, the rate of AE/Viable in fresh and frozen/thawed stallion sperm are similar, and highly associated with the in vivo fertility of stallions.[2] In the current study, we determined some factors that may influence the occurrence of AE/Viable in frozen/thawed stallion sperm. In Experiment 1, to determine the effect of seminal plasma exposure to sperm, ejaculated (EJ) or epididymal (EP) sperm from 10 various stallions (n = 10) were cryopreserved. In Experiment 2, frozen/thawed sperm (n = 14 ejaculates, 7 stallions) were thawed and incubated in Lac-MW, Lac-MW with 50 mM added L-carnitine (Lac/Car-MW), or Lac-MW with 0.5 mM added penicillamine (Lac/Pen-MW). In both Experiments, after thawing, sperm were processed by density gradient centrifugation (40% silica particle solution), diluted to 30 x 10^6 sperm/ml in Lac-MW, and incubated for up to 6 hours at 38.2°C in 5% CO_2. At 0, 2, 4, and 6 hours of incubation, sperm aliquots were analyzed by flow cytometry for viability (% VIAB) and acrosomal status in viable sperm (AE/Viable). Data were rank-transformed for normalization and analyzed using the t-tests, the mixed linear model, and the Tukey-Kramer adjustment test. Statistical significance was set at $p < 0.05$. In Experiment 1, at all-time points, VIAB (0 hours: 52 versus 52%; 2 hours: 50 versus 53%; 4 hours: 49 versus 49%; 6 hours: 50 versus 50%, respectively) and AE/Viable (0 hours: 8 versus 6%; 2 hours: 16 versus 17%; 4 hours: 26 versus 30%; 6 hours: 38 versus 41%, respectively) were similar between EJ and EP groups ($p > 0.05$). In Experiment 2, at all time points, VIAB (0 hours: 63 versus 63 versus 58%; 2 hours: 63 versus 55 versus 60%; 4 hours: 58 versus 59 versus 51%; 6 hours: 50 versus 51 versus 53%, respectively) was similar among Lac-MW, Lac/Car-MW, and Lac/Pen-MW groups ($p > 0.05$). At 0 hours (6 versus 5 versus 4%), 2 hours (21 versus 13 versus 26%) and 4 hours (33 versus 20 versus 23%), mean AE/Viable was similar among Lac-MW, Lac/Car-MW, and Lac/Pen-MW groups ($p > 0.05$); whereas at 6 hours, mean AE/Viable was higher ($p < 0.05$) in Lac-MW than in Lac/Car-MW (42 versus 26%;) and similar ($p > 0.05$) between Lac-MW and Lac/Pen-MW (42 versus 40%). Results indicated that lack of exposure to seminal plasma did not negatively affect the ability of epididymal stallion sperm to undergo lactate-induced AE in viable sperm. Also, adding 0.5 mM penicillamine, a thiol and antioxidant compound recently included in the formulation of a medium for conventional IVF in horses,[3] did not negatively affect the occurrence of lactate-induced AE in frozen/thawed stallion sperm. Current studies are focused on utilizing the

Lac-MW model to achieve conventional IVF of in vitro-matured equine oocytes using frozen/thawed stallion sperm.

Keywords: Stallion sperm, acrosomal exocytosis, frozen/thawed semen, lactate, seminal plasma, penicillamine

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Megestrol acetate medication error induced diabetes mellitus in a cat

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A 2-year Bengal cat (2.73 kg) was evaluated for suppression of estrus, due to cardiac abnormalities and risk of anesthesia for surgical sterilization. Additionally, the cat was to have a cardiology examination. Cat was the offspring of an accidental mating where 2 sibling female cats were produced that had congenital cardiac concerns. On auscultation, there was right sided systolic II/VI murmur; cat appeared clinically healthy. Cardiology examinations confirmed the murmur and identified a dilated aorta concurrent with right outflow obstruction. Various options were discussed with the client including oral progestogens and melatonin implant, but neither was available at examination. Oral megestrol acetate (0.95 mg/kg once weekly) was prescribed through a compounding pharmacy. A compounded solution of 40 mg/ml was prescribed with daily volume of 0.06 ml. Approximately after 2 months of therapy, cat was presented with the complaint of weight gain, ravenous appetite, and intermittent inappropriate urination. Estrus was suppressed. On interviewing the client, it was determined that the cat was given 0.67 ml of the compounded medication weekly, resulting in a tenfold medication error. Based upon clinical history, diabetes mellitus was suspected, and urinalysis of a free-catch specimen confirmed the diagnosis based upon presence of glucosuria. Urinalysis was otherwise unremarkable. Gradual reduction in medication volume was as follows: 0.3 ml once weekly for 2 weeks; 0.15 ml once weekly for 2 weeks; then 0.06 ml once weekly for suppression of estrus. Reduction was reportedly uneventful and follow up was directed with the regular veterinarian. The clinically normal cat returned the following year for examination, for refill of the product that had been utilized seasonally. Surgical sterilization was not performed to date.

Keywords: Cat, estrus suppression, progestogen, diabetes mellitus, glucosuria, murmur, cardiac anomaly.

Clinical management practices of equine endometritis in India

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Endometritis is a major cause of infertility in mares. Limited information is available on the clinical management practices of equine endometritis in India. The objective of this cross-sectional study was to collect information from veterinarians in India on their diagnostic and treatment practices of equine endometritis. The information was collected using a 25-question survey sent electronically to veterinarians in India. Participation in the survey was voluntary, and all responses were collected anonymously. A total of 102 veterinarians from various states and union territories of India responded to the survey. The participants reported encountering acute infectious endometritis the most (41%), followed by persistent breeding-induced endometritis (29%). Most veterinarians used uterine swabs (69%) and transrectal examinations (60%) for diagnosis of endometritis. Uterine biopsy was rarely used as a diagnostic tool (8%), likely due to its perceived negative impact on fertility (35%) or uncertainty about its effects on fertility (33%). Uterine bacterial culture often detected *Escherichia coli* (39%) and *Streptococcus equi* subspecies *zooepidemicus* (34%) associated with endometritis. Although most veterinarians reported using a combination of systemic and intrauterine antibiotics, there was a substantial number of respondents (24%) did not use culture and antimicrobial susceptibility to select antibiotics. This practice constitutes indiscriminate use of antibiotics and can worsen the already concerning antimicrobial resistance in the country. The results of this study suggested that there is a need for increased awareness and education on equine endometritis, more standardized diagnostic and treatment protocols, and more research on the disease in the Indian context.

Keywords: Horse, endometritis, diagnosis, treatment, antimicrobial resistance

Prenatal ultrasonographic diagnosis of kidney abnormality in an equine fetus

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Prenatal ultrasonographic examination is a potent diagnostic tool frequently overlooked during mid-late pregnancy in the mare. The combination of transrectal and transabdominal examination evaluates the fetus and placenta, providing crucial information about pathological conditions. Aim of this report is to present the evolution of a case of congenital kidney abnormalities. An 18-year Warmblood multiparous broodmare was bred with frozen semen in July 2024. No abnormalities were detected on days 14, 25, 45, and 60 of pregnancy ultrasonographic examinations. At 120 days, in a follow-up examination, the fetal kidneys appeared hyperechogenic and dysplastic (length: 34 and 35 mm). Combined uteroplacental thickness (CUPT) was 4.5 mm and fetal parameters, including activity (grade 2 out of 3), heart rate (118 beats per minute), aortic diameter (5.5 mm), fluid depth, and echogenicity (dark grey), as well as the abdominal and thoracic organs, were within normal limits. Day 143 ultrasonographic images revealed widespread kidney cystic structures. Subsequent pregnancy evaluations had progressive enlargement of the renal cystic structures. Initial differential diagnosis included developmental renal cystic disease (DRCD), fetal kidney dysplasia, and congenital hydronephrosis. DRCD is a congenital or sporadic kidney malformation that occurs early in organogenesis due to abnormal differentiation of the metanephric duct system. The presentation has distinct forms, including aplastic, hypoplastic, obstructive, multicystic, and diffuse patterns. The most frequent forms are autosomal recessive polycystic kidney disease (ARPKD) observed perinatally or neonatally in dogs, cats, sheep, and horses, followed by autosomal dominant polycystic kidney disease (ADPKD), most observed in adult dogs and cats. In the event of fetal kidney dysplasia, it can present as abnormal renal size, structure (cysts), or function. The dysplastic changes during fetal development are dynamic, unilateral, or bilateral; therefore, continuous evaluation is advised. With respect to prenatal hydronephrosis, enlargement or dilation of the kidneys, specifically the renal pelvis, is very characteristic. It can occur unilaterally or bilaterally and is often caused by a blockage in the urinary tract or the reflux of urine from the bladder to the kidneys. Ultrasonography typically reveals lobulated kidneys with anechoic content, devoid of renal parenchyma. However, this pathology was ruled out on day 143 of pregnancy based on the ultrasonographic images that exhibited hyperechogenicity and cystic-like structures. Although the present case did not exhibit all the clinical manifestations of ARPKD, it was initially diagnosed as such. This disease typically manifests as bilateral enlargement of echogenic kidneys, accompanied by hepatic cystic development and dysfunction. Since the kidneys have a crucial role in fetal development, as the disease progresses, it compromises the growth of the fetus. Pulmonary hypoplasia can also occur due to oligohydramnios and the increased pressure in the chest cavity caused by the enlarged kidneys. Renal cystic disorders in equine are rare, and the genetic mutation responsible for the condition remains unknown.

Consequently, fetal well-being, development, and uterine examination throughout pregnancy are essential for early diagnosis of congenital disorders. This case emphasized the importance of ultrasonography in detecting high risk pregnancies and the need for the development of genetic testing for horses.

Keywords: Congenital, horse, polycystic, kidney, fetal development

Development of a diagnostic test for nocardioform placentitis

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Nocardioform placentitis (NP) is a form of mucoid placentitis which results in episodic abortions in mares with few external signs of disease. NP is characterized as a mucoid placentitis in which the bacterial infection is limited to the chorionic surface of the placenta without infection of the fetus, with the ecology and biology of the major causative organisms, *Crossiella equi* and *Amycolatopsis spp.* remaining unknown. As NP is notoriously difficult to diagnose, we hypothesized that we could utilize the technology behind tuberculosis testing in humans to develop a sensitive and specific test for NP in horses. With this methodology, viable peripheral blood mononuclear cells (PBMCs) are extracted from heparinized whole blood, then plated on an equine interferon gamma enzyme-linked immunosorbent spot plate at 25 x 10^4 cells/well. Three wells were exposed to media plus Nocardioform-specific antigens (Ag) whereas another 3 were exposed media alone (Con). Mares were considered positive when their PBMCs had > 2-fold increase in the number of IFNy producing cells in Ag-treated wells with a $p < 0.05$. Fetal membranes were evaluated by the University of Kentucky Diagnostic Laboratory. In 2024, 30 mares were successfully evaluated, including 8 with NP. In 2025, 60 mares deemed 'at-risk' by farm managers were enrolled in a prospective study with monthly blood samples. An additional 15 mares have enrolled with suspected disease based on clinical signs or transabdominal ultrasonography results. In 2025, finalized diagnostic reports were available for 19 mares thus far, with the remaining 56 mares yet to foal. Across both 2024 and 2025, the test had 83.3% sensitivity and 88.37% specificity with 10 true positives, 38 true negatives, 5 false positives and 2 false negatives. Currently, work is underway to optimize the test and assess repeatability. Data will be updated as after mares foaling and necropsies of fetal membranes.

Keywords: placentitis, nocardioform, mare, diagnostic test

The unique framework of the equine fetal gonad for the synthesis of estrogen precursors

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In the 11-month equine pregnancy, the fetal gonad delivers androgen precursors for estrogen synthesis to the placenta. Two of these estrogens, estrone and equilin, differ by 1 bond. This structural variation arises from their distinct synthesis pathways: estrone is derived from cholesterol, whereas equilin is derived from its precursor, 7-dehydrocholesterol (7-DHC). Because estrone and equilin synthesis periods overlap but peak at various stages of pregnancy, we hypothesized that the synthesis of their androgen precursors is separated spatially and temporally in the equine fetal gonad. To investigate how the estrogen profile in pregnancy is regulated, we conducted RNA-seq on 21 fetal gonad samples collected between the 4th and 11th months of pregnancy. We examined key elements in cholesterol synthesis (*ACAT2, HMGCS1, HMGCR, MVK, PMVK, MVD, FDPS, FDFT1, SQLE, LSS, DHCR24, CYP51A1, LBR, TM7SF2, MSMO1, NSDHL, HSD17B7, EBP, SC5D, DHCR7*) and early steroid hormone production (*CYP11A1, CYP17A1*). *DHCR7*—the enzyme that converts 7-DHC to cholesterol—was progressively downregulated throughout the pregnancy (Log_2 fold change (FC) = −3.7; $p_{adj} < 0.001$), correlating with decreased estrone synthesis. Additionally, we noted less pronounced downregulation of the HMG-CoA reductase (*HMGCR*), and 24-dehydrocholesterol reductase (*DHCR24*), both involved in cholesterol synthesis, in the 10th versus 6th months of pregnancy (Log_2FC=-2.3 and Log_2FC=2, respectively, $p_{adj} < 0.05$). This suggested their role in the overall decline in steroid hormone synthesis observed towards parturition. No other differential expression was observed. We next asked if the synthesis of estrone and equilin precursors was separated spatially in the hormonally active gonadal interstitium. To test this, we first studied the interstitium for *CYP11A1* and *CYP17A1* expression via immunofluorescence to identify regions responsible for the synthesis of the androgen precursors. Next, we used RNA scope and immunofluorescence to investigate the potential compartmentalization of *DHCR7* expression within this population. RNA scope had the highest mRNA compartmentalization in the 4th of pregnancy, with a decline with the progression of pregnancy, and immunofluorescence had less pronounced compartmentalization in the male samples in the 6th month of pregnancy. These findings are in agreement with our hypothesis and suggest that the expression of *DHCR7* may be a limiting factor of the synthesis of estrone, and that

equilin and estrone precursors are synthesized in separate compartments of the equine fetal gonad interstitium.

Keywords: Estrone, equilin, DHCR7, cholesterol, 7-DHC

Assessment of fetal ultrasound parameters for predicting parturition date in mares

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Foaling is a rapid event and any complications can quickly compromise the foal. This necessitates the presence of skilled personnel to assist or promptly detect dystocia, ultimately improving the survival rates of the offspring. The variable length of pregnancy in mares makes predicting the foaling date challenging. The pH and calcium concentrations of mammary secretions are commonly used but still lack reliability in some cases. We aimed to utilize certain fetal developmental characteristics to enhance the accuracy of foaling date prediction. Healthy pregnant mares (n = 8) with known ovulation dates were monitored weekly from day 310 of pregnancy to parturition by transabdominal and transrectal examinations. Transrectal ultrasonography was performed to evaluate the size of the navicular bone, as well as the diameters of the internal (IOS) and external (EOS) cervical os. Transabdominal ultrasonography was performed to assess the timing of fetal stomach rugae appearance and sustained gastrointestinal peristalsis lasting over 30 seconds. On presentation of mammary secretions, calcium concentrations and pH were measured using a Foal Watch test kit and a commercial pH meter. Regression analysis was performed to evaluate the relationship between the measured parameters and the number of days to parturition. Navicular bone size was significantly associated with the number of days to parturition ($R^2 = 0.52$, $p < 0.001$). Both pH ($R^2 = 0.36$, $p = 0.003$) and calcium concentrations ($R^2 = 0.35$, $p = 0.007$) had moderate association with days to parturition. The diameter of IOS, EOS and GI peristalsis scores were also significantly associated with days to parturition ($p = 0.02$, $p = 0.04$, and $p = 0.005$, respectively). However, their predictive power was weak ($R^2 = 0.1$, $R^2 = 0.1$, and $R^2 = 0.2$, respectively). The presence of stomach rugae was scored on a scale from 0 to 2 (0- stomach rugae not present; 1-stomach wall slightly irregular; 2- stomach rugae present). A significant association was there between the rugae scores and days to parturition ($R^2 = 0.35$, $p < 0.0004$), with mares displaying a score of 2 predicted to foal within 6 days. Results demonstrated that among the examined parameters, navicular bone size was the most reliable predictor of parturition timing, whereas pH, calcium concentrations, and gastrointestinal scores had moderate associations. Further validation is needed to enhance predictive accuracy in equine reproductive management.

Keywords: Foaling, parturition, gestation, mare, ultrasound

Use of endotracheal tube as a long-term stent for chronic pyometra in a mare

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Pyometra in mares is a chronic inflammatory condition characterized by the accumulation of inflammatory fluid in the uterus due to impaired uterine clearance, cervical dysfunction, and/or poor vaginal conformation. Unlike in other species, pyometra is relatively rare in mares but poses a substantial risk for subfertility or infertility. The prognosis for restoring fertility is poor, especially in cases of cervical closure leading to a closed pyometra with extensive intrauterine fluid accumulation. Treatment options include medical management and surgical intervention, with ovariohysterectomy as the most definitive but challenging procedure due to the risk of postoperative complications. A 17-year Warmblood maiden mare was presented with chronic pyometra, with the main complaint being discomfort during riding with frequent urination. On examination, cervical adhesion and abnormalities were diagnosed using digital manipulation and endoscopy. Uterine lavages and cervical dilations had been previously performed multiple times without success. As breeding was not intended for the mare, an endometrial biopsy was not performed. Due to the mare's age, and the cost and risks of an ovariohysterectomy, the owner opted for a nonsurgical option. A sterile 9.5 mm cuffed endotracheal tube was placed inside the cervix after manual dilation, and the cuff was inflated with sterile water. Accurate placement was confirmed via transrectal ultrasonography and verified at 1 week, 3 weeks, 2 months, 6 months, and 1 year. Subsequent reexaminations for up to 1 year revealed no fluid within the uterus, effective uterine drainage and appropriate cervical tube position. This report highlighted a case in which an endotracheal tube was successfully used as a cervical stent following manual cervical dilation to maintain continuous uterine drainage. The mare remained symptom-free for up to 1 year, demonstrating good clinical progress and normal performance. Although potential complications, such as stent loss, exist, the cervical stent provides a simple, cost-effective, and minimally invasive alternative for long-term management of pyometra, particularly in cases where surgery and general anesthesia pose substantial risks or where financial constraints are present.

Key words: Pyometra, mare, cervix, stent, nonsurgical treatment

Vulvar injection of 2.5% iPAAG Hydrogel to improve perineal conformation

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A multiparous barren 10 year old Thoroughbred mare with evidence of vulvar and vestibular trauma was presented for breeding management. There was a dehisced Caslick in place which failed to provide a barrier to the internal reproductive tract. The mare's uterus was completely distended with air to the tips of both uterine horns, preventing transrectal ultrasonographic imaging of the uterus. Multiple small follicles were present on each ovary and the mare was hyperreactive to any vulvar manipulation. Over a one year period, multiple Caslick operations were performed, reinforced, repaired, and ultimately destroyed when the mare aborted.

At the beginning of the following breeding season, 10 milliliters of ArthramidVet 2.5% polyacrylamide hydrogel was injected into the dorsal vulvar lips at the level of the perineal body. A visible bulge was appreciated at the injection sites, and a temporary horizontal mattress suture was placed just dorsal to the perineal body to improve the vulvar seal. The mare was bred via live cover and double ovulation was confirmed. After ensuring uterine fluid clearance, a Caslick operation was performed with additional horizontal mattress sutures to relieve tension on the primary sutures. The mare was confirmed pregnant with twins at 14 days post ovulation and a manual twin reduction was performed; the Arthramid-induced bulges remained visually appreciable at this time (27 days post injection). The vulvar seal remained adequate, as evidenced by lack of intravaginal air seen on repeat ultrasound examinations. The injection site bulges were grossly visible for approximately 90 days. At 120 days following Arthramid injection, the Caslick and horizontal mattress sutures were removed, leaving fully sealed vulvar lips. The injection site bulges were no longer visually appreciable. A pinpoint defect was evident at the level of the perineal body; the edges were freshened and a single suture placed to address the defect. The mare was pregnant as of routine fall examination.

This case describes the use of a hydrogel product to increase the tissue bulk of the vulvar lips. This created enough tissue bulk and vulvar seal to allow for live cover breeding, standard breeding management of the mare, and the Caslick to heal without being under tension. The downsides of this technique are the volume of hydrogel, in that a volume greater than 10 ml would be beneficial but is cost prohibitive for most clients, as well as the finite lifespan of the product, so in some cases re-application may be necessary after 90-100 days. Potential long term effects on the character of vulvar tissue have yet to be evaluated.

Keywords: Equine; mare; Caslick; vulvoplasty; hydrogel

Seminal microbiome and its influence on the mare uterine microbiome

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The functional role of the semen microbiome has been explored in women but not in domestic species; studies demonstrated its interaction with the vaginal and uterine microbiome changes the microbiome composition and effects on physiological functions and fertility. High bacterial richness in semen was linked to dysbiosis in the vaginal microbiome of couples and to cases of subfertility. This study was designed to study the effects of the seminal microbiome on the mare uterine microbiome; we hypothesized that the semen and seminal plasma microbiome of the stallion differs from the jack and that could trigger distinct changes in the mare uterine microbiome. Fertile mares (n = 15) were serially monitored using transrectal ultrasonography to detect signs of estrus. Once in estrus, mares were inseminated once with fertile jack or stallion raw semen (2×10^9 total sperm) with seminal plasma from either male or saline in a crossover design. A sample of uterine fluid was collected before insemination, at 6 and 24 hours after insemination, and at embryo flush (8 days after ovulation). Bacterial DNA was purified from semen, seminal plasma, and uterine fluid with a commercial kit and submitted for PCR amplification and sequencing of the full-length 16S region. Taxonomy assignments were done via DADA2 using the Silva database, and R was used for the analyses. The dataset was filtered and agglomerated per rank to the family level. Alpha and beta diversity indexes were calculated and compared with the Wilcoxon rank-sum test, a linear mixed model, and PERMANOVA; significance was set at $p < 0.05$. Alpha diversity (Faith's PD index) differed ($p = 0.048$) between donkey and horse semen, whereas beta diversity was not different ($p > 0.05$) across species. The most prevalent phyla in mares bred to the donkey were Proteobacteria (50.3%), Actinobacteria (18%), and Firmicutes (29.8%). Similarly, in mares bred to the horse, Proteobacteria (62.5%), Actinobacteria (21.7%) and Firmicutes (12.3%) were the most abundant. Noteworthy, bacteria from the order of Lactobacillales contributed up to 20% of the microbiome composition in mares bred to the donkey and up to 6% in mares bred to the horse. Twenty-four families of bacteria were in common between donkey semen and the uterus after the insemination, and 9 were identified between horse semen and the uterus. Species richness and evenness (alpha diversity) were not different in mares with a positive or a negative embryo flush (Observed ASVs, $p = 0.11$; Chao1, $p = 0.22$; Shannon, $p = 0.91$; Simpson, $p = 0.19$, Faith's PD, $p = 0.70$). The beta diversity of the uterus 8 days after ovulation differed based on the embryo outcome, in both mares bred to the donkey and the horse ($R^2 = 0.069$; F-value = 2.01; $p = 0.02$). These results revealed that seminal microbiome affected uterine microbiome and suggested for the first time in the horse that there is a temporary combined male and female microbiome with consequences on physiological reproductive functions like the establishment and development of an early pregnancy.

Keywords: Interspecies breeding, donkey microbiome

Glucuronide estrone and estradiol secretion in pregnant mare serum: insight from a liquid chromatography tandem mass spectrometry analysis

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The equine placenta produces conjugated estrogens: research mainly focused on sulfonated estrogens, although glucuronide estrogens were observed in early studies. These preliminary investigations were performed using immunoassays that lacked specificity for small molecules. However, the higher analytic accuracy of liquid chromatography tandem mass spectrometry (LC-MS/MS) enables to refine the knowledge about estrogens produced during equine pregnancy. This study aims to describe the evolution of estrone and estradiol glucuronide (E1G and E2G) during equine pregnancy in 2 breeds and to determine if their secretion is linked to native and sulfonated estrone or estradiol (E1, E1S or E2, E2S). Between 2020-2024, serum samples were collected monthly from 18 Warmbloods (WB) and 24 Spanish purebred (SPB) pregnant mares. From 4 months of pregnancy onward, the combined thickness of the uterus and placenta (CTUP) was measured by ultrasonography. Mares with enlarged, heterogeneous CTUP or clinical signs of placentitis pre or postpartum (premature lactation, vulvar discharge, abortion, abnormal macroscopic placenta, foal weakness) were excluded from this study. A dedicated LC-MS/MS method was used to assay serum E1, E2, E1S, E2S, E1G, and E2G. For the 93 pregnancies included, monthly hormone changes were assessed using the Kruskal-Wallis test, and potential concentration differences between breeds were studied with the Mann-Whitney test. Nonparametric Spearman's tests were used to assess correlations between E1G, E2G, E1, E2, E1S, and E2S. Data were reported as median (25^{th} percentile, 75^{th} percentile) with significance set at $p < 0.05$. The peak concentration of E1G (12,068 (6,53015,751) pg/ml) was observed at 2 months, whereas the peak value of E2G was lower (1,692 (1,0362,000) pg/ml) and observed at 4 months. There was a breed effect for both hormones, but profiles differed; E1G concentrations were higher in WB from 6-10 months of pregnancy, whereas higher E2G concentrations were observed in SPB at 3, 7, and 8 months. Concentrations of E1G were poorly correlated with other studied estrogens, with its strongest correlation observed with E1S ($r = 0.47$, $p < 0.0001$). Positive correlations were observed between E2G and E1 ($r = 0.66$, $p < 0.0001$), E1S ($r = 0.71$, $p < 0.0001$), and E2S ($r = 0.70$, $p < 0.0001$). To the best of our knowledge, LC-MS/MS was never used to describe E1G and E2G kinetics during pregnancy, and differences in their concentrations between breeds of different sizes were not reported. Although glucuronosyltransferase genes are constantly expressed by the endometrium during pregnancy, maximum E2G concentrations were observed at 4 months, before the E1, E2, and E1S peaks at 5 months. Surprisingly, the E1G peak occurred earlier in pregnancy, 1 month after the onset of eCG production by endometrial cups, which has an FSH-like effect

normally promoting follicular E2 secretion. This unique E1G kinetic during pregnancy was confirmed by the absence of correlation with E1, E1S, E2G, and E2S, which all correlated, suggesting different pathways of production and effects of E1G. Further studies should confirm the origin of early E1G production in pregnant mares and physiological effects of its secretion after implantation.

Keywords: Liquid chromatography tandem mass spectrometry, sulfonated estrogens, glucuronide estrogens, mare, pregnancy, placenta

Follicular dynamics in insulin resistant mares

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Obesity and insulin resistance have been linked to prolonged interovulatory period, aberrations in the estrous cycle, and continuous reproductive activity during the nonbreeding season. Equine metabolic syndrome has been determined to influence the intrafollicular environment of mare ovaries. In humans, insulin resistance has been linked to polycystic ovaries as part of polycystic ovarian syndrome. A study was conducted to determine 1. the impact of insulin resistance on follicle growth and size at ovulation, and 2. whether predicted ovulatory follicles respond to human chorionic gonadotropin (hCG) treatment in insulin resistant (IR) mares. Mares were selected for the study based on insulin sensitivity (IS) and separated into an IR group (n = 6) and an IS group (n = 6). The ovaries and uterus were examined via transrectal ultrasonography at regular intervals during a spontaneous cycle and a $PGF_{2\alpha}$ shortened synchronized cycle. The dominant follicles (F1) had similar size and F1 size at ovulation between groups. There were more subordinate follicles in IR than IS mares ($p < 0.05$). The second largest follicle (F2) of IR mares was larger in diameter ($p < 0.05$) than the F2 of IS mares, which may signify a lack of dominance by the largest follicle. Treatment with hCG induced ovulation before 48 hours in 2 out of 4 IR mares, whereas 4 out of 4 in IS mares, although difference in time to ovulation after hCG treatment did not differ statistically. Results observed in this study may provide caution to practitioners working with IR mares with regards to numbers and sizes of secondary follicles and the effectiveness of hCG for induction of ovulation. The results of this study may support information that the mare could be used as a model to study human ovarian pathologies.

Keywords: Follicle, dynamic, insulin resistance, mare

Effect of follicle stimulating hormone commercial source and in vitro maturation medium formulation on equine oocyte maturation, cleavage, and blastocyst rates after intracytoplasmic sperm injection

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In vitro production of equine embryos by intracytoplasmic sperm injection (ICSI) of in vitro matured oocytes is a customary procedure in the equine breeding industry. Media utilized to induce oocyte in vitro maturation (IVM) is supplemented with hormones, particularly follicle stimulating hormone (FSH),[1] or combinations of FSH, luteinizing hormone (LH), and estrogens.[2] To date, comparisons among different commercial sources of FSH or IVM medium formulation on the efficiency of ICSI in horses are scarce. In this study, we sought to determine whether differences in the commercial source of FSH or the IVM medium formulation would impact the efficiency of ICSI in equine oocytes. In Experiment 1, 2 commercial sources of porcine FSH (Sioux Biochemical [SI] versus Prospec [PRC]; 5 mU/ml) added to a Medium 199 (M199)-based IVM medium were compared. In Experiment 2, 2 IVM media formulations (M199-FSH versus FSH + LH-ready-to-use commercial medium [IVFBS]) were compared. For all experiments, cumulus oocyte complexes (COCs) were recovered via transvaginal oocyte aspiration from 13 mares, aged 8-17 years, held overnight (12-18 hours) at 22°C, and then incubated in a humidified 5% CO_2 atmosphere at 38.2°C for 30 hours. In vitro matured oocytes were fertilized by Piezo-driven ICSI using frozen/thawed sperm from a single fertile stallion, presumptive zygotes were cultured from days 0-5 at 38.2°C in a 5% CO_2/6% N_2 humidified atmosphere, and cleaved embryos (> 8 blastomeres) were further cultured for up to 5 more days in a 5% CO_2/6% N_2 humidified atmosphere. On days 7-10 after ICSI, blastocyst development was recorded. Differences in IVM, cleavage, and blastocyst rates per injected oocyte were compared by Fisher's exact test (JMP Pro 17). In Experiment 1, 191 follicles were aspirated and 121 COCs were recovered (SI [n = 68]; PRC [n = 53]). In vitro maturation (59% [n = 40] versus 60% [n = 32]), cleavage (65% [n = 26] versus 63% [n = 20]), and blastocyst rates (35% [n = 14] versus 34% [n = 11]) were similar for SI and PRC, respectively ($p > 0.05$). In Experiment 2, 353 follicles were aspirated and 226 COCs were recovered (M199-FSH [n = 113]; IVFBS [n = 113]). In vitro maturation (52% [n = 59] versus 61% [n = 69]), cleavage (64% [n = 38] versus 74% [n = 51]), and blastocyst rates (31% [n = 18] versus 25% [n = 17]) were similar for M199-FSH and IVFBS, respectively ($p > 0.05$). Overall, the results of this study indicated that 2 commercial sources of FSH (SI versus PRC), and 2 IVM medium formulations (M199-FSH vs. FSH + LH ready-to-use commercial medium [IVFBS]) yielded similar oocyte maturation, cleavage, and blastocyst rates following ICSI. These results provided helpful information for ICSI laboratories regarding some factors that may or may not affect the efficiency of ICSI in horses.

Keywords: Horse, oocyte, intracytoplasmic sperm injection, in vitro maturation

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Chronic endometritis in a Thoroughbred mare with *Bordetella bronchiseptica*

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A 3-year, Thoroughbred mare presented for evaluation of subfertility with a history of chronic infectious endometritis. Throughout the breeding season, multiple samples including uterine swabs, low volume uterine lavage (LVL), and a uterine aspirate were submitted for aerobic culture and cytology, revealing growth of *Pseudomonas putida* (*P. putida)* and β-hemolytic *Streptococcus* spp. Despite intrauterine treatments based on antimicrobial sensitivity, no pregnancies were achieved over 2 estrous cycles. Evaluation of the mare's perineum revealed a vertically positioned vulva with two-thirds distal to the pelvic brim. A Caslick was in place; however, the dorsal vulva and perineal body were markedly flaccid. Notable findings on transrectal ultrasonography included fluid throughout the uterine lumen and air present in the cranial vagina. Hysteroscopy showed inflammation of the endometrium with the right horn appearing to have more severe, chronic inflammation. A direct uterine fluid aspirate obtained via hysteroscopy was submitted for aerobic culture and cytology. N-acetylcysteine was infused. Ultrasonographic examination the following day revealed cloudy fluid with swirling debris. Uterine lavage was performed and submitted for aerobic culture and cytology. Cultures were consistent with *Bordetella bronchiseptica (B. bronchiseptica)*. Due to the similarities between *P. putida* and *B. bronchiseptica,* both oxidase-positive and appearing as small, grey, mucoid colonies on agar plates, the samples were submitted for identification. The isolate was positively identified as *B. bronchiseptica* via Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry (MS). After reviewing the mare's historic culture results it was hypothesized that the previously identified *P. putida* samples were *B. bronchiseptica*. Treatment was aimed at correcting anatomical abnormalities and treating infectious endometritis. A Gadd procedure was performed. Intra-uterine ozone therapy utilized a combination of ozonated sterile saline lavage followed by insufflation of the uterus with ozone gas once daily for 5 days while the mare was in estrus[1]. This alternative therapy was chosen due to the mare's history of receiving multiple intrauterine antibiotics. Four weeks later, a uterine swab yielded no bacterial growth on aerobic culture. Unfortunately, *B. bronchiseptica* was detected again several weeks later when the mare was cultured prior to breeding. Mare was treated systemically with chloramphenicol for 2 weeks, and repeat culture resulted in no bacterial growth. Cytology was collected and submitted for genomic mapping. Shotgun metagenomic analysis produced a low number of reads of *Bordetella spp.,* confirming MALDI-TOF results but signaling low confidence that a substantial amount of the bacterium was present within this sample. At this time the mare is due to be bred this season.

B. bronchiseptica is a gram-negative bacterium known as a respiratory disease-associated pathogen in various species, notably dogs and swine. Although detecting it in the uterus is rare, it has been reported twice; in a mare with infertility[2] and an aborted equine fetus[3]. This report adds to our knowledge of this uncommon occurrence. Given its similarities to *P. putida*, *Bordetella* infections may be underreported due to misclassification. This abstract highlighted the need for advanced diagnostics, such as MALDI-TOF MS, to improve accuracy in bacterial identification, essential for appropriate clinical management and treatment.

Keywords: *Bordetella bronchiseptica*, endometritis, mare, ozone, nonantibiotic

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Phenotypic variation in female caprine XX/XY hematopoietic chimeras

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XX/XY hematopoietic chimerism is caused by the anastomosis of placental vasculature between male and female fetuses, resulting in the exchange of hematopoietic stem cells. This phenomenon is common in cattle, with bovine XX/XY blood chimeric females typically being infertile but, presenting with variability in gonadal and uterotubular development due to exposure to male factors. This case report describes 2 female, caprine XX/XY hematopoietic chimeras (A and B) characterized phenotypically through physical examination, hormonal assays, diagnostic assessment of the reproductive tract and pathology, and blood and tissue genotyping. Goat A, was born triplet to 2 male littermates and goat B, was born triplet to a female and male littermate. At birth, both presented as horned, Saanen female goats. At 4-months, Goat A developed secondary male sex characteristics, including an enlarged poll with long hair and a full beard, vulva with coarse, excessive hair and an enlarged clitoris, accompanied by unusually small, short, narrow teats and a blinded end vagina, approximately 4.5 cm in length. Goat A underwent a necropsy which revealed a short vagina, with no discernable uterus and 2 small gonads with a smooth homogenous surface. Both gonads were attached to a tubular structure curving around the gonad from the cranial poll and both were associated with a vascular plexus. Goat B appeared phenotypically unremarkable with no secondary male sex characteristics. A vaginal examination revealed a normal vaginal length of 15.4 cm and the visualization of the external cervical os. Goat B responded to an estrus synchronization protocol and underwent laparoscopic examination, artificial insemination and visualization of uterine horns, oviducts, and ovaries (had follicular activity). However, Goat B failed to conceive after insemination or natural mating on 6 subsequent cycles, although progesterone concentrations were consistent with corpus luteum formation. Antimüllerian hormone (AMH) and testosterone concentrations were measured from birth through puberty along with age-matched male (n = 7) and female (n = 6) herd mates. At birth, both goats had AMH concentrations similar to the male controls. After 3 weeks, Goat A had evidence of endogenous AMH production in the female range. After 7 days, Goat B had AMH concentrations that were at or below the limit of detection. Peripubertal testosterone concentrations of goat A were between the male and female control ranges, whereas goat B had testosterone concentrations in the female control range at all timepoints. XX/XY hematopoietic chimerism was confirmed by PCR for X and Y specific genes. Chimerism was confined to the hematopoietic compartment with DNA isolated from hair follicles being positive for only the X chromosome (based on *AME* genotyping), whereas blood was positive for Y chromosome genes, *SRY* and *AME Y1,* as well as *AME* on the X chromosome. Karyotyping revealed XX and XY lymphocytes in both animals.

Additionally, microsatellite marker testing revealed some loci with 3 alleles identified in blood, but only 2 in hair, consistent with hematopoietic chimerism. This report demonstrated the variable phenotype of caprine XX/XY blood chimeras ranging from substantial masculinization of a female to infertility in an otherwise classic, phenotypic female.

Keywords: Goat, hematopoietic chimerism, anti-müllerian hormone, disorder of sexual differentiation

Extracellular vesicles secreted by mouse oviductal organoids: a model for contraceptive development

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Extracellular vesicles (EVs) are nanoparticles secreted by cells for intercellular communication via the transfer of bioactive molecules, such as lipids, proteins, and nucleic acids. Organoids are 3D, spherical cell clusters formed in vitro that are capable of long-term proliferation and self-organization with similar function to their tissue of origin. EVs produced by organoids are more similar to EVs produced in vivo than those produced in traditional 2D cell culture models because organoids retain more in vivo-like properties. The objectives of this study were to 1. generate mouse oviductal organoids using 2 culture conditions (static versus bioreactor) and 2. isolate and characterize EVs secreted by the mouse oviductal organoids. Our hypothesis was that EVs produced by mouse oviductal organoids share the same protein markers identified in EVs collected from the in vivo mouse oviduct: CD9 and Hsp70. Mouse oviductal cells (n = 3 mice) were used to generate organoids that were cultured for up to 189 days (passaged every 7 days) either in a static organoid culture system or a dynamic CERO (Omni Life Sciences) bioreactor culture condition. Organoids were assessed via brightfield microscopy for maintenance of phenotype throughout the culture period. The spent culture medium from each culture condition was collected for isolation of EVs via differential ultracentrifugation. EVs were assessed for 1. size and concentration using nanoparticle tracking analysis (NTA), 2. morphology using transmission electron microscopy (TEM), and 3. the presence of EV-associated protein markers using a Jess Automated Western Blot system (Bio-techne). Brightfield imaging of mouse oviductal organoids demonstrated round cellular clusters with a central lumen for both static and bioreactor culture conditions, consistent with previous mouse oviductal organoid reports. EVs isolated from the static culture condition spent medium (starting volume = 20 ml) had a median diameter of 134 nm with a concentration of 9.4×10^9 particles/ml, and the EVs from the bioreactor culture condition spent medium (starting volume = 9.5 ml) had a median diameter of 159 nm diameter and a concentration of 13×10^9 particles/ml. Transmission electron microscopy demonstrated the characteristic 'cup-shaped' morphology for the EVs, and Jess analysis demonstrated the presence of EV proteins CD9 and Hsp70 and absence of the cellular contaminant protein CYCS. Data demonstrated that mouse oviductal organoid EVs express proteins expected in EVs secreted by mouse oviductal cells in vivo. Additionally, more EVs were produced per milliliter of spent medium in the bioreactor compared to the static culture condition; thus, the bioreactor appeared better suited for EV production from organoids. Future experiments will use mouse oviductal organoids to test novel contraceptives, such as EVs containing CRISPR-cas9 ribonucleoproteins that target essential genes for fertility, including *PGR* and *OVGP1*. Research reported here was supported by the Office

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Keywords: Fallopian tube, 3D culture, murine, exosome

Alpaca with a history of dystocias

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A 5-year alpaca presented for dystocia on day 366 of pregnancy. The alpaca had a history of 3 previous pregnancies that resulted in dystocia, with 1 cria surviving. Stage 1 labor began the morning before presentation but failed to progress over the next 9 hours. On admission, the alpaca was quiet, alert, and responsive, with normal vital parameters. Clinical examination revealed decreased gut sounds, mildly injected ocular conjunctiva, hyperglycemia, an elongated vulva, a relaxed sacrosciatic ligament and perineum, absence of abdominal contractions, and minimal cervical dilation. Transabdominal ultrasonography confirmed fetal death. To promote cervical dilation, 5 ml N-butylscopolammonium bromide was applied topically to the cervix. Additionally, 10 ml lidocaine 2% mixed with lubricant was given rectally to facilitate transrectal palpation and rule out uterine torsion, which was not diagnosed. Vaginal palpation revealed a fetus in a dorsopubic position with neck flexion. Following substantial obstetrical manipulation, a nonviable 9.98 kg fetus (average; 6.9-8.4 kg[1]) was delivered vaginally. The fetus was submitted for necropsy, and findings were unremarkable. Dystocia occurs in 2-5% of alpaca pregnancies, with < 1% requiring intervention.[2] Common maternal causes include uterine torsion, inertia, and cervical dilation failure.[2,3] Fetal causes primarily comprise of fetal malposition or congenital abnormalities.[3] Recurrent dystocia in this case suggested a maternal factor, though fetopelvic disproportion remains a consideration, given the cria's above average size and the need for obstetrical manipulation. Uterine inertia, secondary to prolonged stage 1 labor, and incomplete cervical dilation, further impeded parturition. Absence of cervical dilation has been noted as a cause of dystocia in alpacas, resembling a condition described in sheep as 'ring womb'.[4,5] This case illustrated the importance of timely obstetrical intervention during dystocia and supported the use of N-butylscopolammonium bromide to promote cervical dilation during parturition.

Keywords: Hembra, alpaca, camelid, dystocia, incomplete cervical dilation

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Effect of endometrial cyst removal via laser on pregnancy rates in the mare

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Lymphatic and glandular cysts arise from collections of lymphatic fluid within the endometrium or myometrium, due to obstructed lymphatic channels or the gravitational effects of a pendulous uterus. The incidence of endometrial cysts in the general mare population has been reported as 1-22%, with subfertile and older mares up to 55%. Endometrial cysts may reflect senility of the uterus by the correlation between the number of cysts and severity of pathological changes. The aim of this study was to determine the effect of endometrial cyst removal ablation via laser on pregnancy rates in barren/subfertile mares. Removal of endometrial cysts should improve embryonic mobility, growth during fixation, and maternal recognition of pregnancy, thereby increasing pregnancy rates. A retrospective study was performed identifying 240 mares with endometrial cyst ablations over a 10-year period. All mares had a history of being bred and not achieving a pregnancy for at least 1 cycle, or of early embryonic loss. Hysteroscopic examination was performed using a 1.68-meter long, 12.8-mm diameter fiberoptic Olympus colonoscope, on mares in diestrus or under the influence of exogenous progesterone or progestins. Uterine dilation was achieved with lactated Ringer's solution allowing both horns to be traversed and visualization of the oviductal papillae. Endometrial cyst ablation was performed using a Ceralas D25 (CeramOptec) 980 mm Nd:YAG laser fiber through the biopsy channel with penetration of the endometrial cysts at 20-22 watts in multiple regions of the cyst until lymphatic fluid was released, the epithelium heated, and collapse occurred. Mare age, number of cysts, hysteroscopic observations, postprocedure treatment, and 14-day pregnancy rates were analyzed. Statistics were performed with SAS 9.4, and all data was assessed for normality and equal variances. The impact of age on pregnancy and number of cysts was evaluated utilizing a Chi-square test. Of the 196 mares with pregnancy data, 62% of mares became pregnant following ablation of cysts. This was not impacted by age ($p = 0.958$). Most mares became pregnant in 1 (31%) or 2 (26%) estrous cycles. This was again not impacted ($p = 0.615$) by age. Age did not influence ($p = 0.276$) the number of cysts but did range immensely. Age of mares with < 2 cysts ranged from 7-21 years with pregnancy rates 17 out of 22 (77%), 3-5 cysts ranged from 11-25 years with pregnancy rates 17 out of 19 (89%), and >5 cysts ranged from 13-24 years with pregnancy rates 6 out of 10 (60%). The above data revealed that laser ablation of endometrial cysts improved pregnancy rates as all mares had been bred prior and failed to become or maintain a pregnancy. In addition, since the average age of the mares was 17.5 years, the pregnancy data are even more clinically relevant.

Keywords: Endometrial cysts, hysteroscopy, laser, blocked lymphatics

Testicular asymmetry reported after vasectomy in a miniature pinscher dog

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A 1-year intact male miniature pinscher was presented for lack of penile erection and testicular asymmetry after elective vasectomy performed at another clinic 2 months before presentation. On physical examination, the only abnormality was testicular asymmetry. On reproductive examination, the penis could be exteriorized to the base of the bulbus glandis without resistance. The left testis (6 x 9 mm) was substantially smaller than the right (12 x 23 mm), and the left spermatic cord was thickened. On ultrasonography, the left testis was hypoechoic; the left epididymis was markedly smaller than the right, and hyperechoic lesions were observed in the left spermatic cord. Color Doppler ultrasonography revealed no blood flow through the left pampiniform plexus and testis. The right testis was normal in size, consistency, and ultrasonographic appearance. As the right epididymis was enlarged on palpation and ultrasonography, *Brucella canis* infection was ruled out by serological testing. Bilateral castration and testicular histopathology were recommended. Although vasectomy can lead to atrophy of the seminiferous epithelium and reduced testicular volume, testicular atrophy in this case was unilateral. It is unknown whether the dog had testicular asymmetry prior to the surgery. The pathology of the left testis and spermatic cord may have resulted from congenital hypoplasia or severe ischemic atrophy following vasectomy due to accidental ligation of the pampiniform plexus. Ischemic necrosis would reduce testosterone production that may explain the lack of penile erections. The owner declined measuring serum testosterone concentrations. The etiology of the right epididymal enlargement remains uncertain and could indicate either a congenital anomaly or a potential complication of vasectomy. Canine vasectomy is generally regarded as an acceptable sterilization procedure that preserves the gonads and hormone production, with rare complications including epididymal distention. Further investigation is underway to determine the underlying cause of this dog's reproductive issues.

Keywords: Testis, atrophy, hypoplasia, spermatic cord, ischemia

First documented case of fetal anasarca in a Karst shepherd dog: a detailed ultrasonographic progression from onset to outcome

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Fetal anasarca is a rare condition characterized by substantial fluid accumulation in fetal subcutaneous tissues and body cavities, causing generalized edema. This report presents a case of fetal anasarca in 4.5-year, healthy, primiparous Karst shepherd bitch that was artificially inseminated intravaginally with fresh semen. Dog was regularly vaccinated, including against canine herpesvirus, and dewormed. Pregnancy was confirmed on day 20 after ovulation and ultrasonography was performed on days 25 and 30, followed by weekly monitoring. In 1 of the 6 fetuses, fetal anasarca was first observed on day 37 of pregnancy, initially manifesting as fluid accumulation in the subcutaneous tissue of the neck region. One week later, a pleural effusion was detected in the affected fetus. On day 54 of gestation, fluid accumulation had further progressed, involving the pleural cavity and to a lesser extent, the pericardium. Two days before delivery, the fetus had no signs of life. Following progesterone concentrations decrease, cesarean surgery was performed. The affected fetus weighed only 220 grams, notably smaller than its littermates (458.6 ± 34 grams). Severe subcutaneous edema and serous fluid accumulation resulted in marked body deformity, with serous fluid filling the abdominal and thoracic cavities. The lungs, liver, kidneys, spleen, and intestines were severely hypoplastic, and the heart chambers were markedly dilated. The morphology and gross lesions were consistent with fetal anasarca. This case is particularly important as it represents the first documented anasarca in the Karst shepherd dog where the progression of fetal anasarca was ultrasonographically monitored throughout pregnancy. The detailed sequential ultrasonographic observations provided a unique opportunity to monitor the dynamic progression of the disease, emphasizing the critical role of regular prenatal ultrasound examinations in detecting fetal pathologies at an early stage.

Keywords: Fetal anasarca, Karst shepherd, ultrasonography, monitoring

Pregnancy toxemia in a dog

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A 2-year pregnant mixed breed Yorkshire terrier-shih tzu was referred from a local clinic with history of hypoglycemia. On presentation, the body condition score was 2/9, dog exhibited dull mentation, vomiting, lethargy, and weakness. The temperature, pulse and respiration were unremarkable, mucous membranes were pale and tacky, and capillary refill time was over 2 seconds. Bloodwork revealed hypoglycemia (33 mg/d;), hypoalbuminemia (1.1 g/dl) and anemia (packed cell volume: 32%, red blood cell concentration: 4.31/µl). Abdominal ultrasonography detected 4-6 fetuses with heart rates > 200 beats per minute (bpm). Urinalysis revealed ketonuria (1 mmol/l). The presumptive diagnosis was pregnancy toxemia. Pregnancy termination was recommended, but the owner elected to continue with pregnancy and supportive care in hospital. Pregnancy toxemia occurs when the dam does not receive adequate nutrition, and the definitive parameters are hypoglycemia, ketonemia, and ketonuria. Dog's treatment plan included an intravenous dextrose bolus (2.5% dextrose [3 ml] in saline [1 ml]) followed by 2.5% dextrose in PlasmaLyte (9 ml/hour, total volume: 45 ml/kg/day) with intravenous 10% calcium gluconate (2 ml once), and intravenous ondansetron (0.5 mg/kg every 8 hours). During hospitalization, dog continued vomiting, developed diarrhea and aborted 2 fetuses. Transabdominal ultrasonography revealed 2-4 fetuses (heart rates > 200 bpm). After 2 nights of hospitalization, blood glucose concentrations stabilized (120 g/dl), but hypocalcemia persisted (ionized calcium: 7.5 mg/dl). The owner elected to continue treatment at home due to financial constraints. On discharge, it was recommended to feed small, frequent portions of pup food and to return to the primary veterinarian if health status deteriorated. Three days after discharge, dog returned due to abortion of more fetuses. Transabdominal ultrasonography confirmed abortion of all fetuses. This case highlighted the critical management of pregnancy toxemia in dogs, emphasizing the importance of adequate nutrition during pregnancy, and client education.

Keywords: Pregnancy toxemia, hypocalcemia, diet, ketonuria

Successful uterine prolapse replacement of extended duration in a Hereford cross cow

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A 2-year Hereford cross cow was presented for evaluation of a postpartum uterine prolapse over 48 hours in duration. Parturition was otherwise uneventful, and the calf was apparently healthy at presentation. The owner replaced the uterine horn in the vagina but failed to reinvert the horn. Subsequently, the cow ruptured through the retention stitches the owner placed. Examination revealed a torn and necrotic vulva consistent with trauma from prolapsing through sutures, and the exposed uterine horn was relatively nonpliable with areas of necrosis. A caudal epidural with 2% lidocaine (100 mg) was given and the exteriorized tissue was cleaned with soap and water. Brown roll gauze was used to manually reduce uterine edema and size with slight effect such that the uterus was unable to be replaced. Intramuscular oxytocin (100 IU) was given and was successful in reducing the size of the uterus, so additional intramuscular oxytocin (200 IU) was given with further size reduction achieved, allowing the horn to be reinverted. This action is contrary to current guidance recommending avoiding the use of oxytocin until after the uterus is successfully replaced.[1] The uterine horn was then successfully inverted and replaced using a wiffle ball bat. During inversion and replacement, large quantities of urine were expelled. Umbilical tape sutures were placed in a horizontal mattress pattern, and transdermal flunixin meglumine was administered. Overnight observation demonstrated the appropriate ability to urinate. No apparent complications were reported. Given the extended duration of uterine prolapse and time of the uterine horn exposed to the environment despite owner efforts, it is notable that this case was able resolved with no immediate mortality due to necrosis or fatal hemorrhage due to uterine vessel rupture.[1]

Keywords: Prolapse, uterine, oxytocin, necrosis, postpartum

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Paraphimosis in a Thoroughbred gelding

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Paraphimosis is the inability to retract the penis into its normal position in the prepuce. As the penis protrudes, the muscle fatigue, swelling, and stretching of the pudendal nerves leads to the inability of the horse to retract the penis. The exposed epithelium becomes inflamed and friable, predisposing the underlying loose connective tissue to bacterial infection. This infection leads to fibrosis and paralysis that permanently inhibit the penis to retract back into the prepuce.[1] However, paraphimosis can be resolved, and normal function can be restored if treated appropriately and promptly. An 8-year Thoroughbred gelding was presented for paraphimosis. The paraphimosis could have been caused by trauma, but a perceived weight loss in the gelding was observed each winter. Body condition score was 2/9; gelding's penis had severe edema with moderate excoriations. Hydrotherapy was performed, followed by an application of Femycin and silver sulfadiazine cream on the extended penis and prepuce. A few hours later, the penis was wrapped with brown gauze and an Esmarch bandage for 15 minutes. A purse string suture was placed around the preputial opening. Once the compression bandage was removed, the penis and prepuce were manually replaced into the preputial cavity. The purse string was tightened to contain the penis, leaving 1 finger width allowing for urination. The following day, the purse string was removed and replaced by a customized bottle to secure the penis in the preputial cavity, tying rubber bands around the gelding's back to keep it in place. During the gelding's 5-day hospitalization, hydrotherapy was conducted twice daily with daily penile compression to reduce edema. Anti-inflammatories and antibiotics were sent home with the bottle to keep on until the gelding could maintain the penis within the prepuce without assistance.

Keywords: Horse, paraphimosis, penis, penile paralysis, gelding

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Congenital encephalocele in a live Friesian foal

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A 6-year Friesian mare was presented for foal watch at 335 days of pregnancy. Pregnancy was confirmed on day 30; transrectal ultrasonography at 4 and 8 months identified a fetus with normal heart rate, fetal fluids, and fetal membranes. On due date (April 15, 2024) the mare had fully developed udder that was streaming milk, with a pH of 6.3, predicting parturition within 72 hours. Fetal heart rate detection was challenging, ranging from 100-120 beats per minute. Mare foaled on day 339 of pregnancy with minimal assistance. Fetus was delivered 13 minutes after chorioallantois rupture in anterior longitudinal presentation, dorsosacral position, with extended legs and neck, but was not breathing. Mare retained fetal membranes that were removed 4 hours after parturition. Filly was unresponsive, displaying abnormal mentation, absent suckle reflex, focal seizures, anisocoria, suspected blindness, and an inability to attain sternal recumbency. During resuscitation attempts, a 1.5 × 4 cm skull defect with a 7.6 × 6.4 × 1.5 cm fluid-filled mass of brain tissue herniating through the frontal bone were identified. Additionally, there was left lateral thoracic scoliosis. Due to severe neurological impairment and congenital encephalocele, euthanasia was performed 5 hours after birth. Postmortem computerized tomography, magnetic resonance imaging, and necropsy confirmed encephalocele, a rare neural tube defect causing cerebral and meningeal herniation through the skull. Although typically congenital, encephaloceles can result from trauma, tumors, iatrogenic injury, or hypervitaminosis A. To our knowledge, this is the first documented case of a live foal born with an encephalocele. Signs of fetal stress were inconsistent, and the only concerns were a delay between the milk pH decrease and foaling as well as prolonged stage 3 of labor. This case highlighted the importance of detailed fetal ultrasonographic evaluation and stress monitoring for early identification of congenital malformations despite their poor prognosis.

Keywords: Encephalocele, neural tube defect, fetal malformation

Gangrenous mastitis in a dog after cesarean surgery

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Mastitis is a disease diagnosed in postpartum lactating dogs where 1 or more mammary glands become infected by opportunistic pathogens.[1] This condition is not common in dogs compared to other species, and only encompasses about 5.3% of reproductive disorders in the dog.[2] A 4-year English bulldog dog was presented 4 weeks after cesarean surgery and ovariohysterectomy with anorexia and a swollen, bruised mammary gland. The referring veterinarian initially prescribed oral prednisone (0.5 mg/kg twice daily) and oral cephalexin (20 mg/kg twice daily), and pups were weaned. Examination of the mammary chain revealed an inflamed left cranial mammary gland with 2 open wounds, red discharge, and a 2-3 inch pocket of purulent fluid in the gland. Results from a complete blood cell count were consistent with an inflammatory/infectious process and a chemistry panel had no overt renal damage. Based on these findings, previous treatments were discontinued, and an alternative treatment plan was created due to minimal clinical response. Oral amoxicillin/clavulanate was empirically prescribed (15 mg/kg twice daily) pending culture and susceptibility results. Oral omeprazole (1 mg/kg twice daily) was also prescribed to prevent gastrointestinal ulceration from the prednisone. Following a 2-day prednisone withdrawal period, oral carprofen (2.2 mg/kg twice daily) was given. Culture results revealed heavy growth of *Escherichia coli* and *Enterococcus faecalis*, with resistance to amoxicillin/clavulanate and susceptibility to fluoroquinolones. Oral ciprofloxacin was then prescribed (10 mg/kg twice daily) and additional wound management with absorbent dressing was implemented as the mammary gland had begun to slough and drain. This case demonstrated the value of correct antimicrobial selection to successfully treat and manage gangrenous mastitis. It is important to swiftly diagnose and appropriately treat this disease as it can cause gangrenous necrosis of mammary tissue and become life-threatening if the animal becomes systemically septic.

Keywords: Dog, mastitis, postpartum, antimicrobial

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Twin pregnancy in a southern tamandua (*Tamandua tetradactyla*)

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Southern tamandua is characterized by a simplex uterus, uniparous reproduction, and a discoid hemochorial placenta, with an average duration of 160 days (range; 130-190 days) of pregnancy. Although twins are possible in this species, they are rare and not well documented. Here, we report the only known case of a twin pregnancy with a live birth in the Association of Zoos and Aquariums population. To the authors' knowledge, there is only 1 other twin pregnancy observed in this population that resulted in abortion. A 10-year, multiparous female, chronically treated with methimazole for hyperthyroidism and previously documented with a 2.1 x 3.3 mm uterine cyst prior to pregnancy, was genetically valuable to the population and paired with a proven male for breeding. Twin pregnancy was diagnosed on day 117 prepartum (PP; 35-42 days after breeding) via voluntary ultrasonography. Fluid was observed around the heart of Fetus 1 (F1) on day 54 PP (98-105 days after breeding), and F1 died in utero 20 days after detection. Twice daily pentoxifylline (10 mg/kg) treatment began on day 46 PP to support the pregnancy through parturition. Fetus 2 (F2) was born alive and hairless between 152-159 days after breeding, but died 8 hours after birth, with no substantial lesions on histopathology. An intact placenta was recovered, having a single chorioallantois containing 2 distinct chambers and a separate blood supply for each fetus. There was an absence of fetal capillary structures and moderate multifocal interstitial hyalinosis of terminal chorionic villi in portions of the placenta that may have contributed to the death of F1. The dual-chambered placenta raised the question of monozygotic or dizygotic twins. DNA samples from both parents and offspring have been submitted to evaluate the genetic similarity between the twins.

Keywords: Southern tamandua, twin pregnancy, hemochorial, pentoxifylline, avascular villi

Validation of a portable computer assisted semen analysis system for evaluating progressive motility and concentration of stallion and bull sperm in field and laboratory settings

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We evaluated a portable computer-assisted semen analysis (PC) system (AndroScope, Minitube) for assessment of progressive motility (PM) and concentration of stallion and bull sperm by comparing the system to reference laboratory and field-based methodologies. Stallion (n = 12) and bull (n = 13) semen samples were collected and diluted using commercial extenders (INRA96, IMV Technologies and Triladyl, Minitube, respectively) or fixed in phosphate buffered saline formalin solution. In the field, extended samples were analyzed for PM by light or phase-contrast microscopy and PC, and for concentration by PC. In the laboratory, extended samples were analyzed for PM by PC and a laboratory-based computer-assisted semen analysis (LC) system (SpermVision, Minitube) as well as for concentration with the PC. Formalin-fixed samples were analyzed for concentration using a hemocytometer. Correlation and agreement between PC and LC, field microscopy, and hemocytometer methodologies for PM and concentration were assessed by Spearman tests and Bland-Altman analyses, respectively. In stallions, PM measured by PC strongly correlated with field microscopy (r = 0.71) and LC (r = 0.90). Conversely, PC underestimated PM by 11.9 ± 13.4% (mean ± SD) compared to field microscopy, and overestimated PM by 1.5 ± 8.4% when compared to the LC. Concentration measured by PC was strongly correlated with the hemocytometer (r = 0.83) but overestimated by 109 ± 64.1 x 10^6 sperm/ml. In bulls, PM measured by PC correlated fairly with field microscopy (r=0.39) but strongly to LC (r = 0.91). However, PC underestimated PM by 7.9 ± 11.0% compared to field microscopy and overestimated by 6.5 ± 5.0% compared to LC. In bulls, PC revealed poor correlation (r = 0.11) and severely underestimated sperm concentration (-908.0 ± 619.0 x 10^6 sperm/ml). Overall, the PC adequately estimated PM, particularly in stallion semen, but overestimated sperm concentration in both species. In practice, care should be taken when interpretating the results from automated semen evaluation methodologies and consider further calibration of these systems to avoid over or underestimation of semen parameters.

Keywords: Semen concentration, progressive motility, CASA, stallion, bull

Effect of knockout serum replacement supplementation in culture medium on bovine blastocyst gene expression after cryopreservation

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Embryos used for bovine in vitro fertilization (IVF) are cryopreserved to protect cell morphology, maintain proper gene expression regulators, and increase longevity. Early embryonic mortality is a major cause of reproductive failure in many species. The medium in which the embryos are cryopreserved can influence the success of embryo survival after preservation. This study investigated the effect of a Knockout Serum Replacement (KSR; Thermofisher) and the connection between preservation medium and embryo survival and competence after cryopreservation. Blastocyst supplemented with KSR were expected to have a greater expression of genes involved in embryo survival compared to those that did not after cryopreservation. Embryos were cultured for 6 days in a medium supplemented with 1 of 3 treatments: 5% KSR, 5% fetal bovine serum (FBS), or 0.6% bovine serum albumin (CON). On day 7, after fertilization, embryos were classified based on their morphology. Quality 1 blastocysts were selected to be frozen, stored and then thawed to test genes associated with growth and development of blastocele re-expansion after thawing identified as aquaporin 3 (AQP3), sphingosine-1-phosphate phosphatase 1 (SGPP1), Bcl-2-associated X-protein (BAX), and glyceraldehyde 3-phosphate-dehydrogenase (GAPDH; housekeeping gene) via real time polymerase chain reaction. Percentage of reexpansion of thawed blastocysts was greater in the CON groups than in the KSR or FBS groups. No significant differences were observed in any of the tested genes among the knockout treatments. Under the described conditions, KSR did not increase the expression of genes associated with embryo survival nor did it increase the survival of embryos after cryopreservation.

Keywords: Embryos, cattle, in vitro fertilization, cryopreservation, blastocyst, KSR

Investigation into hypochlorous acid as a treatment for bacterial endometritis in the mare

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An increase of antimicrobial resistance necessitates alternative treatments to bacterial infections. Investigated in this pilot study; hypochlorous acid (HOCl), an endogenous substance with broad-spectrum antimicrobial activity to treat bacterial endometritis. We hypothesized that HOCl is effective at killing bacteria known to cause equine endometritis and can be used intrauterine at 240 ppm without detrimental effects in the mare. Aims of this study were to: 1. Investigate if HOCl (Wound and Skin Care Liquid, Vetericyn Plus VF. Innovacyn, Inc.) is effective at killing bacteria known to cause equine endometritis; and 2. can 120 ppm and 240 ppm HOCl be safely used for intrauterine irrigation in the mare. Broth microdilution assays were used to establish the minimal inhibitory concentration (MIC) for gram-positive *Streptococcus equi* subsp. *zooepidemicus* and *Enterococcus faecalis*, and gram-negative *Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Six estrous mares received once daily intrauterine infusions of 120 ppm and 240 ppm HOCl (n = 3/group) for 3 days. Endometrial cytology score (neutrophils/100 endometrial cells) and vaginoscopy were performed before, during, 1 and 23 days after treatment. Serum amyloid A (SAA), fibrinogen (Fib) and white blood cell (WBC) were performed before, during, and 1 day after treatment. Endometrial biopsy was performed before, 1 and 23 days after final treatment and assigned Kenney-Doig score. We established a MIC for gram-positives at 200 ppm and gram-negatives at 225 ppm. Further results reported in (median, IQR). No side effects were noted, and no differences between 120 ppm and 240 ppm groups for SAA (< 20 mg/l, 0 mg/l), Fib (266.7 mg/dl, 50 mg/dl; 200 mg/dl, 1.5 mg/dl), WBC (6.4 x 10^3/µl, 0.46 x 10^3/µl; 7.7 x 10^3/µl, 1.18 x 10^3/µl). Five out of 6 mares had a decline in cytology score during treatment whereas 1 out of 6 mares had a gradual increase. Four out of 6 mares had no change in Kenney-Doig score over the study period. One out of 6 mares had an ulcerative lesion that resolved, and 1 out of 6 mares changed from grade 1 to 2B over the study period. In summary, HOCl was effective in killing common bacteria causing equine endometritis and it could be a safe, nonantibiotic treatment in mares.

Keywords: Horse, endometritis, treatment, hypochlorous acid

Polycystic/fibrocystic mastopathy following ovariohysterectomy in a diestrous dog

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A 3.5-year female Pug underwent ovariohysterectomy during diestrus, ~ 4-5 weeks after last estrous cycle. One month after surgery, the dog developed acute mammary gland enlargement, characterized by multiple cystic structures distributed across both mammary chains and bloody mammary discharge. Bloodwork abnormalities included reticulocytosis and mild leukocytosis with neutrophilia and monocytosis. Red blood cells, degenerative neutrophils, lymphocytes, and macrophages with a granular background were noted on mammary discharge cytology. The dog was given a 10-day course of amoxicillin-clavulanate (12.5 mg/Kg PO q12h) but failed to improve. Follow-up mammary discharge cytology revealed neutrophils, lymphocytes, foam cells/macrophages, and few extracellular bacteria. Culture yielded mild growth of *Staphylococcus pseudointermedius*, susceptible to amoxicillin-clavulanate. Dog was then prescribed a 7-day course of amoxicillin-clavulanate (12.5 mg/Kg PO q12h), 5-day course of carprofen (2.2 mg/Kg PO q12h), and cold compresses, with minimal response. On ultrasonography examination, multiple cystic cavities ranging 0.32-3 cm in diameter were diffusely distributed throughout both mammary chains. The cystic cavities contained hypoechoic to mildly echogenic fluid, with larger cysts also containing hyperechoic sediment. There was complete absence of normal mammary architecture. Ultrasound-guided aspirations were performed, yielding 12 ml of serosanguinous discharge and yellow caseous material. Fluid sediment contained numerous poorly preserved neutrophils, foam cells/macrophages within a pink proteinaceous material, and lysed cells. Few macrophages contained hematoidin crystals. No etiologic agents or atypical cell populations were observed. Findings were consistent with polycystic or fibrocystic mastopathy, a typically benign mammary dysplasia rarely observed in dogs. This condition has been associated with hormonal fluctuations, particularly estrogen-progesterone shifts, or progestin supplementation. However, in this case, there was no history of exogenous steroid hormone exposure and hormone concentrations (progesterone: 0.29 ng/ml, LH: > 1 ng/ml) did not support ovarian remnant syndrome. This case presented a firsthand report of polycystic/fibrocystic mastopathy as a complication of ovariohysterectomy performed during diestrus.

Keywords: Dog, mammary gland, blue dome, cysts

Caruncular edema and torsion in an ewe

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A 7-year Icelandic ewe was presented for a 30-day history of frequent straining and hind end bloody discharge. Historically, the ewe successfully bred naturally and lambed uneventfully 3 times. On presentation, the findings of physical examination were within normal limits with the exception of frequent unproductive straining, serosanguinous vaginal discharge, and asymmetrically enlarged udder. Vaginal speculum examination revealed dark red to black serosanguinous discharge from the cervix. Transabdominal ultrasonography revealed enlarged uterus with intraluminal hypoechoic fluid. Ovariohysterectomy was performed under general anesthesia. On gross examination, the uterus was moderately and uniformly distended with an obvious firm mass within the left uterine horn. Incision of the uterus revealed serosanguinous fluid and prominent pinkish caruncles (average 12 mm diameter) throughout with one 25 mm diameter darkened pedunculated mass corresponding to a caruncle. No obvious ovarian abnormalities were noted. Uterus and ovaries were submitted for histopathology examination. Ovaries had follicular development with primordial, primary and secondary follicles along with a corpus albicans in both ovaries. There was marked widespread caruncular edema. The dark mass was a caruncle distended with hemorrhage indicative of coagulation necrosis resulting from torsion of the caruncle's base. There were no other predisposing causes of vascular impairment that would lead to the severe caruncular edema, hemorrhage, and necrosis. According to a recent literature search, torsion of caruncles has not been previously reported, and its etiology remains to be elucidated. The ewe was discharged the same day, and her clinical signs completely resolved immediately following surgery according to the client during a follow up phone call 2 months later.

Keywords: Sheep, caruncle, edema, torsion, ovariohysterectomy

Femoral dysgenesis in a neonatal Weimaraner pup

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A 4-day, Weimaraner male pup, weighing 207 grams, was presented for routine breed-associated surgery; it was considerably smaller than littermates since birth, but was vigorous and gaining weight. On physical examination swelling and pain on palpation of the right femur and stifle were noticed. Based on findings, no further diagnostics or surgery were performed, and he was discharged with injectable buprenorphine and oral amoxicillin-clavulanic acid. Three days later, pup returned due to lack of improvement. Radiographs revealed a misshapen, lytic distal right femoral epiphysis. Based on the constellation of clinical and radiographic findings, humane euthanasia was elected due to lack of response to treatment and poor long-term prognosis. Necropsy was performed, and gross evaluation revealed an eroded distal right femur suggestive of osteolysis. Histology revealed extensive effacement and replacement of the distal femur architecture by densely packed, mitotically active mesenchymal cells, all in various stages of maturity. Aerobic culture of the synovial fluid had no bacterial growth. Given the lack of inflammation or infection, and the persistence of an early mesenchymal tissue core that tightly interdigitated with existing tissue, the pup was diagnosed with congenital developmental dysgenesis of the distal femur. Conditions concerning appendicular skeletal dysgenesis in puppies are rare and scarcely documented.[1] Underlying causes of these defects in dogs have been poorly defined, but are hypothesized to be related to dam nutrition, fetal positioning, and failures of intrinsic embryogenesis and cellular differentiation.[2] Instances of canine axial skeletal malformation are well-documented, with the mechanisms of these defects more clearly delineated. The scarcity of appendicular skeletal malformations in literature suggests the need for greater investigation and testament of them. This case serves as a well-documented clinical, radiographical, and histological example of the lesser-investigated pelvic limb variant of canine appendicular skeletal defects.

Keywords: Dog, neonate, pup, femoral dysgenesis, appendicular skeleton

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Adhesion induced pyometra and subsequent peritonitis in a Miniature Horse

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Pyometra is an infection of the uterus with accumulation of purulent fluid often due to compromise of the cervix and hindered uterine clearance. Pyometra can lead to infertility, but rarely leads to clinical signs of disease in the mare where many cases can be manually drained and medically managed.[1] A 7-year miniature horse mare was presented for evaluation of a corneal ulcer and malodorous vaginal discharge. Transabdominal ultrasonography revealed a distended uterus filled with highly cellular, echogenic fluid. Transrectal palpation revealed a firm, extremely distended uterus with nonpalpable ovaries. Vaginal speculum examination revealed purulent discharge in the caudal vagina and what appeared to be a persistent hymen with an 'os' in the center, and purulent fluid draining from it. Cervix was not palpable. To permit drainage, the vaginal defect was manually dilated, and revealed distinct communication with the peritoneal cavity. There were multiple adhesions covering the opening of the cervix, interfering with drainage of the uterus. Mare began destabilizing the next day and was rushed into emergency surgery for an ovariohysterectomy. Multiple focal adhesions, inflammation of the peritoneum, necrotic tissue and a prior ventral midline surgical incision were noticed during surgery. Uterus was remarkably enlarged and drained prior to removal. Mare's abdomen was closed, and the mare was placed in the Trendelenburg position, to allow for access to repair the vaginal laceration. After several surgical and postsurgical abdominal lavages, the mare recovered uneventfully. It was concluded that the mare had undergone an undisclosed trauma to cranial vagina, potentially a dystocia, that led to a permanent communication between vagina and peritoneum. Although the mare had been able to adapt to this condition over time, it was likely that peritonitis was imminent without this discovery by the reproductive service.

Keywords: Pyometra, cervix, adhesions, ovariohysterectomy, peritonitis

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Hemospermia secondary to cutaneous habronemiasis of the urethral process

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In the summer of 2023, a 12-year Welsh pony stallion was diagnosed with hemospermia secondary to cutaneous habronemiasis of the urethral process. Habronemiasis is a parasitic skin condition in equids caused by *Habronema* larvae. Flies deposit larvae on the horse's skin, particularly around the prepuce and urethral process, leading to granulomatous skin lesions. The diagnosis in this stallion was confirmed via punch biopsy and histopathology. The initial treatment included ivermectin, sexual rest, and topical fly repellents. However, despite these measures, the stallion's urethral process remained mildly irritated and inflamed, and the hemospermia persisted into the 2024 breeding season. Further diagnostics, including urinalysis, ultrasonography of the accessory sex glands and scrotum, and endoscopic evaluation of the urethra and bladder, revealed no other underlying causes for the hemospermia. To obtain a blood-free ejaculate, manual semen collection was attempted. However, the stallion failed to ejaculate, and the inflamed tissue of the urethral process bled. An open-ended Missouri artificial vagina (AV) was subsequently used, allowing fractionation of the ejaculate and direct observation of the urethral process during ejaculation. Using this method, all 3 fractions of the ejaculate were free of blood. Additionally, the open-ended design of the AV minimized urethral trauma during collection. Semen was successfully collected throughout the 2024 breeding season using this technique. Despite the resolution of hemospermia, the urethral process remained inflamed. Steroid therapy was considered to address urethral inflammation but was ultimately avoided due to the stallion's elevated resting insulin concentrations and the associated risk of laminitis. Instead, the stallion received multiple rounds of ivermectin treatment and daily applications of topical fly repellents. After approximately 1 year, the urethral process fully healed.

Keywords: Hemospermia, habronemiasis, open-ended artificial vagina

Presumed fetal anasarca dystocia in a Guernsey goat

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A 2-year, 40 kg, Guernsey doe was presented on emergency for dystocia. The doe was in stage 2 labor and was not progressing; manual delivery was attempted by owners with no success. On the previous day, another presumed anasarca fetus had been delivered on farm. Recommendation to not breed that doe or buck again were provided. The sire of the current dystocia was unknown. On initial examination, the doe was bright, alert and responsive, with visible abdominal contractions every 30 seconds to 1 minute. One edematous limb exteriorized to the level of the fetal carpus was protruding from the vulva. Vaginal palpation revealed an abnormal fetus with edematous front extremities, and an edematous body. Based on these examination findings, a presumptive diagnosis of fetal anasarca was made. Treatment options provided included cesarean surgery or fetotomy, with the latter selected. The doe received intramuscular oxytocin (10 units) and a lumbosacral epidural with 2% lidocaine (1 ml/20 kg). The abnormal fetus was successfully removed in 2 fetotomy cuts, with 1 normal kid delivered after removal. The live doeling was provided with colostrum replacer (at 15% of body weight) and was clinically normal on examination. The doe received subcutaneous oxytetracycline (20 mg/kg once) and meloxicam (1 mg/kg once). This case highlighted the importance of client education surrounding breeding animals that have potential genetic conditions. Due to the series of suspect anasarca kids on this farm, the genetic component could be presumed to be related to the buck. It is important to note the infrequency of this genetic anomaly reported within the caprine species.

Keywords: Edematous fetus, anasarca, fetotomy, dystocia, goat

Callicrate banding failure leading to surgical corrective castration

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Castration via 'banding' is commonly performed by owners and requires care to ensure both testes are fully entrapped. A 10-month, 338 kg Charolais-cross steer was referred for bull-like behavior, 8 months following on farm castration by banding (Callicrate). Prior to presentation, serum submitted to Texas A&M Veterinary Medical Diagnostic Laboratory demonstrated serum testosterone concentrations of 29.10 ng/dl (< 20 ng/dl in castrated animals). On initial examination, the steer was apparently healthy with soft tissue-like structures palpable bilaterally in the inguinal regions. Ultrasonographic imaging of the inguinal structures demonstrated the presence of 2 pampiniform plexuses and surgical castration was recommended. The steer received intravenous flunixin meglumine (1.1 mg/kg once), was sedated with intravenous xylazine (0.05 mg/kg) and midazolam (0.1 mg/kg), and was placed in right lateral recumbency. An incision over the palpable tissue was made to visualize the pampiniform plexus identified ultrasonographically. Two structures resembling pampiniform plexus and testicular tissue were identified and removed. Histopathology of removed tissues confirmed them as retained, atrophied testes and associated structures with presence of spermatic cord that was obliterated in some areas followed by areas of recanalization of the arterial wall, indicating banding castration failure. Recovery was unremarkable; steer received subcutaneous florfenicol (40 mg/kg once) and *Clostridium perfringens* type C and D and *Clostridium tetani* vaccination (Barvac, Boehringer Ingelheim). This case emphasized the importance of precise placement of bands when utilized for castration. The presence of testicular and spermatic cord tissue on the right and left sides indicated either a failure to include the complete testes when placing the band or that the band had not been placed at an appropriate tightness. Appropriate usage of the Callicrate Bander and similar devices is paramount to preventing complications as described in this report.

Keywords: Castration, banding failure, surgery, cryptorchid

Large offspring syndrome in a cow

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Large offspring syndrome is a genetic condition that can arise naturally but most commonly occurs in calves derived from in vitro fertilization (IVF) or somatic cell nuclear transfer.[1] A 7-year Simmental cow previously confirmed pregnant following transfer of an IVF-derived embryo was presented for dystocia at full term. Fetal membranes were protruding from the vulva, although there were no visible indications of labor at or prior to presentation. There was a foul odor indicating the fetus had died and begun decomposing. Vaginal examination revealed minimal uterine fluid and a calf with a large edematous head. Head of the calf was removed via fetotome. After, it was discovered that the entirety of the calf was large and edematous, it was attempted to remove the right forelimb with a fetotome; however, positioning of the fetotome was difficult due to the size of the calf and lack of fluid in the uterus that resulted in inadvertent amputation of the limb at the level of the humerus. Further reduction in fetal size with the fetotome was not deemed a viable option. Cow was not a suitable candidate for a cesarean surgery due to the fetid uterine contents and an assumed reduced mobility of the uterus. This was due to the size of the calf and contraction of the uterus that prevented uterine manipulation to the level of the incision. Therefore, the cow was sedated with xylazine and euthanized with a captive bolt. Assisted reproductive techniques are commonly employed in cattle with IVF-derived embryos being the predominant technique used. This case highlighted the impact of large offspring syndrome and the necessity of research studies that help detect this condition early in pregnancy to allow early interventions.

Keywords: Cattle, dystocia, in vitro fertilization

Reference

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