Reproduction in Domestic Animals

Reprod Dom Anim **47** (Suppl. 5), 58–115 (2012); doi: 10.1111/j.1439-0531.2012.02110.x
ISSN 0936-6768

WORKSHOPS

Workshop 1
Timing of AI
Moderator: Stephen Butler

WS 1.1

Male and female aspects of the timing of AI

ST Butler

Teagasc, Moorepark, Fermoy, Co. Cork, Ireland

Artificial insemination (AI) using semen from daughter proven sires, and more recently sires with genomic proofs, has had a pronounced effect on animal productivity. This has been most notable in dairy cattle, where uptake of AI has been greatest. Successful use of AI requires accurate identification of the timing of oestrus followed by insemination with high quality semen containing sufficient viable sperm at the appropriate time to maximise the likelihood of successful pregnancy establishment. Accurate detection of oestrus has become more difficult in high producing cows as the duration of oestrous expression has become shorter with fewer mounts compared with cows of lower production potential (Lopez et al. 2004). We have recently reported that more cows with poor genetic merit for fertility traits (Fert−) ovulate without showing any behavioural signs of heat compared with cows with good genetic merit for fertility traits (Fert+), and that more Fert− cows failed to ovulate after displaying signs of behavioural heat compared with Fert+ cows (Cummins et al. 2012). There are also challenges related to the male in terms of generating semen straws that contain high quality sperm at the optimum concentration under a variety of different conditions. These conditions include preparation of fresh semen, cryopreserved semen, and whether or not the semen is sorted to result in biased offspring gender. It is critically important that sperm, regardless of the conditions under which it was prepared, are capable of surviving in the female reproductive tract, successfully undergo capacitation, and are capable of fertilizing an oocyte at the appropriate time.

WS 1.2

Female aspects of fertility in relation to timing of AI in lactating dairy cows

MM Herlihy, ST Butler

Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland

The ability of an animal to display behavioural oestrus and ovulate a dominant follicle at the appropriate time is a prerequisite for optimal timing of AI and oocyte fertilisation. Early AI (0 h after oestrus onset) results in lower fertilisation rates (but good embryo quality), whereas, late AI (24 h after oestrus onset) results in greater fertilisation rates but poorer embryo quality due to an aging ovum. There are different estimates for the optimal time of AI that range from 4 to 20 h after oestrus onset. During spontaneous oestrus, overt oestrous behaviour is required to identify cows for AI. Genetic selection for increased milk yield has shortened the duration of behavioural oestrus and increased the incidence of 'silent heat', representing a major challenge to efficient identification of cows for AI. This has led to the development of ovulation synchronisation programmes that tightly control when ovulation occurs, allowing AI to take place at a predetermined time ('Timed AI protocols'). Some of these synchronisation protocols achieve pregnancy outcomes similar to untreated cows inseminated at spontaneous oestrus. In addition, a variety of automated approaches to identify when cows are inoestrus have been developed. These include automated activity meters, continuous monitoring with cameras, in-line progesterone measurement. These approaches have the advantage of being 'always on', and hence may be useful from a management perspective to identify optimum timing of AI.

WS 1.3

New semen processing technologies – no need for optimal insemination timing?

IC Oskam[1], KE Waterhouse[1], AH Alm-Kristiansen[1], IS Grevle[1], J Sunde[1], RT Garmo[1], G Klinkenberg[2]

[1]*Biokapital AS, Hamar, Norway;* [2]*Sintef, Norway*

Enhancement in cattle production is the result of implementation of new biotechnological techniques and production practices. However, many studies report a decline in the fertility of dairy cattle. The causality for this decline is multifactorial and costs to control fertility impairment are expensive. Good herd fertility is based on regular breeding of cows with normal reproductive function. Based on several genetic components of infertility, genetic selection may in some cases be a proper approach for fertility improvement. However, pragmatic reproductive management including a proper AI program is required to keep fertility at an acceptable level and lower costs inherent to infertility. Successful AI requires survival of spermatozoa outside the body, replacement of spermatozoa into the female genital tract and identification of standing oestrus for optimal timing of insemination. Accurate detection of standing heat is one of the most problematic aspects of AI and complicates optimal timing of insemination. Today, reproductive technologies focus on the development of novel semen processing technologies that circumvent critical timing of insemination by extending the fertile life of spermatozoa in the female reproductive tract after insemination. The SpermVital technology intends to extend the shelf life of spermatozoa in the female reproductive tract after AI. Prevalent use of new semen processing technologies requires that they are successful, but also simple during its production and use, as well as being economical worthwhile in comparison to the more traditional AI techniques.

Workshop 2
Reproduction in felids
Moderator: Tom Rijsselaere

WS 2.1

Latest aspects of male feline reproduction

T Rijsselaere

Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

During a long period reproduction in cats was poorly studied although the reproductive cycle of the cat is in many aspects unique such as e.g. the induced ovulation. During the last decade a considerable amount of new information has been published mainly due to the increased interest in cat reproduction in daily practice and due to the possible use of the domestic cat as a research model for endangered wild felids and for the study of several important human pathologies. The aim of the present workshop is to review and discuss the different techniques for semen collection in tomcats and several recently validated sperm assessment techniques. Moreover, an update of the currently used methods for cooling and freezing cat semen will be provided and the techniques for intravaginal and intrauterine insemination and their results will be discussed. Sojka et

al. (1970) described already more than 40 years ago the first successful artificial insemination with fresh semen in cats. Subsequently, birth of kittens has been achieved and documented in cats and wild felids after insemination with cooled and cryopreserved semen. The main reasons to perform an insemination in the cat may be difficulties to conduct a natural mating due to anatomical, physiological or behavioural problems, preservation and transport of genetic material of valuable breeding animals and finally research and subsequent use of the information obtained in the domestic cat for the conservation of endangered wild felids. Insemination in cats is at present however not as commonly performed in daily veterinary practice as insemination in dogs due to the practical difficulties in collecting a sperm sample from the tomcat, the small volume of sperm obtained, the relatively few possibilities to determine the optimal timing of insemination and the need for ovulation induction and sedation of the queen upon insemination. In the domestic cat semen collection is rather difficult and challenging in comparison with several other domestic species and the collected volume of semen is mostly very small frequently limiting the number of possible investigations on the collected semen sample. Consequently, pooling of ejaculates obtained from several males is often required when designing adequate research protocols for e.g. studying new semen extenders for short or long-term conservation or for artificial insemination purposes. Research during the last years elucidated that storage of cat semen (either chilled or cryopreserved) requires the use of appropriate extenders and equipment; the addition of egg yolk, Equex STM or Orvus ES, centrifugation of thawed semen and addition of antioxidants to semen extenders may have beneficial effects in this respect. Finally, although the currently used preservation procedures could definitively benefit from further refinement, promising results were described and litters have been born after insemination with cryopreserved semen.

WS 2.2

Sperm collection and assessment techniques in the cat

T Rijsselaere

Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

In the domestic cat, sperm can be collected by an artificial vagina, by electro-ejaculation under general anaesthesia, by incision of the epididymides after castration, or by urethral catheterization after sedation with medetomidine. Sperm collection by an artificial vagina requires a trained male and a teaser queen in oestrus, which is impractical to perform in daily practice. Epididymal sperm are routinely used in IVF laboratories for research purposes, makes it possible to conserve genetic material even after the unexpected death of a valuable male and may additionally yield important information, which can be used for research in endangered, wild felids. The sperm quality in cats appears to be influenced by the season and by the sperm collection technique used. Practical difficulties related to sperm quality assessment in cats are the relatively low number of spermatozoa that can be collected, the large individual variation, the relatively high number of males with poor sperm morphology and the current lack of cut-off values for sperm quality parameters and their unclear relation with *in vivo* fertility. These factors cause several practical difficulties in designing research protocols for e.g. investigating new sperm diluters for conservation. Sperm quality is mostly performed by assessing the routine sperm characteristics i.e. concentration, motility, morphology, membrane integrity and total sperm output. During the last decade however, several new techniques such as computer assisted sperm analysis and fluorescent staining were validated in cats, which allow for a more detailed sperm assessment.

WS 2.3

Cooling and freezing of feline sperm and artificial insemination in the cat

E Axner

Division of Reproduction, Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

Birth of kittens after AI with preserved spermatozoa has been achieved in cats and wild felids. Refinement of techniques for semen preservation and AI in felids is, however, complicated by the need for sedation/anaesthesia for collection of ejaculates and for intrauterine AI and the comparatively low number of spermatozoa ejaculated by cats. In addition many cats and many species of wild felids produce high numbers of morphologically abnormal spermatozoa. Ejaculated and epididymal cat spermatozoa tolerate cooling to 4°C relatively well while freezing causes damage, especially on the acrosomes. Improvements in the protocol for freezing wild and domestic feline spermatozoa include addition of detergents such as Equex STM or Orvus ES paste, and addition of antioxidants to the freezing extenders. By adjusting the thawing rate and by post-thaw dilution the sperm quality can be further improved. Although births of kittens have been reported after intravaginal insemination it is clear that intrauterine insemination is required for acceptable pregnancy results with frozen-thawed semen. Intrauterine insemination has usually been achieved by surgery but transcervical insemination has succeeded and offers a less invasive alternative. The number of spermatozoa required to achieve acceptable pregnancy rates with cryopreserved spermatozoa is, however, often higher than the number of spermatozoa that can be retrieved in one semen collection. Refinements in the methods for evaluation of follicular maturation, sperm conservation and AI are therefore required in order to routinely achieve acceptable results.

Workshop 3
IVF vs. Superovulation
Moderator: Pat Lonergan

WS 3.1

Superovulation and *in vitro* fertilization: two means to the same end

P Lonergan

School of Agriculture and Food Science, University College Dublin, Dublin, Ireland

In cattle, due to the fact that only one oocyte is normally ovulated during each oestrous cycle and that gestation lasts 9 months, females typically produce relatively few (< 10) offspring in their lifetime. This contrasts with the situations in males where billions of gametes are produced at each ejaculation and where, through artificial insemination, individual elite bulls can sire many thousands of offspring each year. Several methods are available to increase the potential contribution of elite females. Superovulation, involving pharmaceutical induction of multiple ovulations in a donor female, artificial insemination, embryo recovery and transfer to synchronised surrogate recipients, has been around since the 1970s and forms the basis of the international embryo transfer industry in cattle. With the development of *in vitro* embryo production (IVP) on a large scale in the late 1980s and the development of transvaginal ultrasound-guided ovum pick up in the early 1990s, allowing repeated access to the ovaries of animals of high genetic merit, an alternative method of generating large numbers of embryos of high genetic merit was established. According to data compiled by the International Embryo Transfer Society, the number of bovine *in vivo*-derived embryos collected worldwide in 2010 was 732 227; the number transferred was 590 561, of which approximately half (55.5%) were transferred fresh and the remainder transferred frozen. In contrast, 339 685 IVP embryos were transferred in the same year of which the vast majority (93%) was transferred fresh. Relative advantages and disadvantages of both approaches exist.

WS 3.2

In vitro fertilization in Europe: what's new in the age of genomics?

C Ponsart

UNCEIA, National Union of AI Cooperatives, Maisons-Alfort, France

The use of *in vitro* embryo production (IVP) was compared over the last 10 years in Europe; some countries, such as Finland, stopped using

it mainly due to high costs compared to MOET, while others such as Denmark have been reluctant to develop this technology for ethical reasons. In contrast, four European countries developed Ovum Pick Up (OPU) programs in order to intensify embryo production in donor stations. Approximately 90%of IVP embryos produced in Europe are produced in the Netherlands and in Germany, where breeding companies collect oocytes twice per week from non-superovulated females. In Italy, one particular market has been developed with the aim of producing beef embryos from slaughterhouse ovaries. In France, OPU activity declined over the last decade, but four new stations opened since 2009, indicating that genomic selection may change the situation. Indeed, the future development of OPU-IVP in Europe is related to the combined use of IVP and genomic selection. With genomic breeding values, it is possible to produce more embryos (more suitable candidates) from young heifers (as bull dams) combining production of *in vivo* derived and *in vitro* produced embryos during the first trimester of pregnancy. Moreover, the combination of techniques allowing biopsy, freezing and genotyping of embryos enables the selection of embryos for transfer of known sex and breeding values 9 months before birth of a calf. Initial experiments conducted jointly by CRV and UNCEIA showed that amplification of total DNA from the biopsy leads to concordant genomic breeding values between embryos and calves. The future for this combination of technologies is promising.

Workshop 4
Current clinical research in equine reproduction
Moderator: Tom Stout

WS 4.1

Effect of post-breeding dexamethasone treatment on the uterine inflammatory reaction in the mare

TAE Stout, M de Ruijter-Villani

Department of Equine Sciences, Utrecht University, Utrecht, The Netherlands

Insemination of an oestrous mare triggers a physiological uterine inflammatory reaction that aids the elimination of excess sperm and bacteria. In normal mares this inflammation resolves within 1–2 days, whereas in 'susceptible' mares the inflammatory debris persists beyond 48 h and compromises fertility. Recently, it has become popular to treat susceptible mares with high doses of corticosteroids in the immediate pre- or post-mating period in an attempt to suppress the inflammatory reaction and avoid 'persistent post-breeding endometritis' (PBBE). However, field studies have yielded conflicting results on the efficacy of corticosteroid therapy, and it is not yet clear how corticosteroids modify the uterine inflammatory response. In a recent study, we found that a single intravenous injection of 50 mg dexamethasone 1 h before AI reduced oedema 24 h post-AI, but did not prevent intra-uterine accumulation of fluid or neutrophils. Neither did dexamethasone treatment suppress the endometrial expression of inflammatory mediators such as COX-2, LOX5 and NO at 24 h post-AI. On the other hand, recent studies performed in Kentucky suggest that corticosteroid therapy does suppress endometrial inflammatory mediator expression in the acute phase (up to 6 h). However, it also appears that while there is a marked increase in the expression of inflammatory mediators that peaks at around 6 h post-AI in normal mares, it is less intense but more prolonged in susceptible mares. Since elements of the inflammatory reaction are essential to uterine clearance and normal fertility, but may be suppressed by corticosteroid therapy, it is still not clear whether and under what circumstances corticosteroid or other immunosuppressive therapies are really beneficial.

WS 4.2

Differentiating an early corpus luteum from a hemorrhagicous ovulatory follicle in the mare

J Cuervo-Arango

Department of Animal Medicine and Surgery, Veterinary Faculty, University CEU Cardenal Herrera, Valencia, Spain

In equine practice, accurate detection of (normal) ovulation is important for deciding the optimum time for breeding with short-lived semen (e.g. frozen semen or semen from poorly fertile stallions) that needs to be introduced shortly before or soon after ovulation. Correct detection of ovulation is also important for estimating embryo age for embryo flushing and recovery, especially if the embryos are intended for cryopreservation. If the pre-ovulatory follicle fails to collapse and release its oocyte into the oviduct, fertilization is not possible. The most common pathological disturbance of ovulation that occurs spontaneously during the breeding season is the haemorrhagic anovulatory follicle (HAF). Soon after normal ovulation, however, around 60% of mares develop a corpus haemorrhagicum (CH) with a central cavity. This type of corpus luteum may resemble an HAF and thereby complicate the diagnosis of 'normal' ovulation. Approximately, 20% of mares with normal ovulations develop CHs with a central cavity larger than 30 mm in diameter, which may be mistaken for an HAF. Following a normal ovulation, the evacuated follicle remains collapsed (with no central cavity) for at least 12 h post-ovulation. Thereafter, the cavity will refill with blood to form a CH. This blood clots rapidly in a normal CH, i.e. within <16 h. During the first 24 h of central cavity formation, the luteinised border of the CH will be thicker than 5 mm. In contrast, the HAF contents (fresh blood) remain unclotted for 32–72 h, and the luteinised border of the anovulatory follicle will be <3 mm thick. Once the HAF contents organize, they form a cobweb-like network of fibrin. At this stage it may be difficult to distinguish it from a CH with a large organized cavity. However, in this scenario, the overall diameter of the CH should be similar or smaller to that of the original pre-ovulatory follicle, whereas an organized HAF will almost always be larger than the original pre-ovulatory follicle.

WS 4.3

Streptococcus equi subsp. *zooepidemicus* isolates from infectious endometritis belong to a distinct genetic group

CD Rasmussen[1], MM Haugaard[2], MR Petersen[1], M Christoffersen[1], MHT Troedsson[3], H Lehn-Jensen[1], AM Bojesen[4]

[1]*Veterinary Reproduction & Obstetrics, University of Copenhagen, Copenhagen, Denmark;* [2]*Medicine & Surgery, University of Copenhagen, Copenhagen, Denmark;* [3]*Gluck Equine Research Center, University of Lexington, Lexington, KY, USA;* [4]*Disease Biology, University of Copenhagen (KU), Copenhagen, Denmark*

Streptococcus equi subsp. *zooepidemicus* (*S. zoo*) is the pathogen isolated most commonly from the uterus of mares. *S. zoo* is an opportunistic pathogen and part of the resident flora in the caudal reproductive tract. This study genotyped and compared *S. zoo* strains from the uterus of mares with endometritis to isolates from the vagina and fossa clitoridis, using pulsed-field gel electrophoresis (PFGE). Uterine samples were collected using a guarded endometrial biopsy punch. A double-guarded swab was used to recover samples from the cranial vagina, and samples from the fossa clitoridis were collected using a regular sterile swab. Only pure cultures (≥90% of all colonies) were analysed further. If *S. zoo* was present, up to three colonies were selected from each anatomical location (max. nine samples per mare). The bacterial isolates were characterized by PFGE. In 12 mares, *S. zoo* was isolated from the endometrium. A total of 88 *S. zoo* isolates were analysed, 31 from the endometrium, 26 from the cranial vagina and 31 from the fossa clitoridis. Analysis of the banding patterns demonstrated genetic similarity of the *S. zoo* isolates obtained from infectious endometritis, which differed to isolates obtained from the caudal reproductive tract. In conclusion, this study indicates that a genetically distinct group of *S. zoo* is associated with infectious endometritis in the mare.

Workshop 5
Early/late embryo loss
Moderator: Michael Diskin

WS 5.1

Timing and extent of embryo mortality in cattle

MG Diskin

Teagasc, Animal & Grassland and Innovation Research Centre, Mellows Campus, Athenry, Co. Galway, Ireland

Embryo mortality is a major cause of economic loss in dairy production systems. Direct effects of embryonic mortality are reflected in reduced conception rates with consequent effects for efficiency of production and profitability. In heifers and moderate yielding dairy cows, fertilisation rates are of the order of 90–100% following the use of high quality semen. In contrast, for higher-producing dairy cows there is less quantitative information on fertilisation rate. Based on published results it appears that fertilisation rate may be a little lower and more variable in high and moderate producing dairy cows, at least during the hot season. A calculated embryonic and foetal mortality rate (excluding fertilisation failure) of about 40% for moderate-producing cows based on a fertilisation rate of 90% and an average calving rate of about 55% with an estimated 70–80% of losses sustained between days 8 and 16 after AI. The comparative figure for high producing dairy cows, based on a fertilisation rate of 90% and a calving rate of 40%, is 56%. There is evidence that the pattern of early embryo death in the modern high producing cow may be different from that observed in heifers and lower yielding dairy cows. It would appear that early embryo loss is greater in the modern high-producing dairy cow and that a much higher proportion of the embryos die before day 7 following breeding compared with lower producing cows or heifers. There is evidence that the extent of late embryo/foetal mortality (7–10%) recorded in cows on pasture-based systems of milk production is much lower than that reported (15–25%) for cows on more intensive systems of milk production. The causes of these differences are unclear. While the extent of late embryo loss is numerically lower than early embryo loss, it, nevertheless causes serious economic losses, particularly in seasonal calving herds because it is often too late to rebreed cows resulting in increased culling rates.

WS 5.2

Progesterone and embryo survival in cattle

P Lonergan

School of Agriculture and Food Science, University College Dublin, Dublin, Ireland

The steroid hormone progesterone (P4) plays a key role in reproductive events associated with establishment and maintenance of pregnancy. Conceptus growth and development require the action of P4 on the uterus to regulate endometrial function, including conceptus-maternal interactions, pregnancy recognition, and uterine receptivity to implantation. In cattle, the majority of embryo loss occurs very early during pregnancy, around or prior to maternal recognition. A considerable proportion of this loss may be attributable to inadequate circulating P4 concentrations and the downstream consequences on endometrial gene expression and histotroph secretion into the uterine lumen. Low P4 concentrations have been implicated as a causative factor in low pregnancy rates observed in high-yielding dairy cows. Elevated concentrations of P4 in the immediate post-conception period have been associated with an advancement of conceptus elongation, an increase in interferon-tau production and higher pregnancy rates in cattle and sheep. Elevated P4 advances the transcriptomic changes in the endometrium, which normally occur during pregnancy, resulting in enhanced conceptus elongation; interestingly, the embryo does not have to be present in the uterus during the period of P4 elevation in order to benefit from it, supporting the concept that the positive effect on conceptus growth is mediated via P4-induced changes in the endometrial transcriptome. Many strategies have been devised to augment P4 including treatments, which provide an exogenous source of P4 (e.g., intravaginal P4 pessaries, P4 injections) or those which enhance the ability of the CL to produce P4 (e.g., hCG, eCG). The relative merits of such strategies and the consequences for embryo survival are the subject of ongoing studies.

WS 5.3

The influence of genetic background on embryo survival in lactating dairy cattle

ST Butler

Teagasc, Moorepark, Fermoy, Co. Cork, Ireland

Genetic evaluation systems in most countries have now incorporated fertility traits into their national selection index for dairy cattle. Embryo loss in dairy cattle is a major obstacle to efficient milk production, especially in seasonal calving systems. Early embryo mortality is the main source of embryo loss, generally occurring between day 8 and 16 post-insemination, and is manifest as cows returning to oestrus 18–24 days after AI. Late embryo mortality can be described as embryo loss between days 30 and 60 after AI. A Holstein cow genetic model of fertility has been established at Moorepark Research Centre. The cows have similar proportions of Holstein genes and similar genetic merit for milk production, but have extremes of good (Fert+) or poor (Fert−) genetic merit for fertility traits. Conception rates at day 30 after AI are greater and the calving to conception interval is shorter in Fert+ cows compared with Fert− cows, but phenotypic milk production is similar. The Fert− cows have lower circulating progesterone concentrations during the luteal phase. Fertilisation rates after super-stimulation and embryo development to day 7 post-AI appear to be broadly similar, suggesting that subsequent early embryo loss is largely a result of lower circulating progesterone concentrations and associated effects on uterine environment.

Workshop 6
Non-surgical contraception in dogs and cats
Moderator: Sandra Goericke-Pesch

WS 6.1

Non-surgical contraception in dogs and cats

S Goericke-Pesch

Clinic for Obstetrics, Gynaecology and Andrology of Large and Small Animals, Giessen, Germany

Several possibilities are available for non-surgical contraception like progestins, melatonin implants, immune-contraception using GnRH vaccines in cats, GnRH antagonists, and GnRH agonists. The use of slow release GnRH agonist implants for down-regulation of the testicular endocrine and germinative function in male dogs as well as use of such implants in pre-pubertal and adult bitches and the use of GnRH agonist implants in tom cats and queens are in the focus of interest to elucidate whether these implants are safe and the best option for hormonal contraception.

WS 6.2

Non-surgical contraception in dogs and cats – an overview

IM Reichler

Vetsuisse-Faculty, University of Zurich, Zurich, Switzerland

Long-acting or permanent alternatives to spay and neuter are needed to control pet over-population as gonadectomy requires both technical expertise and equipment. However, some pet owners would prefer an effective and reversible contraceptive that eliminates breeding behaviour, but preserves future reproductive potential.

Since the 'Pill' had conquered the market in the '60s', researchers have studied many strategies and technologies to prevent pregnancy in small animals. Progestin treatment, extrapolated from human medicine, was frequently used for reversible control of fertility in dogs and cats. However, it is known that its use may prevent fertility in the future, because it predisposes to diseases like cystic endometrial hyperplasia. Even worse, progestin treatment promotes growth of the mammary gland and thus increases the risk for mammary tumours as well as for fibroadenomatosis in cats. Furthermore, glucose metabolism is altered and adrenocortical function is suppressed, which may result in diabetes mellitus or Cushing's disease. For males, safe products for the permanent or temporal control of reproduction are available. A single intra-testicular or intra-epididymal injection of substances like zinc arginine or calcium chloride resulted in irreversible infertility within 90 days. For fully reversible fertility control in male dogs, a GnRH-agonist implant has been recently released to the market. Although up to now not registered for the use in other species, the treatment with GnRH-agonist implants has been shown to be an effective, temporal contraceptive in tomcats and in queens, as well. On the other hand, the use of slow-release GnRH-agonists in bitches to suppress fertility resulted in treatment failure and side effects such as persistent heat and/or glandular cystic endometrial hyperplasia. Nevertheless, those implants may still be an alternative for young bitches with known high risk for side effects of surgical spaying, as long as owners are aware of the off-label use and potential adverse effects. GnRH-antagonists for suppression of reproductive function either alone or in combination with GnRH-agonist implants are still in the process of development, and up to now there is not a long-acting, effective product available on the market yet. Immunocontraceptive vaccines would be particularly suitable for reproductive control of stray dogs and cats. In contrast to reproduction control in privately owned pets, contraceptives used for population management have to be long lasting, but not necessarily 100% reliable. Ongoing research has already shown promise for the future use of single-dose, long-acting GnRH-immunocontra-ceptives. Further studies are needed to evaluate their side effects, especially in queens, where safety is still a concern. Melatonin implants provide short-term oestrous cycle control in the queen. Melatonin implants are of great interest for cat breeders, as infertility seems to be fully reversible. However, studies in a larger number of queens on the side effects and efficacy of these implants are still missing.

WS 6.3

The use of GnRH agonists implants for contraception in the bitch

A Fontbonne[1], E Fontaine[1], C Maenhoudt[1], F Mir[1], C Navarro[2]

[1]*Centre d'Etudes en Reproduction des Carnivores – UMR 1198 INRA-ENVA, Ecole Nationale Vétérinaire, Maisons-Alfort, Paris, France;* [2]*Medical Department, Virbac SA, Carros, France*

Over the last 10–15 years, long-acting GnRH agonists have become widely available. Most recent studies focused on the use of two compounds developed as subcutaneous implants: azagly-nafarelin and deslorelin. Only the latter has been commercially available in several countries of the EU since 2008 for use in male dogs. Some studies also focussed on application of this compound as well ason GnRH for contraception in bitches. Very few studies have been conducted about the postponement of puberty by GnRH agonists implants. Rubion et al. (2006) used sub-cutaneous (SC) azagly-nafarelin implants in bitches ageing 4.9 months. After removing the implant 1 year after implantation, age of puberty in these bitches was 25.5 months. Trigg et al. (2006) used a 4.7 mg deslorelin SC implant in 4 months old bitches and did not observe any signs of oestrus for the following 36 weeks. Interestingly, in the same study the use of deslorelin implants in bitches ageing ≥7 months systematically induced oestrus. We conducted a study to investigate the use of deslorelin SC 4.7 mg (group1) and 9.4 mg (group 2) implants in the postponement of puberty in bitches < 6 months of age. The implants were administered subcutaneously in the post-umbilical region. The occurrence of the first oestrus was recorded and a clinical examination was performed. No bitch of the first group showed any signs of induced oestrus soon after implant administration. The owners recorded no clinical side effect. Bitches displayed their first oestrus between 13and 24 months post-implantation and had elevated plasma progesterone levels (> 15 ng/ml). None of the bitches that underwent puberty showed any abnormality in the fur, growth or development of external genital organs. No bitch of group 2 showed oestrus signs till the end of the observation period (8–15 months). Adult bitches seem to respond to implantation by an induced oestrus. It may occur whatever the stage of the anoestrus is, including dioestrus with high level of progesterone. The medical prevention of this preliminary induced oestrus is not standardized yet. Megestrol acetate was able to prevent induction of oestrus (Wright et al. 2001). In opposite, Corrada et al. (2006) observed oestrus in 3 bitches between 26 and 51 days after implantation. The treatment with progestagens simply time-shifted the induced oestrus. However, when starting the treatment 4 days before implantation, only 10% of the bitches came into oestrus. Sung et al. (2006) attempted the same experiment, starting the treatment 7 days before implantation, but four of the five bitches expressed oestrus. In a recent study, we implanted adult bitches and concomitantly treated them with the aromatase inhibitor anastrozole 0.1 mg/kg (Group 1, n = 3, Arimidex®; Astrazeneca, France) or the anti-oestrogen clomifen acetate 5 mg/kg (Group 2, n = 8, Clomid®; Sanofi-Aventis, France) during 15 days per os. In Group 1, 2 bitches presented bloody discharge and keratinization of the vaginal epithelium after 5 and 6 days post-implantation. Ovulation was confirmed in these 2 bitches. In Group 2, no bloody discharge was observed in 6/8 bitches but keratinized cells were observed in vaginal smears of all bitches. Ovulation occurred in 5/8 bitches between 16 and 18 days post-implantation. As a result, these compounds cannot be considered as valuable alternatives to prevent the induced oestrus occurring in anoestrous bitches. Therefore, prevention of induced oestrus after implantation of GnRH agonists in adult bitches still has to be further studied. Side effects may be considered like prolonged heat, induced lactation behavioural changes, and miscellaneous problems like cystitis, vomiting, and allergic reactions. Mostly bitches recover after removal of the implant. However, ovario-hysterectomy may be necessary in some cases. Unpublished observation of our laboratory tends to show that there is a good fertility after returning to oestrus, and that bitches are able to give birth naturally, with no sign of hypoluteoidism during pregnancy.

Workshop 7
Perinatal mortality
Moderator: Gaby Hirsbrunner

WS 7.1

Perinatal mortality in calves

G Hirsbrunner

Ruminant Clinic, Vetsuisse Faculty, Berne, Switzerland

The term *bovine perinatal mortality* (BPM) includes calves dying within 48 h of calving. Estimates of calf losses show that almost 66% of all losses up to weaning occur during this time period. Farmers and practicing veterinarians register an increasing rate of BPM. When calvings are unassisted, the reasons for BPM are difficult to define. Lately, data on BPM in different production systems were collected. In the US Holstein population the increase of the dystocia level seemed to be the primary factor. But, is BPM truly increasing? Is management of cows at term getting less professional? Are we simply more aware of BPM? Are there systems where BPM is favoured? Different studies tried to describe and weight the reasons for BPM such as heritability, breed, management, feeding. Diagnostics, incidence and reasons for BPM throughout different countries need to be addressed.

WS 7.2

Still born calves in first calving heifers: a farm survey

G Hardarson

Agricultural University of Iceland, Lbhí - Stóra Ármóti, 800 Selfoss, Iceland

The incidence of stillborn calves has been an increasing problem in Iceland over the last 15 years. A project consisting of five modules

was carried out in the years 2006–2008. (i) Post mortem of stillborn calves; (ii) Trace element status of pre-partum heifers; (iii) Vitamin E concentration in forage; (iv) The calving process; and (v) The effect of Se enriched artificial fertilizer on grass and barley. The main purpose of the project was to gather information, which could lead to a better understanding of possible causes of the condition. The survey included 675 parturitions on 58 farms in the main dairy areas of Iceland. The mean stillborn rate was 22.6%. The survey revealed many factors in feeding and management of the heifers that lead to increased incidence. Most of the variance in incidence of stillborn calves is farm dependant. The incidence on different farms varied from 0% to 50%. The concentration of some trace elements in hay was much below requirements. This was in particular true for selenium (Se) and was further reflected in low blood status in heifers, and 75% of liver samples from stillborn calves were below acceptable levels. Trials with Se enriched fertilizer showed good response in Se concentration in hay. The results furthermore indicate a widespread deficiency in iodine and zinc. The results from the study on the concentration of Vitamin E in hay showed that the concentration decreases rapidly while drying in the field. The storage in round bales did not seem to have much effect on the concentration of Vitamin E. The concentration was below requirements according to NRC 2001. There was a large variation between farms in the feeding and management of heifers. Surveillance of the calving process varied also strongly. In many cases the expected date of parturition was unknown. The main reason for that is the common use of natural services, which account for about 60% in heifers. Calving difficulties mainly due to oversized calves were common. The maximum weight for female calves was 46 kg and for male calves 48 kg. For comparison the mean weight of calves from first calving heifers at the research farm Stóra Ármót is 31.5 or 1.5 kg less than the average weight of stillborn calves in the survey. There was a good correlation between incidence of stillborn calves and calving difficulties and how much calving assistance was provided. Post mortem examination of stillborn calves revealed injury in 35% of calves and 37% suffocated while being born. Muscular dystrophy due to Se deficiency was diagnosed in a few cases. It is concluded that the main reason for calving difficulties is oversized calves. Based on these results, the following guidelines were given to farmers to reduce the high incidence of stillborn calves in Iceland: Feeding in late gestation of first calving heifers should be reviewed. The feeding routine and management on farms with high incidence of stillborn calves should be given a special attention. The use of vitamin and mineral supplements should be increased and their composition should take into account the common composition of hay. Artificial fertilizer with selenium should be available to farmers. They should be advised to increase the supervision of calvings. They should also be advised to use artificial insemination instead of natural services for first calving heifers. Leaflets and seminars on the management of the peri-parturient cow should be available for farmers. The national breeding programme should take into account parameters like the incidence of stillborn calves and gestation length when choosing breeding bulls.

WS 7.3

A Delphi survey of the causes of death (COD) and the diagnostic criteria for COD in cases of bovine perinatal mortality (BPM)

JF Mee

Animal and Bioscience Research Department, Teagasc, Animal and Grassland Research & Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland

The objective of the survey was to elicit opinion from veterinarians on the COD and the criteria used to assign COD in cases of BPM (fullterm calf dying within 48 h of calving). The e-survey commenced in February 2012. Two types of respondents were targeted; veterinarians with expertise in bovine perinatology and veterinarians without particular expertise in bovine perinatology. Of 94 veterinarians contacted in 23 countries, 77 registered to participate. In each round questions were asked about the COD and the answers were collated, summarised and returned to the respondents for further feedback. The aim was to gain a consensus on each COD and to identify and to share non-consensual responses for iterative feedback. This communication reports the results from the first two rounds in which 69 (round 1, 90% of 77) and 55 (round 2, 71%) registrants responded. Round 1 dealt with COD of BPM. There was unanimity consensus on anoxia, dystocia, infection in utero and lethal congenital defects as COD. There was majority consensus on accidents, foetal haemorrhage, infection after calving, IUGR, micronutrient imbalances, premature placental separation and prematurity as COD. Additional COD most frequently suggested included hypothermia, unexplained, intoxications and idiopathic. Round 2 dealt with diagnostic criteria for accidents and anoxia as COD. There was majority consensus on post-parturient trauma (e.g. fractured ribs, liver tear), and aspiration of colostrum (e.g. colostrum in trachea and in lungs ± history of stomach tubing) as accidental COD. Other accidental COD were also suggested. There was majority consensus on subserosal haemorrhages, meconium staining, organ congestion and cyanosis, in that order of frequency, as criteria to diagnose anoxia. Other criteria to diagnose anoxia were also suggested. Further survey rounds are being conducted.

Workshop 8
Second litter syndrome in sows
Moderator: Nicoline Soede

WS 8.1

Second litter syndrome in sows

NM Soede

Adaptation Physiology Group, Wageningen University, Wageningen, The Netherlands

Reproductive performance is supposed to increase with increasing parity, reaching the highest level from parity 3–5. However, around 50% of the sows have a reduced litter size in second parity compared with in first parity. This so called 'Second Litter Syndrome' is often related to (excessive) weight loss during first lactation. Animal experiments, in which first parity sows were fed restricted during lactation, have shown that feed restriction compromises follicle and oocyte development during lactation and weaning-to-oestrus interval. A comprised follicle and oocyte development can lead to a lower embryonic survival and eventually to a lower litter size or even a lower farrowing rate. Up to the mid-nineties, the negative effects of weight loss were mainly expressed as a prolonged weaning to oestrus interval. More recent studies, however, mainly show effects on ovulation rate, embryonic survival and subsequent litter size. The shift from a prolonged weaning to oestrus interval to a reduced ovulation rate and embryonic survival is probably due to genetic selection for a short weaning to oestrus interval in sows. This genetic selection has successfully led to sows that come in oestrus shortly after weaning, but – as a downside – may now have a compromised follicle and oocyte quality at insemination, related with the negative energy balance during lactation. This reduced quality may lead to increased embryo mortality. The increase in litter size in recent years has increased the metabolic demands of sows, whilst feed intake did not increase. This discrepancy increases lactational weight loss, which is specifically problematic for first litter sows that have a limited feed intake capacity and limited body reserves. More insight in (management) factors influencing weight loss during first lactation and effects of lactational weight loss on reproductive performance in second parity sows might lead to tools to reduce weight loss or its effects on reproductive performance.

WS 8.2

Risk factors associated with different 'parity syndrome' profiles in sow herds

S Boulot[1], Y Després[1], B Badouard[1], E Sallé[2]

[1]*IFIP Institut du porc, Le Rheu, France;* [2]*MSD Santé Animale, Beaucouzé, France*

Reproductive disorders are often frequent after 1st weaning. This so-called '2nd parity syndrome' (P2S) includes various signs: delayed oestrus, infertility and small litters. We present results from a recent

study designed to evaluate the occurrence of these different components, and to identify risk factors at herd level. Analysis is based on data from 842 farms (French National Pig Management database). Fertility problems, delayed oestrus (>7 days) and small litters (<11 total born) occurred respectively in 16%, 13% and 19% primiparous sows, with 21% exhibiting a severe 2nd litter size drop (>20% fewer total born piglets). Herd profiles were characterized using threshold values for average 2nd parity results: fertility to 1st service <85%, reduction in total born ≥0.2, and weaning to insemination interval >7 days. P2S is frequent, with at least one of its component occurring in 80% farms. However, one or two signs together (fertility and/or oestrus) was a more frequent occurrence (40% herds) than the full syndrome (<10% herds). Logistic analysis identified factors significantly associated with P2S: herd size, batch management, age at weaning, 1st parity fertility or litter size, fostering. As risk factors vary according to P2S components, efficient prevention may benefit from precise identification of herd profiles. Results suggest that further detailed investigation of farm practices is needed and should focus on key factors such as gilt management, lactation feeding, litter size control and insemination procedures.

WS 8.3

Reproductive performance of second parity sows: causes of variation and relations with subsequent reproduction

LL Hoving[1,2], B Kemp[2], NM Soede[2]

[1]*Varkens KI Nederland, Helvoirt, The Netherlands;* [2]*Adaptation Physiology Group, Wageningen University, Wageningen, The Netherlands*

Around 50% of the second parity sows show reduced reproductive performance compared with their first parity, which negatively affects farm productivity. The aim of this paper is to describe the relation between body weight development during first parity and reproductive performance in second parity and to study if second parity reproductive performance was related to performance in later parities. Data from two experimental farms showed that increase in sow weight from first insemination to first weaning, rather than only weight loss during first lactation, was related with pregnancy rate in second parity (odds ratio 0.7 per 10 kg weight gain). On one farm, where gilts were relatively light and young at first insemination, second parity litter size was also positively related with this increase in weight (+0.4 piglet per 10 kg weight gain). Data from 12 000 sows, from 85 Dutch farms, showed that second parity reproductive performance was related with sow performance in later parities. Repeat breeders in second parity had a lower farrowing rate in 3rd (−4%) and 4th (−3%) parity and sows with a low litter size (≤10 total born) in second parity produced 4.6 fewer piglets in parity 3–5 than sows with a high litter size (≥14 total born) in second parity. This effect of second parity litter size on later performance was reinforced by differences in first parity litter size. In conclusion, farm productivity is related with second parity reproductive performance, which in turn is related with first parity performance.

Oral Communications

OC 1.1

Assessment of fecundity and proof of mendelian dependent germline transmission in two Venus transgenic pig lines produced by Sleeping Beauty transposition

W Garrels[1], S Holler[1], N Cleve[1], H Niemann[1], Z Ivics[2], W Kues[1]

[1]*Department of Biotechnology, Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut, Neustadt-Mariensee, Germany;* [2]*Paul-Ehrlich-Institut, Langen, Germany*

Recently, we described a method for producing transgenic pigs using a non-autonomous Sleeping Beauty (SB) transposon system. The founder animals showed ubiquitous expression of the CAGGS-Venus cassette in almost all cell types, including mature germ cells. To assess whether expression of the Venus reporter fluorophore affects fecundity of the transgenic animals, we analyzed reproductive parameters of two founder boars (Ni 503 and Ni 505). Spermatozoa were found to be Venus positive by fluorescence microscopy and flow cytometry [FACScan; BD Bioscience, Germany; argon laser (488 nm; 15 mW), filter for green fluorescence (530/30 nm)]. Molecular analysis of ejaculated sperm cells suggested three independent integrations of the transgene in both boars. To test the germline transmission of the three monomeric integration sites 14 wild-type sows were artificially inseminated and eight got pregnant. Two pregnant sows were slaughtered at day 30 and fetuses were analysed. Boar Ni 503 got three litters and boar 505 got two litters. The offspring was nursed to sexual maturity and one sow (line Ni503) was inseminated with sperms from Ni 505 and two sows (line Ni 505) were inseminated with sperms from boar Ni 503. A clear segregation of the transgenic trait following the Mendelian rules could be seen in the F_1 and F_2 offspring. The results show that SB-mediated transposition is a promising approach for stable genetic modification in the pig genome. Apparently, the Venus transposon trait behaves neutral and does not affect fecundity and animal health.

OC 1.2

Transcriptomic changes in bovine placentomes with retained foetal membranes (RFM) are associated to apoptosis and remodelling of the extracellular matrix

D Lange

Ludwig-Maximilians-Universität München, München, Germany

The mechanisms underlying the RFM are still unclear; hence we designed a holistic microarray study using Affymetrix® arrays comparing the placental gene expression of cows with and without RFM. The placental samples were extracted immediately after delivery (RFM group: n = 20 cows vs. control group: n = 20 cows). The samples of the feto-maternal contact zone were obtained by systematic random sampling and processed for stereological quantification of the cellular composition and RNA extraction. Statistical analyses (R Bioconductor, FDR 5% and FC 1.5) showed 801 differentially expressed genes (DEG) associated to the biological processes of (i) apoptosis (e.g. FBLN5, PLAU, ADAMTS4, CASP6), (ii) degradation of extracellular matrix (e.g. TIMP1, ADAM19, THBS1, THBS2, ITGB3), and (iii) innate immune response (parallel project), classified using the Functional Annotation Clustering tool of the DAVID Bioinformatics Resources. The higher DEGs are similar to expression patterns of ante partal placentomes. This suggests a placental immaturity in RFM. These findings need further investigation, but it has been found that there are relevant changes in the placental gene expression, which elucidates new aspects of the patho-physiology of RFM and might provide new targets in therapy strategies. (Supported by Pfizer Animal Health)

OC 1.3

Addition of coenzyme Q and vitamin E to Uppsala Equex II extender for canine semen freezing

C Milani[1], V Neagu[2], C Milani[1], H Sontas[3], S Romagnoli[1], A Mantovani[4], A Rota[5]

[1]*Department of Animal Medicine, Production and Health, University of Padua, Padua, Italy;* [2]*Department of Obstetrics, Gynecology and Reproduction, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania;* [3]*Section for Obstetrics and Reproduction, University of Veterinary Sciences, Vienna, Austria;* [4]*Department of Agronomy Food Natural Resources Animals & Environment, University of Padua, Padua, Italy;* [5]*Department of Animal Pathology, Faculty of Veterinary Medicine, University of Turin, Turin, Italy*

The effect of coenzyme Q, in association or not with α-tocopherol, added to Uppsala Equex II extender (UII) was investigated. For each of five dogs, the sperm rich fraction of three ejaculates was collected, divided into three aliquots, centrifuged for seminal plasma removal, and diluted with: UII as control (C); UII added with coenzyme Q 1 mM (Q); UII added with coenzyme Q and tocopherol

1 mM (E). Semen was equilibrated at 4°C for 1 h and diluted with an equal volume of the previous extenders C, Q, E, containing a different glycerol concentration (7% instead than 3%), and 0.5% Equex STM paste. Final spermatozoa concentration was 100 × 106 spz/ml; semen was packaged in 0.5 ml straws and frozen on LN2 vapors for 10 min before being plunged into it. Motility parameters were measured by CASA (Hamilton Thorne Analyzer Ceros 1.2, USA), just before packaging, immediately after thawing at 37°C × 1 min (T_0) and after 2 (T_2) and 4 (T_4) hours of incubation at 37°C. Integral (AI), reacted (AR) and detached (AS) acrosomal status was evaluated using Spermac® stain, both before freezing and after thawing. No differences between Q, E and C were found for parameters analyzed by CASA before freezing, at T0 and T4. A statistically significant difference ($p > 0.05$) was observed between C and E for VCL and ALH indicating a lower hyperactivation for E treatment, although not confirmed by Spermac® stain. Spermac® stain revealed an increase of AS ($p > 0.05$) for Q + E with respect to C before freezing, and of AS ($p > 0.05$) for Q and E with respect to C after freezing.

OC 1.4

Myeloperoxidase as an indicator of endometritis in the mare: preliminary results

S Parrilla Hernandez[1], T Franck[2], J Ponthier[1,2], E Mottart[3], S Deleuze[1,2]

[1]*Equine Clinic, University of Liège, Liège, Belgium;* [2]*C.O.R.D., University of Liège, Liège, Belgium;* [3]*Linalux/Mont-le-Soie, Ciney, Belgium*

Diagnosis of endometritis in the mare is routinely based on the presence of polymorphonuclear cells (PMNs) on endometrial smears. Studies show a relation between PMNs and myeloperoxidase (MPO), an enzyme released by PMNs during degranulation or after cell lysis, in many fluids and tissues. The aims of this study were to assess the presence and concentration of MPO in the mare's uterus, and to investigate its relation with PMNs. Thirty-six cycles from 28 mares (ages ranging from 6 to 22 years) were used. Endometrial cytological samples were obtained with a small volume uterine flush and either a uterine cotton swab or a cytobrush, when a follicle >35 mm was observed by ultrasound. The smears were stained with Diff-Quick® and one or more PMNs per field (400×) was diagnosed as endometritis. The supernatant of the flushes was used to measure MPO concentration with a specific equine MPO ELISA assay. Our results showed the presence of MPO in the equine uterus during oestrus (mean = 2839 + 2785). MPO concentrations were significantly ($p < 0.05$) higher in samples with positive cytological results. Occasionally, some samples with negative cytological results showed high MPO concentration, but the opposite was never observed. Clinical signs of endometritis are not always present, or they may be delayed. An early diagnostic improves the success of treatment. Our results show that high quantities of MPO in endometrial samples indicate the presence of PMNs. Further studies are needed to determine if MPO concentration could be routinely used as a tool of early detection of endometritis.

OC 2.1

Telomerase expression during prolonged culture of pig granulosa cells

E Chronowska[1], T Kott[2]

[1]*Department of Biology of Reproduction, Institute of Animal Science, Prague, Czech Republic;* [2]*Department of Molecular Genetic, Institute of Animal Science, Prague, Czech Republic*

It was suggested that telomerase in granulosa cells (GC) plays an important role for healthy follicle life and that loss of the enzyme activity may be linked to the follicular atresia. Spontaneous apoptotic cell death is the reason for rapid degeneration of primary granulosa cells *in vitro*. The aim of the present study was to analyse the relative transcript abundance of telomerase reverse transcriptase (TERT) gene in pig GC derived from follicles 3–5 mm in diameter and cultured in the presence of leukemia inhibitory factor (LIF), basic fibroblast growth factor (bFGF) and stem cells factor (SCF). Chosen factors are known to be involved in the maintaining of the survivability of different cell types *in vitro* and also play the role in the physiology of the ovary. Granulosa cells were isolated from healthy, non-atretic follicles (estimated on the basis of morphology and proper vascularisation) and cultured for up to 18 days in Knockout™ DMEM medium with supplements. Following concentrations of experimental factors were used: LIF-1000 IU/ml, bFGF-10 ng/ml, SCF-10 ng/ml. Pig TERT expression was determined by Real-Time PCR using glyceraldehyde 3-phosphate dehydrogenase as a reference gene. During the culture cells were harvested for telomerase expression analysis in 72 h intervals (every third day). ANOVA was used to determine the significance of differences. LIF, bFGF and SCF significantly increased ($p < 0.01$) telomerase gene expression in pig GC cultured for 3, 6, 9, 12, 15 and 18 days. The results of the study indicate that enhanced telomerase expression is associated with survivability and proliferation potential of porcine granulosa cells cultured over prolonged period of time.

OC 2.2

Influence of body condition of dairy cows on the oocyte and embryo quality *in vitro*

A Makarevich[1], E Kubovičova[2], L Olexikova[1], Z Hegedusova[2], P Chrenek[1]

[1]*Animal Production Research Centre Nitra, Lužianky, Slovak Republic;* [2]*Research Institute for Cattle Breeding Ltd. Rapotin, Vikyrovice, Czech Republic*

The aim of the study was to investigate effect of cow's body condition score (BCS) on quality of oocytes and *in vitro* produced embryos. Oocytes were collected from ovarian follicles of slaughterhoused Holstein cows with BCS 1, 2 and 3 using a five-point scale. Good quality oocytes (Gordon, 1994) selected for IVF were matured in TCM 199 with glutaMAX, fertilized in Fert-TALP medium and the presumptive zygotes were cultured in the B2 INRA medium on the BRL cell monolayer until blastocyst stage. *In vitro* developmental potential of oocytes was examined upon embryo cleavage and blastocyst yield. Embryo quality was evaluated upon blastocyst cell number (DAPI) and apoptosis (TUNEL-index). More high quality oocytes were collected from the BCS 2 (57.5%) and BCS 3 (60.9%) than from the BCS 1 (43.6%) groups ($p < 0.05$, Chi-square). There were no differences in cleavage and blastocyst rate among BCS 1 (55.9% and 14.5%), BCS 2 (58.3% and 15.06%) and BCS 3 (57.7% and 18.9%) categories, resp. The highest blastocyst cell number ($p < 0.05$, *t*-test) in the BCS 1 group (122.3 ± 6.9), compared to BCS 2 (101.8 ± 3.6) or BCS 3 (105.4 ± 3.7), was accompanied with highest apoptotic index (7.07%; $p > 0.05$, ANOVA) in this group, when compared to BCS 2 (6.54%) or BCS 3 (6.06%), respectively. Our results indicate that BCS affects initial quality of recovered oocytes, but does not influence their developmental potential *in vitro* and embryo quality. Grant support: NAZV QI91A061, APVV-0137-10, MSM LA 09031

OC 2.3

Role of COX-2 related PGE2 and EP2 receptors during gonadotrophin-induced bovine oocyte maturation

W Marei, W Marei, D Wathes, D Abayasekara, A Fouladi-Nashta

Royal Veterinary College, London, UK

Gonadotrophins (Gn) induce oocyte nuclear maturation and cumulus cell expansion in mammals. We and others have shown that during Gn-induced maturation prostaglandin E2 (PGE2) is produced by cumulus oocyte complexes (COCs) and there is increased mRNA expression of cyclooxygenase-2 (COX2), PGE synthase (mPGES1) and EP2 receptors. This study investigated the role of PGE2 in mediating Gn-induced maturation and cumulus cell expansion. In Experiment 1, bovine COCs were cultured for 24 h in serum-free Gn-free media with or without increasing concentrations of PGE2 (50–1000 ng/ml). A Gn-

containing control supplemented with 5 μg/ml FSH and LH was also used. PGE2 (≥100 ng/ml) stimulated mild-to-moderate expansion of cumulus cells and resulted in a concentration dependent increase in maturation rates to reach levels (97 ± 0.36%) higher than the Gn-containing controls (87 ± 1.9%) and significantly higher than Gn-free controls (45 ± 13.5%). Full cumulus cell expansion was only observed in Gn-containing controls. In Experiment 2, COCs were cultured in Gn-containing media treated with or without a specific COX2 inhibitor (NS398; 10 μM) or EP2 receptor antagonist (AH6809; 50 μM). NS398 significantly decreased PGE2 production in spent media as measured by radioimmunoassay (44 ± 10.2% of control; $p = 0.05$). NS398 significantly reduced the maturation rate compared to controls (64 ± 2.5% vs. 85 ± 1.9%; $p < 0.001$), an effect that was completely abrogated by 100 ng/ml PGE2. AH6809 also caused a significant decrease in maturation rate (66 ± 3.6% vs. 91 ± 1.5% in control). Cumulus cell expansion was not affected by NS398 or AH6809. In conclusion, COX2-PGE2 (via EP2 receptor) partially mediates Gn-induced bovine oocyte maturation but not cumulus cell expansion.

OC 2.4

Is impaired *in vitro* fertilization during simulated stress in the pig due to female or male gametes?

A Kumaresan[1], R González[2], A Bergqvist[2], Y Brandt[2]

[1]*National Dairy Research Insitutute (NDRI), Karnal, India;* [2]*Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden*

Simulated stress by supplementing *in vitro* fertilization medium with blood plasma from adrenocorticotropic hormone (ACTH)-treated sows reduced fertilization, cleavage and blastocyst rates due to alterations in the hormonal profile in comparison to plasma from control sows. However, it is unknown if this altered milieu is affecting the oocytes or the spermatozoa. To evaluate this, in experiment 1, *in vitro* matured oocytes (n = 525) were exposed to 10% plasma from ACTH-treated sows (A), control sows (C), or medium without plasma (B) for 1 h in fertilization medium. The plasma was collected at ovulation time (0 ± 2 h; cortisol and progesterone levels were higher in ACTH-group). After treatment, oocytes were fertilized and *in vitro* embryo development was evaluated. In experiment 2, spermatozoa (six ejaculates; two boars) were incubated for 0, 1, 4, or 24 h in A, B or C. Sperm viability, capacitation, acrosome reaction and protein tyrosine phosphorylation (PTP) were examined. Data were analyzed by ANOVA. In experiment 1, cleavage and blastocysts rates were not affected by the treatment. In experiment 2, no effect of treatment was found on sperm viability or capacitation. Alterations in acrosome reaction and PTP patterns were observed in group A from 1 h of exposure to treatment onwards. In conclusion, plasma from ACTH-treated sows did not affect oocyte competence but may affect spermatozoa fertilizing ability through alterations in the acrosome reaction and PTP patterns. Funded by Formas.

OC 3.1

Prevalence of uterine disease in Austrian dairy farms determined by vaginoscopic and cytological examination

I Prunner[1], H Pothmann-Reichl[1], K Wagener[1,2], M Ehling-Schulz[2], M Drillich[1]

[1]*Clinic for Ruminants, Section of Herd Health Management, Vetmeduni Vienna, Austria;* [2]*Institute for Functional Microbiology, Vetmeduni Vienna, Austria*

Numerous studies reported about the occurrence of endometritis on dairy farms in Germany and North America, but the knowledge from countries with smaller agricultural structures is limited. The objective of this study was to determine the prevalence of clinical (CE) and subclinical endometritis (SE) on dairy farms in Austria. The study was conducted on 10 commercial dairy farms in Lower Austria with 35–290 cows per farm. All cows were examined for CE 20–30 days post partum by rectal palpation of the uterus and by vaginoscopy. Intrauterine samples were collected with the cytobrush-technique. Two thresholds from literature for the proportion of polymorphonuclear cells (PMN) in the samples were used as indicative for SE, > 5% and > 18% PMN. The proportions of samples exceeding the threshold value for PMN of > 5% were compared by chi-squared-analysis considering clinical findings of endometritis. Of 100 examined cows 22.0% showed signs of CE based on vaginal discharge. SE was diagnosed in 29.5% (9% with > 18% PMN) of cows free of signs of CE. Overall 55% of the cows were free of clinical and cytological signs of endometritis. The cytological samples of cow with CE showed in 63.6% > 5% PMN. The difference in proportion of cows with > 5% PMN between cows with and without vaginal discharge was significant ($p = 0.003$). Results showed that the prevalence of CE and SE were within the range of prevalence reported from other studies. Further samples will be collected to determine the effects of CE and SE on reproductive performance.

OC 3.2

Pathogenic bacteria influence mRNA expression patterns of pro-inflammatory factors in the bovine endometrium

M Bittel[1], N Scheibe[2], M Drillich[3], W Heuwieser[1], M Jung[2], R Einspanier[4], C Gabler[4]

[1]*Clinic for Animal Reproduction, Freie Universität Berlin, Berlin, Germany;* [2]*Institute for the Reproduction of Farm Animals Schönow, Bernau, Germany;* [3]*Clinic for Ruminants, University of Veterinary Medicine, Vienna, Austria;* [4]*Institute of Veterinary-Biochemistry, Freie Universität Berlin, Berlin, Germany*

Invasion of pathogenic bacteria into the bovine uterus after parturition often causes inflammation of the endometrium resulting in impaired reproductive performance. The aim of this study was to investigate mRNA expression patterns of the pro-inflammatory factors CXC chemokine ligand (CXCL)1, -2 and -3 and their receptor CXCR2 in the endometrium of healthy cows and cows with clinical endometritis (n = 9 each). The influence of an isolated autochthonous *Bacillus* sp. on bovine endometrial cells *in vitro* was also evaluated. Endometrial samples for *in vivo* analysis were collected by cytobrush technique. Total RNA was extracted from *in vivo* as well as *in vitro* samples and subjected to real-time RT-PCR. The mRNA expression of CXCL1 and -2 was 10–20-fold higher in cows with clinical endometritis compared to healthy cows. CXCL3 and CXCR2 were twofold and 50-fold higher expressed in cows with inflamed endometrium compared to the healthy control, respectively. Bovine endometrial cells (n = 3 cows) were co-cultured with *Bacillus* sp. which induced cell death within 24 h. Presence of *Bacillus* sp. resulted in an approximately 10–20-fold increased mRNA expression of CXCL1, -2 and -3 after 2 h of co-culture compared to untreated controls. The expression levels decreased after 6 h. However, endometrial cells of each animal showed individual differences in the response to bacterial load. Transcripts of CXCR2 were not detected in cultivated cells. These results suggest that bacterial infection of the bovine uterus induces the synthesis of pro-inflammatory cytokines resulting in a local inflammatory reaction by attracting neutrophils. Supported by DFG GA 1077/5-1.

OC 3.3

Equid herpesvirus type 1 outbreak with abortion and encephalomyelitis on a warmblood stud in Germany

J Walter[1], C Seeh[2], U Bleul[1], K Osterrieder[3]

[1]*Clinic of Reproductive Medicine, Vetsuisse-Faculty, University of Zurich, Zurich, Switzerland;* [2]*Pferdegesundheitsdienst, Tierseuchenkasse Baden-Württemberg, Fellbach, Germany;* [3]*Institute of Virology, Freie Universität Berlin, Berlin, Germany*

Equid herpesvirus 1 (EHV-1) is an important pathogen for broodmares with the ability to cause big abortion storms. In recent years the equine herpesvirus myeoloencephalopathy (EHM) attained more and more attention as a severe complication of the EHV-1 infection. EHV-1 with a single nucleotide polymorphism in the viral DNA polymerase gene (ORF30; G2254/D752) is associated predominantly with EHM

outbreaks. In this case report we present clinical data of 61 horses collected during a large EHV-1 outbreak with the G2254/D752 variant which caused abortion (n = 6) and EHM (n = 8), in three mares coincidentally. Time curves of all clinical data were documented. Paired serum neutralization tests (SNT; day 12 and 28 after the index case) and virus detection by nested PCR targeting gB from nasal swabs, blood and peripheral blood mononuclear cells were performed in 42 horses. All aborted fetuses were examined by gross pathology and were tested positive for EHV-1 by PCR. Restriction enzyme digestion (*Sal*I) identified the Polymerase genotype. Only twelve (28.6%) of the tested horses showed a significant (≥4-fold) increase of SNT, which may be the result of delayed sample collection. The combined clinical manifestation with abortions and EHM cases on a single equine facility was a unique opportunity to document the clinical disease progression.

OC 3.4

Pyometra in a dog caused by a multiresistant *Escherichia coli* (*E. coli*)

B Walter, C Otzdorff, A Geyer, L Daub, J Braun

Clinic of Small Animal Surgery and Reproduction, Centre for Clinical Veterinary Medicine, Veterinary Faculty, LMU Munich, Germany

A 9-year-old intact female dog (mixed breed) was presented to the clinic because of polydipsia. At the time of first examination the bitch was in oestrus and a mild cystitis was diagnosed after the examination of the urine. No abnormalities of the urogenital tract were found by ultrasonography and blood parameters were in the normal range. A microbiological examination of the urine was performed and a multiresistant *E. coli* was found. Among the 16 tested antibiotics only 6 (Colistin, Chloramphenicol, Doxycyclin, Gentamicin, Imipenem, Nitrofurantoin) were effective. After 9 days treatment with Chloramphenicol the microbiological examination of the urine was negative. Two weeks later the dog was presented because of purulent vaginal discharge. The bitch had a leukocytosis of 67.32×10^9/l. The vaginal cytology contained many neutrophile granulocytes and less epithelial cells. A fluid filled uterus up to 2 cm in diameter was diagnosed by ultrasonography. Ovariohysterectomy was performed and the dog recovered completely. *E. coli* with a similar resistance pattern as the one in the bladder was found in the uterus. In most cases antibiotics like Penicilline or Fluorochinolone are effective in the concomitant treatment of pyometra. Recently, the significance of multiresistant bacteria is increasing and microbiological testing should be performed to choose the right antibiotic treatment.

OC 4.1

Seasonal differences in the ovarian dynamics of weaned sows

J Sanchez-Osorio[1], T Lopes[2], A Bolarin[2], J Vazquez[2], E Martinez[2], J Roca[2], J Roca[1]

[1]*Department of Medicine & Animal Surgery, Veterinary Science, Regional Campus of International Excellence 'Campus Mare Nostrum', University of Murcia, Murcia, Spain;* [2]*Department of Medicine & Animal Surgery, Veterinary Science, Regional Campus of International Excellence 'Campus Mare Nostrum', University of Murcia, Murcia, Spain*

The fertility impairment during the summer/early autumn becomes a limiting factor for productivity in swine farms. The aim of this study was to determine whether there are changes associated with season in ovarian dynamics of weaning sows. Weaned crossbred sows of a farm located in the southeast of Spain were randomly selected during summer (S, July-September; n = 51, 5 batches) and winter (W, January–March; n = 54, 5 batches). Sows showed similar parity (3.31 ± 0.3 vs. 2.87 ± 0.3) and body condition (3.39 ± 0.05 vs. 3.42 ± 0.06) in S and W, respectively. The ovaries were transrectal ultrasonography scanned every 24 h from weaning to onset of oestrus and every 12 h from onset of oestrus to ovulation. At weaning, the diameter of follicles was smaller ($p < 0.01$) during S (0.31 ± 0.01) than W (0.40 ± 0.01). The interval weaning-to-oestrus was longer ($p < 0.01$) during S (≥7 day in 26.2% of sows) than W (≥7 day in 3.9%). At onset of oestrus, the diameter and number of follicles in each ovary were smaller ($p < 0.01$) during S (0.69 ± 0.01 and 13.00 ± 0.37, respectively) than W (0.74 ± 0.01 and 14.10 ± 0.25, respectively). The percentage of sows did not show oestrus signs within 13 days after weaning was higher during S (20.41%) than W (7.41%). These results show seasonal differences in the ovarian dynamics of weaned sows supporting that altered follicular development may explain the reduced reproductive performance during summer/early autumn. Supported by Seneca foundation (04543/07), Murcia, Spain.

OC 4.2

The effect of azaperon treatment on LH and follicles development in weaned sows

TS Schwarz[1], M Murawski[1], A Zięcik[2]

[1]*University of Agriculture, Kraków, Poland;* [2]*Institute of Animal Reproduction and Food Research PAS, Olsztyn, Poland*

The aim of the study was to determine the level of cortisol and LH secretion and ovarian follicular activity in 14 primiparous sows during weaning. One group of seven sows (TG) were treated with azaperon immediately after weaning to inhibit stress. Blood samples were taken every 10 min during the 1st hour, then every hour during the next 6 h and every 4 h until ovulation. USG examination of ovarian activity was conducted daily using Aloka PS2 scanner equipped with 7.5 MHz linear array rectal probe and diameter of 181 growing follicles were measured. Results showed the increase in cortisol to 34.5 (±2.4) ng/ml in 10 min. after weaning in control group (CG), while only 2 h later in TG to the level of 35.7 (±7.4) ng/ml. Daily rate of follicular growth in TG was 1.08 (±0.17) mm and it was lower ($p \leq 0.01$) than in CG – 1.23 (±0.18) mm. Maximum concentration of LH was 11.8 (±1.3) ng/ml in TG and did not differ from CG – 11.1 (±4.4) ng/ml. Ovulation rate (OR) in TG – 13.7 (±0.5) was higher ($p \leq 0.05$) than in CG – 12.1 (±2.2). Mean period of LH surge was 32 (±3.6) hours in TG and was higher ($p \leq 0.01$) than in CG – 26 (±1.8) hours. The correlation between LH surge length and OR was 0.64 ($p \leq 0.01$). Results demonstrated that azaperon treatment did not inhibit postweaning cortisol increase, but working through the sympathetic system delayed it about 2 h. Extended period of ovulatory LH surge led to increase in ovulation rate. To conclude, study showed that azaperon affects follicular dynamics in weaned sows. Supported by a grant from MNiSW no. N N311 370837

OC 4.3

Nodal and receptors Alk4 and Alk7 in equine corpus luteum: mRNA quantification and functional role

A Galvao[1], M Rebordao[1], A Szostek[2], D Skarzynski[2], G Ferreira-Dias[1]

[1]*CIISA, Faculty of Veterinary Medicine, TULisbon, Portugal;* [2]*Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland*

Nodal and its receptors are transforming growth factor beta superfamily members, which have been associated with many processes of vertebrate embryogenesis. Nevertheless, the role of Nodal in the corpus luteum (CL) is not known. The aim of this work was to evaluate (i) the mRNA transcription of Nodal and the receptors Alk4 and Alk7 and (ii) the modulation of prostaglandin (PG) E2 secretion by Nodal, in equine CL, throughout the luteal phase. Blood and CL samples were obtained post mortem from 15 cyclic mares and classified into early (ECL, n = 5), mid (MCL; n = 5) and late (LCL, n = 5) luteal phase CL. The mRNA transcription was quantified by real time PCR. Later, luteal cells from all CL stages were treated with: (i) no factor (control), (ii) Nodal (concentrations of 0.1, 1, 10 and 50 ng/ml) and (iii) equine Luteinizing Hormone (LH 10 ng/ml; positive control), for 6, 24 and 48 h and PGE2 assessed by EIA. Nodal mRNA level was increased in ECL and decreased in LCL ($p < 0.05$), while Alk4 showed the opposite profile, with the highest mRNA level in LCL

($p < 0.05$). Alk7 did not change. Regarding the effect on PGE2 secretion after 24 h, Nodal (1 and 10 ng/ml) decreased PGE2 secretion from LCL ($p < 0.05$). After 48 h, Nodal (10 ng/ml) decreased PGE2 secretion from MCL and LCL ($p < 0.05$). The LH increased PGE2 secretion in MCL 24 h treatment ($p < 0.05$) and after 48 h in ECL and MCL ($p < 0.05$). In conclusion, the stage specific transcription profile of Nodal and its receptors, as well as Nodal modulatory effect on CL secretory function, suggest a putative role of Nodal pathway on the physiologic regulation of equine CL.

OC 4.4

Exposure of murine early secondary follicles to elevated FFA concentrations: implications for follicular development

S Valckx[1], V Van Hoeck[1], M Arias-Alvarez[2], S Andries[1], E Merckx[1], R Cortvrindt[3], P Bols[1], J Leroy[1]

[1]*University of Antwerp, Antwerp, Belgium;* [2]*Universidad Complutense de Madrid, Madrid, Spain;* [3]*EggCentris, Anderlecht, Belgium*

Elevated free fatty acid (FFA) concentrations, due to an upregulated lipolysis, have been proposed as a major link between maternal metabolic disorders and impaired fertility through a decreased oocyte and embryo quality, in the bovine and in humans. We studied the effect of elevated FFA concentrations on early follicular development, as this phase plays a key role in the oocyte's acquisition of developmental competence. Hence, we cultured murine early secondary follicles individually for 12 days (four repeats, $n = 466$). They were exposed to a mixture of 3 predominant FFAs in serum (palmitic, stearic and oleic acid), representing normal and pathological serum concentrations: control ($n = 153$), basal FFA ($n = 159$, 72 μM FFA) and high FFA ($n = 154$, 720 μM FFA). Binary logistic regression indicated that chronic exposure to elevated FFA concentrations tended to decrease Day 12 antrum formation in high FFA exposed follicles (69.5%) compared to control follicles (79.1%, $p = 0.07$). Follicles cultured in basal FFA conditions (77.4%) showed a delayed growth on Day 8 in comparison to control follicles (87.6%, $p = 0.04$), but not on Day 12. There were no other significant effects on antrum formation on Day 12 of culture, nor on the developmental kinetics to reach the antral phase. In conclusion, the negative effect of elevated FFA concentrations on early murine follicular development was limited. Further research will focus on the consequences for oocyte development and follicular physiology.

OC 5.1

Twenty-year trends in AI-boar longevity and reasons for early culling

V Furstoss[1], S Ferchaud[1], S Boulot[2], G Renaud[1], P Guillouet[1]

[1]*INRA UE88 UEICP, Rouillé, France;* [2]*IFIP, Le Rheu, France*

Premature AI-boar culling received little attention, although it may negatively impacts genetic effort and semen production. This study investigated the factors associated with frequency and reasons for early culling among AI-boars over a 20-year period. Data was collected from 973 boars delivered to the INRA boar stud at Rouillé, from January 1989 to December 2011. Early culling refereed to boars discarded within 180 days after arrival in quarantine. The effects of genetic line (four breeds), three periods of entry (1989–1996, 1997–2004, 2005–2011) and interaction were studied using linear or generalized linear models (Splus). Age at entry was higher in the last period (248 vs. 235 days, $p < 0.001$), with a breed × period interaction ($p < 0.01$). The career duration shortened over time, with longer duration from 1989 to 1996 (464.2 days) than in later periods (331.1 and 283.6 respectively, $p < 0.001$). This was associated with the significant effect of the periods of entry on the early culling rate (19.3%, 28.3% and 19.8% for the three periods, $p < 0.01$). Main reasons for early culling ($n = 240$), were semen defects (30%), poor libido (21%), lameness (18%), death (16%), others (10%) and genetic constraints (5%). Semen defects exhibited only transient reduction. Despite year-to-year variability, libido and locomotor troubles remained constant, with slight breed differences (higher values for Pietrain, $p = 0.06$ for lameness). Death rate decreased ($p < 0.01$) but was frequent in Pietrain ($p < 0.03$). Ways for improving the early culling issue in AI-boar are still low. The main causes remain at high frequencies suggesting the need of further investigations on boar management.

OC 5.2

HeatimeTM activity monitoring for heat detection in beef cattle in Norway

P Gillund[1], T Lunde Børsheim[2], P Groseth[2], K Karlberg[2]

[1]*Geno Breeding and AI Association, Hamar, Norway;* [2]*Norwegian School of Veterinary Science, Oslo, Norway*

Beef cattle often show less heat activity than dairy cattle. Activity measurement systems are not commonly used to detect heat in beef cattle. The objective of the study was to test the efficacy of Heatime, a standalone heat detection system, based on increasing motion patterns. The material included 113 estrous cycles in beef cows and heifers of different breeds, housed in four herds. The recommended interval from the threshold activity alarm signal to AI is 15–25 h. Animals were equipped with activity tags on neck band one or two estrus cycles prior to AI to record normal animal and herd activity. Threshold activity value was tested and reduced below the producer's recommendation (5.0) during the study. The farmers performed visual heat detection minimum twice daily. Pregnancy was verified by rectal palpation 6 weeks after AI. The average activity level was 16, and the average interval from alarm signal to AI was 21.9 h (SD ± 7.8). There was lower activity level in Hereford, compared with Charolais and cross breeds. Overall pregnancy rate after AI based on alarm signal was 76%, whereas the pregnancy rate after first AI was 56%. The activity monitoring system revealed estrus episodes not detected by visual observation. More estruses were detected when the threshold activity value was lowered to 3.5–4.0. Visual heat detection was helpful to exclude false positive and false negative alarm signals. The optimal time for AI was close to 22 h after achieved threshold value for alarm signal. Based on this investigation, Heatime is a useful tool for heat detection in beef cattle.

OC 5.3

Comparison of two electronic hand-held devices for detection of hyper-ketonemia in dairy cows

M Iwersen[1], M Pichler[1], I Schwendenwein[2], D Klein[1], M Drillich[1]

[1]*Clinic for Ruminants, Section for Herd Health Management, University of Veterinary Medicine, Vienna, Austria;* [2]*Central Diagnostic Unit, University of Veterinary Medicine, Vienna, Austria*

Subclinical ketosis in dairy cows leads to a higher risk for the occurrence of production diseases and poor reproductive and milking performances. Several cowside diagnostic tests for ketosis in milk and urine are available and have been reviewed elsewhere. The objectives of this study were to evaluate a new commercially available electronic hand-held meter (GlucoMen LX Plus®, Menarini, Italy) for detection of hyperketonemia in blood of dairy cows and to compare the results with an already evaluated device (Precision Xceed®, Abbott, USA). A total of 221 dairy cows (117 Holsteins and 104 Simmental) within the first 3 months of lactation from two dairy farms were used in this study. The blood samples drawn from the Vena coccygea were immediately analyzed for β-hydroxybutyrate (BHB) with both devices. After clotting, blood samples were centrifuged and serum was stored at −18°C until analysis by the Central Diagnostic Unit of the University. Serum concentrations of BHB determined in the laboratory were regarded as the gold standard. The prevalence of subclinical ketosis, based on concentrations of BHB in serum >1.4 mM was 9.1%. The correlation coefficients were highly significant for both devices with 94.0% for the GlucoMen LX Plus (GM) and 98.2% for the Precision Xceed (PX). Sensitivities and specificities of 1.0 and 0.99, respectively for both devices were excellent for a cowside test. The study demonstrates that GM is suitable for detection of cows with

hyperketonemia, with test characteristics comparable to the previously evaluated PX.

OC 5.4

Evaluation of an electronic hand-held device for measurement of β-hydroxybutyrate in ewes

M Pichler[1], J Gasteiner[2], I Schwendenwein[3], A Damberger[1], M Drillich[1], M Iwersen[1]

[1]*Clinic for Ruminants, Section for Herd Health Management, Vetmeduni Vienna, Austria;* [2]*Central Diagnostic Unit, Vetmeduni Vienna, Austria;* [3]*Research and Teaching Center (LFZ) Raumberg-Gumpenstein, Austria*

Hyperketonemia (HKET), defined as a concentration of β-hydroxybutyrate (BHB) in blood serum >1.6 mM, is one of the most common metabolic disorders in ewes carrying more than one lamb. The aim of the present study was to evaluate an electronic hand-held device (Precision Xceed®, Abbott, USA) for blood ketone measurement from the *Vena jugularis* (*V. jug*) in ewes. An additional objective was to test, if the concentration of BHB in a blood drop obtained by minimal invasive venipuncture of an ear vein, is of similar accuracy as the sample taken from the *V. jug*. The study was conducted in Austria with 194 ewes, provided by two commercial and two research farms. Blood samples, taken from the *V. jug* and an ear vein, were analyzed by using the hand-held meter on farm and by the Central Diagnostic Unit of the University (*V. jug*; gold standard). The coefficients of correlation between the gold standard and the concentrations measured with the hand-held device in the samples taken from the *V. jug* and ear vein were 93.9% and 89.1%, respectively. An ROC-Analysis was performed for the rapid test to detect ketotic animals, resulting in threshold values of 1.1 and 1.2 mM for the *V. jug* and ear vein, respectively, with a sensitivity and specificity of 1.00 and 0.99. The results demonstrate that the hand-held device is a useful tool for detection of HKET in ewes.

OC 6.1

Effect of body condition score at calving on reproduction indicators of dairy cows

J Beran, L Stádník, J Ducháček

Czech University of Life Sciences Prague, Prague, Czech Republic

Level of reproduction is expressed by parameters as insemination interval (INT), insemination index (IND), service period (SP) and calving period (CP). These indicators are physiologically affected by negative energy balance caused decline of body condition score (BCS). The BCS of observed cows was evaluated at calving by five point scale with an accuracy of 0.25 points according to the methodology for Czech Fleckvieh breed. Reproduction results were obtained from farm evidence. The 375 Czech Fleckvieh cows were divided into four groups according to cows parity (1st, n = 100; 2nd, n = 110; 3rd, n = 87; 4thand subsequent lactation, n = 78) and into three groups according to BCS at calving (up to 3.75, n = 94; from 4 to 4.25, n = 157; from 4.5, n = 124). The statistical program SAS 9.1. was used for analyzing the data. Level of INT and IND had a tendency to increase with the parity. But significant differences were found only between the 1st and 4th and subsequent lactation (INT +3.78; IND +0.36, $p < 0.05$). The average BCS at calving was 4.16 points. Dairy cows with BCS at calving <3.75 points had a longer INT, SP and CP than with BCS higher than 4 points. Significant differences were found only in the length of CP between BCS≤3.75 and ≥4.5 (+15.91, $p < 0.01$), respectively BCS from 4 to 4.25 and ≥4.5 (+9.48, $p < 0.05$). The opposite trend was found for INT which increased with the higher BCS (+1.48, respectively +0.64, $p > 0.05$). The BCS at calving and parity were found as important effects influencing subsequent reproductive performance of dairy cows. Funded by 'S' grant of MSMT CR and by the NAZV QI91A061.

OC 6.2

Relationship between bacteriological findings in the 2nd and 4th week postpartum in dairy cows and evaluation of laboratory results

A Werner[1], V Suthar[1], J Plöntzke[2], W Heuwieser[1]

[1]*Clinic for Animal Reproduction, Freie Universität Berlin, Berlin, Germany;* [2]*Konrad-Zuse-Zentrum für Informationstechnik, Berlin, Germany*

The most relevant uterine pathogens in dairy cows are *Escherichia* (*E.*) *coli* and *Arcanobacterium* (*A.*) *pyogenes*. Coagulase negative Staphylococci (CNS) and α-haemolytic Streptococci are also highly present, however, they are seen as opportunists. The objective of our study was to evaluate the effect of time postpartum, method of sampling as well as laboratory on the bacteriological results. Bacteriological samples were collected at 10 ± 1 and 24 ± 1 DIM from the uterine lumen using a cytobrush (CY). Vaginal mucus was classified by vaginoscopy. In a subsample bacteriological results of three different laboratories and of CY and cotton swab (CS) were compared. Lochia samples were collected at 10 ± 1 DIM and bacteriological samples taken using CY and CS. Bacteria were cultivated and identified. Animals had a higher risk for an infection with the same bacterial species at 24 ± 1 DIM when infected with *E. coli* (RR = 2.5) or *A. pyogenes* (RR = 3.0) at 10 ± 1 DIM. Risk of being diagnosed with abnormal vaginal mucus at 24 ± 1 DIM increased in cows with *E. coli* (RR = 2.1) or A. pyogenes (RR = 2.7) at 10 ± 1 DIM. Bacteriological results of three different laboratories agreed significantly for laboratory A + B and A + C for CY and CS. The results generated from samples collected with the CY agreed ($p < 0.001$) with those from CS from each laboratory. In conclusion cows infected with *E. coli* or *A. pyogenes* had higher risk for persistent infection and clinical endometritis. CY is useful for bacteriological samples from the uterus.

OC 6.3

Placental development in Holstein cattle is correlated with dam characteristics and β-cell function of the newborn calf

G Opsomer[1], MM Kamal[1], M Van Eetvelde[1], L Vandaele[2], M Hostens[1]

[1]*Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium;* [2]*Institute for Agricultural and Fisheries Research, Animal Sciences Unit, Melle, Belgium*

This study investigated the relation between placental characteristics, intra-uterine calf development and dam characteristics. Therefore, expelled placentas of Holstein heifers (n = 28) and cows (n = 66) were collected and weighed, and all cotyledons were individually measured in length and width. The association between placental outcomes and both calf and dam attributes (including MilkBot® lactation parameters) were investigated. The mean placental weight was 5.3 ± 1.24 kg and significantly correlated with calf weight ($r = 0.54$, $p = 0.001$). Placental efficiency, as determined by calf weight/placental weight, was negatively correlated with dam weight in heifers ($r = -0.39$, $p = 0.04$), but no such correlation was found in cows. The mean cotyledonary area (CotA, 0.56 ± 0.11 m^2) was significantly correlated with calf weight ($r = 0.72$, $p = 0.001$); and was higher in cows than in heifers (0.59 ± 0.11 vs. 0.49 ± 0.08 m^2; $p = 0.001$). In cows, CotA was positively correlated with dam milk production during their third trimester of gestation ($r = 0.29$, $p = 0.02$). The cotyledons were, therefore, less efficient (as calculated on calf weight/CotA) in cows than heifers (7.7 vs. 8.5; $p = 0.001$). The CotA in heifers was also positively correlated with the basal glucose level of the newborn calves ($r = 0.47$, $p = 0.03$); and tended to be negatively correlated with the pancreatic β-cell function as assessed by homeostatic model assessment (HOMA-B, $I0 \times 3.33/(G0-3.5)$, $r = -0.4$, 0.07). The association of placental measures with dam characteristics and with basal metabolic parameters of the newborn calf emphasize its probable importance in programming of the calf well-being.

OC 6.4

Day length and weather pattern effects on outdoor sow reproduction in the UK

A LeMoine, H Miller

Faculty of Biological Sciences, University of Leeds, Leeds, UK

The meteorological causes of seasonal infertility are poorly understood, especially in outdoor sows exposed to varying conditions year round. Therefore the relationships between meteorological conditions around the time of service and subsequent total born litter size (TB) and farrowing rate (FR) were studied in Landrace × Duroc sows under outdoor commercial conditions. Data for 123, 994 farrowings between 2004 and 2009, from 29 UK herds, were analysed by ANOVA. Monthly patterns in FR were observed across all parities, regardless of climatic conditions, and TB had a tendency to be lower from late summer and early autumn matings. A 5% reduction in FR was seen when maximum daily temperatures of 18°C were reached ($p < 0.001$), whereas TB only showed detrimental effects at average temperatures above 24°C ($p < 0.001$). Rainfall, humidity and wind had little effect on FR or TB under varying temperatures but in warm weather, the presence of wind improved TB ($p < 0.001$) and precipitation resulted in reduced FR ($p < 0.001$) and smaller TB ($p = 0.002$). This may be due to moderating effects on humidity, which when high tended to result in smaller TB in hot weather. Long days reduced FR ($p < 0.001$) and periods of rapidly changing day length improved FR ($p < 0.001$). Lengthening days resulted in higher TB ($p < 0.001$). This study suggests that conditions around the time of service are critical to understanding inconsistencies found in sow fertility during summer months. Temperature particularly needs further investigation as the upper critical temperature of lactating outdoor sows may be lower than previously thought.

OC 7.1

Effect of stress-related glucocorticoids on early bovine embryo development *in vitro*

C O'Meara, P Lonergan

University College Dublin, Dublin, Ireland

Under stress, high levels of glucocorticoids (GCs) are released through activation of the hypothalamic-pituitary-adrenal axis. GCs modulate follicular function in cattle but it is not known if they directly affect the embryo. The objectives of the current study were to observe the effects of adding GCs to bovine zygotes *in vitro* using a synthetic, dexamethasone (Dex), or natural, corticosterone (Cort), GC. *In vitro* produced presumptive zygotes, 24 h post insemination (hpi), were exposed to one of 14 treatments, namely: Control; Control (+DMSO); Cort or Dex at 1, 10, 20, 40, 80 or 100 μg/ml (four replicates over two dates with $n \succ 170$/treatment). Cleavage rate was assessed at 48 hpi and blastocyst rate on Day 8 pi. At 1 μg/ml, Cort had a negative effect on cleavage rate (65.0% for treated vs. 77.4 and 75.6% for controls ± DMSO, respectively) and blastocyst rate (5.6% for treated vs. 25.7% and 23.9% for controls ± DMSO, respectively). There was no effect of Cort at concentrations ranging between 10 and 40 μg/ml. At concentrations of 80 and 100 μg/ml cleavage rate declined (53.3% and 61.9%, respectively) and blastocyst development was obliterated (4.4% and 0.0%, respectively) compared to controls. Dex followed a similar trend to Cort where concentrations of 80 μg/ml and 100 μg/ml decreased blastocyst rate (9.2% and 8.3%, respectively; $p < 0.05$). There was a negative effect of high concentrations of GCs on embryo development. Low concentrations of Cort also decreased embryo development suggesting that there is an optimum concentration for bovine embryonic survival.

OC 7.2

Management factors affecting fertility in seasonal-calving Irish dairy herds

E Lane, M Beltman, M Crowe, S More

School of Veterinary Science, University College Dublin, Dublin, Ireland

The analysis of herd management records allows for accurate assessment of the current herd status, a crucial decision making tool to implement effective change. To determine the relative importance of cow and management factors on reproductive indices in moderate yielding Irish seasonal-calving dairy herds, breeding records of 1173 cows were collected on 10 seasonal calving herds between 2007 and 2009. After accounting for the heirarchical nature of the data, backward-stepwise multivariate logistic regression analysis was utilised to determine the effect of cow factors including parity, calving timing, days post partum, heat detection accuracy and herd factors including herd size and heat detection efficiency on key reproductive indices. Mean 6-week pregnancy and end of season not-in-calf rate were 43% (range 18–72%) and 25% (range 3–39%), respectively. Heat detection efficiency ($p < 0.000$), timing of calving ($p < 0.000$) relative to start of breeding, history of abnormal repeat intervals ($p < 0.000$) and length of post partum interval ($p < 0.000$) were each associated with lower 6-week pregnancy rates. While timing of calving ($p < 0.000$) and history of abnormal repeat intervals ($p < 0.000$) were associated with higher not-in-calf rates. Herd size and cow parity were not associated ($p > 0.05$) with either outcome when factors including existing calving pattern and heat detection quality were accounted for. The existing spread in calving pattern was the most influential factor, along with heat detection quality and length of voluntary waiting period that reduced fertility performance in seasonal-calving herds.

OC 7.3

Evaluating stress caused by dry-off in dairy cows

S Bertulat[1], C Fischer-Tenhagen[1], V Suthar[1], E Möstl[2], W Heuwieser[1]

[1]*Clinic for Animal Reproduction, Freie Universität Berlin, Berlin, Germany;* [2]*Department of Biomedical Science, Veterinary University of Vienna, Vienna, Austria*

A sudden dry-off is a common management practice on dairy farms. It is suspected that this procedure increases udder pressure and causes pain. While evaluation of pain is hardly possible estimating stress by measuring fecal cortisol metabolites (5β-11,17 dioxoandrostan – DOA) has been established. The objective of this study was to investigate the effect of dry-off on DOA levels in dairy cows considering udder pressure and milk yield (MY). Seventy-six dairy cows were included and grouped based on daily MY in low (lMY), medium (mMY) and high (hMY) producers. Fecal samples were collected before (D-7, 0) and after dry-off (D2, 3, 5, 7, 9). DOA was determined using an 11-oxoetiocholanolon EIA. Udder pressure measurements were performed daily using a dynamometer (Penefel DFT 14). Udder pressure increased within 2 days after dry-off in all groups ($p < 0.05$) and declined continuously afterwards. DOA increased only in cows with mMY or hMY ($p < 0.05$; lMY $p = 0.11$) and peaked on D2. DOA levels were elevated after dry-off for 8 days in mMY and hMY cows. Udder pressures differed between lMY and mMY cows on D1 and D2 after dry-off and between lMY and hMY from D1 to D8 ($p < 0.05$). While overall there was an effect of milk yield ($p < 0.05$) and pressure ($p = 0.05$) on DOA, for a given day DOA levels did not differ between the three yield groups. Our study showed that a sudden dry-off causes an increase in udder pressure and fecal DOA level. Therefore it can be suspected, that a sudden dry-off is stressful especially for high yielding dairy cows.

OC 7.4

Minor increases in progesterone indicate resumption of ovarian function in cattle

J Taponen[1], M Mikkola[1], T Kokkonen[2]

[1]*Department of Production Animal Medicine, University of Helsinki, Helsinki, Finland;* [2]*Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland*

Blood or milk progesterone (P4) analysis is widely used in the assessment of the resumption of ovarian function (OF) post partum (pp) in cattle. The criterion for the OF is generally the first ovulation pp. This is followed by the rise of P4 concentration, indicating luteal activity. However, various irregularities in the P4 rise are seen. This exacerbates the selection of criteria for the luteal activity (threshold level, duration). For example, minor increases in P4 preceding the first clear luteal activity are seen. These commonly last for one to three samplings, depending on the sampling frequency (generally once in 2 or 3 days) and only scarcely exceed the threshold level. To scrutinize the minor P4 rise, 24 Ayrshire cows were monitored daily with transrectal ultrasound scanning and plasma P4 analyses from 7 days pp to the third ovulation. Four animals showed the minor increase in P4. In all the cases, the increase was preceded by an ovulation and CL formation. However, the P4 dropped and CL disappeared in a few days, and a new ovulation occurred 10–12 days later (a short oestrous cycle). In two cases, the P4 concentration only once exceeded 3 nM (about 1 ng/ml). In the other two cases, P4 remained above 3 nM for four and 6 days. The maximum concentrations were 3.5, 3.6, 5.9, and 8.3 nM. In conclusion, most P4 increases, even very minor, seem to indicate an ovulation and thus, a resumption of OF. With commonly used sampling frequencies and threshold levels, many of these ovulations may be missed and the resumption of OF incorrectly determined.

OC 8.1

Building of biomimetic structures in order to reproduce the outer membrane of bull sperm and to analyse the contact with protective agents used in bull semen extenders

J Le Guillou[1], S Desherces[2], E Schmitt[2], M Ropers[3], E David-Briand[3], M Anton[3], L Amirat-Briand[1], D Bencharif[1], D Tainturier[1]

[1]*ONIRIS (ENVN), Nantes, France;* [2]*IMV Technologies, L'Aigle, France;* [3]*INRA Angers-Nantes, Nantes, France*

The knowledge about the mechanisms of action of protective extenders used to preserve bull semen for frozen or chilled is essentially empirical. The purpose of this study was to create biomimetics structures which can reproduce these mechanisms. So, a structure which reproduces the outer membrane of spermatozoa has been built. As biomimetics structure, the lipid monolayers at the air-water interface formed on a Langmuir balance were chose for analyze, on a controlled subphase, the contact with biomolecules implicated in the protective effect on the semen. First, a lipids mix deposit was done, then the barriers positions was modulated to get the desired molecular compression with a controlled pressure. Then, molecules with protective effect were introduced in the subphase. The monolayer changes were monitored. Each experiment was replicated twice. Miscibility studies at 34 and 8°C shows the formation of homogeneous domains of sphingomyelin and cholesterol, located in fluids domains composed of phosphatidylcholine. Complex biomolecules extracted from egg yolk like Low Density Lipoprotein was incorporated into the monolayer, contrary to other purified molecules like egg phospholipids. Contact tests with the monolayer were conducted with bull seminal plasma. Seminal plasma was injected either alone or associated with protective molecules which seems to inhibit the effect of seminal plasma on the monolayer. This model could be an opportunity for further studies, by varying the monolayer composition whose lipids fluctuate with the species studied, or protective molecules, as well as the composition of the subphase.

OC 8.2

Role of mitogen-activated protein kinase (MAPK) 14 signalling pathway in bovine spermatozoa exposed to heat stress *in vitro*

MB Rahman[1], L Vandaele[2], T Rijsselaere[1], D Maes[1], M Shamsuddin[3], A Van Soom[1]

[1]*Department of Reproduction, Obstetrics and Herd Health, Ghent University, Ghent, Belgium;* [2]*Institute for Agricultural and Fisheries Research, Belgium;* [3]*Department of Surgery and Obstetrics, Bangladesh Agricultural University, Bangladesh, India*

Heat stress has long been recognized as a cause of subfertility in farm animals and the male factor responsibility is considered to be at least 50%. We have recently shown that *in vitro* pre-incubation of spermatozoa at hyperthermic (41°C) condition compromised sperm motility, oocyte penetration and embryo development. Though spermatozoa are transcriptionally and translationally inactive upon leaving the testis, they remain vulnerable for post-translational modification of proteins which may cause functional damage in response to stress. MAPK14 is the key signaling pathway in heat-induced germ cell apoptosis. We investigated the involvement of the MAPK14 signaling pathway to sperm in response to heat stress by using a specific inhibitor SB203580; taking sperm DNA strand breaks, fertilizing capability and embryo development as endpoints. Selective pharmacological inhibition of MAPK14 signaling pathway by SB203580 significantly protected sperm motility (45.8% vs. 26.5%; $p < 0.01$) in comparison with control sperm as evaluated by computer-assisted sperm motility analysis. Moreover, SB203580 significantly prevented DNA strand breaks as detected by terminal deoxynucleotidyl transferase dUTP nick-end labelling (16.9% vs. 23.4%; $p < 0.05$). However, blocking MAPK14 signaling pathway in spermatozoa during heat stress could not improve fertilization (53.9% vs. 46.1%) and embryo development rates (5.5% vs. 6.9%). In conclusion, for the first time we showed the involvement of MAPK14 signaling pathway in heat-induced ejaculated spermatozoa. Further research will focus on the role of other signaling pathways in sperm cell damage and subsequent developmental competence.

OC 8.3

Phosphorylation of serine residues in HSP70 during holding time at 17°C is related with higher resistance of boar spermatozoa to cryopreservation

M Yeste, E Estrada, M Rivera, S Bonet, J Rodríguez-Gil

Autonomous University of Barcelona, Bellaterra, Spain

The present study sought to compare how different holding times at 17°C (4 h vs. 28 h) affected the success of boar sperm cryopreservation. Nucleoprotein structure, chromatin integrity and other sperm functional parameters, like membrane integrity, acrosome integrity and sperm motility, were assessed before freezing and 30 and 240 min after thawing. Sperm cryopreservation after a holding time at 17°C of 28 h post-collection resulted in significant higher percentages of spermatozoa with intact plasma membrane, intact acrosome and intact chromatin than frozen/thawed spermatozoa cryopreserved after a holding time at 17°C of 4 h. In contrast, a different holding time did not affect motility and kinetic parameters of boar spermatozoa either 30 or 240 min after thawing. On the other hand, phosphorylation levels of serine residues in HSP70 were significantly higher after 28 h of storing spermatozoa at 17°C than after 4 h. These results lead us to conclude that a longer holding time at 17°C prior to boar sperm cryopreservation is beneficial for several cell parameters and for chromatin integrity. In addition, phoshorylation of serine-residues in HSP70 during a longer holding time may be related to a higher sperm resistance to freezing/thawing.

OC 8.4

Seminal plasma-spermatozoa interactions in the boar

N Gerard[1], M Gaudin[1], V Labas[1], A Teiceira-Gomes[1], G Tsikis[1], S Ferchaud[2], X Druart[1]

[1]*INRA, Nouzilly, France;* [2]*INRA, Rouillé, France*

We previously demonstrated that hypotonic resistance of boar sperm could be related to their protein composition, characterised by Intact Cells MALDI-TOF Mass Spectrometry. At ejaculation, seminal plasma (SP) interacts with spermatozoa and modifies their hypotonic resistance. We hypothesize that differential ICM-MS sperm profiles are linked to different protein composition of SP. The aim of the present study was to analyse by mass spectrometry profiling the protein composition of SP from boars of different sperm hypotonic resistance (low and high). SP were desalted on Zip Tip C4 before being spotted onto the MALDI sample probe with sinapinic acid matrix, and analysed by linear MALDI-TOF mass spectrometry. This is a rapid and sensitive analytical approach that measures molecular weights of peptides and proteins < 20 kDa that are present in complex biological samples, such as SP. Data processing and statistical analysis were performed using Masslynx and Progenesis softwares, respectively. Among a total of 124 molecular species visualized in the MALDI-TOF profiles, 38 were differentially expressed between boars with high and low hypotonic resistance, according to a probability value < 0.01 with a fold change > 2. Based on molecular weights of the observed peptides (12–16 kDa), we can hypothesize that these proteins include spermadhesins. In conclusion, MALDI-TOF profiling can be used to characterize biomarkers of boar reproductive efficiency.

Poster Abstracts

P1

The effects of oocyte-secreted factors on cumulus expansion and meiotic maturation of bovine cumulus-oocyte complexes *in vitro*

A Abavisani

Department of Basic Sciences, Veterinary Faculty, Ferdousi University of Mashhad, Mashhad, Iran

Oocyte secreted factors (OSFs) have vital roles in key events of reproduction such as regulation of follicular cell functions and developmental processes. Aim of this study was to investigate the effects of native secreted factors of denuded oocytes on *in vitro* maturation of bovine oocytes were examined. Totally, 1211 immature oocytes were collected from slaughtered cattle ovaries, and COCs surrounded with at least three layers of cumulus cells were co-cultured with denuded oocytes (DOs) in a 50 µl droplet of oocyte culture medium (OCM). The selected COCs were randomly co-cultured in four groups of: group 1) COCs cultured alone as control; group 2) COCs co-cultured with DOs in 1:1 ratio; group 3) COCs co-cultured with DOs in 1:3 ratio and group 4) COCs co-cultured with DO in 1:6 ratio. Collected data after maturation period (24 h) were evaluated by Kruskall–Wallis. The results showed that increasing amount of native OSFs does not improve nuclear maturation and cumulus expansion of bovine COCs ($p > 0.05$). As importance of oocyte-secreted factors was speculated on the improvement of bovine oocyte competence and embryo production, it seems that the other aspects of oocyte maturation such as molecular level, fertilization and preimplantation embryogenesis have been affected by OSFs which should be more investigated for better understanding their effects in cattle.

P2

Characterization of the alpha-L-fucosidase in the porcine oviduct by molecular approach

OS Acuña, M Izquierdo-Rico, J Ballesta, M Jiménez-Movilla, M Avilés

Department of Cell Biology and Histology, Faculty of Medicine, University of Murcia, Murcia, Spain

Gamete maturation, fertilization and early embryo development take place in the oviduct of species with internal fertilization. Glycosidases have been detected in the female genital tract of different species and have been related with modification of carbohydrate residues present in the surface of gametes and oviductal cells. They could participate in the regulation of processes like gamete interaction or sperm binding to oviductal cells. Fucosidase activity has been detected in oviductal fluid; however, the type of fucosidase has not been identified. In this study, the gene expression of FUCA1 and FUCA2 was analyzed in the porcine oviduct. For that, oviducts from animals at the late follicular phase of the cycle were obtained ($n = 3$) and total RNA was isolated from luminal epithelial scraping of the ampullary region. cDNA was synthesized and used as template for amplifications by polymerase chain reactions using specific primers for FUCA1 and FUCA2. Two different amplicons of 1086 and 1165 bp were obtained for FUCA1 and two different amplicons of 547 and 585 bp were obtained for FUCA2. Results of this study reveal that FUCA1 and FUCA2 mRNAs are expressed in the pig oviduct. Further studies including Western-Blot analysis are needed to detect these enzymes in the oviductal fluid. This study was supported by Fundación Séneca de la Región de Murcia (0452/GERM/06) and AGL2009-12512-C02-01-02.

P3

Bovine sperm death kinetics: changes in motility, acrosome and plasma membrane

M Ahmad[1], N Ahmad[2], A. Riaz[2], M Anzar[1]

[1]*Department of Veterinary Biomedical Sciences, WCVM, University of Saskatchewan, Saskatoon, SK, Canada;* [2]*Department of Theriogenology, University of Veterinary and Animal Sciences, Lahore, Pakistan*

Extent and timing of alterations in sperm integrity affect the fertilization. Detailed report of kinetics of changes in bovine sperm during pathway to death is not available. The present study dealt with the temporal changes in motility, acrosome and plasma membrane stability in fresh and frozen thawed semen during incubation at 37°C over 24 h. Each pooled sample (3 bulls/replicate, cryopreserved by standard rate) was evaluated for motility with CASA, membrane integrity by FITC-PNA/PI assay and phosphatidylserine externalization by Annexin V/PI assay at 0, 2, 4, 6, 12 and 24 h of incubation with flow cytometer. Data was analyzed using PROC MIXED model as 2 (semen type) × 6 (time) factorial, taking time as repeated measure. Motility and viable sperm were higher in fresh than frozen-thawed till 6 h. Motility and viable sperm declined below threshold (30%) much later (12 h) in fresh and earlier (viable sperm 6 h, motility 2 h) in frozen-thawed. Membrane integrity decreased below 30% at 2 h in both types of semen. Necrotic sperm increased overtime (97%) in fresh while frozen-thawed had both late apoptotic (53%) and necrotic (34%) sperm. In conclusion, the alterations in motility, acrosomes and plasma membrane integrity was slower in fresh than frozen-thawed semen. Fresh semen followed necrosis and frozen-thawed semen underwent apoptosis-like pathways.

P4

Abstract withdrawn.

P5

Evaluation of ciprofloxacin for cryopreservation of buffalo spermatozoa

S Akhter[1], B Rakha[1], N Ullah[1], S Andrabi[1], S Qadeer[1], M Ansari[2]

[1]*Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Rawalpindi, India;* [2]*University of Gujrat, Gujrat, India*

Bacteria isolated from buffalo semen have become resistant to traditional antibiotics therefore; Ciprofloxacin (CP) was evaluated for bacterial control, post thaw quality and fertility of buffalo semen at SPU, Sahiwal, Pakistan. *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus* sp. *Corynebacterium* sp., *Micrococcus* sp. and *Staphylococcus* sp. were isolated from buffalo semen. *P. aeruginosa*, *Corynebacterium* sp and *Micrococcus* sp. were resistant to streptomycin while *P. aeruginosa* and *Proteus* sp. were resistant to penicillin. All bacteria were susceptible to CP. *In vitro* dose toxicity of CP in sodium citrate buffer containing 0, 200–2000 μg/ml, assessed by motility and livability, analyzed by ANOVA indicated that CP up to 1000 μg/ml was non-toxic to buffalo sperm. Buffalo semen frozen in extender containing streptomycin-penicillin (SP;1000 μg/ml–1000 IU/ml) or CP 600 μg/ml was assessed for total aerobic bacterial count (TABC; post thaw), motility, plasma membrane integrity (PMI), viability at 0, 2, 4 h post thaw, and analyzed by ANOVA. At 4 h post-thaw, PMI (%) was higher ($p < 0.05$) in extender containing CP (15.0 ± 1.0) than SP (11.7 ± 1.5). TABC was 0.00 in extender containing CP compared to 0.07×10^4 CFU/ml with SP. Semen (two bulls) frozen in tris-citric extender containing SP or CP was used to inseminate and 400 inseminations (200/group) were recorded. Chi-square test showed higher ($p \leq 0.05$) fertility rate with CP (55%) vs. SP (41%). In conclusion, ciprofloxacin may be used in extender for cryopreservation of buffalo semen.

P6

Effect of 37°C incubation test after thawing of Brown Bear (Ursus arctos) semen cryopreserved with soybean extenders

M Álvarez-Rodríguez[1], M Álvarez[1], S Borragan[2], E Lopez-Urueña[1], S Gomes-Alves[1], F Martinez-Pastor[3], L Anel[1], P de Paz[3]

[1]*ITRA-ULE, INDEGSAL, Animal Reproduction and Obstetrics, University of León, León, Spain;* [2]*Cabárceno Park, Cantabria, Spain;* [3]*ITRA-ULE, INDEGSAL, Cell Biology, University of León, León, Spain*

The use of an incubation test to evaluate alternatives to egg yolk extenders may help to the develop of a species-specific freezing protocol. Electroejaculated semen (six males) was extended TTF-Base (TES-Tris-fructose 300 mOsm/kg, pH 7.1, with 6% glycerol, 2% EDTA and 1% Equex-Paste), supplemented with: EY20 (20% egg yolk), S5-1 (5% soybean lecithin; 24% phosphatidylcholine (PC); Avantis Polar Lipids R, Alabasyer, Alabama, USA) and S5-2 (5% soybean lecithin; 14–23% PC; Sigma, P5638) and frozen in a programmable biofreezer (−20°C/min). Thawed samples (65°C/6 s) were incubated at 37°C (2 and 4 h) and analyzed by CASA and flow citometry (PI/PNA-FITC). Total and progressive motility (%), curvilinear velocity (μm/s) and amplitude of the lateral movement of the head (μm) showed no differences immediately after thawing, but they were higher for EY20 at 2 h and for EY20 and S5-2 at 4 h. However, viable spermatozoa (PI-) were higher for EY20 after thawing (40.8 ± 3.2 vs. 27.5 ± 5.9 vs. 25.6 ± 5.2, at 2 h (16.4 ± 2.2 vs. 1.5 ± 0.3 vs. 6.2 ± 1.4) and at 4 h incubation (13.2 ± 2.4 vs. 1.1 ± 0.4 vs. 3.5 ± 1.1)). The incubation test might provide us with an accurate assay to highlight sub-lethal effects caused by cryopreservation. Further studies should improve the use of these extenders at different lecithin concentrations. Supported partly by CICYT (CGL2010-19213/BOS), Cantur SA. and Ramón y Cajal program (RYC-2008-02560, MICINN, Spain).

P7

Expression of Avian β-Defensins during growth and in response to Salmonella infection in the chicken testis and epididymis

M Anastasiadou, M Avdi, G Michailidis

Department of Animal Production, School of Agriculture, Aristotle University of Thessaloniki, Greece

Avian β-defensins (Avβ D) are antimicrobial peptides that play significant roles in the innate immune system in chickens. Although many studies have reported the expression of Avβ Ds in various chicken organs, including the ovary and oviduct, little is known about the function of these genes in the rooster reproductive tract. The aim of this study was to investigate the expression and the changes in the expression levels of the complete family of the 14 Avβ D genes, during growth and in response to Salmonella enteritidis (SE) infection in the rooster reproductive organs. RNA was extracted from the testis and epididymis of roosters aged between 3 months to 2 years old ($n = 4$ at each age), as well as from SE-infected birds ($n = 4$). Expression of Avβ Ds was examined by RT-PCR and the mRNA levels were quantified using Real-Time PCR. Expression analysis data revealed that 11 and 10 members of the Avβ D family were expressed in the rooster testis and epididymis respectively. Furthermore, a significant up regulation of certain Avβ Ds was detected during growth and in response to SE infection. These results suggest that the avian β-defensins host defence peptides play a significant role in the chicken male reproductive organs and that they function *in vivo* to protect the reproductive organs from *Salmonella* colonisation.

P8

Selection of cryopreserved red deer epididymal spermatozoa using Androcoll-O improves membrane and mitochondrial status

L Anel-López[1], M Álvarez-Rodríguez[1], E López-Urueña[1], M Álvarez[1], P de Paz[1], L Anel[1], J Garde[2], J. Morrell[3], F Martínez-Pastor[1]

[1]*INDEGSAL, University of León, León, Spain;* [2]*IREC, UCLM-CSIC-JCCM, Albacete, Spain;* [3]*Swedish University of Agricultural Sciences, Uppsala, Sweden*

Epididymal sperm can be harvested from hunted red deer, frozen and used in farms. However, dead cells or debris can be a problem for artificial reproductive techniques (ART), such as sex sorting. We tested the effects of selecting thawed deer spermatozoa using the colloid Androcoll-O. Epididymal spermatozoa from three deers were frozen at 108/ml in 0.25-ml straws (TES-Tris-fructose, 20% egg yolk, 8% glycerol). Thawed samples were pooled and selected ($300 \times g$) using: 2 ml of colloid in 15 ml tubes (T2) with 6 or 1 straw (S6, S1), or 1 ml in 1.5-ml tubes (T1) with 1 straw, assessing membrane and mitochondrial status (YO¬PRO¬1/Mitotracker deep red, flow cytometry), after thawing and selection. Selection effects were evaluated by linear mixed-effects models. Sperm recovery was lower in the combination T2/S6 (5.5 ± 1.5% vs. T2/S1: 9.2 ± 1.1% or T1/S1: 12.7 ± 3.4%). Membrane and mitochondrial status were improved after selection, with 47.5 ± 10.0% and 31.4 ± 8.4% after thawing, increasing of 22.6 ± 5.7 ($p < 0.05$) and 23.3 ± 4.7 ($p < 0.01$), respectively. However, methods did not differ. Androcoll-O selected an improved sperm population from the thawed sample, being promising for its use in ART applied to red deer. Supported by AGL2010-15758 and RYC2008-02560.

P9

Reproductive performance of recipients after non-surgical transfer of embryos collected from super-ovulated pure-bred Duroc sows

M Angel[1], M Gil[1], C Cuello[1], J Sanchez-Osorio[1], J Gomis[1], I Parrilla[1], I Colina[1], M Diaz[2], J Reixach[2], J Vazquez[1], J Vazquez[1], J Roca[1], E Martinez[1]

[1]*University of Murcia, Murcia, Spain;* [2]*Seleccion Batalle SA, Girona, Spain*

The study aimed to evaluate the effectiveness of superovulation protocols to improve the efficiency of embryo donors for porcine non-surgical embryo transfer (ns-ET) programs. After weaning (24 h), purebred Duroc sows were treated with 1000 IU (n = 27; T1 group) or 1500 IU (n = 27; T2 group) eCG followed 72 h after by 750 IU hCG. Untreated sows (n = 36) were used as control (C group). Sows were inseminated and subjected to laparotomy on D6 (D0 = onset of estrus). There were no differences in the percentage of sows with normal fertilization among groups (range 88.9–100%). The number of corpora lutea and collected embryos in T2 group was higher ($p < 0.01$) than those in T1 and C groups (31.0 ± 1.7 and 25.4 ± 1.7; 24.5 ± 1.0 and 21.1 ± 1.0; 19.6 ± 0.5 and 16.2 ± 0.8, respectively). The number of transferable embryos was similar in T1 (19.7 ± 1.3) and T2 (22.8 ± 1.9) groups and it was higher ($p < 0.01$) than that obtained in C group (14.2 ± 1.1). Recipient sows on D4-5 were non-surgically transferred deep into a uterine horn with embryos (30/recipient) from T1 (n = 12), T2 (n = 13) and C (n = 9) groups. Farrowing rates (70.6 ± 7.9%) and litter sizes (9.3 ± 0.7) were not different among groups. Our results show that superovulation increases the efficiency of embryo donors and that ns-ET with superovulated embryos allows obtaining successful reproductive performance in recipient sows. Supported by CDTI/S. Batalle (20090686), MICINN (AGL2009-12091) and SENECA (GERM 04543/07).

P10

Thyroid hormones concentrations in foals affected by Perinatal Ashyxia Syndrome

C Antonelli[1], C Castagnetti[1], A Pirrone[1], S Panzani[2], N Govoni[1], M Veronesi[2]

[1]*Department of Veterinary Medical Science, University of Bologna, Bologna, Italy;* [2]*Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Università degli Studi di Milano, Milan, Italy*

The purpose of this study was the evaluation of total plasma thyroid hormones (TH) (T3 and T4) concentration in foals affected by Perinatal Asphyxia Syndrome (PAS) compared to healthy foals. Twenty-one healthy and 24 PAS foals ≤7 days-old were included in this study. Jugular blood samples were collected at birth/admission and every 24 h for 7 days (T0–T7). TH concentrations were analyzed by radioimmunoassay (RIA). Statistical analysis was carried out to evaluate: the time-dependent changes in TH concentrations in healthy and PAS foals, the comparison in TH concentration at admission between surviving and nonsurviving foals, and between healthy and PAS foals evaluated for age at admission. In both groups, T3 concentration significantly decreased at T5 compared to T1 ($p < 0.05$), and T4 plasma levels significantly decreased from T0 ($p < 0.01$). No differences were found in T3 and T4 concentrations at admission between surviving (n.20) and nonsurviving (n.4) foals. Statistical comparison between healthy and PAS foals divided in age groups showed a significantly lower T3 and T4 concentration at T0 in PAS foals ($p < 0.01$). In conclusion, the lower TH concentrations in PAS foals could be due to an 'euthyroid sick syndrome', that represents an adaptive response to reduce metabolic rate and to prevent organ failure, as reported in human neonates. TH concentration showed no prognostic value, maybe due to the small number of nonsurviving foals in this study.

P11

Effect of L-(+)-ergothioneine (EGT) on freezability of Tushin ram semen extended with milk based extender

U Ari, R Kulaksiz, Y Ozturkler, N Lehimcioglu, S Yildiz

Department of Reproduction, Faculty of Veterinary Medicine, University of Kafkas, Kars, Turkey

The aim of this study was to investigate freezability of ram semen extended with different L-(+)-Ergothioneine (EGT) doses. For this aim, semen from four ram were collected with artificial vagina (44°C) and then pooled. Pooled semen was divided five aliquots and extended with skim milk based extender containing 0 mM (EGT0: Control), 1 mM (EGT1), 2 mM (EGT2), 5 mM (EGT5) and 10 mM (EGT10) EGT, respectively. After equilibration (+5°C/2 h), the extended aliquots of semen in straws were cryopreserved in Liquid Nitrogen (LN_2) vapour (−120°C/15 min) and stored in LN_2 (−196°C) until examination date. Totally two straws from 17 replications (trials) in each experimental group were thawed in water bath (37°C/1 min), and percentages of progressive motility, sperm viability, abnormality, acrosome and membrane integrity were determined and statistically assessed with SPSS. In the result, it was determined that different doses of EGT did not affect freezability of ram semen, compared with Control for all parameters in total ($p > 0.05$). However, when separated trials accord with good (≥20% for motility in control groups; eight in 17 replications) and poor (<20% for motility in control groups; nine in 17 replications) freezability were statistically analyzed, beneficial effects of 10 mM concentration of EGT on motility and membrane integrity were determined in poor freezability trials ($p < 0.05$). In conclusion, addition of EGT in semen extenders may be considered to improve freezability of ram semen, if there is a situation of poor-freezability.

P12

Evidence-based animal reproduction – training students by development of critically appraised topics

S Arlt, P Haimerl, W Heuwieser

Clinic for Animal Reproduction, Freie Universität Berlin, Berlin, Germany

In current veterinary education skills like retrieving, critically appraising, interpreting, and applying the results of published scientific studies are hardly taught. The objective of this study was to test the concept of collaborative development of Critically Appraised Topics (CATs) in the course of training Evidence Based Veterinary Medicine (EBVM) in animal reproduction. The development of a CAT comprised the steps: identification of a clinical problem, asking a clinical question, finding, retrieving and evaluation of information and formulating an answer to the clinical question. The 116 participants were in their fifth year and attending the clinical rotation at the Clinic for Animal Reproduction. A total of 18 CATs of various qualities with topics chosen by the students were developed. Preparing the CATs in teams did stimulate discussion on the topic and on the quality of the retrieved papers. The evaluation of the project revealed that training of critical appraisal of information in veterinary education was endorsed by more than 90% of the students. In addition, more than 90% considered the development of CATs an effective exercise for assessing the quality of scientific literature on animal reproduction. In conclusion, this concept is highly valuable for training EBVM. Learning and intrinsic motivation seem to be enhanced by creating a situation similar to veterinary practice since the task is embedded into an authentic clinical problem. This approach of clinical training helps to prepare the students to integrate evidence from literature into practice.

P13

Relationship between mineral composition of seminal plasma and semen quality in various ram breeds

R Asadpour, R Asadpour, R Jafari

Department of Clinical Science, Faculty of Veterinary Medicine University of Tabriz, Tabriz, Iran

The present study was performed to investigate proteins in ram seminal plasma and the correlation between specific proteins and semen characteristics in cross breed ram.

Material, Methods and Results: Sixteen crossbred fertile rams (four Baluchi × Moghani, four Ghezel × Baluchi, four Ghezel × Merino, and four Merino × Moghani) were used in this study. Three ejaculates of each animals were collected during the breeding season from the 16 fertile crossbred rams and examined spermatozoa with light microscopy for motility, concentration, for dead sperm and morphology. Seminal plasma was harvested by centrifugation and then subjected to analysis enzyme, mineral composition and SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Total protein, AST, ALP, LDH, $Na+$, $K+$ and calcium Ca^{2+} were measured. The highest seminal plasma concentration of $Na+$, $K+$ and Ca^{2+} was recorded in Ghezel ×Merinos rams. Spearman's correlation coefficient test was applied to examine the correlation between seminal plasma protein fractions with all the parameters of the semen. The result showed that there was a significant negative correlation between $Na+$ and $K+$ but small correlation was recorded between $Na+$ and Ca^{2+}. The highest plasma concentration of ALP was recorded in Baluchi × Moghani ram and the lowest was recorded in Merino × Moghani ram (8 ± 1.25 and 3.33 ± 0.01 U/ml, respectively). The highest plasma concentration of LDH and AST (Mean ± SEM) was recorded in Ghezel × Baluchi ram (4.68 ± 0.19 U/ml, 19 ± 4 U/ml respectively). Significant correlations were found between LDH levels and sperm viability. The results obtained indicate that ram seminal plasma protein profile is charecterized by many protein bands with a molecular weights, ranging from 8.81 to 95.82 kDa. This study indicates that the relative content of seminal plasma proteins could not be an essential index to evaluate ram semen quality. While some enzyme and mineral composition could be an essential index to evaluate cross breed ram semen quality.

P14

Follicular fluid composition in relation to ovarian status in dromedary camels (*Camelus dromedarius*)

K Attia[1], A Abo-El Maaty[2]

[1]*Faculty of Veterinary Medicine, University of Cairo, Giza, Egypt;* [2]*Department of Animal Reproduction and AI, National Research Centre, Giza, Egypt*

The aim of this study was to evaluate the effects of ovarian status (presences or absence of corpus luteum; CL) on chemical compositions, hormonal profiles and antioxidant capacity of the follicular fluid collected from different sized ovarian follicles in dromedary camels. One hundred ovaries were collected from female dromedary camels at slaughter. The ovaries were collected in pairs from each animal and divided into two groups according to their status; ovaries with CL (CL-bearing group, 25 pairs) and ovaries without CL (non-CL bearing ovaries; 25 pairs). The follicles on each ovary were counted and categorized according to their diameter into three categories; small (1–3 mm), medium (4–9 mm) and large (10–20 mm) follicles. Follicular fluid (FF) aspirated from each follicle category on each pair of the ovaries was analyzed. The results showed that, the average numbers of ovarian follicles per ovary were significantly ($p < 0.05$) higher in non-CL bearing ovaries compared to CL-bearing group (6.4 ± 1.2 vs. 3.6 ± 0.9, respectively). Progesterone concentrations were significantly ($p < 0.05$) higher in the follicular fluid collected from all follicle categories in CL-bearing group than those obtained from non-CL bearing one. However, the concentrations of estradiol 17β were significantly ($p < 0.05$) lower in the follicular fluid collected from medium and large size follicles in CL-bearing ovaries compared to non-CL bearing group. Glucose concentration significantly ($p < 0.05$) increased in the follicular fluid collected from large follicles in non-CL bearing group (64.9 ± 6.1 mg/dl) than those obtained from the same follicle category in CL-bearing ovaries (45.4 ± 4.0 mg/dl). The concentrations of malondialdehyde (MDA) were significantly ($p < 0.05$) higher in the FF collected from small, medium, and large follicles in CL-bearing ovaries than non-CL bearing ones. Total antioxidant capacity (TAC) significantly ($p < 0.05$) increased in the FF obtained from large follicles in non-CL bearing ovaries compared to their counterpart in CL-bearing groups. Taken together, these data indicated that FF composition differ according to the ovarian status. Presence of the corpus luteum on the ovary plays an important role not only in the process of follicle growth and development but also in the concentrations of biochemical metabolites and hormonal profiles in the FF of dromedary camels.

P15

Seasonal reproductive activity and inbreeding in two small Greek Skyros horse populations

M Avdi, G Michailidis

Department of Animal Production, School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece

Skyros is a small-sized horse originating in the Greek island of the same name. The breed is considered endangered, as <200 purebred Skyros can be found throughout the country. As horses exhibit a seasonal variation in their reproductive activity and many differences are observed in the seasonality between different breeds and locations, the aim of this study was to determine the onset and length of the natural breeding period of Skyros breed horses throughout the year, as well as the inbreeding and heterozygosity levels, in 107 mares from two different experimental farms. Estrus was detected with teasing, using adult stallions, over a period of study of 15 years. Estrus was observed from April to August. Mares were teased and parturitions took place from January to August, with the highest percentage observed in March (25.4%) and April (20.3%). Inbreeding and heterozygosity levels were calculated. Twenty-four horses were inbred with average inbreeding coefficient of 0.11 (±0.02). Animals were also genotyped for 18 microsatellite markers. Average number of alleles was 4.11 (±0.43) and 3.72 (±0.39) for the two populations respectively. Average theoretical heterozygosity was 0.56 and 0.54 (±0.06) for the two populations respectively, while average observed heterozygosity was 0.59 (±0.06) for both populations. Genetic diversity levels were reasonable and comparable to results from other breeds internationally.

P16

Collection of field reproductive data from carcasses in the female Eurasian lynx (*Lynx lynx*)

E Axnér[1], R Payan-Carreira[2], P Settergren[1], J Åsbrink[3], A Söderberg[3]

[1]*Swedish University of Agricultural Sciences, Uppsala, Sweden;* [2]*Centro de Ciência Animal e Veterinária (CECAV – UTAD), Vila Real, Portugal;* [3]*National Veterinary Institute, Uppsala, Sweden*

All lynx that are killed or found dead in Sweden are to be sent to SVA. The aim of this study was to collect reproductive data from organs of female lynx and to validate the usefulness of such data by comparisons with published field data. Reproductive organs from a total of 76 lynx females that were killed or died between March 1 and April 1 during 2009 and 2010 were collected and evaluated for structures on the ovaries and the number of placental scars. Females were divided into juveniles (<1 year, n = 12) pubertal (>1 year to <2 years, n = 9) and adults (>2 years, n = 55). Corpora lutea from the present season were morphologically/distinctly different from CL from previous seasons. Females that were 1 year old had not been pregnant the previous season, according to lack of placental scars, but 7/9 had old CL. All 17 animals that were 2-years old had old CL (2–8) indicating that they had ovulated the previous season and some probably also the season before. Nine of 17 (52.9%) 2 year-old females had placental scars vs. 89.5% for older females ($p = 0.002$, chi-square test) indicating a pregnancy in the previous season. The mean number of placental scars was 2.4 (SD 0.76)

and the range 1–4. The number of old CL always equalized or exceeded the number of placental scars. Active ovaries (fresh CL, follicles or both) were found in 19 females that had been killed March 14 or later. Our data corresponds well with published field and zoo data indicating the usefulness of monitoring lynx reproductive organs.

P17

Fibromuscular dysplasia in the arteries of uterine mucosa layer in cows with cytologically determined endometritis

W Barański[1], T Rotkiewicz[2], T Janowski[1], S Zduńczyk[1]

[1]*Department of Animal Reproduction, University of Warmia and Mazury, Olsztyn, Poland;* [2]*Department of Pathology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland*

The aim of this study was to compare the use of histology and cytology examinations for the diagnosis of bovine subclinical endometritis. Cytobrush and biopsy samples were obtained from 33 clinically healthy cows in the fourth week postpartum. As an indicator of cytologically determined endometritis (81.8% of cows), a threshold of >5% polymorphonuclear leucocytes was used. Histological examination (hematoxilin-eosin and Mallory staining) showed slight inflammatory changes in the majority of cows with cytologically determined endometritis (CE). A novel aspect of histological examination was the presence of fibromuscular dysplasia in the arteries of the mucosa layer in 6 CE cows (22.22%); this was not present in the healthy animals. Fibromuscular dysplasia is an idiopathic, non-inflammatory, non-atherosclerotic vascular disease of the arteries, and rarely, of the veins. Until now fibromuscular dysplasia has not been described in the bovine uterus. The age of the cows with dysplasia varied from 2 to 7 years. The parturitions preceding the examinations in all these cows were unassisted. Four cows (66.7%) were inseminated after these parturitions and became pregnant, two (33.4%) cows were culled because of infertility. Further studies are needed to evaluate the prevalence and importance of fibromuscular dysplasia in pathogenesis of endometritis in cattle.

P18

Single-port laparoscopic ovariectomy in Sana Ines ewes using pre-tied loop ligature

F Barros[1], P Teixeira[1], M Silva[1], C Coelho[1], M Lopes[1], A Kawanami[1], D Chung[1], R Ribeiro[2], L Coutinho[1], L Padilha[1], W Vecente[1]

[1]*School of Agrarian and Veterinary Sciences, São Paulo State University (FCAV/UNESP), Jaboticabal, São Paulo, Brazil;* [2]*Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Rio de Janeiro, Brazil*

The aim of the current study was to develop a technique for single-port laparoscopic ovariectomy using a pre-tied loop ligature in seven healthy Santa Ines ewes and assess the feasibility of the technique, postoperative pain and the inflammatory response. A 10-mm operating laparoscope, with a 6-mm working channel, was inserted through an 11-mm trocar, positioned on the ventral midline. The pre-tied Meltzer's knot was employed for prophylactic hemostasis. The slipknot was introduced in the abdomen through a 14G needle and applied around the ovarian pedicle. Mean surgical time was 63 ± 20 min. Time for manipulation, ligature and resection of each ovary was 20 ± 10 min. Anesthesia time was 91 ± 26 min. No bleeding was noted during the surgeries. Using a modified visual analogue scale to assess postoperative pain (ranging from 0, no pain, to 6, severe pain), the ewes showed low scores (0.5 ± 0.5). Plasma fibrinogen was within the normal range for sheep. The females had a significant weight gain in comparison to the basal scaling (1 week before the surgery). The surgical technique was feasible in the ovine specie, which provided great security, minimal postoperative distress and rapid weight gain. However, the surgical time was higher than other laparoscopic techniques described for the ovine specie.

P19

Ovarian remnant syndrome in dogs: 52 cases (2001–2011)

A Becher, C Aurich, N Winter, S Schäfer-Somi

Centre for Artificial Insemination and Embryo Transfer, University of Veterinary Medicine, Vienna, Austria

All cases of dogs presented to the clinic with a history of estrus signs after ovariectomy (OE) or ovariohysterectomy (OH) between 2001 and 2011 were reviewed. Precondition for inclusion was a histologically confirmed ovarian remnant (OR). The patients belonged to 31 different breeds. Mean body weight was 24.7 ± 1.39 kg. In two cases a GCT was found (OH/OE in each >7 years ago). Mean age at diagnosis of OR was 5.1 ± 0.5 years. According to age at time of OE or OH dogs were attributed to groups: 4–12 months (n = 11), >12 months (n = 24). OH was performed in 42 (80.8%) dogs, OE in 6 (11.5%) dogs and in 4 (7.7%) cases this remains unclear. In 20 cases (38.5%) the OR was found on the right side, in 14 dogs (26.9%) on the left. In eight dogs (15.4%) OR were removed on both sides, in 10 cases (19.2%) the location was not given. In 30 dogs, determination of serum estradiol concentration revealed concentrations corresponding with proestrus/estrus in six cases, in 14 dogs basal levels and in 10 dogs values in between. Determination of progesterone concentration demonstrated the presence of a corpus luteum in 18/28 dogs. Statistical analysis was performed with SPSS 19 using non-parametric tests throughout. There was a significant relation between the age of the bitch at diagnosis of OR and the location it was removed (left side 6.3 ± 1 years, right side 3.7 ± 0.6 years, $p = 0.039$) whereas age at surgery and the location of the OR were not related. Interestingly, incomplete removal of the left ovary results in prolonged resumption of ovarian activity in comparison to the right side.

P20

Different patterns in peri-partus fat mobilization and commencement of luteal activity in high producing dairy cows

F Becker, C Hametner, C Weber, H Hammon, W Kanitz

Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany

The study was aimed to characterize different strategies of dairy cows regarding their peripartal fat mobilization in relation to metabolic and reproductive traits. Cows (n = 27; MY > 11 500 kg) were analyzed from dry off up to 12 weeks of their 3rd lactation and were grouped according to liver fat content (LFC), dry matter intake (DMI) and energy balance (EB) with relation to their first luteal activity (FLA). DMI and MY were recorded daily, body weight (BW), back fat thickness (BFT), BCS and concentrations of NEFA, BHBA, glucose, and insulin were measured weekly. Clinical reproductive examinations and estimation of progesterone concentrations in plasma and milk were carried out twice weekly. LFC correlated positively with BW changes ($r = 0.37$; $p < 0.001$), but was not correlated with changes in BFT. Alterations in BW were negatively correlated with DMI ($r = -0.42$; $p < 0.001$). Plasma NEFA concentrations decreased ($p < 0.001$) from dry off to d15 a.p. and afterwards increased in all groups, showing highest concentrations either on day 7 post partum (p.p.) in the group of low LFC or on d14 p.p. (high and medium LFC; $p < 0.01$). Thereafter, plasma NEFA concentrations steadily decreased ($p < 0.01$) in all groups to d63 p.p. However, cows with significant ($p < 0.05$) elevated NEFAs showed a significant earlier occurrence of first luteal activity. It seems that individual postpartal fat mobilization of different depots could have an influence on the FLA. An influence of the EB on the FLA was observed according to the time (2nd vs. 4th week p.p.), but not to the amount of the energy nadir. In a second experiment with 556 cows, a correlation could be shown between the first luteal activity and different subsequent reproductive parameters (intercalving interval, number of services/conception, pregnancy rate). This study was supported by BMBF (FP-REMEDY).

P21

Breeding soundness evaluation of 36 bulls with reduced reproductive performance: a retrospective study

M Beltman[1], M Canty[2]

[1]*Veterinary Sciences Centre, School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Dublin, Ireland;* [2]*Centre for Veterinary Epidemiology and Risk Analysis, University College Dublin, Dublin, Ireland*

The objective was to determine the fertility status of 36 bulls presented with a history of reduced reproductive performance to University Veterinary Hospital, University College Dublin. Eight different pedigree breeds were represented, with the most common breeds Friesian and Charolais. A breeding soundness evaluation (BSE), consisting of a full general clinical exam, assessment of the locomotion system and assessment of the reproductive tract was performed on all bulls by the same clinician. Reproductive tract evaluation comprised measurement of the scrotal circumference followed by assessment of testicles, penis, prepuce and the accessory glands. Semen was obtained by electro-ejaculation. Semen parameters assessed were: volume of the ejaculate (ml), colour of the ejaculate (1 = almost clear, 2 = skim milk, 3 = milk), sperm concentration ($\times 10^6$), sperm motility (1–5), percentage live sperm (%) and percentage normal sperm (%). Results were used to classify animals as fertile, sub-fertile or infertile on the day of examination. A chi-square test was used to assess the unadjusted relationship between fertility classification and colour, and fertility classification and motility. The average age of bulls at examination was 2.7 ± 0.21 years and after the BSE and semen evaluation, 15 were classified as fertile, eight as sub-fertile and 13 as infertile. Colour ($p < 0.001$) and motility ($p = 0.05$) were significantly associated with fertility classification. After converting variables, concentration ($p < 0.001$) and percentage live sperm ($p = 0.002$) were also associated with fertility classification. Of those bulls classified as infertile, motile sperm were found in only three samples examined. In conclusion, BSE was performed on 36 bulls with questionable fertility. Of these 36 bulls, 15/36 (42%) had normal semen. Colour of the ejaculate, sperm motility as well as concentration and percentage live sperm were directly related to overall fertility outcome.

P22

The use of liposomes for chilling canine semen at +4°C, preliminary results

D Bencharif[1], L Amirat-Briand[1], J Le Guillou[1,2], J Delay[1], S Eric[2], S Desherces[2], S Destrumelle[1], A Garand[1], P Barrière[3], D Tainturier[1]

[1]*ONIRIS, Nantes, France;* [2]*IMV Technologies, L'Aigle, France;* [3]*CHU Nantes, France*

Egg yolk is beneficial for storing semen; the LDL (Low Density Proteins) fraction has demonstrated an important role in the cryopreservation of canine spermatozoa (spz). LDL cannot be extracted on a large scale, unlike liposomes, which are produced from phospholipids. The aim of this study was to test the use of these liposomes for chilling canine semen for 48 h or more. Ten ejaculates were collected from seven dogs. The second fraction (spermatic fraction) of each ejaculate was diluted in seven different extenders: basic extender (BE) + 2, 4, 6, 8, or 10% liposomes (IMV Technologies), BE + 6% LDL, and BE + 6% egg yolk plasma (Canixcell; IMV Technologies). The final concentration was 100×10^6 spz/ml. The samples were stored at +4°C. Every day, for 2–4 days, 50 µl were sampled, warmed to +37°C and analysed with an IVOS HAMILTON THORNE analyser. We compared two important indicators of fertility: % motile spz and % progressive spz. The liposome extenders gave good results: the BE + 4% liposomes protected 90% of the dogs for 48 h, and 50% for 72 h after sampling. The motility was >30%, the threshold for artificial insemination in the dog, although the difference was not statistically significant ($p > 5\%$). The statistical test used was the equality of variances: paired results. This made it possible to compare the media against each other. The parameters studied show the benefit of liposomes for chilling canine sperm, enabling storage for at least 48 h after collection. These parameters give no real indication of the fecundity of the chilled spermatozoa, although good motility is an important factor. Further studies will thus include stains and artificial inseminations. Individual differences, possibly due to adnexal secretions, could explain the difference in semen motility between the dogs.

P23

Flow-cytometric evaluation of lectin binding moieties on porcine uterine epithelial cells

A Bergmann, U Taylor, D Rath

Department of Biotechnology, Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut, Neustadt-Mariensee, Germany

After entering the uterus, porcine spermatozoa undergo a transient binding with the endometrium. It is known that the interactions between sperm and oviduct epithelium as well as the zona pellucida are lectin mediated in all species studied. In the pig it has been shown that the binding of sperm within the oviductal reservoir is facilitated by mannose specific binding mechanisms. We established a cell culture model from primary porcine uterine epithelial cells (UEC) to examine lectin binding intensity as a preliminary stage of characterizing the participating surface molecules of sperm-endometrium interactions. We tested 21 lectins (Con A, DBA, DSL, ECL, GSL I, GSL II, AIL, LCA, LEL, PHA-E, PHA-L, PSA, PNA, RCA I, SBA, SJA, STL, UEA, VVA, WGA, sWGA) for their binding properties to porcine UEC. Fluorescence intensity was evaluated flow-cytometrically. Cells were collected from sows slaughtered at estrus and grown in six-well-dishes till confluence. After trypsinisation the suspension was centrifuged for 4 min at $800 \times g$ and RT. The remaining pellet was resuspended in PBS. Subsequently UEC were incubated with the lectins for 15 min at 37°C. Strong binding was observed for ConA, LCA and PSA, PHA-E, PHA-L and RCA I, GSL I, sWGA and WGA, which correspond to Mannose and Glucose, Galactose and *N*-acetylgalactosamine, *N*-acetylglucosamine and sialic acid respectively. These results confirm the existence of oligosaccharide ligands on the UEC surface as potential binding sites for sperm. This knowledge may allow for possible modulations and improvements of existing semen extender in pig reproduction. Supported by BVN, Neustadt/Aisch and IMV-Technologies, L'Aigle, France.

P24

Actin cytoskeleton integrity and mitochondrial patterns in pig blastocysts after simulated stress during *in vitro* maturation (IVM) and fertilization (IVF)

Y Brandt, R González

Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden

Oocyte competence and further embryo development can be affected by culture conditions. Exposure of oocytes during *in vitro* maturation to blood plasma from sows treated with adrenocorticotropic hormone to simulate stress did not affect fertilization, cleavage and blastocyst rates. The same parameters were negatively influenced by simulated stress during IVF compared to controls. However, the quality of the blastocysts was not determined. The purpose of the study was to assess the quality of the resulting blastocysts after simulated stress during IVM or IVF by determining actin cytoskeleton integrity and mitochondria distribution after staining with Alexa Fluor-488 Phalloidin and MitoTracker Orange, respectively. Actin cytoskeleton integrity was graded as good, fair or bad. Mitochondria distribution was scored as even, fair or heterogeneous/lack of staining. Data were analyzed as categorical variables using Cramér's V coefficient and Pearson chi-squared test. Confocal image analysis of blastocysts showed that neither the actin cytoskeleton integrity nor the mitochondrial activity pattern was affected by simulated stress exposure compared to controls during IVM ($p > 0.05$; $n = 140$) or IVF ($p > 0.05$; $n = 71$). To conclude, the quality of blastocysts, as assessed by actin and mitochondrial patterns, was not affected by a brief exposure of

oocytes to simulated stress during IVM or gamete exposure during IVF. The ability to generate offspring from such blastocysts should be explored. Funded by Formas.

P25

Expression of progesterone and estradiol receptors in the equine oviduct

P Bravo[1], A Galvão[1], M Rebordão[1], E Silva[1], R Costa[2], G Ferreira-Dias[1]

[1]*C.I.I.S.A., Faculty of Veterinary Medicine, TU Lisbon, Portugal;* [2]*Coimbra College of Agriculture, Coimbra, Portugal*

The oviduct plays a critical role on reproductive function, allowing for fertilization and providing the adequate environment for early embryo survival. Hypothetically, steroid hormones progesterone (P4) and estradiol (E2) may be involved in those functions. The aim of this study was to evaluate the expression of P4 receptor (P4R) and E2 receptors (ERα, ERβ) in the oviduct during the estrous cycle. Oviducts and blood were collected post mortem from nine cyclic mares classified into: (i) follicular phase (FP; n = 3), early luteal phase (ELP; n = 3); (ii) or mid luteal phase (MLP; n = 3) based on plasma P4 concentration and ovarian structures. Real Time PCR from the infundibulum and ampulla was performed using β2 mibroglobulin as the housekeeping gene. When the oviducts were considered as a whole, P4R showed higher mRNA level in the FP, when compared with ELP and MLP ($p < 0.05$). Nevertheless, no difference was found when the infundibulum and ampulla were considered separately. Also, no difference in ERα and ERβ mRNA level was observed between different estrous phases or between the infundibulum and ampulla. This study confirmed the expression of these ovarian steroid receptors in the equine oviduct. The higher P4R mRNA transcription in the FP is probably related with the lack of negative feedback mechanism, due to the low plasma P4 level. Nevertheless, further studies are needed in order to understand the effect of these steroid hormones in the oviduct function.

P26

The antioxidative effects of hyaluronic acid (HA) and catalase (CAT) on post-thaw boar semen parameters

M Brya, M Trzcińska

National Research Institute of Animal Production, Krakow, Poland

The present study was undertaken to determine the effects of different concentration of HA and CAT supplementation in freezing extender on post-thaw quality of boar semen. The sperm-rich ejaculate fractions collected from five crossbred boars (n = 35) were divided into five aliquots: (i) diluted with lactose egg yolk extender with 9% glycerol (LEYG), as control, (ii) LEYG containing 0.5 mg/ml HA, (iii) LEYG containing 1 mg/ml HA, (iv) LEYG containing 200 IU/ml CAT, (v) LEYG containing 400 IU/ml CAT. The semen was cryopreserved using a standard protocol (Westendorf et al., 1975) with minor modification. The effectiveness of freezing extenders on sperm progressive motility (CASA), plasma membrane integrity (YO-PRO-1/PI) and acrosome integrity (FITC-PNA staining) was assessed at 15 min post-thaw. The results revealed that mean progressive sperm motility (±SD) examined after thawing for extenders I, II, III, IV and V was 63.4 ± 7.3, 69.3 ± 9.5, 76.2 ± 11.1, 68.0 ± 6.4 and 73.1 ± 12.7, respectively. A significantly higher percentage of live spermatozoa (YO-PRO-1-/PI-) was found in extenders III (74.8 ± 9.8) and V (70.4 ± 5.9) than in the other extenders (I: 59.3 ± 6.1, II: 62.4 ± 5.9, IV: 64.3 ± 5.9) (Duncan's test, $p < 0.01$). The percentage of cells with acrosome-intact spermatozoa was significantly higher in the extenders supplemented with antioxidants compared to LEYG extender (Duncan's test, $p < 0.01$). In conclusion, the supplementation of 1 mg/ml HA or 400 IU/ml CAT in the LEYG extender improved progressive motility and plasma membrane integrity of the post-thawed semen qualities. Financed by grant N N311 524840.

P27

Alkaline phosphatase is involved in boar sperm function

D Bucci, E Ferlizza, G Andreani, G Isani, E Giaretta, M Spinaci, C Tamanini, G Galeati

Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy

Alkaline phosphatase (ALP) is diffused in several body tissues; it has also been demonstrated in seminal plasma and sperm plasma membrane from different species such as swine, canine and equine, but its role in sperm function has not been clarified yet. This study was aimed at delineating ALP activity and its possible involvement in boar sperm function. Pools of two ejaculates from five different boars were used. ALP activity was assessed (eight repetitions) in control (CTR) and *in vitro* capacitated (Brackett and Oliphant's medium + 10% FCS, 4 h, 39°C, 5% CO_2) sperm extracts (Cap) by spectrophotometric assay; ALP influence on sperm capacitation (five repetitions) was assessed by adding one unit of purified enzyme to the capacitating medium (Cap + ALP) and comparing CTC staining pattern in CTR, Cap and Cap + ALP. ALP activity was significantly ($p < 0.05$) higher in CTR sperm extracts as compared with CAP samples (8.07 ± 1.33 vs. 1.39 ± 0.26 nmol/min/mg protein, respectively); CTC staining after capacitation showed an increase in B pattern that was significantly ($p < 0.05$) inhibited by ALP (CTR 6.91 ± 2.49%; Cap 26.09 ± 3.15%; Cap + ALP 15.25 ± 3.76%). These data allow us to hypothesize that ALP could be involved in regulating boar sperm capacitation, considering its activity reduction during *in vitro* capacitation and its inhibitory effect when added to capacitating medium. Further studies would better explain the mechanisms involved in these effects.

P28

Characterization of porcine endometrial stromal stem/progenitor cells

J Bukowska, A Ziecik, G Bodek

Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland

It has been proposed that the porcine endometrium contains a population of adult stem cells that can participate in regeneration processes. The present studies were undertaken to characterize the endometrial stromal stem/progenitor cells. Stromal fraction of endometrium was enzymatically isolated from the uterus and collected at Days 2–3 (group A) and Days 5–7 (group B) of the estrous cycle. In order to obtain high purity suspension of endometrial stromal cells, epithelial cells were digested by 0.1% dispase and then, standard enzymatic method based on application of 0.06% collagenase type III was used. To test the clonogenicity, freshly isolated stromal cells were cultured at different clonal density (10, 20, 100, 200, 400 cells/cm^2). After incubation for 14 days, two types of colony forming units were distinguished: large (> 50 cells) and small (< 50 cells). The cloning efficiency for large colonies was 3.07% and 6.16% (group A and B, respectively) and for small colonies 5.13% and 3.45% (group A and B, respectively). Flow cytometry analysis of the endometrial stromal cell culture revealed the presence of two, different size populations: large cells (P1) and small cells (P2). Immunophenotype characterization revealed that population P2 contained cells positive for the expression of two mesenchymal stem cell markers: CD29+ and CD90+ but negative for hemopoietic cell marker CD45−. Stimulation of cells by EGF (epidermal growth factor), IGF1 (insulin – like growth factor 1) and HGF (hepatocyte growth factor) separately or in combination, resulted in reduction of CD90+/29+ and increase of CD90+/29− and CD90−/29− subpopulations. Multipotency examination of endometrial stromal cells showed that freshly seeded stromal cells and cultured clones, are capable of the differentiation into three mesenchymal lineages: adipocytes, chondrocytes and osteocytes, when cultured in differentiation media. These results demonstrate that the porcine endometrium contains cells with the characteristics of mesenchymal stem/progenitor cells, that can participate in the processes of endometrium regeneration during the estrous cycle.

P29

Factors influencing body temperature in early postpartum dairy cows

O Burfeind, V Suthar, S Bonk, R Voigtsberger, W Heuwieser

Clinic for Animal Reproduction, Freie Universität Berlin, Berlin, Germany

Fresh cow protocols are playing an important role for an early detection of sick cows on large dairy farms. The most objective parameter included in these protocols is measuring body temperature to detect infectious diseases (i.e. puerperal metritis). The temperature measured, however, is influenced by several factors (i.e. season, measuring process). Therefore the objective of this study was to identify factors that affect body temperature in freshly calved dairy cows. This might help to better interpret temperature measurements, to make treatment decisions and to avoid errors. A prospective observational study was performed from May to July 2010 on a farm milking 1200 Holstein cows. Temperature loggers were inserted in the vagina from day 2 to 10 after calving measuring temperature every 10 min. A total of 219 cows (Parity: 2.3 ± 1.4) that calved during the trial were enrolled in the study and monitored daily for production diseases. The effects of biological plausible factors on mean vaginal temperature were tested in a repeated measure ANOVA in a linear mixed model with day in milk as the repeated factor. Day in milk (2–10), period of calving (May, June, July), dystocia (assisted vs. unassisted calving), vaginal discharge (foul smelling vs. no foul smelling), age (primiparous vs. multiparous), and BCS (low vs. high) did influence vaginal temperature ($p < 0.05$). The results of this study illustrate that several factors have to be considered when interpreting results of measurements of body temperature in dairy cows.

P30

Determination of peri-follicular blood flow and follicular dynamics in ewes during breeding season

M Catalkaya, M Kuru, R Kulaksız, S Pancarci

Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey

It was aimed to determine perifollicular blood flow (PBF) and follicular dynamics in crossbreed Tuj ewes during breeding season. In the first study, presence of PBF was investigated with transvaginal color Doppler ultrasonography in ewes ($n = 14$) at estrus. Moreover, efficacies of transrectal and transvaginal ultrasonographic examinations were compared for the determination of number of follicles. In the second study, follicular dynamics were monitored with transrectal B-mode ultrasonography every other day from estrus (day 0) until day 14 and then daily until to be detected in estrus in ewes ($n = 9$). In the first study, relative risk ratio estimate to detect PBF was 2.8 (1.4–5.5) times higher ($p < 0.01$) in follicles ≥ 4 mm (14/40; 35.0%) compared to follicles < 4 mm (11/87; 12.6%), and presence of PBF could reveal the viability and dominance of the follicle. For the determination of number of follicles, there was no correlation between rectal and vaginal examinations (R: 0.35; $p > 0.05$), and rectal examination seems to be better for this matter. In the second study, it was determined that estrous cycle length was 15.7 ± 0.5 days, each estrous cycle had two follicular waves, ovulation rate was 2.1 ± 0.3 following the first estrus, diameters of the first wave dominant and Graafian follicles were 4.7 ± 0.7 mm and 4.7 ± 0.6 mm; respectively.

P31

Altering n-3 to n-6 polyunsaturated fatty acid (PUFA) ratios affect prostaglandin (PG) production by ovine uterine endometrium

Z Cheng, C Wathes, R Abayasekara

Royal Veterinary College, Hatfield, UK

N-3 PUFAs are the precursors of 3-series PGs, while n-6 PUFAs produce 2-series PGs. Both PUFA families compete for the same enzymes for PG synthesis and cellular incorporation, so n-3 PUFAs may influence 2-series PG production. Regulation of uterine 2-series PG production is essential for normal reproduction. This study examined the effects of altered ratios of n-3:n-6 PUFAs on uterine PG production. Confluent endometrial cells isolated from cyclic ewes were supplemented with 0, 20 or 100 μM of the n-3 PUFAs α-linolenic acid (18:3, ALA), stereodonic acid (18:4, SDA), eicosapentaenoic acid (20:5, EPA) or different combinations of EPA with arachidonic acid (n-6, 20:4, AA) in serum free medium for 24 h. Stromal and epithelial cell populations were confirmed by immunocytochemistry. PGs were quantified using RIAs and cyclooxygenase (COX) isoforms were measured by qPCR. Treatments were in quadruplicate with cells isolated from ≥8 ewes. ALA and SDA increased PGE2, PGF2α and PGE2:PGF2α ratios significantly ($p < 0.05$–0.01) while EPA alone at all tested concentrations did not affect PG generation ($p > 0.05$). AA at 20 and 100 μM intensively stimulated both PGE2 and PGF2α production ($p < 0.01$). The stimulatory effect of AA was attenuated by up to 80% ($p < 0.05$) when AA was combined with 20 or 100 μM EPA. The combined EPA + AA also increased the PGE2:PGF2α ratios ($p < 0.05$). SDA and EPA decreased COX-1 expression ($p < 0.05$) but did not affect COX-2 mRNA. The study suggests that n-3 PUFA supplementation altered PG production from n-6 PUFAs and the ratios of PGE2:PGF2α. This may affect reproductive processes.

P32

Progesterone priming of seasonally anoestrous ewes alters the expression of angiogenic growth factors in pre-ovulatory follicles

A Christensen[1], W Haresign[1], M Khalid[2]

[1]*Aberystwyth University, Aberystwyth, UK;* [2]*The Royal Veterinary College, Hatfield, UK*

Small dose multiple injections of GnRH induce ovulation in seasonally anoestrous ewes. However, unless primed with progesterone (P4), 2/3 of animals develop defective CL. The mechanism by which P4 priming ensures normal luteal function is poorly understood. This study tested the hypothesis that P4 priming of seasonally anoestrous ewes induced to ovulate with GnRH eliminates defective luteal function by altering the follicular expression of angiogenic factors: VEGF, VEGFR-2, ANG-1, ANG-2 and TIE-2. Twenty seasonally anoestrous ewes were given 500 ng GnRH (iv) at 2 h intervals for 28 h followed by a 300 mg bolus injection of GnRH to synchronize the pre-ovulatory LH surge. Half of the ewes were injected im with 20 mg P4 3 days before the start of GnRH treatment, while the other half served as controls. Ovaries were collected both before and after the LH surge but prior to ovulation, i.e., at 24 and 46 h after the start of GnRH treatment, respectively. Small (2–2.5 mm) and large (≥2.5 mm) follicles were analysed for protein and mRNA expression of VEGF, VEGFR-2, ANG-1, ANG-2 and TIE-2 by IHC and ISH, respectively. In small follicles progesterone priming did not affect expression of angiogenic growth factors, but in large follicles it significantly ($p \leq 0.05$) increased the expression of VEGF, VEGFR-2, ANG-1 and -2 protein and ANG-2 mRNA. These data suggest that P4 priming alters the expression of angiogenic factors in the large pre-ovulatory follicles that might lead to a better vascular template to support early luteal development and function.

P33

Key factors affecting reproductive success of mares on well managed stud-farms

M Crowe[1], M Osborne[2], I Henderson[1], M Bijnen[1], E Lane[1]

[1]*School of Veterinary Medicine, University College Dublin, Dublin, Ireland;* [2]*Forenaghts Stud, Co. Kildare, Ireland*

To evaluate factors contributing to fertility success of Thoroughbred mares, data from 3743 oestrous periods of 2385 mares were collected on a large stud farm in Ireland in 2008–2009. Fourteen stallions (mean age 8.3 years; range 4–15 years) had bred 2385 mares (mean

age 9.4 years; range 3–24 years). Maiden mares accounted for 12%, foaled mares for 64%, and barren/rested mares for 22.3% of the total. The overall mean per cycle pregnancy rate was 67.8% (68.6% in 2008 and 66.9% in 2009). Backward stepwise multivariate logistic regression analysis was utilised to develop two models to evaluate mare factors, including mare age, status, month of foaling, difficulty foaling, month of cover, foal heat, cycle number, treatments, walk-in status, and stallion factors including stallion identity, stallion age, shuttle status, time elapsed between covers and high stallion usage on the per cycle pregnancy rate and pregnancy loss. Cover <20 days after foaling ($p < 0.001$), mare age ($p < 0.001$) and repeat mares ($p < 0.001$) were associated with lowered pregnancy rates. Year ($p = 0.031$), mare age ($p < 0.001$) and barren/rested status ($p = 0.047$) increased likelihood of pregnancy loss. Pregnancy rates and pregnancy loss rates for mares treated for uterine disease were not different to normal mares. Only high usage of stallions was associated with lowered ($p = 0.012$) pregnancy rates. Shuttle stallions were more likely to have lowered ($p = 0.044$) pregnancy loss rates, perhaps reflecting a subgroup of increased fertility. Thoroughbreds can be effectively managed to achieve high reproductive performance in a commercial setting.

P34

Leukotriene C_4 synthesis and its effect on the contractile activity of inflamed porcine uterus

J Czarzasta[1], J Jaroszewski[2], W Markiewicz[2], B Jana[1]

[1]*Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland;* [2]*Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland*

The aim of the study was to estimate the content of LTC4, the level of 5-lipooxygenase (5-LO) and LTC4 synthase (LTCS) protein expression as well as the cellular localization of 5-LO and LTCS in the inflammatory-changed porcine uterus. The level of LTC4 in peripheral blood and its effect on the contractile activity of inflamed uteri were also determined. On day 3 of the estrous cycle (day 0 of the study), 50 ml of either saline or *Escherichia coli* suspension (109 colony-forming units/ml) were injected into each uterine horn of the control and experimental gilts, respectively. The uteri were collected on days 8 and 16 following treatment. Acute endometritis developed in all gilts after the bacterial infusion, however on day 8 of the study a more common severe form of acute endometritis was observed. The bacterial injection caused an increase in the LTC4 content of blood on days 4–16 of the study and in endometrium (ENDO) on days 8 and 16. On day 16 the levels of 5-LO and LTCS proteins were elevated in the ENDO with inflammation. The intensity of 5-LO and LTCS immunoreactions in luminal and glandular epithelium of the inflamed uteri increased on days 8 and 16 and on day 8 in arteries of the ENDO. On day 8 LTC4 increased the contraction intensity in the ENDO/myometrium (MYO) and MYO of the control and inflamed uteri, but more so in the controls. Our data indicate that elevated LTC4 synthesis may have a significant influence on the course of uterine inflammation.

P35

Plasma kynurenic acid in bitches with pyometra

R Dąbrowski[1], T Kocki[2], J Parada-Turska[3], M Szczubia[1]

[1]*Department and Clinic of Animal Reproduction, Faculty of Veterinary Medicine, University of Life Sciences, Lublin, Poland;* [2]*Department of Experimental and Clinical Pharmacology, Medical University of Lublin, Poland;* [3]*Department of Rheumatology and Connective Tissue Diseases, Medical University of Lublin, Poland*

Kynurenine is formed from tryptophan by tryptophan 2,3-dioxygenase and indoleamine 2,3-dioxygenase (IDO). IDO is activated by inflammatory stimuli. Kynurenic acid (KYNA) is produced from L-kynurenine by kynurenine aminotransferases. It has been found as endogenous constituent of various body fluids and tissues, e.g. the urine, saliva, blood serum, gastric and pancreatic juices, kidneys, liver, lungs and muscles. The aim of the present study was to determine KYNA concentrations in serum in healthy bitches ($n = 6$) and those with pyometra ($n = 6$). Blood was sampled from the saphenous vein. KYNA was determined by means of the high performance liquid chromatography with fluorescence detection. The mean serum level of KYNA in bitches with pyometra was 328 pmol/ml and was statistically significantly higher ($p < 0.05$) compared to the values in healthy bitches (40 pmol/ml). Our results show that serum KYNA is significantly elevated during infection of the uterus.

P36

Importance of peri-ovulatory follicle progesterone synthesis on ovine pre-ovulatory follicle function

J Daly[1], V Gath[1], L O'Shea[2], V Maillo[3], C de Frutos Benitez[3], F Carter[1], P Duffy[1], M Wade[1], P Lonergan[1], T Fair[1]

[1]*School of Agriculture and Food Science, University College Dublin, Dublin, Ireland;* [2]*SBBS, University College Dublin, Dublin, Ireland;* [3]*INIA, Madrid, Spain*

Recent work highlights the importance of P4 during the mid-cycle stages of follicle development and oocyte maturation. The objective of this study was to investigate the role of follicular fluid P4 synthesis in preovulatory function in ewes. The oestrous cycles of ewes were synchronised and animals were randomly assigned to one of two treatments: (i) intrafollicular injection of 3 beta-hydroxysteroid dehydrogenase inhibitor, Trilostane, ($n = 12$), or (ii) intrafollicular injection of placebo (PBS) ($n = 6$), at 34 h post-sponge removal (hps). At 54 hps the ewes were either slaughtered and their reproductive tracts retrieved ($n = 18$), or they were mated ($n = 11$) and their tracts collected at Day 7 (Day 0 being day of mating). Serum P4 levels were measured on Days 2, 4, 6 and 7. On recovery, injected follicles were measured, follicular fluid processed for P4 analysis and oocytes were assessed for maturation. For Day 7 recovery, CL formation was assessed. Treatment with Trilostane suppressed follicular P4 (20.2 ± 19.7 vs. 535.8 ± 775 ng/ml, $p < 0.05$), but had no effect on oocyte maturation or ovulation. CL formation was not affected (63 vs. 67%, $p > 0.05$) and serum P4 levels were not different (Day 2 0.29 vs. 0.27, D4 0.7 vs. 0.55, D6 2.7 vs. 2.72, D7 2.53 vs. 3.37 ng/ml, respectively, $p > 0.05$). In conclusion, inhibition of P4 synthesis resulted in lower P4 levels in follicular fluid but had no effect on oocyte maturation, ovulation, CL formation or circulating P4 levels.

P37

Investigation of udder health and milk quality parameters of dairy farms in Northern Cyprus

I Darbaz[1], A Bastan[1], O Tekin[1], D Ozen[1], S Cengiz[2], M Cengiz[2]

[1]*University of Ankara, Ankara, Turkey;* [2]*University of Atatürk, Erzurum, Turkey*

The aim of this study was to investigate the herd udder health and milk quality of dairy farms in Northern Cyprus. For this purpose, 138 dairy farms were evaluated once a month for 1 year. Median somatic cell count and total bacteria count were determined as 521 583 cell/ml and as 227 738 cfu/ml, respectively. As a result of isolation and identification of bacteria; coagulase-negative Staph. as a rate of 22.73%; Bacillus spp 18.68%; *Staph. aureus* 16.55%; Strep. dysgalactiae 11.53%; Strep. uberis 8.14%; Strep. agalactiae 7.62%; *E. coli* 7.44%; *Micrococcus* spp. 1.81%; *Pseudomonas* spp. 1.49%; Enterobacteriaceae 0.9%; *Proteus* spp. 0.85%; *Aeromonas* spp. 0.58%; Yeast 0.53%; *Pasteurella* spp. 0.47%; *Alcaligenes* spp. 0.41% and *Corynebacterium* spp. 0.29% were determined. About 28.5% of bulk tank milk samples had coliform count ≤100 CFU/ml and 71.5% >100 CFU/ml. Antibiotic residues were determined at 2.6% of samples. As a result of milk composition; dry matter as a rate of 12.03%; fat 3.3%; total protein 3.48%; casein 2.61%; lactose 4.56%; free fatty acids 1.13%; solid non-fat 8.75%; acidity 6.56 SH; density 1031.50 gr; freezing point −0.542°H and citric acid 0.13% were determined. Nevertheless, there was a decrease in milk yield during the summer. In conclusion, crucial

udder health and milk quality problems were determined of dairy farms in Northern Cyprus.

P38

Adequate handling improves the fertilizing ability of sex-sorted boar spermatozoa

D Del Olmo, I Parrilla, M Gil, C Maside, M Angel, J Sanchez-Osorio, J Gomis, C Cuello, J Roca, E Martinez, J Vazquez

Department of Animal Medicine & Surgery, University of Murcia, Murcia, Spain

The study aimed to optimize sperm handling protocols during sex sorting procedure in order to increase the fertilizing ability of sorted boar spermatozoa. For this purpose, porcine oocytes were matured *in vitro* and inseminated with spermatozoa sorted using two sheath fluids [PBS and PBS with EDTA (PBS-E)] and three collection media [PBS or PBS-E for PBS and PBS-E sheath, respectively, Tes-Tris-Glucose (TTG) and TTG + 2% egg yolk (TTG + EY)]. Unsorted sperm were diluted to 1×10^6 sperm/ml in PBS and PBS-E and used as controls. After insemination, the oocytes were cultured for 16 h to assess the fertilization parameters. Unsorted samples diluted in PBS and PBS-E showed high penetration (PE) and polyspermy (PO) rates (100%) and high number of sperm per oocyte (SO; 6.5 ± 0.2 and 9.3 ± 0.3%, respectively). Sperm samples sorted in PBS and collected in PBS, TTG and TTG + EY showed PE (range: 8.1 ± 2.4% and 15.1 ± 3.3%) and PO (range: 0.0 ± 0.0% and 5.6 ± 5.5%) lower ($p < 0.004$) than those obtained with sperm sorted in PBS-E and collected in the respective collection media (range: 38.8 ± 4.4–94.3 ± 2.1% and 42.5 ± 7.3–67.0 ± 4.4%, respectively). The PE, PO and SO were higher ($p < 0.02$) for sperm sorted in PBS-E and collected in TTG + EY (94.3 ± 2.1%, 67.0 ± 4.4%, and 2.1 ± 0.1, respectively) compared with all other sorted groups. In conclusion, the addition of EDTA to the sheath fluid and EY to the collection medium increased the fertilizing ability of sex sorted boar sperm. Supported by AGL2008-04127, GERM04543/07 and Sexing Technologies (Navasota, USA).

P39

Expression of anti-apoptotic, DNA repair and apoptotic genes in cryopreserved amniotic fluid stem cells of buffalo (*Bubalus bubalis*)

K Dev[1], A Yadav[1], B Singh[2], M Kumar[3], S Gautam[1]

[1]*Department of Biotechnology, Kurukshetra University, Kurukshetra, India;* [2]*Regional Station, Palampur, India;* [3]*National Institute of Nutrition, Hyderabad, India*

Heterogeneous amniotic fluid contains various cell types and it is believed that some of these cells possess the stem cell properties. This study was conducted to observe the pre and post effect of cryopreservation on expression of DNA repair and anti-apoptotic genes in amniotic fluid stem cells of buffalo (Bubalus bubalis). Expression of DNA repair and anti-apoptotic gene likes BRCA1, Caspase9, Caspase3, Ced9, Max, Parp1, Phb, SH3GLB1, Tdh, TERT and TRF1 were observed from the amniotic fluid stem cells in different passages with RT-PCR amplicon of 100, 197, 120, 349, 199, 332, 233, 426, 253, 425 and 426bp respectively. Pre and post effects of cryopreservation on amniotic fluid stem cells were also studied by scanning electron photograph. More than 87% were in normal shapes and rest of the cells ruptured and shrinks down due to cryo-injuries. There was not any expression of apoptotic genes like Bak1 during 2nd and 3rd passage, but a weak at late 3rd and strong through 4th passage expression of apoptotic genes were observed. Expression of apoptotic genes Bak1 was confirmed by RT-PCR amplicon with 178 bp respectively. But some the expression of anti-apoptotic (Caspase3, Tdh, SH3GLB1 and Phb) and DNA repair (BRCA1 and PARP1) genes were observed at same time. The cells were found to have a normal karyotype during cryopreservation at different passage. We conclude that during cryo-banking of amniotic fluid stem cells some of the cells moves towards the apoptotic pathway, but several genes were present there to over that effect.

P40

Celioscopy as a method of healthy diagnosis of reproductive tract in raptors

A Dogliero, M Mauthe Von Degerfeld, A Ricci, F Scaglione, G Quaranta

Department of Animal Pathology, Faculty of Veterinary Medicine, University of Turin, Turin, Italy

Endoscopy is the clinical method widely used in sex diagnosis in avian species. This procedure can be also proposed in order to evaluate functional status of gonads or, according to many authors, to identify reproductive disorders during sub-clinical phase. Finally celioscopy represent an important diagnostic aid in breeders selection programs. In this study, during the breeding season, 20 wild raptors (three *Accipiter nisus*, six *Buteo buteo*, one *Falco tinnunculus*, three *Asio otus*, four *Athene noctua*, three *Strix aluco*) of unknown sex were evaluated. All the subjects had to be euthanatized because were seriously polytraumatized. The animals were tested by *in vivo* endoscopy and, after the sacrifice, the reproductive tracts were submitted to histopathological examination. During endoscopy birds were anesthetized by isoflurane gas. Celioscopy was performed on the left side, through post-femoral access. The complete procedure (from anesthesia induction to end of endoscopy) covered an average time of 10 min. All the subjects were immediately sacrificed by 1 ml/kg intra-cardiac Tanax® (Intervet, Italy) administration. Reproductive tracts were removed and stored in 10% buffered formalin. Histological samples, were finally colored with ematossilina. The aim of the study was to verify if the magnification of instruments to ensure a diagnostic allow, especially in genital subclinical diseases. Endoscopy results were confirmed in 19 cases (95%) and only in a birds histopathology showed a mild leukocyte infiltration of ovarian stromal tissue, probably not compatible with a reproductive ability. This work represents an initial phase of a larger study of assisted reproduction in Italian wild raptors. Despite the small sampling, endoscopy ensured similar results to histopathology.

P41

Milk fat:protein ratio and its relationships to the incidence of ovarian cysts in cows

J Ducháček, L Stádník, J Beran

Czech University of Life Sciences Prague, Prague, Czech Republic

The objective of this study was to evaluate relationships between milk fat:protein ratio as a indicator of negative energy balance of cows after calving and incidence of follicular (FC) and luteal (LC) cysts. Proportional milk samples were collected the 7th, 14th, 21st and 28th day of lactation and milk fat:protein ratio was determined. Incidence of FC and LC were detected by ultrasound examination of the ovaries at 60th day of lactation. The 375 Czech Fleckvieh cows were divided into four groups according to cows parity (1st, n = 100; 2nd, n = 110; 3rd, n = 87; 4thand subsequent lactation, n = 78) and into three groups according to fat:protein ratio during the first 4 weeks after calving (x = to 1.20, n = 59–113; from 1.21 to 1.50, n = 90–166; from 1.51, n = 54–83). The statistical program SAS 9.1. and procedure ANOVA was used for analyzing the data. The higher cow′s parity the higher incidence of FC was determined, however significant difference ($p < 0.05$) was detected only between the 1st and 4th and subsequent lactation (from +15.63% to +27.35%). The incidence of LC had the opposite tendency. Significantly higher incidence of LC was found in primiparous cows compared to cows in the 4th and subsequent lactation (from +5.47% to +17.53%, $p < 0.05$). Lower incidence of FC (from −14.19% to −0.37%, $p > 0.05$) was found in animals with an average fat:protein ratio in the first 4 weeks of lactation, however no significant trend was detected in the incidence of LC in relation to fat:protein ratio during this period. Funded by 'S' grant of MSMT CR and by Project No. NAZV QI91A061.

P42

Abstract withdrawn.

P43

Availability of computer assisted real time ultrasonography for *in-vivo* and *in-vitro* monitoring ovarian structures in dogs

G Erdogan[1], N Küçük[1], H Kanca[2], M Aksoy[1]

[1]*Faculty of Veterinary Medicine, University of Adnan Menderes, Aydin, Turkey;* [2]*Faculty of Veterinary Medicine, University of Ankara, Ankara, Turkey*

Ultrasonography provides valuable diagnostic information and widely used in small animal practice. The efficiency of B-mode, real-time ultrasonography by means of transabdominal transducers to monitor ovarian structures is not well documented in dogs in contrast to other domestic animals. We expect to differentiate bitches ovaries are in follicular and luteal phase by using B-mode, real time ultrasonography. Thus, the objective of this study was to evaluate efficiency of B-mode, real-time ultrasonography equipped with a 6.6 MHz convex-array transabdominal transducer to monitor ovarian structures in dogs *in-vivo* and *in-vitro*. Ovaries of 36 mongrel dogs, between 1 and 3 years old and 18 and 27 kg of body weight, were scanned for the presence and identification of the ovarian structures both before and after ovariectomy. *In-vivo* and *in-vitro* images were compared by an image analyses software package (ImageJ 1.42q; NIH, USA) for area (total area of ovary), mean (mean grayness value), standard deviation (standard deviation of mean), and pixel range (maximum – minimum pixel value). Furthermore, serum progesterone concentrations were determined via RIA to confirm the stage of the estrous cycle. The dogs were classified either in follicular (5–30.0 ng/ml) or luteal phase (>30.0 ng/ml) based on the serum progesterone concentrations. Accurate classification rates of dogs into two stages (follicular or luteal) according to the images obtained from ovaries were 41.4 (7/17) and 42.1% (8/19), respectively. Average data were presented as mean ±SEM. Independent Samples – *t* test was carried out to compare the preoperative and postoperative data. The scanning plane of ovaries (*in-vivo* or *in-vitro*) significantly altered the image characteristics ($p < 0.05$). Area, mean, standard deviation and range values (mean ± SEM) for *in-vivo* and *in-vitro* captured images were 6776.9 ± 618.9 vs. 6015.1 ± 432.3, 82.8 ± 4.6 vs. 128.1 ± 4.2 ($p < 0.05$), 18.9 ± 0.72 vs. 23.5 ± 1.2 ($p < 0.05$), 98.9 ± 2.8 vs. 111.9 ± 5.5 ($p < 0.05$) in follicular and 4455.0 ± 553.9 vs. 4488.2 ± 609.8, 84.9 ± 3.8 vs. 141.3 ± 4.0 ($p < 0.05$), 16.3 ± 0.5 vs. 19.0 ± 1.2 and 87.6 ± 2.5 vs. 93.1 ± 5.0 in luteal phase, respectively. This data indicate that the difference observed in *in-vivo* and *in-vitro* obtained ovary images was more prominent in dogs in the follicular phase compared to those in the luteal phase and that identification of ovarian structures and phase of the estrous cycle by means of B-mode, real-time ultrasonography connected to transabdominal transducers is elusive in most of the clinical cases.

P44

Fertility of dairy heifers after insemination with sexed semen under field condition in Russia

A Erokhin, M Dunin

Research Institute of Animal Breeding, Moscow, Russia

The goal of this study was to evaluate fertility after insemination with sexed semen of dairy heifer in Russia. Frozen X-bearing sexed semen of Holstein bulls was received from ABS Global Inc., USA. The straws were thawed in a water bath (37°C, 30 s). Post-thaw progressive sperm motility (CASA) and acrosome morphology were evaluated. Sexed semen was used at first service in virgin Holstein heifers during the spontaneous oestrous. Each insemination was performed by one straw containing $2.1–2.2 \times 10^6$ sexed frozen-thawed sperm (SG) or conventional (CG) unsexed semen (15×10^6 motile sperm) and semen was deposited in the uterine body. In total 450 (SG) and 210 (CG) heifers at six farms were inseminated. Pregnancy was detected by rectal palpation between days 60 and 70 after A.I. Statistical analysis was performed using SAS system for Windows. The average progressive sexed sperm motility and acrosome integrity immediately after thawing were 52% and 78%, respectively. The average calving rates in SG were 41.8% (21/82, 13/34, 52/120, 43/97, 22/48, 36/69) vs. 62.6% in CG ($p < 0.05$). The significant variation of fertility rates in SG were observed between farms: it ranged from 25.6% to 52.1% ($p < 0.05$). The proportion of female calves derived from sexed-sperm insemination was 90.9% compare with 50.7% in control ($p < 0.01$). Sexed semen had no affect on the incidence of stillbirth: 5.4% vs. 5.2 in CG ($p > 0.05$). Our results indicated that AI with X-bearing sexed sperm under field condition is effective in producing heifers, but reduced pregnancy rates compare with conventional semen.

P45

Role of coenzyme Q and Vitamin E on stallion semen viability evaluated both pre-freezing and post-thawing

M Falomo[1], R Mantovani[2], H Sontas[3], V Mungioli[1]

[1]*Department of Animal Medicine, Production and Health, University of Padua, Padua, Italy;* [2]*Department of Agronomy Food Natural Resources Animal & Environment, University of Padua, Padua, Italy;* [3]*Section of Obstetrics and Reproduction, University of Veterinary Science, Vienna, Austria*

The objective of this study was to evaluate pre-freezing (PF) and post-thawing (PT) quality of frozen stallion semen comparing a control diluent (Palmer formula + 4% egg yolk + 3% glycerol; C) with C containing 1 mM of Coenzyme Q alone (Q) or 1 mM Coenzyme Q + 1 mM Vitamine E (Q + E). Ejaculates were collected from five stallions, divided in three aliquots extended with EquiproTM (minitübe) 1:1, centrifuged at 1400 g × 14' to remove seminal plasma, and diluted to a final concentration of 100×10^6 sperm/ml with C, Q or Q + E extenders. Kinetic parameters were evaluated with CASA analysis system and acrosomal integrity with Spermac® test both PF and PT. PT CASA analysis at thawing (T0) was repeated after 2 (T2) and 4 (T4) hours of incubation at 37°C. Statistical analysis was carried out with a mixed model on the differences PT-PF, i.e., Δ CASA parameters at T0, T2, and T4. The addition of Q increased progressive motility at T0 (+4.2%), T2 (+6.3%), and T4 (+0.7%); Mean sperm path velocity and linear velocity were increased at T0 (+2.5 μm/s, +5.4 μm/s, respectively); Sperm straightness ($p < 0.05$) and linearity ($p < 0.05$) were increased at T0 (+4.4, and +2.5, respectively). Q + E didn't show any further improvement of CASA parameters as respect to Q. Spermac® test showed no statistically significant differences between the groups. The results of this study suggest that Coenzyme Q improve post-thaw quality of equine sperm.

P46

A 5 year retrospective analysis of the rate of mount refusals among AI-boars

S Ferchaud[1], V Furstoss[1], J Boutin[1], S Boulot[2]

[1]*INRA UE88 UEICP, Rouillé, France;* [2]*IFIP, Le Rheu, France*

Refusals to mount or to ejaculate disturb the management of AI centers, and repeated events may compromise productivity or boar longevity. This retrospective study evaluates the frequency of libido problems among AI-boars over a 5 year period, and some variation factors. Analysis includes data collected from the INRA boar stud in Rouillé (January 2007–December 2011), from 264 boars (four genotypes), during a total of 7203 days of semen collections. The effects of genetic line, year, month or season and age at attempt on the binary event 'successful collection (yes/no)' were evaluated using a generalized linear model (Splus). The average rate of unsuccessful semen collections was 4.5%. Mount refusals (2.5 ± 2.1 per boar) affected 50% boars with single and repeated failures reported for

21% and 29% of the males respectively (extremes 1–13). Month or season had no significant effect on failure occurrence, but it varied significantly ($p < 0.001$) between years (3.1%, 3.6%, 1.7%, 4.1% and 1.4% from 2007 to 2011) and breeds (Landrace 3.6%, Pietrain 3.8%, Large White 1.6%, Large White × Pietrain 2%). Mount refusal depended on age ($p < 0.001$) with the highest frequency for1 year-olds or less (3.9%) vs. 2.7% under 3 years and 1.6% from 3 years onward. Collection failure was associated with a higher rate of culling for libido problems and with only slight differences for other reasons (less aging and semen defects and more locomotor disorders). This analysis suggests that prevention of libido failures should focus on individual factors with more detailed records of behavioural or clinical parameters.

P47

Role of cytokines on NO production and eNOS activity in equine corpus luteum

G Ferreira-Dias[1], D Skarzynski[2], A Galvao[1]

[1]*CIISA, Faculty of Veterinary Medicine, TULisbon, Portugal;* [2]*Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland*

The involvement of nitric oxide (NO) on equine corpus luteum (CL) function modulation is well established. The aim of the present study was to assess the role of cytokines tumor necrosis factor alpha (TNF), interferon gamma (IFNG) and Fas Ligand (FASL) on NO production and endothelial NO synthase (eNOS) gene expression modulation, in equine CL. After enzymatic cell isolation from early (ELP, n = 5), mid (MLP, n = 9) and late luteal phase (LLP, n = 5) CL, *in vitro* cultures were treated with: (i) no exogenous factor (control); (ii) TNF (10 ng/ml); (iii) INFG (10 ng/ml); (iv) FASL (10 ng/ml); or (v) TNF + INFG + FASL (10 ng/ml). The production of NO in culture media was determined by EIA and mRNA transcription of eNOS was quantified by real time PCR in luteal cells, throughout luteal phase. In addition, eNOS protein expression was quantified by western blot in luteal cells from MLP. In ELP, both mRNA level of eNOS and NO production were increased by TNF ($p < 0.05$), while the same effect was produced in the MLP by cytokine association TNF + INFG + FASL ($p < 0.05$). Also in MLP, eNOS protein expression was increased by TNF + INFG + FASL ($p < 0.05$). In LLP, NO was lowered by TNF treatment ($p < 0.05$), whereas TNF + INFG + FASL increased both eNOS mRNA and NO production ($p < 0.05$). The present findings clearly demonstrate cytokines role on eNOS mediated NO production by equine luteal cells.

P48

Ovarian activity and sex ratio in Hereford cows

N Fjerdingby[1], K Karlberg[2]

[1]*School of Veterinary Science, Oslo, Norway;* [2]*Norwegian School of Veterinary Science, Oslo, Norway*

In beef cattle, limited data are available regarding how ovulations alternate between ovaries in following heats and also concerning sex ratio of the offspring in relation to the uterine horn of gestation. The objective of the study was to record which ovary had ovulation during a number of subsequent heats and to determine the sex ratio of calves gestated in the right and left uterine horn. A total number of 59 Hereford cows were palpated during the 2-year study. In a number of 100 heats 63 ovulations were in the right ovary (RO) and 37 in the left ovary (LO). Among the 56 interovulatory intervals, there were 18 ovulations in RO and 4 in LO both times. Ovulations alternated from RO to LO in 12 heats and from LO to RO 22 times. Thus, 61% (no 34) of the ovulations changed side. A total number of 65 pregnancies (50 cows) were studied. Because of twins, three pregnancies were excluded in the part of the study concerning sex ratio of the offspring in relation to the uterine horn of gestation. In the 62 remaining pregnancies, 41 calves were in the right uterine horn and 21 in the left and it was only 46.8% bull calves. In the right horn the proportion of males was 44% and the proportion of females was 56%. In the left uterine horn there were 52% males and 48% females. Among 15 cows with known side of pregnancy and calf sex for two following years, nine were pregnant in the same uterine horn both years; seven of them in the right horn and only two in the left. Two of the cows with corpus luteum graviditatis (CLg) in the right ovary for two subsequent years, had twins the second year but only one CLg. Only four of the 15 cows delivered a calf of same sex the following year.

P49

Global endometrial transcriptomic profiling reveals enrichment of immune pathways during early involution in healthy beef cows

C Foley[1,2], A Chapwanya[2], C Chris[1], C O'Farrelly[2], K Meade[1]

[1]*Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Co. Meath, Ireland;* [2]*Comparative Immunology Group, Trinity College Dublin, Ireland*

The innate immune response is instigated by postpartum bacterial contamination of the uterus. However approximately 30% of cows acquire the uterine disease endometritis associated with persistent bacterial infection and subclinical inflammation. Mechanisms required to achieve a physiologically functioning endometrium postpartum while avoiding disease remain elusive. This study aimed to characterise uterine involution in healthy cows at a pan-transcriptomic level in endometrial biopsies using mRNA-Seq. Histological analysis showed that endometrial inflammation – based on leukocyte infiltrate, was greater at 15 days postpartum (DPP) and subsequently decreased by 30 DPP. At a molecular level, there were 1107 significantly differentially expressed genes, 73 of which were increased and 1034 decreased at 15 relative to 30 DPP (adjusted p-value < 0.1). The immune system was the predominant KEGG system enriched by immune pathways with up-regulated genes at 15 DPP. An independent assessment of gene expression by real-time RT-qPCR was done with candidate genes in additional cows. Greater cell and tissue proliferation and remodelling were evident by the significant elevated expression of candidate genes GATA2, IGF1, SHC2, and UTMP at 30 DPP ($p < 0.01$). Thus, in the absence of pathology, the inflammatory immune response plays a fundamental physiological role in the uterus during involution.

P50

Influence of season on super-ovulatory response and embryo yield of Santa Ines ewes submitted to FSH treatment started near the time of emergence of the last follicular wave

M Franco Oliveira[1], C D'Amato[1], F Barros[1], A Perini[1], M Lima[1], L Oliveira[1], S Bicudo[1], J Fonseca[2], W Vicente[1]

[1]*UNESP, Jaboticabal, Brazil;* [2]*EMBRAPA, Juiz de Flora, Brazil*

Thirty ewes were equally divided into three groups according to season (NB: Non-breeding; T: Transition and B: Breeding). All ewes received a CIDR device for 12, 12 and 13 days, respectively. In Transition, the CIDR was replaced by a new one on D7 (D0 = onset of protocol). There were administered 200 mg of FSHp in eight decreasing doses started on D10, 10 and 11 for NB, T and B seasons, respectively. All ewes received an injection of 37.5 μg of D-cloprostenol on D0 and at CIDR removal. A single i.m. dose of 200 IU of eCG was given at CIDR removal. Ewes were mated by a fertile ram. Embryo collections were done 7 days after CIDR removal. Data were analyzed by GLIMMIX ($p < 0.05$; SAS). The number of ovulations no differ among seasons (NB: 12.6 ± 1.9; T: 14.0 ± 1.8 and B: 12.5 ± 2.6). There were differences among seasons for ovulation rate (NB: 83.5 ± 6.2%b; T: 93.1 ± 2.2%a and B: 88.5 ± 4.4%ab) and number of luteinized unovulated follicles (NB: 3.1 ± 1.6 a; T: 0.9 ± 0.3 b and B: 1.2 ± 0.4 ab). Furthermore, there were no differences among seasons in proportion of ova/embryos recovered (NB: 51.7 ± 7.5%; T: 67.0 ± 6.1% and B: 49.3 ± 8.5%), number of ova/embryos recovered (NB: 5.9 ± 0.9; T:

9.1 ± 1.2 and B: 6.3 ± 1.1), number of viable embryos (NB: 1.8 ± 0.8; T: 5.7 ± 1.4 and B: 3.4 ± 0.8). In conclusion, there were differences in SOV response, however, the embryo yields did not differ among seasons when FSH treatments were initiated near the emergence of the last wave in Santa Ines ewes. Financial support: FAPESP

P51

Genetic and environment factors as leverage effects on fertility at first service in Holstein cows

S Fréret[1], P Salvetti[2], J Gatien[2], P Humblot[3], C Ponsart[2]

[1]*INRA UMR 85 Physiologie de la Reproduction et des Comportements, Nouzilly, France;* [2]*UNCEIA, Maisons-Alfort, France;* [3]*Department of Clinical Sciences, SLU, Uppsala, Sweden*

In order to quantify factors influencing fertility at first artificial insemination (AI) in Holstein cows, a study based on 4621 first AI's was conducted in 135 French dairy herds. Progesterone concentration was measured in milk on the day of AI and 21–23 days later and pregnancy was assessed between day 45–75 using transrectal ultrasonography or palpation or PSPB (Pregnancy Specific Protein B) assay from blood, allowing to determine conception rate (CR, 47.1%) and incidence rates of AI performed in luteal phase (LPAI, 4.5%), non-fertilization or early embryonic death (NF-EED, 36.9%), prolonged luteal phase or late embryonic death (PLP-LED, 31.9%). Individual data were recorded by breeders and AI technicians, milk production data and breeding values were extracted from the national database. Multivariate analysis showed that cows' fertility breeding value ($p \leq 0.001$), calving to 1st AI interval ($p \leq 0.02$), parity ($p \leq 0.01$), first 3 months milk production ($p \leq 0.01$) and somatic cell count ($p \leq 0.01$), calving conditions ($p \leq 0.01$), type of estrus sign used by the breeder when asking for AI ($p \leq 0.01$), estrus detection to AI interval ($p \leq 0.02$) and feeding management ($p \leq 0.01$) strongly affected CR. Multivariate analyses were also performed on LPAI, NF-EED and PLP-LED. Better breeding management from time of calving to AI and increased use of fertility genetic information should contribute to improve reproductive efficiency in Holstein cows.

P52

First insights into *in vitro* models for effects of negative energy balance on viability of reproductive bovine epithelial cells

C Gabler, K Haase, A Hahn, M Bittel, A Bondzio, R Einspanier

Institute of Veterinary-Biochemistry, Freie Universität Berlin, Berlin, Germany

A correlation has been suggested between increasing milk yield and decreasing fertility rates in dairy cows. The influence of negative energy balance (NEB) on reproductive parameters has not yet been elucidated in detail. The aim of this study was to evaluate the effects of different concentrations of glucose and/or β-hydroxybutyrate (β-HB) on the metabolism of epithelial cells obtained from the female bovine reproductive tract. Twenty-four hours after the first passage, oviductal cells were treated with DMEM containing glucose in concentrations of 0.33, 0.66 or 1 g/l, either with or without addition of 1 mM β-HB. Mitochondrial activity (WST-1 assay), lactate dehydrogenase (LDH) release, ATP content and proliferation rate were measured after 12, 24, 36 and 48 h, respectively. Endometrial epithelial cells were treated with the same medium conditions, and the WST-1 assay was performed. A positive correlation was observed between the glucose concentrations and the mitochondrial activities as well as the proliferation rates of oviductal cells, especially after 36 or 48 h. In oviductal cells, LDH release and ATP content were not influenced by different glucose amounts. Supplementation with β-HB did not affect any of these parameters. In addition, low glucose concentrations impaired cell viability of endometrial cells. This negative effect was diminished in parts by addition of β-HB. Oviductal cells seem unable to compensate low glucose levels when compared with endometrial cells. However, β-HB is not an adequate substitute for glucose. These findings may prove helpful in explaining reduced fertility under NEB. Supported by BMBF and DFG (GA1077/5-1).

P53

Changes in histone H4 acetylation and histone H3 lysine 9 methylation (H3K9) induced by vitrification in pig oocytes

G Galeati, C Vallorani, D Bucci, C Tamanini, M Spinaci

Department of Veterinary Medical Sciences (DSMVet), University of Bologna, Bologna, Italy

In order to determine the effect of vitrification on epigenetic status of pig MII oocytes, hyperacetylation of H4 and dimethylation of the lysine 9 residue of histone H3 (H3K9) were assessed by immunofluorescence in control oocytes, after cryoprotectant treatment and after vitrification at two time points, immediately after warming (0 h) and after a post-warming incubation for 2 h at 39°C in modified NCSU37. While no changes in the immunopositivity for the epitopes were recorded after cryoprotectants, the percentage of negative oocytes for dimethyl H3K9 increased immediately after devitrification (0 h) as compared to control (11.6% vs. 1%, $p < 0.01$). The post-thaw incubation for 2 h significantly ($p < 0.01$) increased the acetylation status of H4 as compared to 0 h (27.8% vs. 1.6%). Moreover, 2 h after warming an increase of the percentage of oocytes exhibiting a strong H3K9 positivity (28.7% vs. 3.3%, Vitr 2 h vs. Vitr 0 h respectively, $p < 0.01$) and that of H3K9 negative oocytes (26.1% vs. 11.6%, Vitr 2 h vs. Vitr 0 h, $p < 0.01$) was observed. In conclusion, acetylation of H4 and methylation of H3K9 are altered by vitrification procedure that may lead to an aberrant epigenetic presentation of female chromatin to the fertilizing event and may be, at least in part, responsible for the reduction of developmental competence of vitrified pig oocytes. Work supported by 'Fondazione del Monte di Bologna e Ravenna'.

P54

In vitro growth of pre-antral follicles from isolated and or *in situ* cryopreserved caprine ovarian tissue

A Gangineni, G Aruna Kumari, K SadasivaRao, M Ragasudha, R Amin

College of Veterinary Science, Sri Venkateswara Veterinary University, Hyderabad, India

The aim of the present study was to evaluate the effects of temperature and time of incubation on morphology, viability and ability to grow in *in vitro* of caprine preantral follicles (PFs) during short-term preservation at low temperatures. Isolated and or cortical tissue enclosed (*In Situ*) goat early stage follicles were incubated in tissue culture medium (TCM) at 4°C for 24, 48 h and for 1 WK in −196°C (LN_2) with 1.5 M dimethyl sulfoxide as cryoprotectant. Viability was tested by using trypan blue, Calcein-AM. The percentage of viability was 88.23%, 75% and 78.57% in *in situ* and 83.33%, 66.66% and 75% in isolated follicles at 24, 48 h and −196°C. Isolated and or cortical tissue enclosed follicles were frozen/thawed in the presence of the cryoprotectant in case of liquid nitrogen preserved follicles and then cultured for 6 days in TCM incorporated with 2 mIU/ml of Growth Hormone. The development of cultured follicles were assessed by the proportions of follicles exhibiting growth (60%, 22.5% and 50.6%), increase in follicular diameter (8 microns, 2.87 microns and 17 micrones) and antrum formations (40%, 50% and 60%) *in vitro* at 4°C for 24, 48 h and for 1 week in −196°C respectivly. The study indicated that cryopreserved goat preantral follicles were underwent growth and antrum formation *in vitro*. This study appears to be the first report that it is possible to culture the preantral follicles from goat ovaries for short term preservation at low temperatures.

P55

Potential alternatives to traditional ram sperm cryopreservation

W Garcia, A Tabarez, M Palomo

Universidad Autonoma de Barcelona, Bellaterra, Spain

Our aim was to study potential extenders free from additives of animal origin and also the efficiency of trehalose as an alternative of glycerol on ram sperm cryopreservation. Therefore, we tested the inclusion of 0.6 mM of butylated hydroxytoluene (BHT) or 1% (w/v) soybean lecithin or 15% (v/v) powered egg yolk supplemented with 5% glycerol or 100 mM of trehalose in a Tris-based media. Briefly, fresh ejaculates from eight young rams (1 year old) were collected by artificial vagina and immediately mixed in equal quantities. Pooled semen was washed by centrifugation and the pellet was split into six equal aliquots and re-suspended in one of the six different extenders before freezing. No significant differences were found in post-thaw sperm viability, determined by eosine-nigrosine stain (mean ± SD, n = 6), between the egg yolk (25.9 ± 6.3; 23.2 ± 3.6) and soybean lecithin (26.2 ± 6.2; 18.5 ± 6.9) based media supplemented with glycerol or trehalose, respectively. Likewise, post-thaw sperm viability was similar for BHT based media (20.0 ± 7.6) supplemented with trehalose, but significant lower (15.4 ± 5.4, $p < 0.05$) when the BHT based media was supplemented with 5% glycerol. These preliminary results suggest that lecithin and BHT could be used as substitutes for egg yolk depending on the combination of crioprotectants in the extenders. However, the sperm motion parameters, analysed by a computer-assisted sperm analysis system (ISAS®), were different ($p < 0.01$) between extenders, suggesting that more analysis should be done. Supported by INIA (RZ2009-00008-00-00), Generalitat de Catalunya (2009SGR0621) and Fundacion Carolina.

P56

Behavioural changes during the 12 h before calving and predictors of dystocic delivery in Holstein cows

J Gatien[1], M Le Broc[2], J Philipot[2], P Salvetti[1]

[1]*UNCEIA, Maison-Alfort, France;* [2]*CREAVIA, Rennes, France*

In order to better understand behavioural changes arising before calving and behavioural signs predicting dystocic delivery, a study including 20 parturient Holstein cows was led. Continuous observations from video recordings were used to quantify frequencies and durations of behaviours during the 12 h prior to the calf being completely expelled. Six of the 20 deliveries needed farmer assistance. Average duration of the complete expulsion of the calf was 57.0 ± 44.0 min. During the 12 h prepartum, cows spent on average 51.5 ± 13.0% of time lying, 30.3 ± 13.1% standing motionless, 10.6 ± 6.9% eating, 4.7 ± 2.9% stamping and 2.9 ± 1.4% walking. Significant behavioural changes were observed 6 h before calving, with a decrease of the time spent lying ($p < 0.05$, respectively 56.2 ± 18.0% of time during the first 6 h and 41.0 ± 17.7% during the last 6 h) and an increase of time spent stamping ($p < 0.0001$, respectively 1.6 ± 2.8% and 7.9 ± 5.4%), time spent tail raising ($p < 0.001$, respectively 0.8 ± 0.4% and 40.3 ± 21.3%), frequency of abdominal contractions ($p = 0.0004$, respectively 0.1 ± 0.6 and 36.9 ± 19.4 signs per hour). Associations of behavioral signs have shown that dystocic delivery could be predicted by more than 15 tail raises with a minimal duration of 2 min, with a sensitivity of 77.0% and a specificity of 100%. This study has shown interesting prospects that should be confirmed with more cows, for specific detection of dystocic delivery, unrealizable at this time by any system.

P57

Isolation, culturing and characterization of amniotic fluid stem cells in buffalo (*Bubalus bubalis*)

S Gautam[1], K Dev[1], S Giri[1], A Yadav[1], B Singh[2]

[1]*Department of Biotechnology, Kurukshehtra University, Kurukshehtra, India;* [2]*Indian Veterinary Research Institute (IVRI), Palampur, India*

Amniotic fluid cells are usually obtained for prenatal analysis and can be enlarged *in vitro*; however modern information on their source and properties are narrow. Heterogeneous amniotic fluid contains various cell types. The aim of this study was to phenotypical and genotypical characterizated amniotic fluid stem (AFS) cells in buffalo (*Bubalus bubalis*). The amniotic fluid (AF) cells were cultured without feeder cells, in DMEM containing 16% FBS, 1% penicillin/streptomycin and 1% L-glutamine in 5% CO_2 in humidified air at 38.5 ± 0.5°C. After 6 days of culture different types of cells viz., star shaped (62.7%), spherical without nucleus (1.9%), spherical with nucleus (26.4%), pentagonal (0.4%) and free floating/rounded cells (8.3%) were observed. Most of the cells started anchorage-dependent growth after day 7 of the culture. Oct-4-positive amniotic fluid cell samples also express stem cell factor (SCF), alkaline phosphatase (ALP) and 18s rRNA. Expression of Oct-4, SCF, ALP and 18s rRNA was observed in the cells at different passages. Using species-specific primers, a PCR amplicon of 314, 216, 180 and 162 bp were observed for Oct-4, SCF, ALP and 18s rRNA respectively. The cells were found to have a normal karyotype at different passages. The further research is going on for studying their differentiation and signaling pathway. These results may contribute towards establishing non-embryonic pluripotent stem cells for various therapeutic and reproductive biotechnological applications in the species.

P58

NMR metabolic signatures of follicular fluid of mare, sow and cow

N Gerard[1], S Fahiminiya[1], L Nadal-Desbarats[2]

[1]*INRA, Nouzilly, France;* [2]*PPF ASB, University of de Tours, France*

Follicular fluid (FF) is the *in vivo* extracellular environment of the oocyte, and reflects the physiological status of the follicle. Nevertheless, its metabolic composition is still relatively unknown. The aim of the study was to analyze by 1H-NMR spectroscopy, the biochemical profiles of cow, mare and sow FF. This metabolomic approach allows to target over 70 compounds in complex biological fluids such as FF, and provides opportunities for obtaining qualitative and quantitative data. FF were collected from dominant follicles and added with D2O before being analysed by 1H NMR (Bruker DRX-500). Spectra were processed and integrated using WinNMR, and quantification used the ERETIC signal previously calibrated. Spectral 1H assignments were achieved according to the Human Metabolome Data Base. Statistical tests were performed using SigmaStat and SIMCA P+ softwares. PLS-DA analysis allowed the separation between the three species. Concentrations in glycolysis intermediates and several amino-acids were significantly different between species. Pyruvate was only detected in sows FF, whereas citrate was never detected in this species. Lactate, pyruvate alanine, creatine-creatinine and N-acetyl groups were the most discriminating metabolites of the sow's FF. High values of histidine, acetate, citrate, cis-aconitate and glucose characterized the mare's FF. Similar analysis were performed with subordinate FF, where PLS-DA also discriminated the three species. In conclusion, follicular status is determined by some physiological and species-specific metabolomics changes in the FF that can be characterized by 1H NMR profiling.

P59

Freezing canine semen – a comparison of different sealing and freezing methods and the use of thawing medium

S Goericke-Pesch, D Klaus, A Wehrend

Clinic for Obstetrics, Gynecology and Andrology of Large and Small Animals, Gießen, Germany

The importance of artificial insemination in the dog has steadily increased within the last decades and frozen semen offers the unique possibility of preservation of genetic material. The aim of this study was to compare different sealing methods, SM (S1: manual, S2: heat, S3: ultrasound), different freezing regimes, FR (F1: rigid rack, straws 10 cm about liquid nitrogen, F2: swimming rack, straws 4 cm about liquid nitrogen) and the addition of thawing medium (TM) for freezing of canine semen. Sperm-rich fractions of 11 males were processed using CaniPro Freeze (Minitüb, Germany) and 0.5 ml straws; an equal number of straws were sealed using one of the SM and frozen with either F1 or F2. Twenty-four hours after storage in liquid nitrogen, one straw of each SM and FR was thawed for 1 min in 37°C water and equal parts of the semen were either diluted (1:1) with TM or left native (all stored at 37°C). Examinations of thawed semen were performed immediately after thawing, after 10 and 60 min and included conventional (% progressive motility, VW, % living sperm, liv) and CASA analysis (total motility, TM, progressive motility, PM, viability, via, pathomorphology, patho). Time of incubation had a significant influence on VW, TM, PM, liv and via ($p < 0.00001$). SM had a significant effect on PM ($p < 0.05$), FR on VW ($p < 0.05$) and patho ($p < 0.05$); addition of TM influenced TM, PM and via (each $p < 0.05$). Our results clearly show that SM and FR, but also addition of TM have a significant influence on post-thaw results in frozen canine semen.

P60

Ultrasonographic comparison of feline mammary masses using grey-level histogram and image texture analysis

A Gogny, C Mangold, M Fusellier, M Gogny

ONIRIS, Nantes, France

Eighty-five percent of feline mammary tumours are malignant. Identifying the type of the tumour before the surgery would avoid to perform a complete mastectomy, recommanded in every instances despite the resulting pain and potential complications. The aim of this study was to evaluate the ultrasonographic differences of mammary masses in cats, using image analysis.

Material and methods Ultrasonographic images of 23 mammary masses and 11 healthy mammary glands of cats were acquired using a 12.5 MHz probe (Ixos Esaote® Pie Medical). After mastectomy, the masses were submitted to pathology analysis. The size, volume, shape, appearance of the margins, echogenicity and echotexture of each mass were evaluated. Grey-level histogram width and normalized cumulative histograms curves were measured (ImageJ® software). Texture analysis was based on the measure of Haralick features such as energy, homogeneity, entropy, contrast, variance and correlation. Results were compared using a ANOVA test (R®, Free software foundation).

Results and discussion Pathology identified 13 malignant tumours, 5 dysplasias, 7 fibroadenomatosis and 1 fibroadenoma. The margins of the malignant tumours differed from those of the other masses ($p < 0.05$). Grey-level histogram width was lower in dysplasia and in healthy mammary gland, compared with malignant tumour and fibroadenomatosis ($p < 0.05$). The normalized cumulative histogram curve was different in each type of mammary mass ($p < 0.001$). Texture analysis revealed a difference between malignant tumours and other tumours ($p < 0.05$). This demonstrates that mammary gland ultrasonography, followed by image analysis, may help to identify the nature of the mammary masses in cats.

P61

SOPS-vitrification of *in vivo* derived porcine zygotes

J Gomis, C Cuello, J Sanchez-Osorio, M Gil, M Angel, I Parrilla, J Vazquez, J Roca, E Martinez

Department of Medicine & Animal Surgery University of Murcia, Murcia, Spain

The aims of this study were to evaluate the post-warming *in vitro* viability of intact porcine zygotes vitrified by Superfine Open Pulled Straw (SOPS) method and to investigate whether the lipid polarization by centrifugation (CT) or the equilibration in a high-osmolality medium followed by CT (HCT) increases their cryotolerance. Zygotes ($n = 317$) were obtained from superovulated sows ($n = 13$) on day 2 (D0: onset of estrus) and were assigned to the following groups before vitrification: (i) CT ($n = 90$): zygotes centrifugated for 20 min at $15\,000 \times g$; (ii) HCT ($n = 90$): zygotes equilibrated in hyperosmotic medium (400 mOsm) for 6 min followed by CT and (iii) Untreated zygotes ($n = 94$). Zygotes were then vitrified and warmed. Some fresh zygotes ($n = 43$) were not vitrified. Vitrified/warmed (VW) and fresh zygotes were cultured for 5 day to evaluate the blastocyst formation (BF) rates and the total cell number in blastocysts (CNB). There were no differences in the BF rates among the vitrification groups (range: 34.2 ± 5.3–48.2 ± 5.6%). Fresh zygotes showed higher ($p < 0.001$) BF rates (87.5 ± 5.3%) than those from VW zygotes. The CNB was similar among all groups (range: 34.9 ± 2.8% to 44.1 ± 2.8%). Our results indicate that SOPS-vitrification is a promising method for cryopreservation of untreated *in vivo*-derived porcine zygotes and that lipid polarization by CT or HCT had no effect on their post-warming *in vitro* ability to develop to the blastocyst stage. Supported by MICINN (AGL2009-12091) and SENECA (GERM 04543/07).

P62

Effect of simulated stress *in vitro* on blastocyst development and gene expression profile in the pig

R González[1], E Pericuesta[2], A Gutiérrez-Adán[2], Y Brandt[1]

[1]*Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden;* [2]*Institute Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain*

Stressful situations may change the embryo environment, subsequently affecting early embryonic development. A vulnerable period is the four-cell stage when the major embryo genome activation occurs in the pig. An *in vivo – in vitro* system was used to test if an altered milieu around this stage could affect embryo development and blastocyst quality. Oocytes were matured and fertilized *in vitro*. Afterwards, presumptive zygotes ($n = 1263$; 14 replicates) were exposed for 24 h to blood plasma collected after ovulation (12 ± 2 h; known cortisol and reproductive hormone levels) from adrenocorticotropic hormone (ACTH) or non-ACTH treated sows (control). Embryos were cultured up to the blastocyst stage. The mRNA transcripts for various genes important for embryo development were quantified by qRT-PCR in the resulting blastocysts. These genes are involved in blastocyst formation, metabolism, apoptosis, de novo methylation, pluripotency, mitochondrial function and cell proliferation. Data were analyzed by ANOVA. Cleavage (ACTH: 54 ± 3; control: 57 ± 3%) and blastocyst rates (ACTH: 19 ± 2; control 21 ± 2%) were similar. In expanded blastocysts, quality assessed by morphologic criteria was better in the control than in the ACTH group ($p < 0.05$), but none of the genes studied differed significantly in transcription levels. Therefore, a brief exposure to simulated stress *in vitro* may not have any harmful consequences for the pig embryo. Funded by Formas and the Spanish MICINN.

P63

A comparative study of sperm viability in bull semen determined by flow cytometry and NucleoCounter® SP-100

I Grevle, K Waterhouse, J Sunde

BioKapital AS, Hamar, Sweden

Semen quality evaluation is an integral part of semen production for AI. Quality parameters routinely assessed at AI centers are typically sperm concentration and pre- and post-thaw motility. In contrast, advanced cell evaluation technologies such as flow cytometry (FC) are rarely used. However, simpler instruments, such as NucleoCounter® SP-100TM, estimates sperm concentration and viability using similar technology – 'live/dead' propidium iodide (PI) staining. These provide alternatives to traditional microscopy methods, and will give a more objective assessment of sperm quality. Assessment of bull sperm viability was compared using SP-100 and FC with cryopreserved semen from 16 Norwegian Red bulls. PI incubation before FC was done at room temperature for either 2 min (PI-short), comparable to the SP-100 protocol, or for 10 min (PI-long), which is more common for FC assays. The FC assay also included a 10 min incubation with the membrane-permeant SYBR® 14 (SY) nucleic acid stain to ensure discrimination of non-sperm events. One ejaculate pr bull was assessed in duplicate with each method. SP-100 viability estimates ranged from 42.2% to 69.0%, with a mean of 54.9 ± 6.3 (SD) %. In comparison, viability for PI-short and PI-long was 52.1 ± 7.6% and 47.5 ± 9.4%, respectively. All three methods showed good precision, with SP-100 showing highest correlation with PI-short ($r^2 = 0.7091$). SP-100 on average were 2.8% higher than PI-short. In conclusion, SP-100 was found to be a reliable instrument for measuring sperm viability. However, when compared to FC, PI incubation time was found to influence accuracy between the methods.

P64

Low-dosage intra-fallopian-transfer of sex-sorted sperm in cattle (SIFT)

R Grossfeld[1], H Dobson[2], M Strothmeyer[1], D Rath[1]

[1]*Department of Biotechnology, Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut, Neustadt-Mariensee, Germany;* [2]*Department of Veterinary Clinical Sciences, University of Liverpool, South Wirral, UK*

Sperm intra fallopian transfer (SIFT) is an alternative and novel method to regular AI. The attempt of a first experiment under field conditions with lactating cows and heifers was to reduce the number of sex-sorted/frozen-thawed sperm to about 5×10^5 spermatozoa/per transfer. In total six cows and 18 heifers were synchronized with PG2α (two injections, 14 days apart). Sperm were transferred non-surgically directly into the oviduct isthmus, ipsilateral to the follicle bearing ovary 10–14 h after onset of standing heat. Ovaries were controlled by ultrasonography and the transfer was performed with a specially designed catheter under visual control. In average the transfer procedure took 8–15 min. Pregnancy rates were detected 35–40 days after SIFT by ultrasonography. In total 21.7% of the animals became pregnant, indicating that the SIFT method has the potential to produce pregnancies after transfer of very low concentrations of sex sorted spermatozoa. Further research, especially on timing of the transfer and the requirements of synchronization is currently performed. There is strong evidence that SIFT after spontaneous ovulation increases pregnancy rates significantly. We acknowledge the skilled assistance of Mr. Sander, Mr. Hadeler, Mr. Poppenga, Dr. Junge and Mr. Dobson.

P65

Abstract withdrawn.

P66

Therapy of bovine endometritis with PGF2α: a meta-analysis

P Haimerl, W Heuwieser, S Arlt

Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

Since uterine infections, frequently diagnosed in cows after calving, are stated to have an enormous negative impact on reproductive performance, applicable and successful treatment is essential. Regarding PGF2α treatment, one of the commonly used drugs in this context, there is a wide discordance between research results. Therefore, a meta-analysis of the efficacy of the treatment of bovine endometritis with PGF2α was conducted. A comprehensive literature search was performed utilizing online databases revealing a total of 2338 references. In addition, five articles were retrieved by reviewing citations. After applying specific exclusion criteria, a total of four publications, comprising five trials, were eligible for further analysis. Data for each trial were extracted and analysed using meta-analysis software Review Manager 5.1. Estimated effect sizes of PGF2α were calculated on calving to first service and calving to conception interval. PGF2α treatment of cows with chronic endometritis had a negative impact on both reproductive performance parameters. Heterogeneity was substantial for calving to first service and calving to conception interval [I^2 (measure of variation beyond chance) = 99% and 89%, respectively]; therefore, random effects models were used. Subgroup analysis of the effect sizes showed that the performance of randomization was influential in modifying effect size of PGF2α treatment. The funnel plot illustrates a publication bias towards smaller studies that reported a prolonged calving to conception interval after a PGF2α treatment. We concluded that there is a shortage of comparable (high quality) studies investigating reproductive performance after PGF2α treatment of cows with chronic endometritis. Furthermore, the investigation of this subject by means of meta-analysis did not reveal an improvement of reproductive performance after treatment with PGF2α.

P67

Effect of two different anaesthetic protocols used during bitch Caesarean sections compared to vaginal delivery on the Apgar score and survivability of puppies

J Hajurka, G Kredatusova, T Vitkova

University of Veterinary Medicine and Pharmacy, Kosice, Slovakia

This study compares the effect of two different anaesthetic protocols used during induction of an emergency Caesarean sections (CS) on the Apgar score (AS) and the survivability of the puppies. It then compares CS to vaginal delivery under the same parameters. Twenty-five bitches were examined. Eleven delivered spontaneously, while 14 had dystocia of < 12 h duration and needed a Caesarean section. The latter group was subsequently divided into two different anaesthetic protocols. One group was induced with diazepam (0.3 mg/kg i.v.) and propofol (6 mg/kg i.v.group DP) and the other group with diazepam (0.3 mg/kg i.v.) and ketamine (5 mg/kg i.v. group DK). After intubation anaesthesia was maintained with isoflurane during the CS procedure. The unpaired *t*-test was used for statistical analysis. After spontaneous vaginal delivery, 48 puppies were also evaluated and an AS obtained for those who survived. Of group DP, 87.5% received an AS of 7.3 ± 3.0 ($p < 0.0001$) and of group DK, 88.3% received an AS of 5.7 ± 2.9 ($p < 0.0001$). After spontaneous vaginal delivery, 97.9% of the puppies were born alive with an AS of 9.5 ± 0.9 and 2.1% were born dead. The differences between the two anaesthetic protocols were nonsignificant ($p = 0.1124$). In addition the CS procedure had the effect of increasing the number of stillborn and dead puppies within 24 h after delivery (19.5% after CS vs. 2.1% vaginal delivery; $p < 0.0001$). Funding: KEGA Grant 013 UVLF – 4/2012

P68

In vitro developmental competence of prepubertal goat oocytes cultured with recombinant activin-A

S Hammami, R Morato, M Catalá, M Paramio, D Izquierdo

Facultat Veterinària, Universitat Autònoma de Barcelona, Bellaterra, Spain

The aim of this study was to evaluate the effect of recombinant human activin-A added to the IVM and IVC media on the blastocyst rate of prepubertal goat oocytes. Cumulus Oocyte Complexes (COCs) were matured in groups of 25–30 COCs in two types of IVM media: conventional IVM medium (CM) (TCM199 supplemented with 10% Donor Bovine Serum, 10 μg/ml FSH, 10 μg/ml LH, 1 μg/ml 17β-oestradiol and 100 μM cysteamine), and CM + 10 ng/ml activin A. After IVM, oocytes were fertilized and after 24 h presumptive zygotes were cultured in two media (synthetic oviduct fluid (SOF) and SOF + 10 ng/ml activin-A) during 8 days. The experimental treatments were: (i) COCs matured in CM and cultured in SOF; (ii) COCs matured in CM and cultured in SOF + 10 ng/ml activin-A; (iii) COCs matured in CM + 10 ng/ml activin-A and cultured in SOF; and (iv) COCs matured in CM + 10 ng/ml activin-A cultured in SOF + 10 ng/ml activin-A. The cleavage rate was evaluated at 48 h post insemination and blastocyst percentage at the final of *in vitro* embryo culture. The results of cleavage did not show differences between treatments A, B, C and D (50.81%, 56.50%, 47.51% and 52.06% respectively, $p > 0.05$). However, when activin-A was present during IVC (Treatment B), COCs showed the highest percentage of blastocyst development compared to treatment A and D (11.43% vs. 5.84% and 5.65% respectively, $p < 0.05$). In conclusion, the presence of activin-A during IVC of prepubertal goat oocytes improve its developmental competence.

P69

Effects of size of ova and number of corpus luteum on the amount of recovered embryos and embryo duality in dairy cows

Z Hegedüšová[1], Y Zhang[2], A Dufek[1]

[1]*Research Institute for Cattle Breeding, Vikyrovice, Czech Republic;* [2]*Anhui Agricultural University, Hefei, China*

The aim of our study was to evaluate effect of size of ova and number of corpus luteum (CL) on the amount of recovered embryos and embryo quality. The size of ova and number of CL was recording by palpation per rectum in 567 animals. Donors were divided into three groups according to the recorded size of ova: 1–3 cm, 4–8 cm and above 9 cm and the average number of total flushed out embryos was 4.3 (SD ± 7.9), 9.01 (SD ± 6.9), 14.6 (SD ± 9.01) and suitable embryos for embryo transfer 1.9 (SD ± 5.1), 5.2 (SD ± 5.03), 8.1 (SD ± 6.9) in the groups, respectively. Further, donors were divided into four groups according to the diagnosed number of CL. The average number of total flushed out embryos in the group of donors with the max. 4 CL was 6.6 (SD ± 2.9) and the number of suitable embryos for embryo transfer was 4.05 (SD ± 2.4). Higher numbers of embryos were found out in the group of donors with 5–6 CL. The average number of total embryos was 7.2 (SD ± 4.6), 9.8 (SD ± 5.3), 17.2 (SD ± 8.3) in the group of donors with the 7–8, 9–10 and more than 10 diagnosed CL, respectively and suitable embryos for embryo transfer 3.8 (SD ± 3.3), 5.8 (SD ± 4.6), 9.2 (SD ± 6.3), respectively. The effects of size of ova and number of CL on the total number of embryos and the number of suitable embryos for embryo transfer were significant at the level $p < 0.05$. We can conclude that the number of total recovered embryos increases with the size of ova and the recorded number of CL. However, the higher size of ova, the lower ratio of suitable embryos for embryo transfer. Further, higher number of CL indicates higher ratio of suitable embryos for embryo transfer. Supported by projects: LA 09031, ME09081.

P70

Developmental competence of bovine two-cell stage embryos is predicted by the expression level of genes related to oxidative stress response

E Held, D Salilew-Wondim, D Tesfaye, E Tholen, K Schellander, M Hölker

Institute of Animal Science, Animal Breeding and Husbandry Group, University of Bonn, Bonn, Germany

Several studies in different species revealed separated blastomeres of two cell stage embryos to be able to develop to the blastocyst synchronal. Thus, we expected a single blastomere, with a developmentally competent sister blastomere, to exhibit a certain transcriptomic profile. Therefore, we pooled single, separately frozen blastomeres, according to the development of their sister blastomeres in to three groups. The first group did not cleave after separation (2CB), the second group stopped cleaving at the four cell stage (8CB) and the blastomeres of the last group reached the blastocyst stage (BL). Transcriptome profiling was conducted using the EmbryoGENE 4*44K Chip. Seven hundred and seventy-one genes were differentially regulated between BL and 2CB (fold change ≥1.5, $p \leq 0.05$, FDR ≤ 0.1), of which 413 genes were up-regulated and 358 down-regulated in BL. Similarly 190 genes were differentially regulated between BL and 8CB, 79 were up-regulated and 97 were down-regulated. Several genes including NDUFS1, CAT, PRDX1, PRDX6 and MAPK14 were found to be involved in oxidative stress response. Further validation discovered these genes to be differentially regulated in two cell stage embryos, selected based on the time of first cleavage post fertilisation, which is known to be a marker for developmental competence. The present study revealed, that expression levels of genes related to oxidative stress response determine the developmental competence of bovine two cell stage embryos.

P71

Female aspects of fertility in relation to timing of AI in lactating dairy cows

M Herlihy, S Butler

Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland

The ability of an animal to display behavioural oestrus and ovulate a dominant follicle at the appropriate time is a prerequisite for optimal timing of AI and oocyte fertilisation. Early AI (0 h after oestrus onset) results in lower fertilisation rates (but good embryo quality), whereas, late AI (24 h after oestrus onset) results in greater fertilisation rates but poorer embryo quality due to an aging ovum. There are different estimates for the optimal time of AI that range from 4 to 20 h after oestrus onset. During spontaneous oestrus, overt oestrous behaviour is required to identify cows for AI. Genetic selection for increased milk yield has shortened the duration of behavioural oestrus and increased the incidence of 'silent heat', representing a major challenge to efficient identification of cows for AI. This has led to the development of ovulation synchronisation programmes that tightly control when ovulation occurs, allowing AI to take place at a predetermined time ('Timed AI protocols'). Some of these synchronisation protocols achieve pregnancy outcomes similar to untreated cows inseminated at spontaneous oestrus. In addition, a variety of automated approaches to identify when cows are in oestrus have been developed. These include automated activity meters, continuous monitoring with cameras, in-line progesterone measurement. These approaches have the advantage of being 'always on', and hence may be useful from a management perspective to identify optimum timing of AI.

P72

Relations between luteinizing hormone pulsatility and vena cava and jugular progesterone concentrations on day 14 after insemination in sows

L Hoving[1], N Soede[1], O Peltoniemi[2], B Kemp[1]

[1]*Adaptation Physiology Group, Wageningen University, Wageningen, The Netherlands;* [2]*Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland*

This experiment evaluated LH and progesterone concentrations in the caudal vena cava at day 14 of gestation. On day 13 after first insemination, seven second parity sows received a catheter in de caudal vena cava as well as an ear vein catheter. One day later, blood samples were taken from the vena cava every 15 min and from the jugular vein every hour. Both LH and progesterone were released in a pulsatile manner. The mean number of LH pulses was 4.0 ± 1.1 per 10 h, with an amplitude of 0.86 ± 0.3 ng/ml. Basal and average concentrations of LH were, respectively, 0.35 ± 0.07 and 0.68 ± 0.1 ng/ml. The pattern of progesterone concentrations in the vena cava was very irregular and pulses were hard to define. The number of surges above 100 ng/ml was 4.7 ± 1.1 per 10 h. Basal and average progesterone concentrations were, respectively, 33.6 ± 13.1 and 65.5 ± 19.8 ng/ml. The average concentration of progesterone measured in the jugular vein was 27.6 ± 1.5 ng/ml. On average 1.5 (range 0–3) LH pulses were followed by a progesterone surge above 100 ng/ml within 15–60 min after maximum LH. Average jugular vein progesterone concentrations were negatively correlated with LH concentrations ($r = -0.95$, $p = 0.01$). In conclusion, at day 14 of pregnancy, progesterone release, as measured in the vena cava, shows a very variable pattern, which only seems partly related to LH release.

P73

Effect of cumulus cells on the results of ultra-rapid vitrification of canine oocytes

K Jaroszek[1], R Faundez[2], P Jurka[2], Ç Çebi[3]

[1]*InviMed, European Center of Motherhood, Warsaw, Poland;* [2]*Faculty of Veterinary Medicine, Warsaw Life Sciences University, Warsaw, Poland;* [3]*Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, University of Ankara, Ankara, Turkey*

The aim of this study was to assess the morphology, survival rates, *in vitro* maturation and fertilization by intracytoplasmic sperm injection (ICSI) of bitch oocytes after ultra-rapid vitrification. Cumulus oocyte complexes (COCs) and denuded oocytes were both vitrified in LN_2 at −196°C or in slush nitrogen (SN_2) at −208°C using a super-cooling ultra-rapid vitrification device Vitmaster (IMT United Kingdom). All COCs were matured *in vitro* (IVM) in modified synthetic oviduct fluid (mSOF) for 48 h. Propidium iodide was used to assess the vitality of oocyte. Recovery rates of canine oocytes with normal morphology after vitrification with LN_2 and SN_2 did not differ significantly (89.9% and 83.3% respectively $p < 0.05$). Survival rates of COCs vitrified by SN_2 were lower than those by LN_2 (18.2% and 50.0% respectively $p < 0.05$) Vitrification in LN_2 of denuded oocytes did not affect survival rate (54.4%). When vitrified in SN_2 survival rates of control oocytes were higher than those denuded oocytes (18.1% and 3.2% respectively $p < 0.05$). After IVM and ICSI no cleavage was observed at 48 h after ICSI, in vitrified oocytes both in LN_2 and SN_2 groups in comparison with control (18.6%). Vitrification in LN_2 provided higher survival rates compared with SN_2 and the presence of granulose cells showed only a slight positive effect on survival of canine oocytes after vitrification. Further investigations are required to clarify the reasons of low development of vitrified canine oocytes.

P74

Effect low of steroids on the levels of spermatogenesis stimulating hormone and interstitial cell stimulating hormone after castration in the local male goat

D Jassim AL-Delemy, BH Abed Ali

College of Veterinary Medicine, University of AL-Qadisiya, AL-Qadisiya, Iraq

The current study was conducted for the purpose of identification the effect of the low levels of steroid after castration in male goats on levels of pituitary hormones (SSH and ICSH), and have included (20) males ranged from ages between (1.5–2.5) years old and was free of disease and reproductive problems, that housed in same conditions of nutrition and management. The levels of reproductive hormones include [Spermatogenesis Stimulating hormone (SSH), Interstitial cell stimulating hormone (ICSH), progesterone, testosterone and Estradiol-17β] were estimated two times before castration and in intervals of 2 weeks between examined, Experimental animals were examined after castration all hormones 2 weeks after castration, then after 4 weeks from castration. The method of analysis Radioimmunoassay (RIA) was use to measure the levels of reproductive hormones in animals blood serums. The results revealed that there are significant increased in the level of ICSH in experimental animals after castration (0.345 ± 0.0413) mIU/ml bout aren't significant effect in the level of SSH, while the level of hormones testosterone and estradiol-17β significantly decrease in experimental animals after castration and the results were (0.0225 ± 0.0036) ng/ml and (0.93 ± 0.15) pg/ml, respectively. While castration did not show any significant effect on the levels of progesterone, and lead decreased levels of steroids to increased level of ICSH significantly and increased SSH levels calculated.

P75

Anatomical study of reproductive tracts of feral feline queens in Iraq

D Jassim, AL Delemy, T Thaier, A Abid

College of Veterinary Medicine, University of AL-Qadisiya, AL-Qadisiya, Iraq

The present study is carried out to investigate some aspects of reproduction in Iraqi feral cats. Forty-six female genital specimens from sexually mature queens were trapped during the period from December 2008 to December 2009. The specimens were classified into pregnant and non pregnant. The non pregnant were further classified into reproductive systems with or without obvious pathological changes. During the entire study period, the results showed that the means of length, width and thickness of right and left ovaries were $9.8 \times 5.03 \times 3.95$ and $9.56 \times 4.8 \times 3.05$ mm respectively, with no significant variation ($p \geq 0.05$) between the dimensions of right and left ovaries or between dimensions of the different seasons. The means of length of right and left uterine tubes were 54.4 and 55.7 mm respectively, with no significant variation ($p \geq 0.05$) between the dimensions of right and left uterine tubes or between dimension of different seasons. The means of length and diameter of right and left uterine horns were 48.44×5.41 and 54.43×5.14 mm, with significant variation ($p \leq 0.01$) between dimensions of right and left uterine horns and between the dimensions of different seasons. Also, the result showed that the means of the length and diameter of uterine bodies and cervix were 22.75×5.29 and13.2×8.72 mm respectively with significant variation ($p \leq 0.01$) between dimensions of different seasons.

P76

Mitochondrial status of bovine and porcine oocytes with different meiotic competence during their maturation

M Jeseta, D Knitlova, K Hanzalova, S Hanulakova, M Machatkova

Veterinary Research Institute, Brno, Czech Republic

It was documented that mitochondrial morphology and function can be used as markers of oocyte cytoplasmic maturity. The study was designed to characterize mitochondrial cluster formation and ATP production changes during maturation of meiotically higher competent (MHC) and less competent (MLC) oocytes from medium and small ovarian follicles respectively. Mitochondria was detected on confocal microscope after MitoTracker Orange staining and ATP content was measured using FL-ASC assay kit (pmol per oocyte). In these experiments were evaluated 569 bovine oocytes (MHC n = 174; MLC n = 395) and 401 porcine oocytes (MHC n = 160; MLC n = 241). The proportion of bovine oocytes with clusters increased from 2.9% to 39.4% in MHC- and from 0.6% to 19.6% in MLC-oocytes between stages GV and MII. Mitochondrial clusters were concentrated around the endoplasmic reticulum and formed a characteristic pericortical network. In pig the proportion of oocytes with clusters increased from 5.2% to 44.6% in MHC- and from 12.4% to 29.4% in MLC-oocytes between stages GV and MII. In contrast to bovine oocytes, mitochondria encircled lipid droplets in the so-called metabolic units. The ATP content was higher in bovine than porcine oocytes, both at GV and MII stage. However, in both species, ATP production was higher in MHC- (GV: 2.58 pmol in pig; 2.70 pmol in cattle; MII: 1.85 pmol in pig; 3.30 pmol in cattle) compared to MLC- (GV: 2.05 pmol in pig; 2.31 pmol in cattle; MII: 1.04 pmol in pig; 3.01 pmol in cattle) oocytes. In conclusion, bovine and porcine oocytes differ in some measure in morphology and function of their mitochondria but in both species mitochondrial status is associated with meiotic competence of oocytes. Supported by grants NAZV QI 91A018, QI 101A166 and MSMT INGO LA 09018.

P77

Effect of Icariin on development and apoptosis of porcine IVF embryos *in vitro*

G Jianming, X Jie, Z Zhao, Z Ju, X Rongke, L Xiaoran, W Bo, S Yaran

Beijing Agricultural University, Beijing, China

The objective of this study was to improve the culture system of porcine embryos *in vitro*. Icariin, a Chinese traditional medicine rich in flavanols, was used to evaluate the efficacy of porcine *in vitro* embryo production. IVF 1-cell zygotes were selected as the experimental material in this study. In the first experiment, embryos were cultured in NCSU-23 culture medium as control group. Three experimental groups were added with Icariin in varying concentrations of 0.6, 1.2 or 2.4 μg/ml (Ica1, Ica2, Ica3) to screen the optimal concentration of Icariin for embryo development. About 50 oocytes were used in every group, and had six replicates. A second experiment studied the effect of the optimal concentration of Icariin on embryo apoptosis. In experiment 1, only Ica1 differed from control; the percentages of two-cells at 48 h and blastocysts at 168 h were significantly higher (77.2 ± 2.7 vs. 65.8 ± 4.9, 26.2 ± 4.2 vs. 15.9 ± 1.9, $p < 0.05$) and also the cell numbers of blastocysts (62.2 ± 2.56 vs. 40.8 ± 2.9, $p < 0.01$). Experiment 2 showed that the index of apoptosis of blastocysts was much lower in Ica1 than in the control group (7.0 ± 0.7 vs. 13.7 ± 1.0, $p < 0.01$). To conclude, addition of 0.6 μg/ml Icariin to NCSU-23 can remarkably improve the development of porcine *in vitro* embryos, and inhibite the apoptosis of IVF embryos. But the mechanism of the effect is unknown, we will research on it in the future.

P78

Single layer centrifugation can improve the quality of buck ejaculates collected by electroejaculation

P Jiménez[1], M Ramón[1], O García-Álvarez[2], A Maroto-Morales[2], P Álvaro-García[1], M Pérez-Guzmán[1], A Johannisson[3], J Garde[2], A Soler[2], J Morrell[4]

[1]CERSYRA, Valdepeñas, Spain; [2]IREC (CSIC-UCLM-JCCM), Albacete, Spain; [3]Anatomy Physiology and Biochemistry, SLU, Uppsala, Sweden; [4]Clinical Sciences, SLU, Uppsala, Sweden

To date, no sperm selection techniques have been carried out in order to improve the sperm quality after cryopreservation in buck semen collected by electroejaculation (EE). In this work, we evaluated the effects of selecting sperm samples by Single Layer Centrifugation (SLC) on sperm quality after cryopreservation. Ejaculates collected by EE were divided in two aliquots. One of them (unselected) was diluted with Biladyl® by two-step method and frozen over nitrogen vapors (Group I). The other aliquot (Group II, selected before freezing) was extended in PBS to 100×10^6 spz/ml and 4 ml were layered over 4 ml colloid. The centrifugation process was performed at $300 \times g$ for 20 min and the resulting pellet was extended and frozen as described above. Two unselected straws were thawed and used for SLC (Group III, selected after thawing). At thawing, all samples were assessed for total (TM) and progressive (PM) motility by CASA and for viability by flow cytometry. All evaluated parameters showed higher values for samples selected after thawing (Group III) in relation to unselected (Group I) and selected before freezing (Group II) samples (TM: 86% vs. 67% vs. 62%; PM: 80% vs. 58% vs. 54%; viability: 80% vs. 32% vs. 12%; Groups III, I and II, respectively). In conclusion, sperm quality in samples collected by EE is improved when they are selected by SLC after thawing.

P79

Optimization of two serum-free bovine 'single' *in vitro* embryo production methods

E Jorssen, S Valckx, J Leroy, E Merckx, S Andries, P Bols

University of Antwerp, Antwerp, Belgium

Although *in vitro* group embryo production is routinely used, it does not allow individual embryo evaluation during culture, which could reveal new insights on oocyte quality parameters to predict developmental competence. Therefore, two single bovine IVP systems were optimized, without serum and with or without homologue cumulus cell co-culture. COCs from 2 to 6 mm diameter follicles were randomly allocated to treatment groups (n): GGG (631), GGS (207), SSG (180), SSS (196) or SSSc (163); three replicates (Group or Single: maturation, fertilization, culture; c: cumulus co-culture). COCs were matured in groups of 50 in 500 μl or singly in 20 μl TCM 199 with 20 ng/ml EGF for 24 h and subsequently fertilized in groups of 100 in 500 μl or individual in 20 μl fertilization medium for 20 h (5% CO_2, 38.5°C). Presumptive zygotes were denuded and cultured in groups of ±25 in 50 μl or individual in 20 μl SOF with ITS (10 μg/ml insulin, 5.5 μg/ml transferrin, 6.7 ng/ml selenium) and 2% BSA (5% O_2, 5% CO_2, 38.5°C). Comparable cleavage and blastocyst rates were obtained in treatment groups GGS (68.1% and 31.4%) and SSSc (63.8% and 28.8%) compared to GGG (69.9% and 31.2%). Cleavage ($p = 0.022$) and blastocyst rate ($p = 0.010$) were significantly lower when embryos were produced SSS (60.7% and 21.4%) and tended to be lower in SSG (63.3% and 24.4%) ($p = 0.093$ and 0.083) as compared to GGG. In conclusion, embryos can be produced fully single without serum and by the addition of autologues cumulus cells the embryo development to blastocyst stage increases.

P80

Different mammary tumours, ovarian cyst and uterocervical stump inflammation in a hysterectomised bitch: a case report

P Jurka, J Borkowska

Warsaw University of Life Science, Warsaw, Poland

Hysterectomy is chosen over OH in order to eliminate overweight any behavioral disorders after castration in the bitch. However, hysterectomy doesn't eliminate influence of ovarian hormones. The aim of the study was to present complex side effects after hysterectomy. Nine years old bitch was hysterectomized 3 year ago. Clinical examination showed mammary tumor in L5 and vaginal purulent leakages. Abdominal USG, chest RTG (lateral and peroneal), immunofluorescent assay of P4 and E2 and histological examination were performed. Residual ovaries with cyst and stump inflammation were observed on USG. The level of P4 – 16.4 ng/ml and E2 – 65.1 pg/ml. RTG of the chest was without any changes. Complications were removed by laparotomy and semi-mastectomy. Histological examination showed adenocarcinoma without any changes in inguinal lymph node. After 5 months four new mammary gland tumors in L3 and L4 positions were found. The level of P4 – 0.6 ng/ml and E2 – 25.3 pg/ml. RTG of the chest was without any changes. Histological examination after semi-mastectomy showed complex, ductal, intraductal papillary carcinoma and arising mixed tumor without any changes in inguinal lymph node. Castration with retaining of ovaries may lead to ovarian cysts and mammary tumors. Partial removal of uterus can cause uterocervical stump inflammation. The side effect of ovaries removal are harmless and easy to prevent. In contrast, hysterectomy complications may be complex and hard to treat or even untreatable. Removal of ovaries after tumor development doesn't prevent tumor progression.

P81

Pancreatic β-cell function is positively correlated with the size of newborn Holstein calves

M Kamal[1], M Van Eetvelde[1], L Vandaele[2], G Opsomer[1]

[1]*Department of Reproduction, Obstetrics and Herd Health, Ghent University, Merelbeke, Belgium;* [2]*Institute for Agricultural and Fisheries Research, Melle, Belgium*

Newborn calves (40 males and 38 females) of Holstein heifers (n = 28) and cows (n = 50) were used to evaluate the association between their body size and pancreatic β-cell function. The calves were weighed and specific body sizes were measured in the morning of their 3rd day of life following an overnight fast. At the same time an intravenous glucose-stimulated insulin secretion (GSIS) test was performed. For this, blood samples were taken before and exactly 10 min after the calves had been given an intravenous glucose bolus of 150 mg/kg. The body weight, heart girth and diagonal length of the calves were 41.2 ± 5.03 kg, 80.1 ± 3.62 cm and 70.5 ± 3.56 cm, respectively. Although the basal glucose (G0, 5.9 ± 0.68 mM/l vs. 6.0 ± 0.83 mM/l) did not differ between calves of primiparous vs. multiparous dams (p = 0.73); the basal insulin (I0) of calves of primiparous dams tended (p = 0.14) to be lower (8.5 ± 6.47 mU/l vs. 10.5 ± 8.83 mU/l). The I0 was positively correlated with the weight (r = 0.22, p = 0.05), heart girth (r = 0.21, p = 0.06), and diagonal length (r = 0.24, p = 0.03) of the calves. The insulin sensitivity index (ISI, I0/G0) was also positively correlated with the weight (r = 0.24, p = 0.05), heart girth (r = 0.21, p = 0.08), and diagonal length (r = 0.29, p = 0.01) of the calf. The insulin secretion following a standard glucose bolus as quantified by the insulinogenic index [IGI, (I10-I0)/(G10-G0)] tended to be positively correlated with the weight (r = 0.17, p = 0.16), heart girth (r = 0.22, 0.07), and diagonal length (r = 0.15, p = 0.23) of the calf. These preliminary data suggesting a significant correlation between the β-cell function and the size of a newborn Holstein calf warrant further investigation with a higher number of calves.

P82

Abstract withdrawn.

P83

Long-term storage of porcine spermatozoa in chemically defined extenders

T Khalifa[1], C Rekkas[1], I Tsakmakidis[2], A Zdragas[1], F Samartzi[1], A Lymberopulos[3]

[1]*NAGREF, Veterinary Research Institute, Thessaloniki, Greece;* [2]*School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece;* [3]*Alexander Technological Educational Institute of Thessaloniki, Thessaloniki, Greece*

Two experiments were carried out over a 5-month period to evaluate efficiency of 16 media for preservation of quality traits of liquid-stored boar semen. Whole ejaculates of $\geq 0.2 \times 10^9$ sperm/ml and 75% sperm progressive motility were collected by the 'gloved hand' technique from fertile boars (n = 7; 2–3 years old) and split-diluted to $15–40 \times 10^6$ sperm/ml with citrate-, Tris-, Hepes- and Mops-based extenders. Semen was packaged under anaerobic conditions in 15-ml tubes and examined during a 13-day storage period at 18°C for sperm kinematics, agglutination (AG), abnormal acrosomes (AA), live morphologically normal sperm (LMNS), bacterial colony counts (BCC), pH, osmolarity, plasma membrane integrity (PMI), mitochondrial membrane potential (MMP), lipid peroxidation (LPO) and chromatin instability (CI). Data were analyzed using ANOVA, Duncan multiple range test and linear regression. The results showed that Mops-based extenders were superior to other extenders in terms of higher values of sperm motility and velocity along with lower proportions of AG, CI and LMNS (p < 0.03–0.001). Mops-and Hepes-treated semen had lower incidences of sperm with LPO and AA compared with Tris-and citrate-treated semen (p < 0.005–0.001). Mops- and Hepes-diluted semen maintained pH values (6.83–7.03) lower than those (7.45–7.5) of Tris-and citrate-diluted semen. Mean values of osmotic pressure (mOsm/kg) in Mops- (301.6) and Hepes- (303) extended semen differed significantly from those of Tris- (292–298) and citrate- (314–374) extended semen. PMI, MMP and BCC were not significantly influenced by semen extenders. In conclusion, Mops-based media are suitable milieu for long-term storage of porcine semen. (Supported by Nidacon International, Mölndal, Sweden).

P84

Chromatin instability (CI) of caprine spermatozoa

A Khalifa[1], A Lymperopoulos[2]

[1]*NAGREF, Veterinary Research Institute, Thessaloniki, Greece;* [2]*Department of Animal Production, ATEI, Thessaloniki, Greece*

Five experiments were conducted over a 9-month period to investigate sources of variation in CI of goat spermatozoa. Ejaculates (n = 182) with $\geq 1.5 \times 10^9$ sperm/ml and 70% sperm motility were collected with an AV from crossbred bucks and processed either for liquid storage at various dilution titers (2–20-folds) and temperatures (18–5°C for 48 h) or for frozen storage in plastic straws (0.25–0.5 ml) using static liquid nitrogen (LN) vapor and a programmable freezer (PF). Fluorescence-based analysis of acridine orange-stained sperm nuclei was used to assess incidence of CI in fresh and preserved semen. Data were analyzed using factorial ANOVA, Duncan's multiple range test,

Pearson correlation coefficient and linear regression. A high proportion ($p < 0.05$) of CI was found in ejaculates of non-breeding season compared with those of the breeding season. Ejaculation sequence and semen dilution titer had no significant effects on CI. Storage of semen in a liquid or frozen (0.5 ml straws in LN vapor) state caused a significant increase in CI. Decreasing storage temperature of liquid semen from 18 to 5°C was associated with a pronounced reduction of CI ($p < 0.001$). Semen dilution method (1 vs. 2 steps), seminal plasma removal and semen freezing method (LN vapor vs. PF) did not significantly influence CI. Nonetheless, in LN vapor-frozen semen, spermatozoa packaged in 0.5-ml straws showed higher values ($p < 0.05$) of CI than those packaged in 0.25-ml straws. In conclusion, goat buck, season, temperature of liquid-stored semen and straw size of LN vapor-frozen semen contribute to stability of sperm chromatin structure. (Supported by Nidacon International, Mölndal, Sweden)

P85

Secretion of prolactin is under the regulation of leptin, orexin A in seasonally-breeding sheep

K Kirsz[1], E Molik[1], M Szczesna[1], T Misztal[2], K Romanowicz[2], D Zieba[1]

[1]*Agricultural University, Krakow, Poland;* [2]*Polish Academy of Sciences, Jablonna, Poland*

The study examined the effects of interaction of season (long-; LD vs. short-day; SD), recombinant ovine leptin (roleptin), ovine ghrelin and orexin A (ORXA) on secretions of prolactin (PRL) in sheep. To determine the role of leptin in those interactions the ovine super-active leptin antagonist (LA) was used. Twenty-four ovariectomized and estradiol-implanted ewes were utilized in a replicated switchback design. The ewes were assigned randomly to one of six treatment groups, and the treatments for groups 1–3 were infused into their third ventricles three times at 0, 1 and 2 h, with 0 h being at dusk. In groups 5 and 6, leptin antagonist was centrally infused twice at 0 and 1 h, and the ghrelin or ORXA were infused at 15 and 60 min after LA. The treatments were as follows: (i) control, Ringer-Locke buffer; (ii) leptin, 0.5 μg/kg BW; (iii) ghrelin, 2.5 μg/kg BW; (iv) ORXA, 0.3 μg/kg BW; (v) LA, 50 μg/kg, then ghrelin, 2.5 μg/kg BW; and (vi) LA, 50 μg/kg, then ORXA, 0.3 μg/kg BW. Blood samples (5 ml) were collected at 10-min intervals for 4 h. Results supported our earlier data that PRL is inversely regulated by leptin during SD and LD, with higher PRL secretion observed on LD ($p < 0.001$). However, using LA and completely blocking leptin signal the increased in PRL secretion ($p < 0.001$) after ORXA treatment during both photoperiods was noted. We conclude that ORXA and leptin play together on the level of anterior pituitary and regulate PRL secretion in seasonally breeding sheep. Supported by grants MNiSzW NN 311 318436 and DS/KHiOK/3242/10.

P86

Follicle size at ovulation may affect fertility in the sow

S Kothe, S Vehmas, O Peltoniemi

Department of Production Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland

The follicle size at ovulation is known to be between 6 and 9 mm in the pig. Little is known if this size affects the subsequent fertility. In the present study, the connection between the size of the follicles at ovulation and the size of the live born litter is investigated. Sows ($n = 93$) from a sow pool system, that had farrowed at least once and had a WOI of 4–6 days were included. Starting at day 3 after weaning, sows were checked for standing oestrus in the presence of a boar twice a day. When in heat, ovaries were ultrasounded one-sided transabdominally twice a day until ovulation. The three largest follicles were repeatedly measured to assess timing and size at ovulation. For the analysis, a multiple linear regression model was used. Additionally to the follicle size, the parity, breed, number of inseminations, the boar and whether or not the inseminator got informed about the time of ovulation, were considered as explanatory variables. Out of the variables, only parity 2.9; $p \pm (4.8 < 1.8; p \pm 0.05)$ and follicle size at ovulation ($7.3 < 4.3$). Follicles ± 0.05) influenced the live born litter size (11.1 ovulating at the size of 7–8 mm resulted in larger live born litters than follicles ovulating at < 7 or > 8 mm. These results indicate that there is an optimal size of follicles at ovulation. Ovulation at too small or too large follicular size may be linked to inadequate hormonal control of ovulation. This mechanism is likely to include LH and oxytocin secretion. Work is underway to explore this mechanism.

P87

Effect of Aflatoxin B1 administration on blood serum progesterone and oestradiol-17β concentrations of goats during anoestrus

G Kourousekos[1], E Theodosiadou[2], S Belibasaki[3], C Boscos[1], A Lymberopoulos[4]

[1]*Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece;* [2]*Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece;* [3]*Veterinary Research Institute, NAGREF, Thessaloniki, Greece;* [4]*Department of Animal Production, Technological Educational Institute of Thessaloniki, Thessaloniki, Greece*

This study investigates the effect of aflatoxin B1 (AFB1) administration on blood serum progesterone (P4) and oestradiol-17β (E2) concentrations of the goats during anoestrus. Thirty Greek indigenous goats were used; AFB1 was administered, per os, in 20 goats (10 goats received 50 μg and 10 goats received 100 μg aflatoxin B1/day/head respectively, for 1 month), while 10 goats served as controls. Blood samples were collected from each goat every 3 days, for a period of 1 month before and 1 month after AFB1 administration. Serum P4 and E2 concentrations were determined by radioimmunoassay. Before AFB1 administration no statistical differences were noticed among groups regarding P4 or E2 concentrations. In contrast, after AFB1 administration, linear regression analysis revealed a dose dependent positive relation between P4 and group ($F = 4.061$, df = 265, $p < 0.05$; Constant = 0.155 ± 0.016, $t = 9.884$, $p < 0.001$; group = 0.015 ± 0.007, $t = 2.015$, $p < 0.05$) or E2 and group ($F = 3.909$, df = 265, $p < 0.05$; Constant = 24.413 ± 5.445, $t = 4.483$, $p < 0.001$; group = 4.993 ± 2.526, $t = 1.977$, $p < 0.05$). In conclusion, AFB1 administration increases progesterone and oestradiol-17β concentrations in blood serum of the goats during anoestrus, in a dose dependent manner.

P88

Quality of cryopreserved bull spermatozoa selected by swim-up and Percoll procedures at two different temperatures

A Krogenæs[1], AH Alm-Kristiansen[2], KE Waterhouse[2]

[1]*Department of Production Animal Sciences, Norwegian School of Veterinary Science, Oslo, Norway;* [2]*BioKapital, Hamar, Norway*

The purpose of the present study was to compare quality of bull spermatozoa selected by swim-up (SU) or Percoll (PC) at 27 and 38°C throughout the whole procedures. Semen straws from one ejaculate of nine Norwegian Red bulls were used. Three straws from each bull were pooled and split-sampled for use with both procedures ($n = 3$ at each temperature). Prior to and after selection sperm density was

determined by NucleoCounter® SP-100™, progressive motility by phase-contrast microscopy and sperm viability (YO-PRO 1), acrosome-integrity (PNA-Alexa 488) and plasma membrane (PM)-fluidity (Merocyanine 540) by flow cytometry. Sperm output increased with both procedures at 38°C ($p < 0.05$), and as expected output was higher after PC than SU ($p < 0.01$). Sperm motility was improved ($p < 0.05$) without effect of temperature after SU, whereas PC only slightly improved motility at 27°C ($p > 0.2$), and in fact motility was poorer after selection at 38°C ($p < 0.05$). Sperm viability and acrosome-integrity was improved for both SU and PC ($p < 0.01$), with SU being superior. Moreover, PC negatively affected results at 38°C compared to 27°C ($p < 0.01$). The number of viable acrosome-intact spermatozoa with high PM-fluidity did not change after PC; however this parameter increased after SU at 38°C and was higher than for PC ($p < 0.001$). In conclusion; sperm output was improved after selection at 38°C for both selection procedures, but output was superior after PC. Nevertheless, sperm viability and motility was poorer after PC than SU. Although PM-fluidity of acrosome-intact viable spermatozoa were affected after SU, sperm quality was better than after PC selection.

P89

The effect of different glycerol concentrations on freezability of semen from Angora, Kilis and Saanen goats

R Kulaksız[1], U Arı[1], A Daskin[2], A Üner[3]

[1]*Kafkas University, Kars, Turkey;* [2]*Ankara University, Ankara, Turkey;* [3]*Adnan Menderes University, Aydın, Turkey*

The aim of this study was to investigate effect of different glycerol concentrations on freezability of semen from Angora, Kilis and Saanen goats. Three male goats from each breed were selected and ejaculates were collected with artificial vagina. Three ejaculate from each breed were pooled and extended with skim milk based extender containing 10% (v/v) egg yolk and 0%, 3%, 5%, 7% and 9% (v/v) glycerol (G0, G3, G5, G7 and G9, respectively). Extended semen from different goat breeds was equilibrated, cryopreserved and then stored in liquid nitrogen. The best post-thaw motility for Angora (51.6%) and Kilis (75.0%) goats was obtained with G5 concentration while the best post thaw motility for Saanen goat (61.6%) was obtained with G7 concentration ($p < 0.001$). Similar results were determined for percentage of live spermatozoa for Angora (58.1% in G5), Kilis (78.5% in G5) and Saanen (64.0% in G7) ($p < 0.001$). The lowest abnormal spermatozoa percentages were obtained with G5 concentration for Angora (34.3%), Kilis (30.8%) and Saanen (40.8%) ($p < 0.001$). While glycerol concentrations for goat breeds were considered, it was determined that suitable glycerol percentages for Angora, Kilis and Saanen were 5%, 5–9% and 7%, respectively. It was concluded that glycerol concentrations for different goat breeds was important factor affecting freezability of goat semen.

P90

Unconventional anti-mastitis treatment by endo-mammary platelet concentrate infusion in cattle

A Lange Consiglio, C Spelta, R Garlappi, A Bignotti, F Cremonesi

Large Animal Hospital, Reproduction Unit, Università degli Studi di Milano, Lodi, Italy

Mastitis is a global problem as it adversely affects animal health and economic of milk production. Antibiotic treatments are widely used but a growing problem of antibiotic resistance is more often faced. Non-antibiotic approaches would be necessary. Platelet concentrate (PC) is used in surgery because it contains over 30 growth factors which stimulate the healing processes. Moreover, according to *in vitro* experiment, PC showed antimicrobial effect. In this view, we investigated the activity of PC in the treatment of bovine mastitis as an unconventional therapy. The PC was prepared from blood of healthy cows by a double centrifugation to obtain a standard concentration of 1×10^9 platelet/ml. We used PC alone (15 quarters) or in combination with antibiotic (23 quarters) in respect to controls (only antibiotic: 14 quarters), for three consecutive days by endo-mammary administration of 5 ml of PC at each inoculation. Our data show that the synergistic action of antibiotic + PC performed significantly better than antibiotic alone either for the recovery of the affected mammary quarters or for the somatic cell reduction (86.96% vs. 56.14% of ameliorated quarters with a somatic cell counts < 400 000 respectively; $p < 0.05$). In the same way, the association antibiotic + PC shows significantly less relapses compared to the antibiotic alone (5% vs. 37.50% respectively; $p < 0.05$). The PC may be useful for a reliable resolution of the inflammatory response playing an essential role in limiting the tissue damages to the gland parenchyma.

P91

Chemical castration by intra-testicular injection of a calcium chloride in alcohol solution in dogs

R Leoci, G Aiudi, F Silvestre, G Lacalandra

Department of Animal Production, Università di Bari, Bari, Italy

An affordable and effective non-surgical technique for achieving male dog sterility is needed to solve the problem of overpopulation. The efficacy of 20% calcium chloride in pure alcohol solution, injected into the testicular parenchyma, as a method for chemical castration, was evaluated. Twenty-one dogs of mixed breed, 4.7 ± 1.23 years old, 20 ± 5.84 kg of body weight, with good clinical conditions and normal reproductive parameters, were lightly sedated and injected into the dorsocranial portion of both testes with a solution of 20% calcium chloride dihyrdate in ethanol (95%). The dose injected corresponds with the testicular width (19–22 mm receive 0.8 ml; 23 and above 1 ml). Semen evaluation was performed by CASA (Computer Assisted Sperm Analysis) system at day 30–60–90. The animals in the control group received a single bilateral intratesticular injection of 1 ml sterile saline solution (testicular width 23 mm and above). Forty-eight hours after the injection, dogs showed very light discomfort at palpation and testicular tumefaction, which regressed within 3 days. At day 30, testicular ultrasonography revealed bilateral more dense nodular lesions; prostatic volume and parenchyma were normal. Semen evaluation showed azoospermia at day 30–60 and 90. The sperm count was decreased significantly ($p < 01$) in all the $CaCl_2$ treated dogs in comparison to saline solution control animals. At day 90 testicles were shrunk at palpation. An intratesticular injection of 20% calcium chloride in pure alcohol solution, as a method for chemical castration, was effective and economical for the sterilization of male dogs. It is free from pain and chronic stress and will contribute to a simple alternative method to surgical castration. The dogs of this study are under evaluation to study this solution long term effect (1 year).

P92

Flow cytometric and NIR (near-infrared) Raman spectroscopic investigation of sperm quality in the stained, sorted and frozen-thawed buffalo spermatozoa

X Li[1,2], M Wang[2], X Yang[2], Q Li[2], H Yang[2], S Lu[2], Y Lu[2], M Zhang[2], K Lu[2]

[1]*College of Animal Science and Technology, Guangxi University, Nanning, China;* [2]*College of Animal Science and Technology, Henan University of Science and Technology, Luoyang, China*

Flow cytometry and Laser Tweezers Raman Spectroscopy was used to investigate buffalo sperm cells from different phase (fresh, stained,

sorted and frozen-thawed) of Flow-sorting process in order to optimize sperm sex sorting procedures. The results of the Flow cytometer analysis showed that the percentage of live spermatozoa (67.48%, 58.04% vs. 77.81%) and spermatozoa with active mitochondria (69.58%, 63.71% vs. 83.08%) was lower ($p < 0.05$) for stained and frozen-thawed than that of fresh spermatozoa, while the percentage of reacted spermatozoa was not affected by the sorting process. The results of the spectroanalysis showed that stained and frozen buffalo sperm displayed a higher intensity at all Raman spectra than fresh sperm (except for 1301 and 1661/cm). Moreover, PCA and DFA analysis based on optical data is able to distinguish sperm cell from different phase (fresh, stained, sorted and frozen-thawed). In conclusion, the damages of flow-cytometry-sorted buffalo sperm mainly induced by sorting and freezing-thawing procedures, and the Raman spectroscopy should be a valuable tool in assessing the quality of sperm cells.

P93

In vivo heat stress affects spermatogenesis in rabbits

M Lopez-Bejar[1], M Sabes[1], N Planell[2], E Torres-Mejía[1], E Taberner[1], M Maya-Soriano[1], L Tusell[3], J Ramon[3], M Piles[3], M Lopez-Bejar[1]

[1]*Department of Animal Health and Anatomy, Universitat Autonoma de Barcelona, Bellaterra, Spain;* [2]*Bioinformatics Platform, CIBER-EHD, Barcelona, Spain;* [3]*Unitat de Cunicultura, IRTA, Caldes de Montbiu, Spain*

Heat stress in mammals is linked to deterioration of spermatogenesis and it can cause infertility. The aim of this study was to evaluate the effect of heat stress on the viability, morphology and motility of rabbit sperm cells using an *in vivo* model. Sixty New Zealand White rabbits were housed in two temperature-controlled rooms. Temperature for control group (30 animals) was maintained at 17°C. Temperature for heat stress (HS) group (30 animals) was increased from 17 to 31°C for 3 h at the central part of the day simulating a summer circadian cycle, applied for at least 3 months and a maximum of 1.5 years (maximum and minimum temperatures were determined by data from three previous summers). Eosin-nigrosin staining was performed to evaluate sperm viability and morphology. Motility parameters were obtained using a CASA system (Proiser, Valencia, Spain). HS samples showed significant ($p < 0.05$; Wilcoxon test) lower percentages of alive spermatozoa (80.45% vs. 75.92%) and spermatozoa with proximal cytoplasmatic droplets (5.82% vs. 4.8%) and higher percentages of sperm cells with acrosome abnormalities (23.08% vs. 32.82%) and tailless spermatozoa (8.02% vs. 12.05%) than control samples. Using the CASA system, progressive motility was significantly higher in the HS group than in the control one (26.18% vs. 29.02%; $p < 0.05$). Daily summer cycles applied in a continuous way provoked severe anomalies in spermatogenesis compatible with sperm cell aging.

P94

Evaluation of motility patterns from ram sperm long-term solid storage at 5°C up to 24 h

E López-Urueña, M Alvarez, S Gomes-Alves, C Martínez-Rodríguez, L Anel-López, P de Paz, F Martínez-Pastor, J Boixo, L Anel

University of León, León, Spain

Solid storage could decrease cell metabolic rate and deleterious changes in extender which would improve preservation of sperm quality for a long-term storage. Our aim was to assess motility patterns by 37°C-incubation of 5°C cooled sperm up to 24 h in gelatin. Four ejaculates were collected from four Assaf rams by artificial vagina. Each ejaculate was divided and extended with INRA96 (IMV Technologies), alone (control-C) or supplemented with 1.5% gelatin (G). Sperm was cooled (0.25°C/min) up to 5°C and sampled after 1 and 24 h at 5°C; at these times, samples were incubated at 37°C up to 60 min. Motility patterns were assessed (CASA) at 10, 30, and 60. Data was analyzed using linear mixed-effect models (R). At 1 h, C showed higher TM and velocity (VAP, VCL and VSL) but similar progressive movement (PM, LIN and ALH). After 24 h, TM was similar for both, except at 10 min of incubation (G: 81.8 ± 6.2 vs. C: 74.9 ± 7.5; $p < 0.05$); PM was higher for G, while VAP and VCL were higher for C (for 10, 30 and 60 min, $p < 0.05$), although VSL was similar. G showed higher LIN and lower ALH than C, although significant differences were observed only at 60 (LIN: 56.1 ± 5.7 vs. 48.3 ± 6.4) and 10 min (ALH: 4.1 ± 0.2 vs. 4.5 ± 0.2; G vs. C, respectively). Our findings suggest that solid storage improve progressive movement but no velocity, when spermatozoa are stored for 24 h. Supported by INIA (RZ2010-00005), Junta de Castilla y León (LE019A10-2) and Diputacion de León.

P95

Investigation of sperm quality in the stained, sorted and frozen-thawed buffalo spermatozoa using flow cytometric and NIR (near-infrared) Raman Spectroscope

K Lu[1], X Li[1,2], M Wang[1], X Yang[1], Q Li[1], H Yang[1], S Lu[1], Y Lu[1], M Zhang[1], K Lu[1]

[1]*College of Animal Science and Technology, Guangxi University, Nanning, China;* [2]*College of Animal Science and Technology, Henan University of Science and Technology, Henan, China*

Flow cytometry and Laser Tweezers Raman Spectroscopy was used to investigate buffalo sperm cells from different phase (fresh, stained, sorted and frozen-thawed) of Flow-cytometric sorting process in order to optimize sperm sex sorting procedures. The results of the Flow cytometer analysis showed that sperm viability and percentage of spermatozoa with functional mitochondria were not affected by the sorting process, while the percentage of reacted spermatozoa was lower ($p < 0.05$) for stained (67.48% and 69.58%) and frozen-thawed (58.04% and 63.71%) than that of fresh spermatozoa (77.81% and 83.08%). The results of the spectroanalysis showed that stained and frozen-thawed buffalo sperm displayed a higher intensity at all Raman spectra than fresh sperm (except for 1301 and 1661/cm). Moreover, principal component analysis (PCA) and distinguish function analysis (DFA) analyses based on optical data is able to distinguish sperm cell from different phase (fresh, stained, sorted and frozen-thawed). In conclusion, the damages of flow cytometric-sorted buffalo sperm mainly induced by sorting and freezing-thawing procedures, and the Raman spectroscopy should be a valuable tool in assessing the quality of sperm cells.

P96

Melatonin enhances buffalo oocyte maturation and embryo development *in vitro*

X Luo, E Zuo, Q Li, X Yang, S Lu, Y Lu, M Zhang, K Lu

College of Animal Science and Technology, Guangxi University, Nanning, China

Three experiments were carried out to investigate the effects of Melatonin (MLT) on *in vitro* maturation of oocytes (COCs) and embryo development in buffalo in this study. Experiment 1, COCs were matured in TCM 199 medium supplemented with different concentrations of MLT (10^{-10}, 10^{-9}, 10^{-8} and 0 M) for 24 h and only oocytes with polar body were counted for maturation rate. Experiment 2, COCs were matured in medium with (10^{-9} M) or without MLT and cultured in medium with different concentrations of MLT (0, 10^{-8}, 10^{-9}, 10^{-10} M) after IVF. Experiment 3, Reactive oxygen species (ROS),

produced by COCs matured in medium with or without MLT for 24 h, was measured using 2′, 7′-dichlorodihydrofluorescein diacetate, The results showed that maturation rate of oocytes cultured with 10-9M MLT was higher ($p < 0.05$) than that with control group (46.3% vs. 32.9%), but there was no difference ($p > 0.05$) among the treatment groups (43.4%, 46.3% vs. 43.2% for 10^{-10}, 10^{-9}, 10^{-8} M, respectively). In the group of COCs matured without MLT, the blastocyst rate was higher ($p < 0.05$) in the group of embryos cultured with 10^{-8} M MLT than that of control (34.3% vs. 20.6%), meanwhile, in the group of COCs matured with 10^{-9} M MLT, the blastocyst rate was higher ($p < 0.05$) in the group of embryos cultured with 10^{-9} M MLT than that of control (28.9% vs. 15.4%). MLT-treated (10^{-9} M) COCs had significantly lower level of ROS than control group ($p < 0.01$). In conclusion, MLT can enhance buffalo oocyte maturation and embryo development *in vitro* due to its function in scavenging ROS.

P97

Medetomidine and dexmedetomidine decrease ovarian artery supply in dogs

B Macias Garcia[1], M Facchin[1], F Duque[2], F Peña[1], R Barrera Chacón[2], G De Benedictis[3]

[1]*Department of Reproduction, University of Extremadura, Extremadura, Spain;* [2]*Department of Internal Medicine, University of Extremadura, Extremadura, Spain;* [3]*Department of Animal Medicine, Productions and Health, University of Padova, Italy*

The aim of this study was to evaluate the effect of medetomidine and dexmedetomidine administration on the ovarian artery blood flow in dogs. Two groups of ten healthy bitches each were studied. In the 1st group (GR1) medetomidine (10 μg/kg IM) was administered, and in the 2nd group (GR2) dexmedetomidine (5 μg/kg IM) was used. Ovarian artery flow was assessed using duplex Doppler ultrasonographic examination; peak systolic velocity (PSV), end diastolic velocity (EDV) and resistive index (RI) were assessed in the right and the left ovary independently before and 20 min after sedation. Arterial blood pressure and heart rate were also recorded before and after sedation. A paired one-tail *T*-test was used to contrast the values obtained before and after sedation, and a statistically significant decrease compared to control on heart rate (57.4% GR1; 62% GR2), arterial blood pressure (12.5% in GR1; 12.5% GR2), PSV (44.2% in GR1; 37% in GR2) and EDV (35.2% GR1; 26.4 GR2) were found in both groups after sedation. No variations were found in the RI or between the right and the left ovary. Medetomidine or dexmedetomidine administration had a similar effect on each of the variables. Medetomidine and dexmedetomidine have potent effects on ovarian perfusion in dogs. As reported in this study, there is a significant decrease of ovarian blood supply after medetomidine and dexmedetomidine administration. This finding must be taken into account when ovarioectomy or ovarian neoplasia surgery are performed, as these drugs may be useful in reducing the risk of hemorrhage.

P98

Calmodulin, calmodulin kinase II and extracellular calcium are implicated in control of hyperactivated motility in stallion sperm

B Macías García[1], L González Fernández[1], SC Loux[1], DD Varner[2], K Hinrichs[1]

[1]*Department of Veterinary Physiology and Pharmacology, Texas A&M Universtity, College Station, TX, USA;* [2]*Department of Large Animal Clinical Sciences, College of Veterinary Medicine & Biomedical Science, Texas A&M University, College Station, TX, USA*

Previous results from our laboratory demonstrated that 5 mM procaine, pH 8.5 medium and 4 mM 4-aminopyridine induce hyperactivated motility [HyM; significant increases in curvilinear velocity (VCL) and amplitude of lateral head displacement (ALH)] in stallion sperm. Here we investigated the role of known calcium pathways in control of HyM induced using these agents. Sperm were incubated at 37°C for 15 min in the presence of 50 μM W-7 (calmodulin inhibitor), 12.5 μM KN-93 (calmodulin kinase II inhibitor), 10 μM PKI (protein kinase A inhibitor), 1 μM xestospongin C (IP3 receptor inhibitor) or 100 μM ryanodine (ryanodine receptor inhibitor), in modified Tyrodes containing 2 mM Ca^{2+}, 0 mM Ca^{2+}, or 0 mM Ca^{2+} + 2 mM EGTA. Motility was assessed by CASA at 1, 15 and 30 min after addition of hyperactivating stimuli. Both W-7 and KN-93 inhibited HyM under each stimulus, however, PKI did not significantly affect response. In 0 mM Ca^{2+}, HyM was observed at 1 min for procaine and 4-aminopyridine, followed by a rapid decline in motility. HyM was not reached consistently with any stimulus in 0 mM Ca^{2+} + EGTA. HyM was not affected by either xestopongin C or ryanodine. These results indicate that extracellular Ca^{2+} and the calcium-calmodulin-calmodulin kinase II pathways are involved in modulating HyM in stallion sperm, whereas PKA does not appear to be involved. There was no evidence for involvement of internal calcium stores in regulation of HyM.

P99

Evaluation of serum fibrinogen and total protein in prepubertal sheep subjected to laparoscopic ovum pick-up

P Maia Teixeira[1], M Machado Silva[1], L Padilha[1], A Lima da Silva[1], F Barros[1], L Coutinho[1], R Mendes Medeiros[1], M Brito[1], M Da Conceição[2], M Kirnew[1], W Vicente[1]

[1]*UNESP Jaboticabal, São Paulo, Brazil;* [2]*FRA Belém, Pará, Brazil*

The aim of the current study was to evaluate the serum fibrinogen and total protein response in prepubertal sheep submitted to ovum pickup. Ten sheep aging 2–3 months old, submitted to laparoscopic ovum pickup (LOPU) using a three-port technique, were studied. The sheep were premedicated with xyliazine chloride (0.05 mg/kg) and anesthetized with propofol (6 mg/kg) and maintained with isoflurane in 100% oxygen. In addition, local anesthesia was performed at the port sites using lidocaine chloride (0.4 ml/port site). The pneumoperitoneum was accomplished using 8 mmHg intra-abdominal pressure, and 5 L/m flow rate, using the open technique. Surgical time was recorded during each procedure. In order to evaluate the inflammatory response caused by the surgical procedure, serum samples were obtained and stored before the surgical procedure and 2, 4, 6, 8, 10, 14 and 16 days following the LOPU for further assessment of fibrinogen and total protein dosage. The data were expressed as mean (±SD). The results were evaluated using the non-parametric ANOVA one-way test and the Tukey post-test was used to compare the results obtained among the pre and post-op moments. Mean surgical time was 23.1 (±3.2). There was no difference regarding the serum fibrinogen and total protein ($p > 0.05$). In conclusion, the laparoscopic approach for ovum pickup did not induced significant raise on the systemic inflammatory response. Moreover, the such approach should be properly considered for ovum recovery, without inflicting any kind of distress to the patients.

P100

Effects of season and male's age on brown bear (*Ursus arctos*) mating length

P Manrique[1], E Lopez-Urueña[1], M Alvarez[1], S Borragan[2], S Gomes-Alves[1], M Nicolas[1], P de Paz[3], F Martinez-Pastor[3], L Anel[1]

[1]*ITRA-ULE INDESGAL, Animal Reproduction and Obstetrics, University of León, León, Spain;* [2]*Cabarceno Park, Spain;* [3]*ITRA-ULE INDESGAL, Molecular Biology, University of León, León, Spain*

Mating in brown bear may take from a few seconds to 1 h and is usually preceded by a short courtship. The aim of this study was to determine the

influence of season and male's age in the length of matings in brown bears. Brown bear matings (n = 77) were timed (8–9 AM) at Cabarceno Park (a half-freedom regime with 29 males and 32 females in 36 ha). Length was ranked as: short (1–4 min), medium (4–8 min) and long (≥8 min). Matings were categorized by month (April–July). 'Young males' (n = 9) were considered from 3 to 6 years old and 'adult males' (n = 15) from 7 years. Data was analyzed using FREQ and CATMOD (SAS). No seasonal differences were observed in mating duration for both young (4.6 ± 1.3, 6.9 ± 3.2, 3.7 ± 1.8 vs. 2.5 ± 1.3) and adult (4.3 ± 0.8, 8.2 ± 1.8, 6.3 ± 1.9 vs. 7.2 ± 2.5; April, May, June and July, respectively) males. Mating duration was higher for adult males (6.7 ± 0.9 min) than for young ones (4.6 ± 1.0 min). Age showed differences among three ranges and trend test was significant. In short length range, 63% were young male's matings, while in long length range, 65% were adult male's ($p < 0.05$). The mating length of brown bears seem to depend on male's age but not on season, though other factors [population density, social hierarchy, female behavior (specially relevant in half-freedom regime)] may also influence. Supported by MICINN (CGL 2010-19213/BOS) and CANTUR SA

P101

Effects of lipopolysaccharide on the expression of Toll-like receptors in porcine granulosa cells

A Marantidis[1], G Michailidis[1], S Chadio[2], D Kalogiannis[2], M Avdi[1]

[1]*Department of Animal Production, School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece;* [2]*Department of Animal Science and Aquaculture, Agricultural University of Athens, Athens, Greece*

Bacterial infections can perturb ovarian follicle function, follicular growth and fecundity in vertebrate species. Granulosa cells appear to have roles in the innate immunity of ovarian follicles. The innate immune system senses bacteria through pattern recognition receptors such as the Toll-like receptors (TLRs), which bind highly conserved microbial molecules, called pathogen-associated molecular patterns (PAMPs). The aim of this study was to examine whether porcine granulosa cells use TLRs to detect one of the most common PAMP, the component of the cell wall of Gram-negative bacteria lipopolysaccharide (LPS) and initiate an immune response. Granulosa cells from small (< 3 mm) and large (> 3 mm) follicles were cultured *in vitro* and were stimulated with 1 μg/ml LPS at different time courses (0, 6, 12 and 24 h) in order to study the early response of the cells treated with LPS. Quantitative Real-Time PCR analysis revealed a significant up-regulation in the expression of four members of the porcine TLRs family, namely TLR1, 2, 4 and 5, in RNA extracted from LPS treated granulosa cells from both small and large ovarian follicles. These data suggest that porcine granulosa cells initiate an innate immune response to LPS via the TLR pathway, which probably participates in the protection of ovarian tissues from invasive pathogens.

P102

Effects of the AMP-activated kinase activator metformin in the quality of extended boar semen after long-term storage at 17°C

D Martín, A Hurtado, M Gil, M Bragado, L García

University of Extremadura (SINTREP), Cáceres, Spain

AMP-activated kinase AMPK acts as a sensor that detects the cell energy state and subsequently regulates metabolism. Our previous results show that AMPK protein is highly expressed in boar spermatozoa and that the storage of semen at 17°C significantly increases AMPK activity. Our objective was to study the effect of a well known AMPK activator, metformin (Metf, 1 and 10 μM) in extended boar semen (12 ejaculates from six boars) during long-term storage at 17°C. Sperm motility parameters were evaluated by the ISAS® program and cell viability, plasma membrane fluidity, acrosome reaction and mitochondrial membrane potential (MMP) were analyzed by flow cytometry. Metf treatment induced significant decrease in the percentage of spermatozoa with high MMP (75.8 ± 1.8% vs. 60.9 ± 6.0%, $p < 0.05$ BTS vs. Metf 10 μM after 4-day storage) and in cell viability (87.4 ± 1.8% vs. 77.1 ± 5.3%, $p < 0.05$ BTS vs. Metf 10 μM after 10-day storage). After 1-day storage, Metf induced significant decrease in the percentage of motile and progressive spermatozoa (73.6 ± 3.0% vs. 43.8 ± 2.1% and 28.0 ± 4.3% vs. 9.9 ± 0.9%, respectively, $p < 0.05$ BTS vs. Metf 10 μM) and in sperm velocity (VCL, μm/s: 79.3 ± 4.1 vs. 56.5 ± 2.9, $p < 0.05$ BTS vs. Metf 10 μM) and significant increase in sperm hyperactivated motility (7.8 ± 2.1% vs. 11.4 ± 2.3%, $p < 0.05$ BTS vs. Metf 10 μM). No male effect was observed in our experiments. Our results suggest that AMPK activator Metf has adverse effects in spermatozoa during storage of extended boar semen at 17°C. Supported by JUEX PRI09A07, FSE, GR10156 and AGL2010-15188.

P103

Sperm ejaculate selection using single-layer centrifugation improves boar sperm freezability

MJ Martínez-Alborcia[1], JM Morrell[2], I Parrilla[1], I Barranco[1], JM Vazquez[1], EA Martinez[1], J Roca[1]

[1]*Department of Medicine & Animal Surgery, Faculty of Veterinary Science, University of Murcia, Murcia, Spain;* [2]*Department of Clinical Sciences, Division of Reproduction, Swedish University of Agricultural Sciences, Uppsala, Sweden*

The nonfunctional sperm present in the boar ejaculates negatively influence the freezability of functional sperm. This study evaluated the effectiveness of single-layer centrifugation (SLC) using 15 ml of Androcoll-P-Large prior freezing for removing nonfunctional sperm of boar ejaculates and hence for improving sperm freezability. Twenty-four semen samples (SS) were divided into two groups according to their initial semen traits (IST): standard (n = 15) and sub-standard (n = 9). Each SS was split in two aliquots, one remained untreated (C samples) and the other was single-layer centrifuged (SLC samples) at $500 \times g$ for 20 min. The yield of total, motile (CASA) and viable (cytometrically assessed after staining with H-42, PI and FITC-PNA) sperm after SLC was higher ($p < 0.05$) in standard than sub-standard samples. Semen samples were cryopreserved using a standard 0.5-ml straw freezing protocol. Post-thaw sperm motility and viability were higher ($p < 0.05$) in SLC than C samples, irrespective of IST. SLC samples showed lowest ($p < 0.05$) levels of MDA (BIOXYTECH MDA-586 Assay Kit) after thawing. The thawed viable sperm of SLC samples showed lower ($p < 0.05$) levels of intracellular ROS generation (CM-H2DCFDA) and decreased ($p < 0.05$) plasma membrane fluidity (Merocyanine 540) from those of C samples, when sustain *in vitro* capacitation. These findings indicate that SLC using Androcoll-P-Large prior freezing improves the freezability of boar sperm. Supported by MICINN (AGL2008-04127/GAN) and Seneca Foundation of Murcia (GERM04543/07), Spain.

P104

Refrigeration of ram semen in presence of seminal plasma affects sperm chromatin structure without shortening telomeres

M Mata-Campuzano[1], L Ordás[1], F Cartón-García[1], P de Paz[1], V Robles[1], X Druart[2], F Martínez-Pastor[1,2], F Martínez-Pastor[1]

[1]*INDEGSAL, University of León, León, Spain;* [2]*Station de Physiologie de la Reproduction et des Comportements, INRA, Nouzilli, France*

Refrigeration at 15°C in skim milk is a routine method for preserving ram semen before artificial insemination. We have tested the effects of refrigeration and supplementation with seminal plasma on chromatin status and telomere length in ram semen. Semen was obtained from three Lacaune rams, pooled, washed with PBS and adjusted to 0.7×10^8/ml with skim milk (control) or skim milk with 40% seminal plasma. The tubes were stored at 15°C for 24 h. Analyses were carried out at 0 and 24 h. Chromatin status was assessed by SCSA, and telomere length was assessed by measuring the average ratio of telomere (T) repeat copy number to a single copy gene (S). Sperm DNA was extracted by the phenol-chloroform method, performing real-time PCR with specific primers for telomeres and for beta-actin (S), estimating telomere length as the T/S relation of 2-Cq values. The effects of time and seminal plasma were tested by linear mixed-effects models. No changes were detected by refrigeration alone. At 24 h, the proportion of spermatozoa with altered chromatin (SCSA) significantly increased in the samples incubated with seminal plasma (8.6 ± 1.3%), comparing with the control (3.1 ± 0.8%). However, no telomere shortening was detected in any case. Incubation with seminal plasma might induce changes in sperm chromatin, but not DNA fragmentation. Supported by AGL2010-15758 and Ramón y Cajal program (RYC2008-02339, RYC2008-02560).

P105

Optimization and validation of a practical method to isolate and identify bovine colostral monomorphonuclear leukocytes

V Meganck[1], B Goddeeris[2], S Piepers[1], E Cox[2], G Opsomer[1]

[1]*Faculty of Veterinary Medicine, Department of Obstetrics, Reproduction and Herd Health, Merelbeke, Belgium;* [2]*Laboratory of Immunology, Faculty of Veterinary Medicine, Merelbeke, Belgium*

Administration of a sufficient amount of high quality colostrum during the first 6 h of life is one of the most efficient measures against all kinds of infections in calves. 'High quality' colostrum has traditionally been defined as colostrum with a high antibody content. However, increasing evidence exists that colostrum is also an important supplier of leukocytes. Different protocols have been described to isolate bovine colostral leukocytes, but none of them were validated and a further differentiation into leukocyte subpopulations is only sporadically included. Colostral monomorphonuclear leukocytes (CMLs) were isolated from cows through the use of a density gradient. Subtypes were identified by CD markers with a flow cytometer and the proliferative capacity of the cells was verified with a H3-thymidin-proliferation assay. On average, 26.3 + 16.9% of the CMLs were T- and 2.9 + 2.9% were B-lymphocytes while 31.5 + 11.6% were monocytes. Geometric means (minimum to maximum) for counts per minute after stimulation with medium and ConA were 4310 (179–266 671) and 16237 (11–769 616) respectively. The above described methods were proven to be repeatable through the use of agreement indexes and Bland-Altman plots.

P106

Effect of estradiol benzoate on estrous response and fertility in CIDR treated crossbred heifers'

U Mehmood, A Sattar, A Qamar, U Bashir, N Ahmad

University of Veterinary and Animal Sciences, Lahore, Pakistan

The objectives of the present study were to understand the effect of estradiol benzoate (EB) on estrus response, intensity and fertility in CIDR treated crossbred heifers. Crossbred heifers with average body condition of 2.5 ± 0.5, placed at four different farms near Lahore, Pakistan. Crossbred heifers with EB (Group I) (n = 44) or without EB (Group II) (n = 39). All the heifers were treated with Controlled Internal Drug Releasing Device on day 0 and were injected PGF2α, on day 6 followed by removal of CIDR on day 7. Estradiol benzoate was administered 24 h after the CIDR removal. Estrus detection was carried out by visual observation twice daily for at least 30 min. All heifers were fixed time inseminated with semen 48 and 60 h after CIDR removal, respectively. Pregnancy diagnosis was done between days 30 and 40 post AI. Estrus response and pregnancy rate were analyzed using Chi-square, while estrus intensity scored by Mann–Whitney test. Estrus response was 100% in both groups. Estrus intensity was highly significant in group I 2.97 ± 0.149 compared to group II 2.07 ± 0.75 ($p < 0.01$). Estrus intensity was scored at the time of AI as 1 = Low, 2 = Medium, 3 = High based upon the heat signs. Pregnancy rate was 61.36% (27/44) in group I and 41.02% (16/39) in group II heifers ($p = 0.066$). It is concluded that estradiol benzoate in CIDR protocol intensified signs of estrus and pregnancy rate. It is implied that CIDR could be a good tool to enhance fertile estrus.

P107

Changes in the expression of Toll-like receptors in response to lipopolysaccharide in chicken Sertoli cells

G Michailidis[1], M Anastasiadou[1], P Froment[2]

[1]*Department of Animal Production, School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece;* [2]*INRA, CNRS Université de Tours Haras Nationaux, Physiologie de la Reproduction et des Comportements, Nouzilly, France*

Sertoli cells play an important physiological role in the testis, as they support, nourish, and protect the germ cells. As Toll-like receptors (TLRs) play crucial roles in mediating innate and adaptive immunity and because protection of the developing spermatozoa is an emerging aspect of reproductive physiology, the objective of this study was to examine the expression of TLRs and their functional responses to lipopolysaccharide (LPS) in chicken Sertoli cells. Sertoli cells were isolated from pubertal roosters and cultured *in vitro*. The cells were stimulated with 1 μg/ml LPS at different time courses (0, 6, 12, 24 and 48 h) in order to study the response of the cells treated with LPS. Expression analysis data revealed that all 10 members of the chicken TLRs family were expressed in the chicken Sertoli cells. Quantitative real-time PCR analysis revealed that LPS treatment resulted in a significant induction in the expression levels of six TLR genes, namely TLR1-1, 2-2, 4, 7, 15 and 21. These findings provide evidence that Sertoli cells respond directly to bacterial ligands, and represent an important component of the immune system of the chicken genital tract, and a distinctive constituent of the protective repertoire of the testis to ascending infections.

P108

Antioxidants have a beneficial effect on cumulus expansion and viability of vitrified pig oocytes

I Miclea[1,2], A Hettig[2], N Pacala[3], M Zahan[2], V Miclea[2]

[1]*Veterinary Medicine, Cluj-Napoca, Romania;* [2]*University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania;*[3]*Banat's University of Agricultural Sciences and Veterinary Medicine Timişoara, Romania*

Our goal was to establish the effect of certain α-tocopherol (TOC) and ascorbic acid (AA) concentrations, added together or separately on polar body formation, cumulus oophorus expansion and improvement of swine oocyte viability after cryopreservation. TOC is believed to act in the lipid-soluble compartment of the cell and it can be regenerated from tocopheroxyl radicals by ascorbic acid. Pig oocytes (n = 660) from a commercial slaughterhouse were cultured for 45 h in M199 and divided in four groups: control (CTRL), 5 μM TOC, 250 μM AA or 5 μM + 250 μM TOC-AA. Cumulus expansion was examined and oocytes were vitrified by the Superfine Open Pulled Straw method using a 40 s equilibration time in purified water containing 8% phosphate buffered saline 10×, 20% foetal bovine serum, 45% ethylene glycol and 0.50 M trehalose. After thawing, viability and the presence of the first polar body were assessed by staining with fluorescein diacetate, propidium iodide and Hoechst 33258. Of the oocytes matured in TOC-AA, 65.24% had fully expanded cumulus, compared to 49.47%, 63.39% and 63.39% in TOC, AA and CTRL, respectively ($p > 0.05$, Tukey test). However, all treatments had a negative effect on first polar body formation (CTRL: 63.09%, TOC: 59.32%, AA: 54.48%, TOC-AA: 54.43%). Post-thaw viability was improved beyond the levels for control (86.65%), TOC (85.37%) and AA (88.81%) by culture in TOC-AA supplemented medium (90.14%, $p > 0.05$, Tukey test). Results suggest that although M199 contains antioxidants, a higher concentration can improve cytoplasm ability to withstand freezing stress by protecting lipids from oxidation, but their effect on nuclear maturation needs further investigation. Acknowledgements: This work was supported by project POSDRU/89/1.5/S/62371 and UASMV Cluj-Napoca, grant 1215/12/6.02.2012.

P109

Seminal plasma to control induced acute endometritis by frozen semen in jennies

J Miro, J Miro, K Vilés

Veterinary Faculty, Autonomous University Barcelona, Bellaterra, Spain

Semen cryopreservation in donkeys results in a good posthaw spermatozoa survival percentage. However, AI with frozen semen in jennies shows very bad results, possibly due to an exacerbating acute endometrial response. During the freezing process seminal plasma is removed but it could have a role in the control of induced endometritis by insemination. Endometrial status of six jennies was evaluated in oestrous (C), 6 h after insemination with frozen semen (T1), and 6 h after insemination with frozen semen and 10 ml of seminal plasma (T2). To control the endometrial status cytology, by low volume uterine flushing, and biopsy were performed. Polymorphonuclear neutrophils (PMN) in the recovered fluid were counted by flow citometry. On the other hand, an aliquot of T1 uterine fluid was incubated 1:1 in a water bath at 37°C with raw semen, diluted semen and frozen semen. Spermatozoa motility by CASA and PMN-spermatozoa binding by optic microscopy were analyzed at 0, 60, 120, 180, 240 min. Uterine cytology and biopsy showed a large amount of PMN population after AI indicating endometritis. No significant differences were seen between T1 and T2. Significant higher values of sperm-PMN binding percentages and loss of progressive motility was observed in frozen-thawed semen compared with pure and diluted fresh semen samples throughout the incubation time. Addition of seminal plasma to frozen-thawed insemination dose not successfully reduced the acute inflammation caused by frozen semen. However, seminal plasma reduces de sperm-PMN binding and, in this way, can increase the fertility of frozen semen in donkeys.

P110

Cortisol and progesterone profiles in hair from birth to 90 days of age in foals

M Montillo[1], T Peric[1], M Faustini[2], F Cairoli[3], A Prandi[1], A Comin[1]

[1]*Department of Food Sciences, University of Udine, Udine, Italy;* [2]*Department of Veterinary Sciences and Technologies for Food Safety, University of Milan, Milan, Italy;* [3]*Department of Veterinary Clinical Sciences, University of Milan, Milan, Italy*

Cortisol (C) and Progesterone (P4) are extremely important in equine gestation, parturition and newborn foal. The aim of this study was to investigate the profile of these hormones in foals hair from birth to 90 days of age. The study was carried out on nine foals from the same farm, subjected to the same managerial factors. Each foal was submitted to four hair sampling, always from the same area, at 30 days intervals; samples were collected with clippers from the withers shaved to the level of the skin. All samples were analyzed by RIA. A significant trend of decreasing C levels was detected among sampling times: at birth hair cortisol concentrations (mean ± SD) was 54.23 ± 12.16 pg/mg; at 30 days of age 29.57 ± 5.17 pg/mg ($p < 0.0001$); at 60 days 18.41 ± 2.01 pg/mg ($p < 0.0001$); at 90 days 13.84 ± 2.53 ($p < 0.001$). This trend seems to suggest the progressive adaptation of foals during the first 3 months of growth. P4 levels, instead, remained rather constant from birth until 90 days of age: birth 556.31 ± 79.18 pg/mg; 30 days 476.42 ± 22.47 pg/mg; 60 days 490.83 ± 30.94 pg/mg; 90 days 478.25 ± 10.21 pg/mg. Will be extremely interesting to understand the meaning of these levels of P4 in pre-puberal animals.

P111

Freezing stallion semen: trial of an extender made with low density lipoproteins (LDL) from chicken egg yolk

D Moreno Garcia[1], D Bencharif[1], L Amirat-Briand[1], S Destrumelle[1], E Schmitt[2], M Anton[3], P Barriere[4], D Tainturier[1]

[1]*Laboratory of Biotechnology and Pathology of Reproduction, ONIRIS, Nantes, France;* [2]*IMV Technologies, L'Aigle, France;* [3]*Laboratory of Biopolymers Interactions Assemblies, Unit Interfaces and Dispersed Systems, INRA, Nantes, France;* [4]*Department of Reproductive Pathology, Mother and Child, CHU Hôtel Dieux, Nantes, France*

The aim of this study was to determine the best concentration of low-density lipoproteins (LDL) in a semen extender to improve the percentage of motile spermatozoa in equine sperm after freezing and thawing in comparison with standard extenders. Ten extenders were compared: one with 2% egg yolk (EY), eight with different concentrations of LDL (0.25%, 0.50%, 0.75%, 1%, 2%, 3%, 4%, and 5%), and one with INRA96®; all of the extenders contained 2.5% glycerol. Fourteen ejaculates were collected from four stallions. The first dilution was made at +37°C, centrifuged (600 g/10 min), and re-suspended in the corresponding extenders to obtain a final concentration of 100×10^6 spz/ml. The resulting mixture was cooled at +4°C for 1 h, then packed into four 0.5 ml straws before being left for a further 30 min at +4°C. Finally, the straws were frozen in nitrogen vapours for 10 min before being immersed in liquid nitrogen at −196°C. The extenders made with 2% and 3% LDL gave superior spermatozoal motility rates (35.5% and 35.3% respectively) compared

with the EY (33.3%) and INRA96® extenders (22.1%). The best results of hypoosmotic test to assess the integrity of the plasma membrane; were obtained with the 0.5%, 2%, and 3% LDL extenders and the EY. The best results of FITC/PSA test were obtained with the 0.5%, 0.75%, and 3% LDL and INRA96 extenders, but the difference was not significant in comparison with the 2% LDL. No significant difference was observed of the acridine orange test and Spermac STAIN between the various extenders.

Conclusion: The 2% LDL extender gave the best post-thaw percentage of motile spermatozoa, and superior results of the *in-vitro* fertility tests.

P112

Molecular evidence for the presence of ZP1 and ZP4 in the Bennett's wallaby (*Macropus rufogriseus rufogriseus*)

C Moros[1], M Izquierdo-Rico[1], D Esteban[1], M Alicchio[1], J Ballesta[1], W Holt[2], M Avilés[1]

[1]*Department of Cell Biology and Histology, School of Medicine, University of Murcia, Murcia, Spain;* [2]*Institute of Zoology, London, UK*

The zona pellucida (ZP) is an extracellular coat that surrounds mammalian oocytes. This matrix is implicated in gamete interactions, induction of the acrosome reaction and block to polyspermy. The marsupial egg has been considered surrounded by a ZP made of at least three glycoproteins. However, the recent description of four glycoproteins (ZP1, ZP2, ZP3, and ZP4) in the ZP of some species of eutherian (rat, human, hamster, rabbit) suggests the need for a reanalysis of the ZP composition in marsupials. The aim of this study was to analyse the expression of ZP1 and ZP4 in the Bennett's wallaby using molecular techniques. Total RNA was isolated from ovaries and cDNA was synthesized with oligo-dT as primer to identify the ZP gene expression. Specific primers for ZP1 and ZP4 were designed based on ensembl sequences (ENSMEUT00000000742 and ENSMEUT00000012954). After that, PCR amplifications resulted in the complete amplification of the open reading frame (ORF) of ZP4 (1516 bp) and a partial amplification of the ORF of ZP1 (508 bp). This result demonstrates that ZP1 and ZP4 genes are expressed in the wallaby ovary. Moreover, taking in consideration that ZP2 and ZP3 are present in all vertebrates studied to date and that they have never suffered pseudogenization, these results suggest that the wallaby ZP matrix is composed of four glycoproteins. Supported by a grant from Fundación Séneca de la Región de Murcia (0452/GERM/06).

P113

Effect of systemic progesterone on availabilities of amino acids and glucose in the bovine uterus

M Mullen[1], F Bazer[2], G Wu[2], M Parr[1], A Evans[3], M Diskin[1], M Crowe[4]

[1]*AGRIC, Teagasc, Galway, Ireland;* [2]*Department of Animal Sciences, Texas A&M University, College Station, TX, USA;* [3]*School of Agriculture and Food Science, University College Dublin, Dublin, Irelnad;* [4]*School of Veterinary Medicine, University College Dublin, Dublin, Irelnad*

Uterine histotroph provides nutrients and growth factors to the early developing conceptus during the preimplantation period. The objective was to examine the effects of stage of cycle and systemic progesterone (P4) on the quantity of amino acids and glucose in histotroph during early conceptus development. Following oestrus, heifers were assigned to low, control or high P4 groups (N = 6). The uterine horn ipsilateral to the CL was flushed on either Day 7 or 13. This study determined quantities (nmol) of 24 amino acids and glucose in the flushings using HPLC and fluorometry, respectively. Heifers in the low P4 group had lower plasma P4 throughout the cycle and heifers in the high group had higher P4 between Days 4–7 compared with controls ($p < 0.05$). Total recoverable neutral (Ser, Gln, Gly, Thr, Cit, β-Ala, Tau, Ala, Tyr, Trp, Met, Val, Phe, Ile, Leu, Pro and Cys) and basic (His, Arg, Orn and Lys) amino acids were all higher on Day 13 compared with Day 7 ($p < 0.05$). The amounts of amino acids recovered on Day 7 were similar across treatment groups. On Day 13, the amounts of His and Thr were lower in the low P4 heifers compared with controls ($p < 0.05$). Quantities of glucose were not altered by stage of cycle or P4 treatment. In conclusion, the stage of cycle and P4 play important roles in modulating histotroph amino acid composition, a potentially critical factor for early embryonic survival.

P114

Sperm concentration affects liquid storage of bull semen

C Murphy[1], A Shafat[2], S Fair[1]

[1]*Department of Life Sciences, University of Limerick, Limerick, Ireland;* [2]*Department of Physiology, National University of Ireland, Galway, Ireland*

In Ireland, liquid bull semen contains approximately 5 million sperm per insemination dose and is used within 60 h of collection. The hypothesis of this study was that reducing the sperm number per dose would enable sperm to be stored for longer. Semen was collected at a commercial AI centre (five collections with 3–4 bulls per collection; collection = replicate) and diluted to 5 (T5), 4 (T4), 3 (T3), 2 (T2) and 1 (T1) million sperm per 0.25 ml dose in Caprogen diluent. On Days 0, 1, 2, 3, 4 and 5 post collection, mitochondrial activity (Rhodamine 123), oxidative stress (CM-H2DCFDA), glucose consumption (Commercial Glucose Kit), viability (Propidium Iodide) and motility were assessed. Data were transformed where appropriate and analysed using repeated measures in SPSS. There was an effect of day and treatment on sperm cell viability ($p < 0.001$) with percentage live highest in T1 and lowest in T5 on all days. The level of glucose in Caprogen declined with time ($p < 0.001$) and was lowest in T5 and highest in T1 on Day 5. The percentage of live sperm positive for Rhodamine 123 ranged between 91.9% and 94.8% and was not affected by treatment. Oxidative stress in live sperm increased with duration of storage and was affected by treatment ($p < 0.001$), being highest in T5 and lowest in T1 on all days (Day 5: 56.4 ± 2.76% and 28.8 ± 1.22%, respectively; mean ± SEM). In conclusion, higher concentrations of sperm have detrimental effects on viability, increase oxidative stress but have no effect on sperm metabolism. Supported by IRCSET.

P115

Blue light to a single eye advances the breeding season in mares

B Murphy[1], C Walsh[1], R Prendergast[2], E Woodward[3], L Fallon[4], M Troedsson[3]

[1]*School of Agriculture and Food Science, University College Dublin, Dublin, Ireland;* [2]*School of Electrical, Electronic and Mechanical Engineering, University College Dublin, Dublin, Ireland;* [3]*The Maxwell H. Gluck Equine Research Center, University of Kentucky, Lexington, KY, USA;* [4]*Hagyard Equine Medical Institute, Lexington, KY, USA*

The use of artificial light to advance the breeding season of mares is common practice. We developed light masks for horses that direct 50 Lux blue light at a single eye to inhibit the production of melatonin. Six non-pregnant, healthy Thoroughbred mares, aged 4–17 years were used. On Dec 1st 2011, Group 1 (n = 16) were housed indoors in individual stalls under barn lighting (250 Lux) that remained on until 11 pm daily. Group 2 (n = 26) wore light masks programmed to turn on from 4.30 pm until 11 pm daily and were maintained outdoors as a herd in a large pasture. Group 3 (n = 19) were maintained outdoors under the natural photoperiod

as controls. All mares were maintained on a farm in Lexington, KY. From mid-Dec, and continuing at 2-week intervals until mid-Feb, all mares received rectal ultrasound examinations and blood was collected by jugular venipuncture for progesterone hormone analysis. Oestrous cyclicity was defined as the presence of follicles >20 mm diameter detected in conjunction with serum progesterone >1 ng/ml. On Feb 10th the number of mares exhibiting oestrous cyclicity was 14/16 (87.5%) in Group 1; 20/26 (76.9%) in Group 2; and 4/19 (21%), in Group 3. Pairwise comparison of groups revealed no significant difference in the number of mares determined to be reproductively active between Group 1 and Group 2 (χ^2 test, $p = 0.242$). Significant differences were observed between Group 1 and Group 3 (χ^2 test, $p < 0.0001$) and Group 2 and Group 3 (χ^2 test, $p < 0.001$). We conclude that low intensity blue light to a single eye from head worn light masks is as effective at advancing the breeding season in mares as the practice of maintaining mares indoors under barn lighting, but with economic benefits for the breeder.

P116

Abstract withdrawn.

P117

A role in reproduction for a novel bovine β-Defensin gene cluster

F Narciandi[1], A LLoyd[1], K Meade[2], C O'Farrelly[1]

[1]*Comparative Immunology Group, School of Biochemistry and Immunology, Trinity College Dublin, Ireland;* [2]*Animal & Grassland Research and Innovation Centre, Teagasc, Grange, Ireland*

β-Defensins are small cationic effector molecules of the innate immune system. In addition to their antimicrobial activity, a broader range of roles have been ascribed including a role in reproduction. This study focuses on a cluster of 19 novel bovine defensins on chromosome 13 originally discovered by our group (Cormican, Meade et al. 2008). Using RT-PCR, we show that these genes are expressed almost exclusively in the reproductive tract in six male and 10 female reproductive tracts. Differential site-specific expression of the 19 defensins across the epididymus and Fallopian tube suggests a role in fertility, local defence and/or reproductive immunology. Selected genes were sequenced in Norwegian Red (NR) and Holstein-Friesian (HF), two breeds of cattle with significantly different fertility rates for population genetic analysis. Sequence alignments identified 17 novel SNPs: seven non-synonymous, six synonymous and four non coding. Using this information, 30 animals from each breed were used for genotyping. Significant SNP frequency differences were found between the two breeds in β defensins 115, 117, 121, and 122 ($p < 0.05$). Genotyping data showed the presence of two haplotypes in the cluster. Sperm penetration assays were used to determine the effect of selected SNPs on sperm motility. Emerging data suggest that bulls with the truncated β-defensin 117 have reduced sperm motility *in-vitro*. This study documents the exclusive gene expression of bovine Chromosome 13 β-defensin gene cluster in the reproductive tract of the bovine. Breed associated genetic variation between (NR) and (HF) cattle breeds may result from divergent selection pressures for fertility. Functional analysis of these proteins is being performed to determine their role in reproduction.

P118

Combined effect of DHA and α-tocopherol supplementation during bull semen cryopreservation on sperm characteristics

A Nasiri, A Towhidi

Department of Animal Science, University of Tehran, Tehran, Iran

The aim of this study was to investigate the effect of adding n-3 fatty acids source and α-tocopherol (vitamin E, VE) to semen extender on freezing ability of Holstein bull sperm. Semen was collected from 10 mature Iranian Holstein bulls using an artificial vagina and pooled. In this experiment, semen was divided into 12 groups (in a 3×4 factorial design) including four levels of n-3 FA (0, 0.1, 1, 10 ng/ml) and three levels of VE (0. 0.1, 0.2 mmol). The treatment 0 ng/ml n-3 FA and 0 mmol VE as a control group in 37 and 5°C and after thawing. Sperm characteristics such as motility, progressive motility and viability in ten replicates at 37 and 5°C and after thawing were determined. The treatment of 0.2 mmol VE and 10 ng/ml n-3 FA had the best *in vitro* characteristics after thawing of sperm ($p < 0.01$). Results suggested that adding a source of DHA accompanied with an antioxidant to an extender can improve freezing ability by increase in the motility, progressive motility and viability after thawing of bull sperm.

P119

Stallion sperm parameters from different segments of the cauda epididymidis

S Neuhauser, S Rheinfeld, J Handler

Clinic for Horses, Freie Universität Berlin, Berlin, Germany

Freezing epididymal sperm could be important in sudden death or accidents of breeding stallions. We evaluated stallion sperm from three segments of the cauda epididymidis (E7, E8, E9 – cranial to caudal) from 10 stallions immediately after routine castration. Segment E9 was flushed with PBS, E8 and E7, were sliced and incubated with PBS for 10 min at 37°C. Total sperm count was measured, semen was extended using a commercial skim milk extender and motility was analysed. Total sperm count (0.9 ± 0.7, 1.6 ± 0.7, 12.0 ± 0.7 billion; $p < 0.05$) and percentage of total (30.0 ± 6.2, 38.6 ± 6.2, 52.2 ± 6.2; $p < 0.05$) and progressive motility (24.1 ± 5.8, 33.6 ± 5.8, 44.9 ± 5.8; $p < 0.05$) showed significant differences among segments E7, E8, E9, but not between left and right epididymis. Total sperm count was significantly different among stallions in E7 (0.5 ± 0.3–3.2 ± 0.3; $p < 0.05$) and in E8 (0.5 ± 0.4–4.5 ± 0.4; $p < 0.05$), but not in E9 (7.6 ± 3.0–18.8 ± 3.6; ns). Among stallions percentage of total and progressive motility differed significantly (E7: 5.6 ± 8.3–68.7 ± 8.3 and 3.0 ± 7.9–58.8 ± 7.9; E8: 4.2 ± 11.8–81.6 ± 11.8 and 1.7 ± 11.5–76.2 ± 11.5; E9: 3.0 ± 11.8–83.8 ± 11.8 and 1.2 ± 11.0–76.7 ± 11.0; $p < 0.05$). In contrast to E8 and E9, total and progressive motility differed significantly in E7 with the age of the stallions (15.3 ± 7.3 to 68.7 ± 12.6 and 10.7 ± 6.5 to 58.8 ± 11.3; $p < 0.05$). In summary, the present study revealed major differences in epididymal sperm characteristics among stallions and cauda epididymidis segments, but not between left and right epididymis.

P120

Does urea influence gene expression in bovine endometrium?

C Oguejiofor, T Swangchan-Uthai, S Carr, Z Cheng, C Wathes

Royal Veterinary College, Hatfield, UK

High (>7.5 mmol) and low (<4.5 mmol) circulating urea concentrations can be associated with reduced fertility in dairy cows but mechanism(s) of action remain obscure. This study examined the relationship between urea and endometrial function. In Experiment 1 we utilized results of a study in which two groups of dairy cows were managed by differential feeding and milking to produce mild or severe negative energy balance after calving [1]. Gene expression in endometrium from 12 cows at 2 weeks postpartum was measured by 24 K Affymetrix arrays. The mean circulating urea concentrations after calving ranged from 3.2 to 6.6 mM. From 1310 probes whose expression correlated with urea at $p < 0.05$, 833 genes were mapped and analysed by Ingenuity Pathway Analysis. The Top Network identified was 'Endocrine system development and function, lipid metabolism, molecular transport', score 43. To validate this approach, Experiment 2 investigated expression of five candidate and one housekeeping gene measured by qPCR in primary bovine endometrial cell cultures treated with urea (0, 2.5, 5.0 and 7.5 mM, equivalent to low, medium and high circulating values). The experiment was repeated twice with three replicates each. There was no significant effect (mixed model analysis using ANOVA) of urea treatment for expression of HSPA5, INSR, IGF1R, PGFSL2, IL17RB or 18SrRNA. These results indicate that, although there are differences in endometrial gene expression in cows with varying urea concentrations, the effect is not direct. The circulating urea may instead be indicative of metabolic differences between animals which influence fertility by another mechanism.

Reference

[1] Wathes DC et al. (2009) Physiol Genomics 39:1–13.

P121

Long duration of farrowing may be associated with subsequent follicles development in sows

C Oliviero, S Kothe, J Yun, A Valros, O Peltoniemi

Department of Production Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland

Previous findings from our research group showed that sows with longer duration of farrowing have a significantly increased risk of rebreeding. In the present study we hypothesise that the association between longer duration of farrowing and increased rebreeding rate would be due through an effect on follicular activity or development. We selected a group of sows with a duration of farrowing ≤200 min (SHORT, n = 7) and a group with a duration of farrowing ≥300 min (LONG, n = 12). Ovaries were scanned with ultrasound through the abdomen on the day of weaning (day 0), on day 4 and twice a day on day 5, 6 and 7 until ovulation. Five largest follicles were measured on one ovary to assess growth and time from weaning to ovulation. Parity, lactation length, total born piglets and stillborn tested with a logistic regression model against LONG and SHORT were found not significant. The average size of the largest follicle was 5.1 mm (SHORT) vs. 6.4 mm (LONG, p = 0.005) on day 4 and, 7.0 mm (SHORT) vs. 7.5 mm (LONG, p = 0.16) at ovulation. Time from weaning to ovulation was 126 h (SHORT) vs. 119 h (LONG, p = 0.26). Larger follicles size on day 4 and the tendency at ovulation may indicate that they have grown beyond the optimal time of ovulation. Further investigations, with a larger sample size, are needed to clarify the effect of long duration of farrowing on follicular activity, and possibly on the reproductive hormonal balance.

P122

Effects of different transport temperatures on *in vitro* development of queen oocytes

O Ozdas[1], A Baran[1], A Sandal[1], G Bakirer[1], C Tek[2], S Enginler[2], M Gunduz[2], G Kasikci[2], K Ak[1]

[1]*Department of Reproduction and Artificial Insemination, Istanbul University, Istanbul, Turkey;* [2]*Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Istanbul University, Istanbul, Turkey*

Today, many species, especially feline are endangered. For this, domestic cats are used as a model for *in vitro* culture studies. So many investigators have problems in transporting ovaries to their laboratories. In this study effects of different transport temperatures on *in vitro* maturation of feline oocytes were investigated. Ovaries were collected from 12 ovariectomised queens of 2–3 year old, four of which were at oestrus and eight at anoestrus. One ovary of each pair was brought to the laboratory in PBS at 4°C and the other one at 37°C. Two main groups as oestrus and anoestrus were established and each were divided into further two subgroups as 4°C and 37°C. Oocytes were collected in TCM-199 medium and matured for 24 h under 5% CO_2 at 38.5°C. Matured oocytes were fertilized with fresh semen at a final concentration 1×10^6/ml in Sperm-Talp medium under 5% CO_2 for 18–24 h. After removing cumulus oophorus cells, zygotes were divided into groups and *in vitro* cultured for 72 h in 100 µl SOF medium under 5% CO_2, 5% O_2 and 90% N_2 gas mixture. At the 48th hour of incubation, the best cleavage was 44.4% (8/18) at 37°C oestrus group, and the lowest was 14% (7/50) in the oestrus group at 4°C. These rates were 22.72% (15/66) and 28.57% (8/28) respectively for the anoestrus group. At the 72th hour of culture, in 37°C oestrus group 7 embryos stayed at 4–8 blastomere stage and 1 embryo reached 16–32 blastomere stage. This result was significant when compared to the other groups ($p < 0.001$). In 4°C anoestrus group only five embryos have reached 4–8 blastomere and no significant difference among the results was observed. It is concluded that cat ovaries in oestrus are better transported at 37°C, while anoestrus ovaries could be carried at 4°C.

Key words: Queen, transport, temperatures, oocyte, *in vitro* fertilization.

P123

Effect of the viewing support and drop volume on the results of CASA sperm motility in the ram

I Palacin, S Viciente-Fiel, P Santolaria, J Yániz

Institute of Environmental Sciences (IUCA), University of Zaragoza, Zaragoza, Spain

This study was designed to evaluate the effect of different viewing chambers, drop sample volume, and number of sperm per field on CASA sperm motility parameters in the ram. Two different experiments were conducted. In the first trial, no differences between the two 5 and 10 µl drop volumes were found in the slide-coverslip and Makler chamber supports. In the second experiment, significant differences between different viewing supports evaluated (slide-coverslip, Makler and ISAS chambers) were observed for sperm motility parameter, with a higher proportion of motile sperm in Makler chamber. ISAS chamber caused an important decrease in the percentage of motile and progressive sperm. Sperm velocity variables were different in the three systems with slide-coverslip > Makler > ISAS. An inverse relation was found between the number of sperm per microscopy field and motility parameters into each supports. It was concluded that the type of viewing chamber used for analysis of CASA sperm motility in the ram had a considerable influence on results, precision and accuracy,

whereas the drop volume had a little effect. The type of chamber used and conditions should be clearly described in semen quality analysis including CASA sperm motility assessment. Supported by MEYC (IPT-010000-2010-33 and AGL2011-30353-C02-01).

P124

Buffer, cryoprotectant and antioxidant effects on ram sperm cryopreservation

M Palomo[1], W Garcia[1], A Tabarez[1], A Tabarez[2]

[1]*Department of Animal Medicine and Surgery, Universitat Autonoma de Barcelona, Barcelona, Spain;* [2]*Universitat Autonoma de Barcelona, Barcelona, Spain*

Our aim was to test simultaneously Tes-Tris buffer system (TEST) and Tris and citric acid buffer system (TRIS) in a 1% (w/v) soybean lecithin or in a 15% (v/v) powered egg yolk-based media supplemented both with 5% glycerol for sperm cryopreservation. Also we assessed the inclusion of 5 mM of butylated hydroxytoluene (BHT) as antioxidant. Briefly, fresh ejaculates from eight young rams (1 year old) were collected by artificial vagina and immediately mixed in equal quantities. Pooled semen was washed by centrifugation, and the pellet was split into eight equal aliquots and re-suspended in one of the eight different extenders before freezing. No differences were found in post-thaw sperm viability determined by eosine-nigrosine stain (mean ± SD, n = 6) between the extenders containing TRIS system, supplemented or not with BHT respectively, (24.8 ± 4.1; 23.5 ± 6.0) in egg-yolk or in soybean lecithin (21.3 ± 4.4; 21.1 ± 3.4) based media samples. Likewise, post-thaw sperm viability was similar in extenders containing TEST system with or without BHT respectively (16.7 ± 5.7; 16.8 ± 4.0) in egg-yolk or in soybean lecithin (18.0 ± 2.9; 19.6 ± 6.7) based media. Only the samples preserved in egg-yolk based media with TRIS and BHT showed a higher viability ($p < 0.05$) than in both soybean lecithin based media containing TEST system. Nevertheless the sperm motion parameters, analysed by a computer-assisted sperm analysis system (ISAS®), were different ($p < 0.01$) between extenders, suggesting that more analysis should be tested. Supported by INIA (RZ2009-00008-00-00), Generalitat de Catalunya (2009SGR0621) and Fundacion Carolina

P125

Efficacy of CIDR in Cosynch and oestrus synchronization protocols in lactating cows

S Pancarci[1], K Gurbulak[2], A Zonturlu[3], F Soykan[4], R Kulaksız[5]

[1]*Division of Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Balikesir, Turkey;* [2]*Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey;* [3]*Faculty of Veterinary Medicine, Harran University, Sanlurfa, Turkey;* [4]*Pfizer Animal Health, Kayseri, Turkey;* [5]*Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey*

It was aimed to compare efficacy of CIDR in Cosynch and estrous synchronization protocols based on pregnancy rates following the first service at 45–160 days postpartum in lactating cows. In Group I (Cosynch-CIDR, n = 100), CIDR® (progesterone, 1.38 g) was inserted intravaginally concurrently with GnRH injection (Receptal®, buserelin acetate, 0.02 mg, i.m.). Seven days later CIDR was removed and PGF2α (Dinolytic®, dinoprost tromethamine; 25 mg, i.m.) was injected followed by timed artificial insemination (TAI) and GnRH injection 56 h later. In Group II (Control-CIDR, n = 81), CIDR was administered for 7 days. One day before CIDR removal, PGF2α was administered. During 5 days following CIDR removal, artificial insemination (AI) was performed following heat detection based on AM/PM rule. Pregnancies were diagnosed with transrectal ultrasonography 40–45 days after AI or TAI. Pregnancy rates did not differ between Cosynch-CIDR (35%; 35/100) and Control-CIDR (38.3%; 31/81) groups. Submission rate for AI was higher ($p < 0.01$) in Cosynch-CIDR group (100%; 100/100) compared to Control-CIDR group (82.7%, 67/81). In Control-CIDR group, frequencies of cows detected in heat following 1, 2, 3, 4, 5 days after CIDR removal were 19.8% (16/81), 37.0% (30/81), 17.3% (14/81), 7.4% (6/81), 1.2% (1/81); respectively. Whereas, 17.3% (14/81) of cows were not detected in estrus during 5 days after CIDR removal in Group II. In conclusion, use of CIDR in Cosynch protocol provides similar pregnancy rates but higher submission rate compared to CIDR based estrous synchronization protocol in lactating cows.

P126

Effect of the time of artificial insemination on fertility of short-term progestagen pre-treated Karagouniko and Chios ewes

S Papadopoulos[1], I Valasi[2], C Deligiannis[1], E Theodosiadou[2], D Kantas[1], P Goulas[1]

[1]*TEI Larissas, Greece;* [2]*Veterinary Faculty, University of Thessaly, Karditsa, Greece*

The objective of the present study was to examine the effect of the time of fixed-time artificial insemination on fertility of Karagouniko and Chios ewes subjected to short-term progestagen pretreatment. Oestrus synchronization was performed using intravaginal progestagen pessaries (60 mg MAP, Veramix, Upjohn/Veterin) remained *in situ* for 6 days (groups K1, K2, X1, X2). All ewes received 400 IU equine chorionic gonadotrophin (eCG, Intergonan, Intervet Hellas) at pessaries' removal. Intracervical artificial insemination was performed, without oestrus detection, 48 h after pessaries' removal in groups K1 (n = 28) and X1 (n = 20), while in groups K2 (n = 20) and X2 (n = 16) at 54 h, using fresh diluted semen (300×10^6 spermatozoa/dose). Pregnancy diagnosis was done by ultrasonography 45 days later. Pregnancy rate was greater ($p < 0.05$) in groups K2 (40%) and X1 (40%) compared with K1 (14.3%), with no difference detected between groups X1 and X2 (50%). Litter size was 1.6, 1.2, 2.3, and 2.0 for group K1, K2, X1 and X2, respectively. These preliminary results indicates that fertility rate in ewes subjected to short-term progestagen pretreatment depends on time of fixed-time AI in relation to breed. Further experiments are underway to define the appropriate fixed-time AI protocol in Karagouniko and Chios ewes subjected to short progestagen pretreatment.

P127

Optimization of protocols for Iberian Red Deer sperm sex sorting by flow cytometry

I Parrilla[1], D Del Olmo[1], A Bisbal[2], JA Ortiz[3], A Maroto[2], O Garcia-Alvarez[4], J Roca[1], EA Martinez[1], JJ Garde[2], JM Vazquez[1]

[1]*Department of Animal Medicine and Surgery, University of Murcia, Murcia, Spain;* [2]*UCLM, Albacete, Spain;* [3]*Medianilla SL, Cadiz, Spain;* [4]*CERSYRA, Valdepeñas, Spain*

This study evaluated the effect of different handling methods on the sortability of Iberian Red Deer sperm. Semen was obtained by electroejaculation from 11 stag, during the rut, centrifuged, and processed as follows: (i) diluted to 800×10^6 sperm/ml in Tris Medium (TM), incubated 50 min at 34°C with 2.6 or 5.2 µl (groups G1 and G2) of H-33342 (H-42), diluted to 400×10^6 sperm/ml with TM + 20% EY (TMEY) and transported (8 h at 21°C) to the sorting facilities (SF); (ii) diluted to 400×10^6 sperm/ml (groups G3 and G4) and 800×10^6 sperm/ml in TMEY (groups G5 and G6), transported to the SF, then re-diluted to 100×10^6 sperm/ml with TM and incubated

with 1.3 μl (G3 and G5) or 2.6 μl (G4 and G6) of H-42. Sortability for Y-sperm was evaluated in all groups. Results showed that handling method did not affect non-viable cells, orientated sperm and sorting rates (range: 8.5–11.3, 57.2–61.1 and 4287 ± 4656, respectively). The percentage of samples showing split was higher ($p < 0.05$) in G5 (83.9 ± 10.8%) and G6 (99.9 ± 9.2%) compared to the rest of the groups (range: 15.4–75.7%). Split ratio was also higher for G5 (0.55 ± 0.03) and G6 (0.52 ± 0.03) being different only respect to G2 (0.41 ± 0.03). Conclusion: transport of unstained samples at 800×10^6 sperm/ml in TMEY provides the best sortability results in Iberian Red Deer. Supported by CDTI (2008/0478-2008/0825), Spain; Seneca Foundation (GERM, 04543/07), Spain and Sexing Technologies (Texas, USA).

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Insulin-like growth factor I plasma concentrations in newborn healthy donkey and horse foals

T Peric[1], S Panzani[2], I De Amicis[2], A Comin[1], M Montillo[1], A Carluccio[2], T Meloni[2], A Prandi[1]

[1]*Department of Food Science, Università degli Studi di Udine, Italy;* [2]*Department of Veterinary Clinical Sciences, Università degli Studi di Milano, Italy*

In different species the insulin-like growth factor I (IGF-I) is an important regulatory factor in fetal and neonatal growth, especially in skeletal and gut development. This study was carried out to assess plasma IGF-I profile in newborn healthy donkey and horse foals during the first 2 weeks of age. The study was conducted on 32 horse and 19 donkey foals, born at term, after spontaneous parturition, mature and viable. Blood samples were collected from the jugular vein at 10, 20 and 30 min, 3 and 12 h and at day 3, 7, 10, 14 of life. IGF-I levels were evaluated by RIA. IGF-I showed an increasing profile in both species, with lower levels ($p < 0.05$) during the first 3 days of life. Plasma IGF-I levels were found to vary widely between subjects both in donkey and horse foals. Mean plasma levels were higher ($p < 0.001$) in horses than in donkeys during the first week. However donkey foals showed a greater mean percentage increase (681%) than horse foals (114%). These results evidenced increase of IGF-I concentrations during the first 2 weeks of life that could be associated with a number of physiological challenges in adapting to extrauterine life. Moreover, although horses and donkeys belong to the same genus, these results seem to indicate the presence of some differences in their somatotropic axis activation.

P129

Effect of artificial insemination site on post-mating endometritis in mares

J Ponthier, C Rodier, J Ponthier, S Parrilla-Hernandez, S Deleuze

Equine Clinic, University of Liege, Liege, Belgium

Aim of the study was to determine the effect of artificial insemination (AI) location and volume on post-mating endometrial inflammation 1 and 6 days after AI. Six mares with ages ranging from 12 to 23 years were inseminated with a dose of eight 0.5 ml straws of frozen semen from a same ejaculate, on three consecutive cycles. Mares were inseminated with the following procedures in a random order: (i) Deep uterine insemination with 4 ml of semen; (ii) Horn bifurcation with 4 ml of semen; (iii) Horn bifurcation with 4 ml of semen and 6 ml of extender to assess the effect of larger volume of AI. During each cycle, Cotton (C) and Brush (B) swabs were collected at four different moments: mid-dioestrus, mares with a 35 mm follicle, 24 h and 6 days after AI. Swabs were smeared on slides, fixed and stained (Diff-Quick®) before examination under light microscopy. Proportions of inflammatory and epithelial cells were determined and differences were studied with Kruskal–wallis test for non-parametric data. Distensions of uterine lumen due to intraluminal fluid observed during ultrasound exams were measured and recorded. Quality of slides was better ($p = 0.0006$) with B swabs than C swabs with 97% vs. 65% of slides readable. B swabs were associated with higher percent of endometrial cells retrieved ($p = 0.0323$), making them a better tool for endometritis diagnosis, which is consistent with our previous reports. Volume of intraluminal fluid and percent of inflammatory cells, both on B and C swabs, were not influenced by AI location regardless the timing of sampling. Small volume deep uterine AI did not significantly affect inflammatory response by the endometrium in our experiment.

P130

The effects of season and FSH injection on OPU results of buffalo

G Qin[1], B Yang[1], X Liang[1], Z Tan[1], C Pang[1], F Huang[1], H Jiang[2]

[1]*Guangxi Key Laboratory of Buffalo Genetic, Reproduction and Breeding, Nanning, China;* [2]*College of Animal Science & Technology, Guangxi University, Nanning, China*

The season effect on the recovery and morphology of Ovum Pick up were determined in the present study. Buffalo donors were treated with FSH in March–May (SG-FSH: spring group N = 3), and in August-September (AG-FSH:autumn group N = 3), or seved as controls (SG-CT and AG-CT: control group N = 3 and N = 4) respectively. The donor buffaloes were injected with 10 mg FSH on the first day, and 5 mg/day in the following 2 days. Ovum pick up (OPU) was conducted on the fourth day. The OPU was repeated five times in SG group with a 3 days interval. In AG group, the OPU was repeated for seven times with a 3 days interval. The number and size of follicles was recorded before puncture. The recovered oocytes were graded according to their morphological appearance following IETS criteria. Only oocytes of Grade A and B were classified as usable oocytes. The recovered oocytes and usable oocytes were counted and analyzed with SPSS according Duncan Continuous variables. The results showed there was no significant difference in the SG-FSH and SG-CT group in the average recoved oocytes (4.36 ± 0.34 vs. 5.15 ± 0.49) and usable oocytes (3.54 ± 0.31 vs. 3.73 ± 0.38). The recovered oocytes of AG-FSH group was similar to that of AG-CT (5.91 ± 0.31 vs. 5.40 ± 0.63). However, the usable oocytes of AG-FSH group was significant lower than the AG-CT group (1.86 ± 0.30 vs. 4.06 ± 0.52, $p < 0.001$). Comparing SG-FSH and AG-FSH groups, they were not significant different in recovered oocytes (4.36 ± 0.34 vs. 4.06 ± 0.52), and were significant higher in usable oocytes (3.54 ± 0.31 vs. 1.86 ± 0.30, $p < 0.005$). Comparing SG-CT and AG-CT groups, there were no significant different in revovered ooctyes and usable oocytes (5.15 ± 0.49 vs. 5.40 ± 0.63 and 3.73 ± 0.38 vs. 4.06 ± 0.52 respectively). In conclusion, there were no significant different in OPU results of buffalo between spring season and autumn season. It is a surprise that the injection of FSH decreased the recovered ooctyes and usable oocytes in buffalo OPU. This work was supported by the fund of International technical cooperation projects (2008DFA30320) and the Guangxi scientific research Projects (0833072, 1123005-3).

P131

Effect of breeding season on *in vivo* oocyte recovery in China River buffaloes (*Bubalus bubalis*)

G Qin[1], X Liang[1], M Chen[1], Z Tan[1], J Huang[1], C Pang[1], F Huang[1], B Yang[1], H Jiang[2]

[1]*Guangxi Buffalo Research Institute, Chinese Academy of Agricultural Science, Nanning, China;* [2]*College of Animal Science & Technology, Guangxi University, Nanning, China*

The present study was carried out to examine the effect of season on *in vivo* oocyte recovery in China river buffaloes during 2006–2010. Ovum pick up (OPU) was conducted every 3 days during peak (August–February) and low (March–July) breeding season. The donors were selected in peak breeding season (n = 75) and low breeding season OPU (n = 60). OPU was performed using ultrasound equipment with a 7.5 MHz transvaginal transducer, a 19-gauge, 60-cm needle and a constant 50 mmHg vacuum pressure. The number and size of visible follicles was determined and graded before puncture. The recovered oocytes were graded according to their morphological appearance following IETS criteria. Only oocytes of Grade A and B were classified as usable oocytes. The recovered oocytes and usable oocytes were counted and analyzed with SPSS according Duncan Continuous variables. The results showed there was significant difference in the total number of follicles observed per animal per session ($p < 0.01$) between animals or between puncture sessions in both low and peak breeding seasons (7.63 ± 0.14 vs. 8.27 ± 0.13). Higher ($p < 0.01$) number of middle (0.50 ± 0.02 vs. 0.31 ± 0.02) and large follicles (1.13 ± 0.05 vs. 1.13 ± 0.05) were observed during peak breeding season when compared to low breeding season. However, there was no difference in oocytes recovered (4.36 vs. 4.22), usable oocytes (3.04 vs. 2.99) and usable rate of COCs (63.75% vs. 65.07%) per animal per session ($p > 0.05$) between peak breeding season and low breeding season. In conclusion, the season did not significantly affect the percentage of oocytes suitable for IVP (grade A + B). This work was supported by the fund of Chinese International technical cooperation projects (2008DFA30320) and the Guangxi scientific research Projects (1123005-3).

P132

Sinus arrest associated with ruptured pyometra in a dog

R Ramirez Uscategui, J Vitti Moro, W Russiano Vicente, B Macenti, R Ribeiro Gutierrez

FCAV, UNESP, Jaboticabal, Brazil

Sinus arrest is the most common manifestation of end-stage sinus node disease in dogs. A female Shar Pei, 9 year old, was examined at the Obstetrics Service of 'Governador Laudo Natel' Veterinary Hospital (FCAV-UNESP-Jaboticabal-Brazil), with clear signs of shock. The support therapy was established, with Gelatin Solution 3.5% 5 ml/kg 15 min bolus and NaCl 0.9% CRI in order to maintain medium arterial pressure at 65 mmHg as a minimum and stabilize the patient hemodynamic status. Abdominal ultrasound, blood cells count as well routine biochemical analysis, was performed confirming the diagnosis of ruptured pyometra, peritonitis and acute renal failure. Laparotomy, ovariohysterectomy and abdominal flushing were performed. Premedication with tramadol (4 mg/kg) and isoflurane induction and maintenance was applied as anesthetic protocol. During the surgical procedure intermittent pauses were evident among the ECG complex, between 2 and 5 s, confirmed by absence of palpable pulse and loss of SpO2 signal, diagnosed as sinus arrest; which did not require any medical treatment due to the blood pressure, cardiac frequency and capillary refill time remained normal. ECG disturbances disappeared and all parameters were normal during 96 h of evaluation, being after 18 h from establishment of the support treatment with metronidazole 15 mg/kg bid, enrofloxacin 5 mg/kg sid, flunixin 0.1 mg/kg sid, tramadol 4 mg/kg tid, colloids infusion (if hypotension were present) and 0.9% NaCl 120 ml/kg/24 h. It has been listed as causes of Sinus arrest: pressure in carotid artery sinus, cardiac or pharynx surgical manipulation, degenerative or dilatory heart disease, inflammation or cancer of the heart, sick sinus syndrome, abnormal levels of potassium in blood, digoxin toxicity, and pneumothorax. Although bacterial myocarditis was reported as a cause in dogs, never this have been relates with uterine or abdominal infection, as in this case.

P133

Endometrial steroid, oxytocin, 20α-HSD and COX-II receptors on days 2 and 5 after ovulation in induced short and normal oestrous cycles in dairy cows

MH Rantala, J Taponen

Department of Production Animal Medicine, University of Helsinki, Helsinki, Finland

Short estrous cycles can be induced in cyclic dairy cows with prostaglandin F2α (PG) and GnRH given 24 h apart, and premature PG release has been shown to cause this phenomenon. On day 8 after ovulation (day 0 = day of ovulation), 14 pre-synchronized dairy cows were given 0.15 mg of dexcloprostenol and 0.1 mg of gonadorelin 24 h apart. Animals were bled once daily for progesterone and estradiol-17β (E2) analysis. Endometrial biopsies were taken on days 2 and 5 for steroid, oxytocin, 20α-HSD and COX-II receptor analysis with immunohistochemistry (IHC) and RT-PCR. Ovulations and ovarian structures were followed with ultrasound daily until the next ovulation. After excluding one case of incomplete luteal regression post-PG, short estrous cycles occurred in 8/13 cases (duration 8–12 day). From one day before until 5 days after ovulation, E2 concentration was higher in normal than in short cycles ($p < 0.05$). In the semi-quantitative IHC analysis, no difference was discovered in endometrial estrogen, progesterone, 20α-HSD or COX-II receptor concentrations between the normal and short cycle groups. In the real-time RT-PCR analysis, no difference in relative gene expression in endometrial estradiol, progesterone, oxytocin, 20α-HSD or COX-II receptors between normal and short cycles was detected. In conclusion, despite the fact that premature PG release has been shown to cause induced short cycles, differences in the above-mentioned receptor activities do not seem to explain this release.

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Oxytocin receptors (OXTR) in the equine placenta: a preliminary study of their location and expression

A Rapacz-Leonard[1], A Ras[1], J Calka[2], T Janowski[1]

[1]*Department of Animal Reproduction with Clinic, Faculty of Veterinary Medicine, Warmia and Mazury University, Olsztyn, Poland;* [2]*Department of Clinical Physiology, Faculty of Veterinary Medicine, Warmia and Mazury University, Olsztyn, Poland*

The objective of this study was to describe the location of placental OXTR in mares, and find how OXTR expression in mares with retained placenta (RP) differs from that in mares without RP. One to 2 h after delivery, placenta biopsies were taken from 12 mares: four control mares, and eight mares with RP (four RP mares had secondary atony of the uterus). Biopsies were frozen and immunocytochemically stained using anti-OXTR antibodies. OXTR were visualized using the Vectastain® ABC Kit. Less than 100 precipitates in the whole 25 mm^2 sample were considered either a trace amount of OXTR or an artifact of the staining process. The number of precipitates in all positive samples was at least an order of magnitude greater. OXTR were found in both epithelial and endothelial cells. Epithelial OXTR were found on and between the allantochorial villi, and in the endometrial crypts. Endothelial OXTR were found in the allantochorial vessels, and in the endometrial vessels and glands. The receptors were expressed most

intensely in places where the endometrium contacts the allantochorion, which suggests that expression of OXTR might indeed play an important role in the process of placenta detachment. Moreover, OXTR were not found in mares with RP due to secondary atony of the uterus, or found in only trace amounts. These results suggest that insufficient expression of OXTR might be a cause of uterine atony leading to RP in mares.

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Ovarian follicular dynamics around estrus in Beetal and Teddy goats

H Riaz, N Ahmad

Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore, Pakistan

Goats, in Pakistan are mostly kept by small holder farmers for whom these animals are important for survival – with the Beetal breed offering a high potential for meat and milk production to alleviate the need. There has emerged an interest in the application of reproductive biotechnologies, including the synchronization of estrus and ovulation in order to maximize meat production. Therefore, it is imperative to study the ovarian physiology around estrus in goats. The current study tested the hypothesis if the ovarian follicular population (number of small, medium and large follicles), size of the ovulatory follicle, and ovulation rate, using transrectal ultrasonography are different between Beetal and Teddy goats of Pakistani origin. Beetal ($n = 6$) and Teddy ($n = 8$) does were synchronized using double PGF2α injections 10 days apart and were scanned on Days −2, 0 (estrus) and +2. The onset of estrus was assessed by aproned bucks. Mean number of small follicles were higher ($p < 0.05$) in Beetal goats, compared to Teddy goats, on Days −2, estrus and +2. However, no significant difference ($p > 0.05$) was detected in the medium and large follicles On the Days of examination. The ovulatory diameter (7.3 ± 0.3 mm vs. 6.9 ± 0.5 mm), and ovulation rate (1.8 ± 0.7 vs. 1.8 ± 0.4) were found non-significant ($p > 0.05$) between the Beetal and Teddy goats, respectively. It is concluded that Beetal have greater population of small follicles around estrus compared to Teddy goats thus, holds a potential candidateship for the future reproductive studies.

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Post-trauma severe testicular degeneration in dog: a case report

RR Ribeiro Gutierrez, A Rossi, B Macente, C Menegasso Mansano, W Russiano Vicente

College of Agricultural and Veterinary Sciences, UNESP, São Paulo State University, Jaboticabal, Brazil

The seminiferous epithelial degeneration is considered an important cause of the minimization of the male dog fertility. Some its causes are follow: cryptorchidism, high temperature, infection, trauma, nutritional, vascular lesions, epididymal obstruction, scrotal bag dermatitis, self-immune, hormonal factors, among other idiopathic origin causes. When the degeneration is chronic, the testicle can suffer intense changes, like atrophy and fibrosis formation, and in some cases the tissue mineralization may occur. A Weimaraner dog, male, 2 year-old, was treated at Animal Reproduction and Obstetric Service in the Veterinarian Hospital, UNESP Jaboticabal having a testicular trauma historic at 6 months of age with a big local swelling and, after recovering, it was noticed the lack of it in the scrotal bag, without evidences of contralateral, even in the inguinal region. With the abdominal ultrasound evaluation it was impossible the visualization related to an intra-abdominal ectopic testicle. Following, the exploratory laparotomy was done, finding a rudimentary structure consistent to a spermatic cord lateral to prostate, following up to the inguinal region, where there was a suggestive formation into its sequence of a degenerated ectopic testicle. This structure was collected and sent to histopathologic analysis, with an appraised report of fibered tissue, hemorrhage focus, necrosis, and dystrophic calcification, without seminiferous tubules apparent. Thus, it may be concluded that the trauma suffered by the so-young dog caused the degeneration, as well the testicle necrosis, loosing completely this structure.

P137

Prevalence of infectious bovine rhinotracheitis in repeat breeder cows

A. Risvanli, S Alparslan

University of Firat, Elazig, Turkey

In this study, prevalence of Infectious Bovine Rhinotracheitis (IBR) was studied from repeat breeding cows. Repeat breeder, defined as cows failure to conceive from three or more regularly spaced services in the absence of detectable clinical signs. The repeat breeder in the area of study was a very big problem in older animals. However, the problem of infertility in young animals was not very common. Blood samples were collected from 108 cows at 3–10 years old. The presence of IBR in the samples was determined using Enzyme Linked Immunosorbent Assay (ELISA) antibody kits. The results of the ELISA tests indicated that 52 (48.15%) of 108 animals were seropositive and others (51.85%) were seronegative. Although there was no significant difference between the groups in the distribution of results by breed, and age, IBR seropositivity in 7 years (86.67%) and older animals group were found significantly higher ($p < 0.01$). As a result, IBR seropositivity in repeat breeding cows was high and, this ratio was more increased in the older animals. Therefore, it is concluded that the preventative measures should be taken against IBR to improve the efficiency of progeny in the region. The IBR may be a reason of highly prevalent of repeat breeder. However, the study should be supported by further studies. The study was supported by Firat University Scientific Research Projects Unit (FÜBAP 2097).

P138

The relationship between ovario-hysterectomy and anti-HSP70 in dogs

A Risvanli, M Yuksel, N Saat

University of Firat, Elazig, Turkey

The present study aimed to determine the effects of ovariohysterectomy on blood serum levels of HSP70 and anti-HSP70 antibodies in dogs. The relationship of HSPs with immune system has been shown in dogs as well as in other species. For this purpose, 87 female stray dogs were used. Ten milliliters of blood was taken from the animals just before surgery (preoperative) and 24 h after surgery (postoperative). The amounts of extracellular HSP70 (Uscn Life Sciences, PRC) and anti-HSP70 antibodies (Enzo Life Sciences, USA) in blood serums were measured by using commercial ELISA kits. Subsequently, the preoperatively and postoperatively obtained data were compared. The statistical analyses were performed by using the paired student *t* test in SPSS 11.5 software. As a result, the amounts of HSP70 (4.86 ± 0.99 ng/ml) and anti-HSP70 (109.77 ± 16.64 ng/ml) antibodies in the dogs' blood samples taken after ovariohysterectomy were found to be lower when compared to the preoperative amounts (7.22 ± 1.30 ng/ml for HSP70; 143.67 ± 20.81 ng/ml for anti-HSP70 antibodies). This has led to the hypothesis that ovariohysterectomy reduces HSP70 concentra-

tions in dogs, which weakens the immune system and precipitates the development of various postoperative complications. The study was supported by Firat University Scientific Research Projects Unit (FÜBAP VF.10.03).

P139

The effects of anti-IL–10 and anti-TGFβ on conception rates in mice

A Risvanli, A Godekmerdan

University of Firat, Elazig, Turkey

The present study aimed to determine the effects of post-mating administration of neutralizing antibodies developed against IL–10 and TGFβ with significant impacts on pregnancy in females upon conception rates in mice and the blood serum and uterine fluid concentrations of IL-2, IL-4, IL-6, IL-10, IL-17, IFNγ, TNFα, and TGFβ. In the study, 21 BALB/C strain female mice were mated and randomly grouped in three. The mice in the first group were selected as the control group. The second group of animals was injected with 0.5 mg anti-IL-10 after mating, while those in the third group were intraperitoneally injected with 0.5 mg anti-TGFβ. The animals in all groups were decapitated on the 13th day after mating and their blood samples were taken. The uteruses were removed to determine pregnancy. The mice's uterine irrigation fluids were also obtained. The multiplex immunoassay technique was used to determine the cytokine concentrations in the uterine fluids and blood serums. The study found no intergroup difference with respect to conception rates. A comparison of the cytokine concentrations in the uterine fluids of pregnant mice revealed higher TGFβ concentration ($p < 0.01$) in group 2 injected with anti-IL-10 antibody when compared to the other groups. Yet, no difference was detected in pregnant animals with regard to both uterine fluid and blood serum concentrations of other cytokines. Therefore, we concluded in our study that post-mating administration of anti-IL-10 and anti-TGFβ antibodies in mice did not have any effect on conception rates. The study was supported by the Scientific and Technological Research Council of Turkey (110S232).

P140

Preliminary results of the B-mode ultrasound cerebral biometry and assessment of the medial cerebral artery of canine foetuses using the Triplex Doppler

M Rossi Feliciano, A Nepomuceno, D Cardilli, R Crivelaro, M Silva, W Vicente, F Barros

School of Agrarian and Veterinary Sciences, UNESP, Jaboticabal, Brazil

The aim of the current study was to evaluate the cerebral biometry and vascular index of the median cerebral artery of canine fetuses using B-mode and Triplex Doppler ultrasound. Six pregnant bitches, weighting 5–15 kg, were assessed. The area and volume of fetal brain, the volume (IMCV) and the area brain index (IMCA) were measured during the 5th and 8th gestational weeks using the B-mode ultrasound. The Triplex Doppler was employed to assess the medial cerebral artery for peak systolic velocity (PSV), end diastolic velocity (EDV), vascular resistance (RI) and pulsatility (PI) index on the 7th and 8th gestational week. Although there was a physiologic increase in brain mass, the values of cerebral biometry showed no significant difference among the gestational weeks ($p > 0.05$). Regarding IMCA and IMCV, no significant difference was found among the weeks of evaluation ($p > 0.05$). Concerning the Triplex Doppler mode of the cerebral artery, there was no significant difference for PSV and EDV between the 7th and 8th weeks ($p > 0.05$). The values of IR and PI increased during the 7th week of pregnancy ($p < 0.05$), which indicates a raise on the blood flow to the fetal brain, probably due the normal development of brain tissue and metabolism. In conclusion, the vascular index and brain biometry are suitable for the assessment of cerebral development of canine fetuses and for the diagnosis of congenital abnormalities, such as hydrocephalus and hemodynamic changes. The authors would like to acknowledge FAPESP for the financial support to the current research and for post-doctor scholarship support (processes 2010/16913-7 and 2011/06011-9).

P141

Effect of the addition of catalase and/or Trolox® to a canine freezing extender

A Rota, A Accascina, C Sabatini

Veterinary Clinic Department, University of Pisa, Pisa, Italy

During cryopreservation, Reactive Oxygen Species may cause damages to spermatozoa. The aim of this study was to evaluate if the addition to the freezing extender of two antioxidants, catalase and Trolox® (a water-soluble tocopherol analogue), alone or in association, would better preserve motility, plasma membrane integrity and acrosome morphology. Semen of nine dogs was frozen in two steps in a Tris, citrate and fructose extender with a final concentration of 5% glycerol and 0.5% Equex STM paste (CONTR) to which 200 UI/ml catalase (CAT), 200 μM Trolox® (TROL) or 100 UI/ml catalase and 100 μM Trolox® (CA-TR) were added. One straw/treatment was thawed and evaluated after 0, 1, 2 and 3 h of incubation at 37°C with a Computer Assisted Sperm Analyser; while plasma membrane integrity (HOS-test) and acrosomal status (Spermac-stain®) were evaluated at hours 0 and 2. Differences in motility at hour 0, in % decrease from hour 0 to 1 or 3, and as area under the curve (AUC) were evaluated by Wilcoxon matched pairs signed rank sum test. The same test was used for the other parameters at hour 0 and 2. At hour 0 there were no differences between treatments, but between hours 0 and 1 there was a tendency for a larger decrease in total motility in CAT compared to CONTR ($p < 0.06$). Overall, during incubation, there were no significant differences between treatments and control in motility parameters (AUC). However, CAT tended to have a lower total and progressive motility compared to CA-TR ($p < 0.1$). The proportion of intact plasma membranes and normal acrosomes were also never significantly different between groups. However, there was a tendency for CAT to have a lower proportion of normal acrosomes at hour 2, compared to TROL ($p < 0.06$). In conclusion, the inclusion at the tested concentrations of catalase and/or Trolox® in the freezing extender did not improved canine semen characteristics post-thaw.

P142

Where is pig sperm α-L-fucosidase synthesized?

S Ruiz[1], A De Ondiz[2], M Avilés[3], S Ruiz[1]

[1]*Department of Physiology, University of Murcia, Murcia, Spain;* [2]*Sciences Veterinary Faculty, University of Zulia, Maracaibo, Venezuela;* [3]*Department of Cell Biology and Histology, University of Murcia, Murcia, Spain*

Sperm α-L-fucosidase could have some functions during gamete interaction. The aim of this work was to determine, by immunolocalization, if the origin of α-L-fucosidase present in pig sperm was epididymis or testis. Pig sperm were isolated by cutting and mincing of specific sections of epididymes and testis. The samples were classified in three groups: (i) cauda epididymis sperm (CES), (ii) caput epididymis sperm (CpES) and (iii) testicular sperm (TS). Formaldehide fixed samples were incubated with a primary antibody (anti-human α-L-fucosidase 1 -FUCA1-, produced in rabbit, 1:100, 1 h) and incubated with a secondary antibody (anti-rabbit IgG FITC, produced in chicken, 1:400, 1 h). Immunolocalization pattern was defined in 200

sperm with four replicates, by imaging analysis (Leica Qwin V3.4.0). No differences were observed in the percentaje of immunostained sperm in the different groups analyzed: CES (90.3 ± 2.17), CpES (91.8 ± 1.96) and TS (89.9 ± 3.52), respectively. In the three different samples, the fluorescent signal was evenly distributed over the sperm acrosomal region. Results indicate that the origin of sperm α-L-fucosidase is the pig testis. The enzyme is located in plasma membrane of acrosomal region of pig sperm. Origin and specific distribution of α-L-fucosidase in sperm suggests that this enzyme has a potential role in recognition and initial interaction of gametes during fertilization in pig. Granted by MEC AGL2009-12512-C02-01 and CARM 0452/GERM/06, 08752/PI/08.

P143

Different influence of ovine oestrus synchronization treatments over caruncular early angiogenesis

I Ruiz-Gonzalez, M Sanchez, R Garcia-Fernandez, P Garcia-Palencia, B Sanchez, J Flores

Department of Animal Medicine & Surgery, Veterinary School, Complutense University of Madrid, Madrid, Spain

In the pregnant sheep, maternal caruncles and foetal cotyledons develop into the placentomes, where the metabolic inter-exchange will take place. Thus, extensive angiogenesis occurs in both sides to ensure the increasing metabolic needs of the foetus. Impact of assisted reproductive treatments over endometrial angiogenesis has been hypothesized. In the ewe, progestagens and prostaglandin analogues protocols are commonly used for estrus synchronization. Although differences have been demonstrated between both treatments, no data is available regarding their influence over endometrial vascular expansion in early placentation. Caruncular vascular development was evaluated on days 15 post coitus (pc), 17pc and 21pc (day 0 = day of estrus), through immunohistochemical analysis of Vascular Endothelial Growth Factor (VEGF), CD31 and Von Willebrand Factor (vWF) in uterine samples obtained from ewes synchronized with progestagens (group P, n = 15) or prostaglandin analogues (group PG, n = 13). Each factor was assessed by Total vascular density (total positive blood vessels/mm^2), Capillary vascular density (positive blood capillaries/mm^2) and Arteriolar vascular density (positive arterioles/mm^2). VEGF-capillary density ($p = 0.045$) was higher in group P. Vascular CD31-positivity decreased significantly in both groups within days of pregnancy (total density, $p = 0.007$; capillary density, $p = 0.014$). vWF showed no significant differences between treatments or days pc. Observations pointed out in this analysis indicate a different influence of progestagen- and prostaglandin analogues-based synchronization treatments over early caruncular angiogenic development in the pregnant ewe.

P144

Abstract withdrawn.

P145

The utilisation of roscovitine as a novel agent for stimulation of nuclear-transferred pig oocytes derived from foetal fibroblast cells

M Samiec, M Skrzyszowska

National Research Institute of Animal Production, Balice n. Kraków, Poland

The present study was undertaken in order to explore the reliability and feasibility of R-roscovitine (R-RSCV; specific MPF-related CDK1 kinase inhibitor) for efficient activation of *in vitro* embryo developmental programme of nuclear-transferred (NT) pig oocytes. In Group I, *in vitro*-matured oocytes that had received the foetal fibroblast cell nuclei underwent sequential electrical and chemical activation (SE-CA). The new method of SE-CA involved treatment of NT oocytes with DC pulses and subsequent treatment with 7.5 μM calcium ionomycin, followed by their incubation with 30 μM R-RSCV, 0.7 mM 6-dimethylaminopurine and 3.5 μg/ml cycloheximide. In Group II, reconstituted oocytes were subjected to the standard method of simultaneous fusion and electrical activation (SF-EA). The proportions of cultured embryos that reached the morula and blastocyst stages were 147/235 (62.6%) and 84/235 (35.7%) or 103/201 (51.2%) and 49/201 (24.4%) in Groups I or II, respectively. In summary, the inventive strategy of SE-CA, which, to our knowledge, was applied for the first time to the activation of pig oocytes reconstructed by somatic cell cloning, led to the achievement of considerably higher preimplantation developmental competencies of cloned embryos than the standard protocol of SF-EA. This study was conducted as a part of research projects Nos. N R12 0036 06 and N N311 315936, which were financed from 2009 to 2012 by the National Centre for Research and Development in Poland and the Polish Ministry of Science and Higher Education, respectively.

P146

VEGF system detection in feto-maternal interface in Iberian pig implantation

M Sanchez, P Garcia-Palencia, B Sanchez, A Palomo, I Ruiz-Gonzalez, R Garcia-Fernandez, J Flores

Department of Animal Medicine & Surgery, Veterinary School, Complutense University of Madrid, Madrid, Spain

The Iberian pig is an autochthonous Mediterranean breed characterized in reproduction by a lower prolificacy. Recent studies pointed to embryo loss like the major cause of this problem. The aim of our study is to analyse the expression of Vascular Endothelial Growth Factor (VEGF) system in Iberian viable and no viable attachment site in feto-maternal union. Thus, we analyze by real-time RT-PCR the mRNA expression of VEGF and its receptors, VEGFR1 and VEGFR2, in arresting (n = 9) and viable (n = 19) porcine attachment sites (based in size, weight and vascular characteristic of embryo) at days 32–35 pc in 10 Iberian pigs. mRNA levels of VEGF (99.54 ± 12.89), VEGFR1 (65.38 ± 10.09) and VEGFR2 (108.75 ± 17.51) in endometrium's viable attachment sites were higher than in arresting areas (91.94 ± 14.09 VEGF mRNA; 34.43 ± 6.06 VEGFR1 mRNA; 83.62 ± 13.91 VEGFR2 mRNA), nevertheless these differences were only significant in VEGFR1 mRNA ($p < 0.05$). Similarly, placenta from viable embryos showed better levels of mRNA of each factor (64.73 ± 21.56 VEGF mRNA; 34.69 ± 7.87 VEGFR1 mRNA, 48.64 ± 7.26 VEGFR2 mRNA) compared to arresting embryos (51.66 ± 6.57 VEGF mRNA; 17.53 ± 3.34 VEGFR1 mRNA, 42.80 ± 5.88 VEGFR2 mRNA), but no significant differences. VEGF receptors correlate positively in viable ($r = +0.77$ $p < 0.01$) and arresting sites ($r = +0.73$ $p < 0.05$) in the endometrium. However, VEGF correlates mildly with VEGFR2 only in placenta from healthy embryos ($r = +0.69$, $p = 0.05$). Results point out lower levels of mRNA VEGF system, especially in arresting attachment sites, suggesting a possible relation between inappropriate vascularization and low viability and growth of the embryos.

P147

Crocin improves frozen/thawed bovine sperm motility and viability

V Sapanidou[1], I Taitzoglou[1], I Tsakmakidis[1], Z Abas[2], I Zervos[1], S Lavrentiadou[1], M Tsantarliotou[1]

[1]*School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece;* [2]*School of Agricultural Development, Democritus University, Nea Orestiada, Greece*

Crocin, a carotenoid constituent of spice Crocus sativus L. (saffron), is known for its antioxidant activity both *in vivo* and *in vitro*. During assisted reproductive techniques, oxidative stress affects motility and viability of spermatozoa and reduces their fertilization capacity. The aim of the present study was to evaluate the effect of crocin on bovine sperm motility and viability, *in vitro*. Frozen/thawed spermatozoa of four different bulls were pooled and incubated with three different concentrations of crocin (0.5, 1 and 2 mM), at 37°C and evaluated in three different time points (0, 120, 240 min) by CASA (Computer Assisted Sperm Analyzer). The experiment was repeated six times. The parameters 'Rapid', 'Medium', 'Slow', 'Static', 'Progressive Motility', VCL, VSL and VAP were analyzed as repeated measurements with the evaluation of semen viability included. The results indicate an interaction between time and the 1 mM concentration of crocin. Specifically, the percentage of 'Rapid' spermatozoa showed statistical significant increase ($p < 0.05$) in the presence of 1 mM of crocin. Moreover the incubation of spermatozoa with 1 mM of crocin for 120 min showed the greater number of live spermatozoa with intact acrosome. The rest of the motility parameters ('Slow', 'Progressive motility', VCL, VSL and VAP) showed a similar trend to increase, while the percentage of static spermatozoa showed a trend to decrease. We suggest the beneficial role of crocin on bovine sperm viability and motility in the media of assisted reproductive techniques.

P148

Abstract withdrawn.

P149

Early pregnancy detection using ultrasound imaging of uterine and corpus luteum characteristics in dairy cows

S Scully[1], M Mullen[2], M Diskin[2], A Evans[3], M Crowe[1]

[1]*School of Veterinary Medicine, University College Dublin, Dublin, Ireland;* [2]*AGRIC, Teagasc, Galway, Ireland;* [3]*School of Agriculture and Food Science, University College Dublin, Ireland*

To assess the accuracy of early pregnancy diagnosis using uterine echotexture and CL characteristics, reproductive tracts of dairy cows ($n = 22$) were examined from days 18 to 21 following AI by transrectal ultrasound using a Volusoni scanner fitted with a 12 MHz probe. A pregnancy diagnosis and certainty score (1–3) was based on uterine echotexture (homogeneity and contrast), CL tissue area and Vascularity [blood flow area, BFA)] at each scan. Blood samples were collected for progesterone (P4) analysis. Cows were retrospectively allocated to either pregnant (P. $n = 13$) or non-pregnant (NP. $n = 9$) following a final ultrasound exam between days 30 and 40 after AI. Diagnostic data were analysed using the N2 statistic. The mixed procedure of SAS was used for image analysis data. Visual diagnostic specificity increased numerically ($p = 0.86$) from 62.5% on day 18 to 87.5% on day 21. Certainty scores ranged from 2.5 to 3 on all days for both P and NP cows. Sensitivity remained between 71.4% and 78.6% for all days ($p < 0.05$). Sensitivity and specificity for cows were 76.7% and 62.5%, respectively. Homogeneity and contrast of the uterus were not different ($p > 0.05$) between P and NP on any day. Both CL tissue area and blood flow area were higher ($p < 0.05$) in P cows on days 20 and 21 following AI. P4 was higher ($p < 0.001$) in P compared to NP on all days. Specificity was most accurate on day 21 while image analysis on day 21 showed that NP cows had lower ($p < 0.05$) values for CL area and BFA than P cows.

P150

Effects of addition of Omega-3,6,9 fatty acids to the semen extender on the characteristics of frozen-thawed bull Sperm

M Sheikholeslami[1], J Arshami[1], A Abavisani[2]

[1]*Department of Animal Sciences, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran;* [2]*Department of Basic Sciences, Veterinary Faculty & Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran*

It has been shown that the presence of unsaturated fatty acids in the sperm membrane increases membrane fluidity and its resistance to freezing. In this study, several levels of combination of omega-3,6,9 fatty acids were added to the semen diluents to investigate its effects on motility, viability and morphology of bull frozen-thawed sperm. To emulsify the oil in semen extender, polyethylene glycol (PEG) was added and solution was finally sonicated. For experiment: in treatment 1, PEG was added alone to the samples and in treatments 2, 3 and 4 three different concentrations of omega-3,6,9 fatty acids (1%, 2.5% and 5%) were added to the semen extender. Five proven bulls were randomly selected and parameters of collected semen were recorded. Then semen samples were packed into 0.5 ml straws and were typically frozen. Samples were measured in terms of Motility, viability and morphology after 1 month. Motility and other dynamic parameters were analyzed by computer aided sperm analyzer (CASA). The results were evaluated by repeated measure ANOVA and $p < 0.05$ was considered significant. Motility, Viability and normal morphology were 32.24%, 10.38%, 11.83%, 9.71% and 8.02%; 65.5%, 17.5%, 21.25%, 15% and 15.67%; 87%, 77.75%, 80%, 78.25% and 78.67% for control, treatment 1, 2, 3 and 4 respectively. Our results showed that PEG had some detrimental effects on sperm and the combination of omega-3,6,9 fatty acids could not significantly improve frozen-thawed sperm motility and viability. In conclusion, it seems that this fatty acid combination could not improve sperm membrane resistance to freezing *in vitro*.

P151

Derivation of pluripotent stem cell-like cells from nuclear transferred cloned bubaline (Bubalus bubalis) embryos

B Singh[1], S Gautam[1], V Verma[2], S Singla[3]

[1]*Indian Veterinary Research Insitute, Regional Station Palampur, Kurukshetra, India;* [2]*National Heart Centre, Singapore;* [3]*National Dairy Research Institute, Karnal, India*

Stem cell technology has undergone remarkable metamorphosis, and has been shown to have enormous significance in livestock assisted reproduction, conservation of animal genetic diversity, regenerative medicine and various veterinary health applications. This study reports derivation of pluripotent stem cell-like cells from the inner cell mass (ICM) cells of nuclear transfer cloned bubaline embryos. The cultured cumulus cells at passage 6, were trypsinized and vitrified (−196°C) for 30 days. The oocytes were aspirated from abattoir-derived fresh ovaries, and matured *in vitro*. The IVM oocytes were denuded and enucleated, and cytoplasts were electrofused with re-cultured cumulus cells for producing somatic cell nuclear transfer (SCNT) cloned embryos. The SCNT morulae and blastocysts were used as sources of obtaining ICM cells by micromanipulator-guided glass capillary holding- and suction-pipettes. The ICM cell colonies were dome

shaped when grown on mitomycin C-inactivated fetal skin-fibroblast feeder monolayer, and found to exhibit stem cell-like pluripotency markers, namely elevated expression of alkaline phosphatase, Oct-4, prolonged survival *in vitro*, normal chromosomal profiles at various intervals. Also, the cells were observed to forma embryoid bodies in suspension, and differentiated into various cell lineages under different milieus. It is inferred that cumulus cells could be cryopreserved using economical vitrification protocols, and reprogrammed for deriving pluripotent stem cell-like cells, and that micromanipulator-guided derivation of ICM cells could offer valuable means of obtaining selective and homogenous pluripotent cells for various applications in the species.

P152

Capability of bi-transgenic fibroblast cell nuclei to complete *in vitro* development of porcine cloned embryos to blastocyst stage

M Skrzyszowska, M Samiec

National Research Institute of Animal Production, Balice n. Kraków, Poland

The purpose of our study was to assess the *in vitro* developmental potential of double-transgenic pig embryos generated by somatic cell cloning for the purposes of xenotransplantology. The enucleated *in vitro*-matured oocytes were subzonally-injected with the cultured/trypsinized fibroblast cells that had been derived from ear skin biopsies of a non-chimeric genetically-engineered postnatal gilt robustly and ubiquitously expressing such recombinant human immunoenzymes as α-galactosidase A (α-GLA) and α-1,2-fucosylosyltransferase (H-transferase; α-1,2-FT/HT). Ooplast-somatic cell couplets were simultaneously fused and electrically activated by two consecutive DC pulses of 1.5 kV/cm for 30 μs. Afterwards, clonal cybrids were cultured in NCSU-23/BSA/FBS medium for 144–168 h up to morula and blastocyst stages. From among 202 enucleated oocytes, 193 (95.5%) were successfully fused/activated and intended for *in vitro* culture. The frequency of cloned embryos that exhibited cleavage activity was 159/193 (82.4%). Out of 193 cultured embryos, 77 (39.9%) and 35 (18.1%) reached the morula and blastocyst stage, respectively. Altogether, the abilities of cell nuclei originating from bi-transgenic postnatal cutaneous fibroblasts to direct the preimplantation development of nuclear-transferred pig embryos to morula/blastocyst stages were maintained at the relatively high level. This work was conducted as a part of research project no. N R12 0036 06, which was financed from 2009 to 2012 by the National Centre for Research and Development in Poland.

P153

Sorted sperm decreased the sperm quality and subsequent embryonic development after *in vitro* fertilization of oocytes in buffalo

X Song, X Yang, M Jiang, W Meng, Q Li, D Li, Y Lu, M Zhang, S Lu, K Lu

College of Animal Science and Technology, Guangxi University, Nanning, China

Flow-cytometric sorting of X- and Y-sperm based on their different DNA contents is a promising technology for altering the sex ratio and accelerating the genetic improvement of swamp buffalo. However, the sorting system still has low efficiency, possibly as a result of damage to sperm during or after the sorting process. The aim of this study was to investigate the sperm quality and subsequent embryo development after IVF of oocytes with frozen-thawed sorted and unsorted buffalo sperm. Firstly, two kinds of semen mentioned above were assessed with computer assisted sperm analysis (CASA) for progressive motility, and other parameters such as apoptosis, DNA integrity and membrane integrity were analyzed by flow cytometry or fluorescence microscopy. Secondly, we explored the effects of sorted sperm on *in vitro* embryo development after IVF. The results showed that the quality of sorted frozen-thawed sperm and their subsequent cleavage rate (39.3%) and blastocyst development (11.1%) after IVF was significantly decreased ($p < 0.05$) compared to the unsorted frozen-thawed sperm (49.0%, 20.8% for the cleavage and blastocyst rate, respectively). The results of this study demonstrate that the current sorting procedure for buffalo sperm affects its motility, apoptosis, DNA integrity, membrane integrity, and its subsequent embryonic development after IVF.

P154

SLC-induced capacitation-like changes in porcine semen

M Spinaci[1], D Bucci[1], C Tamanini[1], C Vallorani[1], R Guidetti[2], G Galeati[1], J Morrell[3]

[1]*Department of Veterinary Medical Sciences (DSMVet), University of Bologna, Ozzano Emilia, Italy;* [2]*Inseme S.P.A., Modena, Italy;* [3]*Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden*

Single layer centrifugation (SLC) of semen with a species-specific colloid is a useful technique to preselect the most resistant spermatozoa for artificial insemination or other reproductive biotechnologies. Beneficial effects on sperm viability, motility and fertilizing ability have been demonstrated following SLC with Androcoll-P for boar semen. Moreover SLC has been shown to separate spermatozoa from seminal plasma. Since cholesterol has been identified in the colloid after SLC, the present study was aimed at determining possible changes in sperm capacitative status after SLC with Androcoll-P, by CTC staining and detection of tyrosine phosphorylated proteins. Each experiment was repeated five times and the results are expressed as mean ± SD. The CTC staining showed an increase in B pattern, typical of capacitated spermatozoa, (from 15.7 ± 2.1 to 39.3 ± 4.1%, $p < 0.05$) and, as a consequence, a decrease in F pattern (from 83.7 ± 2.2 to 59.2 ± 3.7%, $p < 0.05$). Interestingly immunolocalization for tyrosine phosphorylated proteins did not evidence any increase in capacitated pattern. Moreover, western blotting did not show any change in phosphorylation status, with the exception of a 25 kDa band, significantly ($p < 0.05$) increased after SLC. In conclusion, SLC with Androcoll-P of boar spermatozoa seems to change membrane organization toward capacitation, as demonstrated by CTC staining. Nevertheless, it does not activate the downstream pathways involving protein tyrosine phosphorylation.

P155

Effect of single-layer centrifugation through colloid on post-thawed motility and viability of ram spermatozoa

N Šterbenc[1], M Kosec[1], M Šavc[1], J Morrell[2], P Klinc[1]

[1]*Clinic for Reproduction and Horses, Veterenary Faculty, University of Ljubljana, Ljubljana, Slovenia;* [2]*Department of Clinical Sciences, Division of Reproduction, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Science (SLU), Uppsala, Sweden*

The present study was performed to evaluate the effects of centrifugation through a single layer (SLC) of a glycidoxypropyltrimethoxysilane-coated silica colloid (Androcoll-O) on post-thawed motility, viability and membrane integrity of ram spermatozoa. Ejaculates from four crossbreed rams were frozen according to the standard procedure with Tris-egg yolk extender. After thawing the semen samples were diluted and divided into SLC (colloid centrifugation at 285 g for

20 min) and control group (group C). Analyses of samples were performed 0, 6 and 12 h after thawing. Motility and the viability (Viadent®) of the semen were analysed with Hamilton Thorne Biosciences, Version 12.3 and membrane integrity with HOS (hypoosmotic swelling test). Differences between groups were analysed with paired *t*-test. Percentage of motile spermatozoa was significantly higher in SLC group in comparison to group C respectively for 0 h ($p = 0.010$), 6 h ($p = 0.004$) and 12 h ($p = 0.003$). Analysis of viability also revealed significantly higher ($p \leq 0.001$) percentage of viable spermatozoa in SLC compared to group C respectively for all times. Percentage of HOS positive spermatozoa was also significantly higher in SLC compared to C group respectively for 0, 6 h ($p \leq 0.001$) and 12 h ($p = 0.002$). The results indicate high positive effect of SLC on quality of frozen-thawed ram spermatozoa.

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Bovine caruncular tissue shows immune competence *in vitro*

K Streyl[1], D Streyl[1], N Hambruch[2], C Pfarrer[2], H Zerbe[1]

[1]*Clinic for Ruminants with Ambulatory and Herd Health Services, LMU Munich, Germany;* [2]*Department of Anatomy, University of Veterinary Medicine, Hannover, Germany*

The mechanisms of physiological release of the foetal membranes in cows are incompletely understood. We could show the participation of a local immune response in previous studies (Streyl et al. 2012), which presumably leads to an influx of immune effector cells into the intra partal placentome. We hypothesize that the maternal components of the placentome, namely the caruncular epithelium and caruncular stroma, fulfil an immunological function by recruiting leukocytes into the placentome. To test this hypothesis *in-vitro* the immune competence of bovine caruncular epithelial and fibrocyte cell cultures were investigated by stimulating the cell culture with LPS, a representative of the pathogen-associated molecular patterns (PAMPs) and hydrocortisol as classical immunosuppressive. The cell culture supernatants were used in transmigration assays to test the chemoattractive potency of the cells. mRNA expression was analyzed by qPCR. The stimulation of both cell types results in a significant increase of migrated PMNs in the transmigration assay and increased mRNA expression of e.g. IL8, CCL20 and TNFalpha. These results show the potential of maternal caruncular epithelial cells and fibrocytes to recruit leukocytes into the placentome. Clarifying the immunological competence of these cell types in the process of foetal membrane release will also lead to better understanding of the retention of the foetal membranes necessary to develop new therapeutic approaches in treating this disorder. (Supported by Pfizer Inc.)

P157

Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows

V Suthar[1], J Canelas Raposo[2], A Deniz[2], W Heuwieser[1]

[1]*Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Germany;* [2]*Bayer Animal Health, Monheim, Germany*

There is a dearth of information on prevalence of subclinical ketosis (SCK) considering the diversity of European dairy farms. The objective of this study was to determine prevalence of SCK and relationships with postpartum diseases such as metritis, clinical ketosis (CK), displaced abomasum (DA) and lameness in European dairy farms. From May to November 2011 a convenience sample of 510 dairy herds from Croatia, Germany Hungary, Italy, Poland, Portugal, Serbia, Slovenia and Spain was studied. Blood β-Hydroxybutyrate (BHBA) was measured in a total of 5012 cows with a handheld meter within 2–15 days in milk and relevant information was recorded. Overall prevalence of SCK was 23.8% (14.8–36.6%) considering a threshold for blood BHBA ≥ 1.2 mM. Using receiver operator characteristic curve, blood BHBA thresholds ≥1.1, ≥1.3, ≥1.4 and ≥1.7 mM were determined for occurrence of lameness, CK, metritis and DA ($p < 0.01$). Plausible factors such as parity, effect of other diseases, herds, months and countries were tested in logistic models for disease of interest. The models demonstrated that cows with SCK have 1.5, 9.0, 5.3 and 1.8 times greater risk for development of metritis, CK, DA and lameness, respectively ($p < 0.01$). Overall, we concluded that prevalence of SCK is high in some European countries. Elevated BHBA within 2–15 days of milk is associated with an increased risk of metritis, lameness, CK and DA.

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Effect of egg yolk–based extenders on motile sperm population characteristics during goat sperm cryopreservation

A Tabarez, W Garcia, M Palomo

Autonomous University of Barcelona, Bellaterra, Spain

Our aim was to assess the replacement of fresh by pasteurized powered egg yolk on sperm motion characteristics, analysed by a computer-assisted sperm system (ISAS®). We also studied the profits of the fresh clarified egg yolk, by centrifugation of egg yolk twice at 10 000 × *g* for 45 min at 4°C, and the effect of the seminal plasma of young males on the motile sperm population. Briefly, fresh ejaculates from six bucks (1 year old) were collected by artificial vagina and immediately mixed in equal quantities. Pooled semen was split into two samples. One sample was washed by centrifugation (twice at 600 *g* for 10 min) and then the pellet was split into three equal aliquots and re-suspended in an extender containing 15% (v/v) of powered, fresh or fresh clarified egg yolk supplemented with 5% glycerol in a Tris-based media. The other semen sample was directly split into three equal aliquots and re-suspended in an extender containing 2% (v/v) of the same different type of egg yolk. Motility data were analyzed with the clustering procedure FASTCLUS, dividing the thawed motile sperm population in four separate subpopulations (SP), showing significant differences ($p < 0.01$) in their motion characteristics. The distribution of these subpopulations was similar (SP1 = 58.2%; SP2 = 10.6%; SP3 = 2.9% and SP4 = 28.4%; $p > 0.05$) between treatments, independently of their total motility, suggesting that the thawed motile sperm population shows similar behaviour in the different samples. Supported by INIA (RZ2009-00008-00-00), Generalitat de Catalunya (2009SGR0621 and CUR-DIUE) and FSE.

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The expression of steroid hormone receptors and inhibin-α in equine endometrial epithelial and stromal cells *in vitro*

T Theuß[1], D Böttcher[1], A Sobiraj[2], C Ellenberger[1], H Schoon[1]

[1]*Institute of Pathology, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany;* [2]*Large Animal Clinic for Theriogenology and Ambulatory Services, University of Leipzig, Leipzig, Germany*

In vitro systems often lead to a loss of essential cellular properties. This could be evident in endometrial cells by a loss of steroid hormone receptors or regulatory proteins (e.g. inhibin-α) which are necessary for studies on cyclical changes or influences of steroids. The aim of this study was to examine oestrogen (ERα) and progesterone (PR) receptors and inhibin-α in cultured equine endometrial cells with special focus on the former endometrial cycle. This study includes all endometrial cycle stages of the mare (proliferation, secretion, physi-

ological inactivity, pregnancy). For *in vitro* culture, the tissue was dissected and digested in collagenase, followed by a separation of the cells on percoll. The prevalence of the three proteins was determined immunocytochemically and the findings were compared to those *in situ*. In addition, the proliferation intensity according to the endometrial cycle was ascertained. ERα and PR were demonstrated in both cell types *in vitro*. However, the expression was stronger in the epithelial cells and exclusively seen intracytoplasmic. Moreover, inhibin-α could be detected *in situ* and *in vitro*. The proliferation intensity *in vitro* was directly dependent on the former endometrial cycle. Summing up, a fairly differentiated cell type of equine endometrial cells can be facilitated with the use of the described *in vitro* culture, as specified by the expression of steroid hormone receptors and inhibin-α *in vitro*.

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Effect of GnRH analogue deslorelin acetate on multiple ovulations (MO) in the thoroughbred mare

D Trundell, F Novotny

Clinic of Horses, University of Veterinary Medicine and Pharmacy, Kosice, Slovak Republic

The use of hormonal therapy to induce ovulation has become widespread in the mare and allows synchronisation of mating and ovulation. Many reports exist to support a link between human chorionic gonadotropin administration and multiple ovulations (MO) in the mare. However similar reports on the effect of other ovulation-inducing drugs such as deslorelin acetate (a GnRH analogue) on MO are somewhat scarce. The aim of this study was to evaluate the effect of deslorelin acetate on incidence of MO in the mare. A retrospective study of breeding records of thoroughbred mares boarded at one stud farm in England over a 2 year period were examined. Six hundred and twenty-seven ovulations resulting in 103 MO and 524 single ovulations (SO) were included for analysis. Ovulations resulting in MO were divided into two groups; those in which deslorelin acetate was administered (n = 77; 74.8%) and those where deslorelin acetate was not administered (n = 26; 25.2%). A Chi-square test was used to analyze the data. A significant difference was found between the MO linked to deslorelin acetate administration and those that were not (χ^2 = 24.28 1 df $p < 0.001$). It is therefore concluded that the use of the GnRH analogue deslorelin acetate may increase the incidence MO occurring. MO can establish as twins and this is of potential concern. In the mare, twins potentially lead to loss of the entire pregnancy and reduce the mare's overall reproductive performance. Funded by Ministry of Education of Slovak Republic, VEGA 1/0498/12

P161

Effects of butylated hydroxytoluene (BHT) supplementation on quality of cryopreserved boar semen

M Trzcińska, M Brya, P Gogol

National Research Institute of Animal Production, Krakow, Poland

The aim of this study was to evaluate the effects of different concentrations of BHT supplementation in freezing extender on post-thaw quality of boar semen. A total of 45 ejaculates from five crossbred boars were divided into five groups according to the compositions of the freezing extender used: (i) lactose egg yolk extender with 9% glycerol as control (LEYG), (ii) LEYG with 0.5 mM/ml BHT, (iii) LEYG with 1.0 mM/ml BHT, (iv) LEYG with 1.5 mM/ml BHT, (v) LEYG with 2.0 mM/ml BHT. The semen was cryopreserved using a standard protocol (Westendorf et al., 1975) with minor modification. Sperm progressive motility (CASA), plasma membrane integrity (YO-PRO-1/PI) and lipid peroxidation (chemiluminescence method) were assessed at 15 min post-thaw. Mean sperm progressive motility (±SD) examined after thawing for extenders I, II, III, IV and V was 62.4 ± 6.5, 71.7 ± 9.7, 73.0 ± 11.7, 75.1 ± 8.9 and 78.4 ± 8.3, respectively. The percentage of live sperm (YO-PRO-1-/PI-) was significantly (Duncan's test, $p < 0.01$) higher in the samples cryopreserved in extender V compared with extenders I, II, III and IV (75.0 ± 3.9% vs. 59.7 ± 9.4%, 62.4 ± 6.3%, 63.6 ± 7.1%, 66.8 ± 2.6%). Moreover, it was shown that semen extender V had a significantly lower peroxidation level than the other extenders ($p < 0.01$). The Integral parameter (counts/integration time) calculated for extenders I, II, III, IV and V was 14.7, 3.7, 3.1, 2.1 and 0.4, respectively. Our results showed that the freezing extender containing 2.0 mM/ml BHT improved the survival of boar spermatozoa after thawing and reduced the oxidative damage to sperm. Financed by grant N N311524840.

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Relationship between oocyte maturation and the activity of two glycosidases [β-N-acetyloglucosaminidase (NAGASE), α-mannosidase (α-MAN)] in bovine follicular fluid

T Tsiligianni[1], F Samartzi[1], E Dovolou[2], E Vainas[1], G Amiridis[2], C Perreau[3], P Mermillod[3]

[1]*NAGREF, Veterinary Research Institute, Thessaloniki, Greece;* [2]*Veterinary Faculty, University of Thessaly, Karditsa, Greece;* [3]*INRA, UMR6175 Reproductive Physiology and Behavior CNRS, Nouzilly, France*

Glycosidases are involved in cumulus cells dispersion, zona pellucida binding and penetration and polyspermy block. No data are available on the presence of glycosidases in bovine follicular fluid (FF). Here we report on the activity of NAGASE and α-MAN in FF of different sized bovine follicles and its relationship with oocyte maturation. Approximately 800 COCs were aspirated from abattoir ovaries, divided into two groups according to the follicular size (visual examination) (Small: 2–5 mm, Large: 6–8 mm) and into three groups according to their quality (grade: A, B, C and D). After a 24 h of maturation, oocytes were fixed and stained (orcein 2%) to evaluate nuclear maturation. Glycosidases activity was determined spectrophotometrically. More grade-A and BC-COCs were collected from small and large follicles, respectively ($p < 0.05$). The activity of both glycosidases was higher in small follicles compared to large ones ($p < 0.05$). Maturation rate was similar in both size groups. In small follicles NAGASE activity was positively associated with the ratio of grade A oocytes and negatively with BC oocytes. In oocytes from small follicles maturation rate was positively related to a-MAN activity. Our results showed a relationship between glycosidases activity in FF and oocyte maturation. This work was financed by GSRT and ERDF (Greece) and French Ministry of foreign affairs (France).

P163

Effect of treatment of GnRH and non-selective inhibitor of phosphodiesterase on fertility of ewes

I Valasi[1], S Ppapadopoulos[2], S Chadio[3], E Theodosiadou[4], D Kalogiannis[3], V Mavrogianni[4], S Spanos[4], G Fthenakis[4], G Amiridis[4]

[1]*Veterinary Faculty, University of Thessaly, Karditsa, Greece;* [2]*Department of Animal Production, Technological Educational Institute of Larissa, Larissa, Greece;* [3]*Department of Animal Science and Aquaculture, Agricultural University of Athens, Athens, Greece;* [4]*Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece*

This study was undertaken to evaluate the effects of 3-isobutyl-1-methylxanthine (IBMX), an inhibitor of c-AMP and c-GMP, on pituitary responsiveness and fertility rate of GnRH-treated ewes. Twenty-five

ewes were allotted into four groups (Controls: n = 7, A1: n = 6, A2: n = 6, A3: n = 6). Oestrus synchronisation was performed by intravaginal progestagen sponges for 7 days. Thirty-six hours after sponges' removal, ovulation was induced by GnRH; ewes in groups A1, A2 or A3 received simultaneously an injection of 2.5, 5.0 or 25.0 mg IBMX, respectively. Ewes were intracervically inseminated with fresh semen (300×10^6 spermatozoa per dose) 9 h later. Blood samples were collected, 0, 45, 75, 105, 135, 165, 195, 225 and 255 min after GnRH for LH assessment, and for 17 days after sponges' removal for progesterone assessment. LH concentrations peaked ($p < 0.05$) 135–165 min after GnRH injection. Albeit lack of significant differences in LH between groups higher levels were detected in A1 group ($p > 0.13$). The number of pregnant ewes were 1/7, 4/6, 2/6 and 2/6 for groups C, A1, A2 and A3, respectively ($p = 0.086$, C vs. A1). These preliminary results show a possible involvement of cyclic nucleotide-dependent intracellular mechanisms in control of sheep reproductive functions and also indicate that IBMX could act as an enhancer of reproductive efficiency in sheep.

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Pancreatic β-cell function differs between newborn Belgian Blue and Holstein calves

M Van Eetvelde[1], MM Kamal[1], L Fiems[2], G Opsomer[1]

[1]*Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, University of Gent, Merelbeke, Belgium;* [2]*Institute for Agricultural and Fisheries Research, Animal Sciences Unit, Melle, Belgium*

The activity of pancreatic β-cells was compared between newborn double-muscled Belgian Blue (BB, n = 42) and Holstein Friesian (HF, n = 38) calves. After an overnight fast, 3 days old calves were weighed and their withers height was measured. Blood samples were obtained before and 10 min after the infusion of a standardized glucose bolus (150 mg glucose/kg body weight). Body weights were 51.2 ± 9.03 kg for BB and 41.1 ± 5.56 kg for HF calves. A significant breed effect was found regarding the body mass index (BMI, body weight/height^2) of the calves, which was 95.5 ± 11.33 for BB and 70.2 ± 7.33 for HF ($p < 0.001$). BMI was significantly correlated with basal glucose (G0) and insulin (I0) levels ($r = 0.45$, $p = 0.003$ and $r = 0.32$, $p = 0.04$ respectively) in BB but not in HF calves. BB calves had significantly ($p < 0.001$) lower G0 and I0 levels (4.3 ± 0.67 mM and 5.10 ± 3.56 mU/l respectively), when compared to HF calves (5.9 ± 0.86 mM and 8.7 ± 5.81 mU/l). After infusion of the glucose bolus, a comparable increase in glucose was seen for BB (1.8 ± 0.63 mM) and for HF (1.6 ± 0.56 mM). On the other hand, the increase of insulin was significantly higher in HF (27.6 ± 17.43 mU/l) than in BB (18.9 ± 18.9 mU/l) calves ($p = 0.04$). As a result, the insulinogenic index [IGI, (I10-I0)/(G10-G0)], used as measure of early insulin secretion, was significantly higher in HF than in BB calves ($p = 0.008$). The higher IGI for HF calves could point to an innately lower insulin sensitivity in these calves or may be due to the higher amount of muscle fibers of the BB calves.

P165

The impact of elevated NEFA concentrations on mitochondrial function during bovine oocyte maturation

V Van Hoeck[1], K Schnorbusch[1], S Valckx[1], S Andries[1], R Sturmey[2], P Bols[1], J Leroy[1]

[1]*University of Antwerp, Antwerp, Belgium;* [2]*Hull York Medical School, UK*

Elevated non-esterified fatty acid (NEFA) concentrations arise as a result of increased lipolysis associated with obesity, type II diabetes or negative energy balance. It is known that elevated NEFA concentrations in the blood of females are a key factor in the pathogenesis of subfertility as it may directly affect the growing and maturing oocyte, hampering oocyte development and the oxidative embryo metabolism. Based on findings in somatic cells, we hypothesized that exposure to elevated NEFA concentrations during *in vitro* oocyte maturation affects mitochondrial function. To investigate this, we matured COCs under control and HIGH NEFA conditions (425 μM, a combination of stearic, palmitic and oleic acid). Resultant zygotes (18 h, n = 62, 3 repeats) were stained with JC-1 to measure the ratio of high vs. total polarized mitochondrial membranes. Images were analyzed by determining data on pixel intensity in the FITC and RITC channel using ImageJ. The % of highly polarized membranes out of the total polarization only showed a subtle trend to be higher in HIGH NEFA zygotes compared to controls for the top plane (55.0 ± 1.2% vs. 51.2 ± 2.6%, respectively; $p = 0.09$) and for the average of both top and midequatorial planes (53.8 ± 1.2% vs. 50.9 ± 2.4%, respectively; $p = 0.09$). We therefore propose that mitochondrial membrane function is not compromised in zygotes originating from HIGH NEFA oocytes, but we cannot exclude that a minor shift in metabolic strategy might lead to the mitochondrial dysfunction at later embryonic stages.

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Addition of caffeine and Ca^{2+} to boar semen through a novel insemination catheter improves litter size

J Van Leeuwen-Ibarrola[1], E Schmitt[1], D Reicks[2]

[1]*IMV Technologies, L'Aigle, France;* [2]*Swine Vet Center PA, St. Peter, MN, USA*

Sperm motility can be stimulated by the addition of caffeine to the preservation medium, but storage for a few days in such a combined medium ultimately leads to lower sperm motility. *In vitro* studies from our lab show that the addition of caffeine to semen after preservation also leads to increased motility. The objective of this study was to evaluate whether addition of caffeine and Ca^{2+}, as this is required for capacitation, at the moment of insemination led to improved fertility in a commercial swine herd. Caffeine and Ca^{2+} were dissolved in a gel, which was used to prefill a novel insemination catheter. Sows were inseminated with the Caffeine/Ca^{2+} catheter ($CaCa^{2+}$; n = 209) or with a catheter only containing the gel (no active ingredients) (Ctrl; n = 205). Data were analyzed using the mixed models procedure in SAS. Farrowing rate did not differ between both treatments ($p = 0.22$). Total born piglets tended ($p = 0.06$) to be higher for $CaCa^{2+}$ compared to Ctrl (13.5 ± 0.3 vs. 12.7 ± 0.3 piglets, respectively) and live born piglets was higher ($p < 0.04$) for $CaCa^{2+}$ compared to Ctrl (12.4 ± 0.3 vs. 11.6 ± 0.3 piglets, respectively). The results show that the addition of caffeine and Ca^{2+} to boar semen at the moment of insemination using a novel insemination catheter improves litter size. The authors would like to thank W.W. and M.J. Thatcher for the data analyses.

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A comparative study of the sperm nuclear morphometry in four species of domestic artiodactyls

S Vicente-Fiel[1], I Palacín[1], P Santolaria[1], C Hidalgo[2], M Silvestre[3], F Arrebola[4], F Quintín-Casorrán[5], E Sevilla-Mur[5], J Yániz[1]

[1]*Institute of Environmental Sciences (IUCA), Department of Animal Production, University of Zaragoza, Huesca, Spain;* [2]*Area de Genética y Reproducción, Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA), Gijón, Spain;* [3]*Departament de Biologia Funcional i Antropología Física, Edifici d'Investigació, Universitat de València, Spain;* [4]*Instituto de Investigación y Formación Agraria y Pesquera (IFAPA), Córdoba, Spain;* [5]*Área Técnica de Producción, Selección y Reproducción (ATPSYRA), Gobierno de Aragón, Movera, Zaragoza, Spain*

This study was designed to compare the nuclear sperm morphometry of four species of domestic artiodactyls (cattle, sheep, goat and swine). Samples of 20 males of each species were collected. After semen collection, sperm concentration and motility were measured, and samples were prepared to morphometric determinations. Smears were fixed with 2% glutaraldehyde, stained with Hoechst 33 342 and photographed. At least 200 spermatozoa per sample were processed using the Image J analysis open software. For the majority of the primary morphometric parameters the relationship between the four species for the sperm head dimensions can be described as follows: bull > ram > boar > goat. However, ram sperm heads were broader than in the bull. For the secondary morphometric parameters, ram sperm nucleus were clearly less elliptical and elongated. In addition, different sperm morphologies were observed in bulls and boars, whereas the morphology was more uniform in small ruminants. In the bull, there were two predominant sperm morphologies, normal and elongated, usually present in different ejaculates. In the boar three different morphologies, normal, pyriform and macrocefalic, were observed in the same ejaculate. Supported by MEYC (IPT-010000-2010-33 and AGL2011-30353-C02-01).

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Effects of eCG on preovulatory follicle size, CL and embryonic development in a fixed time AI protocol in Nelore cows

L Vincenti[1], P Pitaluga Costa e Silva Filho[2], A Ricci[3], J Sá Filho[4], A Dogliero[3], P Sampaio Baruselli[4]

[1]*Clinic for Ostetrics and Veterinary Gynaecology, Faculty of Veterinary Medicine, University of Torino, Torino, Italy;* [2]*Department of Scienze Zootecniche, University of Torino, Torino, Italy;* [3]*Department of Animal Pathology, Faculty of Veterinary Medicine, University of Torino, Torino, Italy;* [4]*Department of Animal Reproduction, Faculty of Veterinary Medicine and Zoology, University of São Paulo, Brazil*

The objective of this study was to evaluate the effects of eCG on preovulatory follicle size, CL and embryonic development. A total of 124 Nelore cows, in the state of MT-Brazil, received on Day 0, 2 mg of estradiol benzoate (Gonadiol®) and P4 intravaginal device (CIDR®1.9 gr). On Day 8, the device was removed and all cows were given 0.15 mg of D-cloprostenol (Prostaglandina Tortuga®) and 0.5 mg of estradiol cipionate (ECP®), randomly assigned to two groups: eCG (n = 60), were cows received 300 UI of eCG (Novormon®) and control (n = 64), without eCG. All cows were submitted to an ultrasonography exam to determine the large follicle (LF) diameter on days 8 and 10 and CL diameter on days 15 and 20. Cows were inseminated at D10 and were slaughtered on D26. CL weight, CL diameter, embryonic recovery (ER) and embryonic length (EL) were determined on D26. Embryos were recovered by uterine flushing and measured. CL were recovered by ovarian dissection, weighed and measured using a caliper rule. Data were analyzed by GLIMMIX of SAS. The eCG treatment tended to increase the LF 10 (12.2 mm vs. 11.0 mm; p = 0.06). Also, increased CL 15 (16.3 mm vs. 14.7 mm; p = 0.03), CL 26 (19.6 mm vs. 17.9 mm; p = 0.003) and CL weight (2.80 g vs. 2.45 g; p = 0.04). But was not effective on EL (118.5 mm vs. 98.1 mm; p = 0.23) and ER (29.7% vs. 30.0%; p = 0.97). In conclusion, the eCG increases the CL diameter, CL weight, wich, while having no effect on EL.

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Dynamics of the uterine bacterial flora in postpartum dairy cows monitored by means of FTIR spectroscopy

K Wagener[1], I Prunner[1], M Drillich[1], M Ehling-Schulz[2]

[1]*Section for Herd Health Management, Clinic for Ruminants, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Austria;* [2]*Institute of Functional Microbiology, Department of Pathobiology, University of Veterinary Medicine Vienna, Austria*

Fertility problems due to bacterial uterine infections are one of the most important causes of economic losses in dairy cattle farming. During the first 3 weeks after parturition a broad diversity of bacteria can be isolated from the bovine uterus, including potential pathogens. For instance, *Escherichia coli* and Trueperalla pyogenes (former name: *Arcanobacterium pyogenes*) are well known etiological agents of bovine endometritis, but also other bacterial species have been isolated from the uterus of infected cows. Some studies have shown that early postpartum *E. coli* infections facilitate subsequent T. pyogenes infections, but information on the dynamic and mechanism of the infection course is rather limited, and the role of other uterine bacteria in this process is largely unknown. The objective of this study was to differentiate the most frequently detected bacteria of the bovine uterus and to illustrate the dynamic process of uterine clearance during the first 3 weeks after calving. Over a 3 week period postpartum 40 cows were sampled using a cytobrush-technique. Bacteria were isolated and subjected to Fourier-transform (FTIR-) infrared spectroscopy followed by chemometric analysis. Hierarchical cluster analysis was performed to identify the major bacterial groups and bacteria were identified using spectral libraries as well as additional molecular methods. *E. coli* (25.5% positive samples) and *Bacillus* spp. (21.0%) followed by *Streptocccus uberis* (18.5%), coagulase-negative Staphylococci (11.5%) and *T. pyogenes* (10.5%) were the most frequently detected aerobe microbes. This investigation confirmed that uterine clearance is a highly dynamic process, during which the different groups of microorganisms show a distinct pattern of progression.

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Hyporesponsiveness to intramammary LPS-infusion in post partum cows

K Waldmueller, H Zerbe, W Petzl

Clinic for Ruminants, Ludwig-Maximilian University Munich, Munich, Germany

Postpartum cows carry the highest risk of severe systemic response syndrome caused by intra mammary or intra uterine infection. The aim of this study was to compare the initial immune response of multiparous periparturient cows to primiparous midlactating cows. LPS (1 μg/5 ml 0.9% saline) was infused intra mammarily in all udder quarters of five multiparous cows 36 to 48 h after calving. In addition, the same procedure was performed in five midlactating primiparous cows (86–112 days in milk). The animals of both groups

were clinically healthy and had no history of mastitis or metritis. Signs of a local or systemic response were assessed by monitoring body temperature, general condition and local inflammatory parameters of the udder [milk somatic cell count (SCC), swelling, udder edema, signs of pain] in an interval of 3 h. Despite the high risk of a severe and peracute *E. coli* mastitis, postpartum cows showed only an absent or mild inflammatory reaction to the LPS infusion. Solely a slight increase in the SCC could be detected [starting from 183.5 (119.5–475.5)*10^3 cells/ml to 2338.5 (1613–5360)*10^3 cells/ml milk]. The midlactating cows however showed a faster and stronger inflammatory reaction with a significant higher ($p < 0.05$) increase of the SCC (5597.2 ± 433.2), occasionally few clots in milk, and elevated body temperature (39.6 ± 0.2). These results indicate that the observed compromised immune response might be causative for the reduced pathogen clearance and high bacterial burden in the post partum cows leading to increased incidence and severity of postpartum diseases.

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The practice of intrauterine glass spheres to suppress the oestrus of mares – myth, inflammation or pseudopregnancy?

V Waltl[1], K Müller[1], H Schoon[1], M Rivera del Alamo[2], T Katila[3]

[1]*Institute of Pathology, Faculty of Veterinary Medicine, Leipzig, Germany;* [2]*Unit of Reproduction, Faculty of Veterinary Medicine, Barcelona, Spain;* [3]*Department of Production Animal Medicine, Faculty of Veterinary Medicine, Helsinki, Finland*

Intrauterine devices (IUDs) suppress signs of oestrus in mares. The mechanism is still unknown. However, hypotheses like placebo effect for mare owners, pseudopregnancy and endometritis are discussed. The aim of this study was to investigate COX-2, Uterocalin (UC) and Uteroferrin (UF) expression on endometrial biopsies (day 15 post-ovulation) of 15 mares with IUDs, compared to the expression of 13 mares after artificial insemination (AI) using immunhistochemistry. The mares were subdivided into four groups: G1: AI, pregnant (n = 8); G2: AI, cycling (n = 5); G3: Ball, prolonged luteal phase (n = 7); G4: Ball, normal luteal phase (n = 8). Inseminated mares showed expected expression patterns: the presence of the conceptus blocked the COX-2 expression, UC appeared maximal and UF revealed weak expression (G1), while in cycling mares (dioestrus) a COX-2 expression occurred in the luminal epithel, UC expression was low and UF detection observed maximal (G2). In contrast to the findings in pregnant and cycling mares, animals with balls (G3, G4) showed variable expressions of the proteins and COX-2. The results indicate that the IUDs change protein and COX-2 expression, so a placebo effect seems implausible. As pregnant mares and IUD-mares with prolonged luteal phase stained significantly different for COX-2 ($p < 0.01$) it can be supposed that pseudopregnancy is unlike.

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Effects of treatment with N-acetylcysteine on uterine mucus and endometrium of mares

T Witte[1], E Melkus[1], W Heuwieser[1], C Aurich[2]

[1]*Clinic for Animal Reproduction, Freie Universität Berlin, Berlin, Germany;* [2]*Centre for Artificial Insemination and Embryo Transfer, University of Veterinary Sciences, Vienna, Austria*

Increased amounts of intrauterine (i.u.) mucus can lead to low fertility in mares. *N*-acetylcysteine (NAC) is known to exhibit mucolytic properties. We investigated the effect of an i.u. and oral NAC-treatment on i.u. mucus and the endometrium. In experiment (EXP)1 healthy estrous mares (n = 12) were randomly assigned to a treatment (TM) or a control (C) group and received an i.u infusion of 5% NAC or saline, respectively. Endometrial biopsies were collected 24 h (n = 5) or 72 h (n = 7) after treatment. In EXP2 healthy estrous mares (n = 12) were randomly assigned to a TM (10 mg/kg NAC p.o. on day 1–4) or a C group (no treatment). On day 1 and 5 i.u. mucus was collected and its rheologic properties were accessed. On day 5 endometrial biopsies were obtained. Biopsies of both EXP were evaluated for integrity of the epithelium, number of polymorphonuclear neutrophils (PMN) and expression of cyclooxygenase2 (COX2). The epithelium was not affected in both EXP. At 72 h number of PMN was significantly higher in C compared to TM mares in EXP1 (3.9 ± 0.6 vs. 2.3 ± 0.2 PMN/field; $p < 0.05$). In EXP2, mean number of PMN was significantly lower in TM mares compared to C mares (1.9 ± 0.3 vs. 4.8 ± 0.4; $p < 0.05$). In both EXP, COX2 was significantly lower in TM compared to C mares ($p < 0.05$). In EXP2, viscosity of mucus increased significantly between d1 and 5 ($p < 0.05$) in TM, but not in C mares. The present study demonstrates that NAC does not decrease viscosity of mucus, but has an anti-inflammatory effect on the endometrium.

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Isolation, cultivation and identification of spermatogonial stem cells from adult buffalo testis

H Yang[1], W Zhang[1], F Luo[2], H Zhao[1], L Hu[1], E Zuo[1], S Lu[1], Y Wu[2], K Lu[1]

[1]*College of Animal Science and Technology, Guangxi University, Nanning, China;* [2]*Key Laboratory of China Education Ministry for the Research of Mammal Reproductive Biology and Biotechnology, Inner Mongolia University, Hohhot, China*

The aim of this study was to explore the feasibility of obtaining SSCs from buffalo testis. The testis tissue obtained from a 104-days old Nili-local hybridized buffalo. The SSCs were co-cultured with STO cells in serum-free medium plus several growth factors following the isolation using differential attachment method and the SSCs clusters were appeared on the third day after co-cultured. The characteristics of SSCs of different passages were analyzed by RT-PCR and a comparison of immunofluorescence staining between the cultured SSCs and the SSCs *in vivo* was made to analysis expression of some SSCs marker proteins. RT-PCR analysis revealed that Oct-4 and PGP9.5 mRNA were transcribed in the cultured SSCs. The positive expression of SSC marker proteins such as C-KIT, PGP9.5 and CDH1, were equally observed in the SSCs cultured *in vitro* and the SSCs in the testis tissue of frozen section indicating that these *in vitro* cultured cells were commendably maintained the characteristics of *in vivo* stem cells. These results indicate that we have preliminarily established *in vitro* culture system of the buffalo spermatogonial stem cells of which can facilitate its practical application and theoretical research in the future.

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Comparison of four fluorochrome combinations for sperm membrane integrity assessment in ram

J Yaniz[1], J Yániz[1], I Palacín[1], S Vicence-Fiel[1], J Gosalvez[2], C López-Fernández[2], P Santolaria[1]

[1]*Universidad de Zaragoza, Huesca, Spain;* [2]*Universidad Autónoma de Madrid, Spain*

This study was designed to compare the results of ram sperm membrane integrity using different fluorochromes combinations (carboxyfluorescein diacetate, CFDA/propidium iodide (PI); SYBR-14/PI; Hoechst-33342; HOE/PI, and acridine orange, AO/PI). A first trial was designed to study the optimal dye concentration and the minimum incubation time required to achieve optimum fluorescence intensities

and contrast for each fluorochrome combination using ram sperm samples. The optimal dye concentration required to achieve optimum fluorescence intensities and contrast for each fluorochrome combination were: 10 μM CFDA, 0.3 μM SYBR-14, 5 μl/ml AO solution from the DUO-VITAL kit, and 32.4 μM of H342. In all cases, a 15 μM PI solution was enough to achieve good fluorescence images identifying dead sperm. SYBR/IP and AO/IP combinations allowed a direct assessment of sperm membrane integrity, whereas a minimum of 4 and 6 min of incubation at 37°C was necessary with the CFDA/IP and HOE/IP groups, respectively. In the second experiment, we compared the results of sperm membrane integrity using the four fluorochrome combinations. The proportions of plasmalemma-intact sperm determined by AO/IP and SYBR/IP were greater ($p < 0.0001$) than the proportions of intact sperm determined by CFDA/PI and HOE/PI stains.

Supported by MEYC (IPT-010000-2010-33 and AGL2011-30353-C02-01).

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Improving quality of frozen-thawed Mangalitsa boar semen using different antioxidants supplementation

M Zahan, F Varo-Ghiuru, A Hettig, I Miclea, V Miresan, I Roman, V Miclea

University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania

In the present study we evaluated the effect of antioxidant supplementation of freezing extender on boar semen characteristics in order to reduce oxidative stress induced by reactive oxygen species produced in the time of freezing. For these purpose, 200 μM Trolox, 10 μM lutein and the mix between 200 μM ascorbic acid and 400 μM Trolox were added to lactose-egg yolk extender, containing a final concentration of 4.5% glycerol and 0.75% Equex-STM. Antioxidant concentrations were chosen on the basis of our previous studies. Eight ejaculates from two Mangalitsa boars were cryopreserved using the straw-freezing procedure described by Westendorf et al. (1975). After cryopreservation semen was thawed at 50°C for 12 s and was evaluated for progressive motility, acrosome integrity, plasma membrane integrity by hypo-osmotic swelling test (HOST) and DNA fragmentation index (DFI) using the Sus-Halomax® kit. Data were analyzed by one-way ANOVA. The results showed better motility (at 30 min after thawing) and acrosome integrity for Trolox (58.88% and 77.25%), lutein (63.75% and 78.50%) and the mix of vitamins (65.25% and 79.13%) in comparison with the control group (49.16% and 64.38%; $p < 0.001$). In addition, sperm membrane functionality and DNA integrity were better protected by lutein (20.88% and 14.12%) and the mix of vitamins (19.13% and 13.75%) in comparison with Trolox (16.00% and 18.62%) and the control (14.88% and 20.37%). In conclusion, the analysis of sperm characteristics showed that these new antioxidants (lutein and mix between ascorbic acid and Trolox) used in boar semen cryopreservation can improve the quality of spermatozoa, with a significant benefic effect on motility, protects the acrosome and plasma membrane integrity and also reduces DNA damage. Based on our data, such approaches should improve boar semen quality after freezing storage. Supported by ADER 2020 project no. 115/2011 and USAMV grant no. 1215/9/2012.

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Cumulus cell layers as a critical factor in meiotic competence

S Zeinoaldini, N Dadashpour Davachi, H Kohram

Tehran University, Karaj, Iran

A critical stage in the optimization of *in vitro* maturation (IVM) is the selection of good quality oocytes. There exists a relationship between the size of the cumulus investment and the *in vitro* developmental ability of the cumulus–oocyte complex (COC), which provides a basis for the selection of the COCs. This study was designed to evaluate the effect of the number of cumulus cell layers which enclose the oocytes, on the *in vitro* maturation, cytoplasm quality and cumulus expansion of the ovine oocytes. Ovaries were obtained from an abattoir and transported to the laboratory within 1–2 h, at 37°C. Oocytes ($n = 535$) were recovered by means of an aspiration pump (set at a flow rate of 10 ml H_2O/min), with a disposable 20 G needle attached. Oocytes were divided into four classes (classes I–IV – with more than 5, 3–4, 1–2 and no cumulus cell layers, respectively) and separately cultured in a TCM199 medium for 24 h. The morphology of oocytes was evaluated following *in vitro* culture (IVC) to assess cumulus expansion, cytoplasm quality (score I with a homogenous cytoplasm and II with granulated cytoplasm) and nuclear maturation stage. The percentage of maximum cumulus expansion for classes I to III oocytes were 53.0 ± 1.0%, 36.3 ± 2.2% and 16.3 ± 1.8% respectively. The rate of meiotic resumption of oocytes in classes I to IV were 77.0 ± 2.7%, 77.2 ± 1.9%, 53.0 ± 2.1% and 2.7 ± 1.1% respectively. The proportion of oocytes with a cytoplasm quality I in oocyte classes I to IV were 62.8 ± 1.5%, 59.4 ± 1.2%, 36.4 ± 2.1% and 0.5 ± 1.1%, respectively. Results showed that the presence of ≥3 cumulus cell layers in the COC prior to IVM led to a better ($p < 0.05$) cumulus expansion, meiotic resumption and cytoplasmic maturation rate. Thus the morphological grading of immature ovine oocytes may be an appropriate selection criterion regarding their developmental ability.