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Oral communications

OC 1.1 | Milk fatty acids can predict delayed commencement of luteal activity in dairy cows

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Early commencement of luteal activity (CLA) after calving will result in a high conception rate, decreased proportion of cows with extended lactations and less culling due to reproductive disorders. We investigated milk fatty acids (MFA, indicators of lipolysis) as predictors of CLA in 2 groups of dairy cows (Holstein $n = 37$, Swedish Red breed $n = 49$). Concentrations of MFA were analysed by Fourier transform spectroscopy twice weekly until week 6 post-partum and their proportion of the total milk fat was calculated. Blood samples for insulin-like growth factor-1 (IGF-1), β-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) analyses were collected once weekly. The CLA was defined as milk progesterone concentrations >3 ng/ml for 2 successive measurements. Cows were categorized as early ($n = 42$) or late ($n = 44$) CLA, using the median day 21 as cut-off. Frequency analysis and mixed linear models were used to analyse data (SAS 9.4). Cows with delayed CLA had lower IGF-1 (92.9 ± 7.9 vs. 114.1 ± 7.9, ng/ml; $p = .05$), higher BHB (0.6 ± 0.03 vs. 0.7 ± 0.03, mmol/L; $p = .05$), higher NEFA (0.26 ± 0.01 vs. 0.31 ± 0.01, mmol/L; $p < .001$), lower C14:0 (10.4 ± 0.2 vs.11.5 ± 0.2 g/100 fat; $p < .01$) and higher C18:0 (9.6 ± 0.2 vs. 8.9 ± 0.6 g/100 fat; $p < .01$) and C18:1 cis-9 levels (24.9 ± 0.4 vs. 23.5 ± 0.4 g/100 fat; $p < .05$). Approximately, 4 out of 5 cows with delayed CLA could be predicted week 2 post-partum based on C18:0 or C18:1 cis-9 concentrations. Concentrations of C18:0 and especially C18:1 cis-9 were the most suitable variables and a potential future tool for early prediction of delayed CLA compared with blood plasma biomarkers, with implications for management of negative energy balance which is important for herd management and decisions for treatment and prevention on individual and herd level.

OC 1.2 | Metabolic predictors of reproductive tract disease in seasonally calving pasture-based cows

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Metabolic stress during the transition period in dairy cows causes immunosuppression, predisposing to the development of reproductive tract disease (RTD) [1]. The objective of this prospective observational study was to investigate which predictors of metabolic disturbance had the greatest risk for the development of RTD at 3 weeks post-partum. Data generated from 402 spring calving dairy cows from 5 herds that were enrolled in an observational clinical trial [2] were analysed. The hypothesis was that some indicators of metabolic stress may be more predictive of RTD development than others. Blood samples were collected in the pre-partum and early post-partum and analysed for serum calcium, non-esterified fatty acids (NEFAs), beta-hydroxybutyric acid, urea and glutathione peroxidase. Cows were examined for RTD at 23 ± 5 and 32 ± 4 days post-partum using both visual assessment of vaginal discharge and transrectal ultrasonographic evidence of endometritis. Statistical analyses were performed using univariable and then multivariable logistic regression, with herd included as a random effect. The metabolic indicator that showed the greatest risk for RTD was increased NEFAs above a threshold ≥0.4 mmol/L in the 15 to 5 days prior to calving. Dairy cows with blood NEFAs above this threshold were at much greater risk of having a positive diagnosis of RTD by either Metricheck or ultrasonography at 23 ± 5 days post-partum [adjusted odds ratio (95% CI) = 2.7 (1.6–6.2) or 4.0 (1.8–9.0), respectively]. The result of the present study reflects the importance of nutritional management during this critical period to reduce the incidence of reproductive tract disease. [1] Dubuc et al. 2010; J Dairy Sci 93(12): 5764; [2] McNally et al. 2014; Theriogenology 82(9): 1263.

OC 1.3 | Activation of hyaluronan synthases in equine endometrium affected by perivascular and degenerative endometrial fibrosis

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Degenerative endometrial fibrosis (endometrosis) is a major problem in equine reproduction, and affects endometrial glands as well as blood and lymphatic vessels. The study aimed to show correlations between the transcription of three hyaluronan synthases and the area of perivascular fibrosis concerning the degrees of equine endometrosis. From corpus uteri ($n = 24$), two endometrial samples were collected in liquid nitrogen and formalin. In frozen samples, the mRNA expression of hyaluronan synthases (HASs 1–3) was measured using qPCR, whereas formalin-fixed samples were histologically processed, cut and stained with Masson's Trichrome (MT) and haematoxylin–eosin (HE). The area of perivascular fibrosis (PF; μm^2) was quantified on MT-stained slides using TissueFaxsPlus and HistoQuest software. Endometria were classified into I, IIA, IIB and III categories on HE-stained slides, and results were compared between categories using the Kruskal–Wallis test. Values (mean ± SEM) of PF (III: 4313 ± 897 μm^2; $p < .0001$), as well as transcript levels of HAS 1 (III: 2.57 ± 0.35 AU; $p = .02$) and HAS 3 (III: 4.76 ± 0.76 AU; $p = .002$), but not HAS 2 (III: 2.30 ± 0.30 AU; $p = .25$) were higher in category III than in other categories (I, IIA and IIB). The measures did not differ between I, IIA and IIB endometrium categories; thus, the mean values were estimated as PF: 1264 ± 171 μm^2, HAS 1 1.93 ± 0.22 AU, HAS 2 1.83 ± 0.18 AU and HAS 3 2.47 ± 0.28 AU, respectively. Spearman correlations between PF and HASs were significant only for PF and HAS 1 in category IIB ($\rho = .57$; $p = .04$), PF and HAS 1 in category III ($\rho = .51$; $p = .03$), as well as PF and HAS 3 in category III ($\rho = .64$; $p < .0001$). The endometrosis-related perivascular fibrosis in mares' endometrium may be mediated by hyaluronan synthases 1 and 3.

OC 1.4 | The role of the fractal dimension domain in IRT-based noninvasive pregnancy differentiation in mares

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Infrared thermography (IRT) is suggested as noninvasive tool for pregnancy detection in horses, concerning monitoring the free-range wild equids. Conventional IRT imaging of the mares' abdomen gives little information about the month of pregnancy apart from pregnant and non-pregnant mares' differentiation. Therefore, this study aimed to transform the IRT image intensities of the mares' abdomen to the fractal dimension (FD) domain for pregnant mares' differentiation based on the texture analysis of the fractal features. A total of 40 Konik Polski mares were divided into two groups: (i) non-pregnant (NP, $n = 14$) and (ii) pregnant (P, $n = 26$). IRT images were taken once a month from 4th to 11th months of pregnancy. The regions of interest (ROIs) representing the lateral surface of the mares' abdomen were manually segmented and digital IRT images were transformed to FD values for each pixel. Then, two fractal features were computed: (i) the FD average (FDavg), which is the maximum selected from an average FD value in each ROI, and (ii) lacunarity (LAC), which represents the degree of nonhomogeneity. The NP and P groups (Mann-Whitney test) differed significantly based on both fractal features (mean ± SD) (FDavg: NP = 1.72 ± 0.02, P = 1.69 ± 0.02, $p < .0001$; LAC, NP = 0.07 ± 0.005, P = 0.06 ± 0.007, $p < .0001$). Moreover, pregnant mares' detection was possible from 5th month of pregnancy based on both FDavg ($p = .004$) and LAC ($p = .026$). Within the P group, a gradual decline (Kruskal–Wallis test) in FD values was observed from 6th month for FDavg ($p = .0001$) and 8th month for LAC ($p = .030$). Conventional IRT aims in late pregnancy detection, while the FD assesses the potential for mares' abdomen fractal features through IRT images to provide a differentiation of both pregnancy state and months of pregnancy.

OC 2.1 | Evaluation of short-term storage of canine semen at room temperature

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Keeping dog raw semen at room temperature would prove convenient for practitioners, but validated information regarding short-term storage of semen is scarce. 0.4 ml of sperm-rich fraction from 10 healthy beagles (age 1–7 years) were collected by digital manipulation, pooled and divided into 3 groups: (1) dilution 1:3 with homemade Tris-fructose 20% egg yolk, (2) dilution 1:3 with a commercial egg yolk-based extender (CaniXCell®) and (3) a sample left undiluted. One half of samples were stored at room temperature (RT, 22°C) in the dark and the other half in the incubator at 37°C. Samples were submitted to microscopy and CASA (IVOS IITM), at 6 time points post-collection: 0, 12, 18, 24, 36 and 48 h. Progressive Motility (PM) was assessed at all times and percentage of wobble movements was evaluated, as a marker of hyperactivation, if PM was higher than 30%. ANOVA 2 model was used to assay effect of time and medium on different CASA parameters. The initial PM at T0 averaged 85%. PM dramatically dropped below 6% in all groups at 37°C and further evaluation was discontinued. At RT, PM was still higher than 70% and 50% at 12 and 24 h, respectively, in all groups. Surprisingly, non-diluted semen still showed 18% of PM after 48 h, while it was nil in both diluted groups. Percentage of wobble movements was significantly lower at 24 h in non-diluted semen than in diluted groups. The premature hyperactivation of diluted semen probably explains the shorter survival time in these samples. No bacterial contamination was observed under microscopy throughout the experiment. These results suggest that short-term storage of good quality undiluted semen at room temperature in the dark may be adequate to inseminate a bitch within 12 h.

OC 2.2 | Relative abundance of mRNA for porcine protamines 1 and 2 following bicarbonate-triggered in vitro sperm capacitation

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Protamine replacement of sperm histones and formation of disulphide bridges during epididymal maturation compact and protect sperm chromatin. Alteration in these processes decrease fertility. This study tested the effect of in vitro capacitation, triggered by bicarbonate, on the expression of porcine sperm protamine mRNA (PRM1 and PRM2). Commercial semen doses from 3 boars of proven fertility (5 batches each) were incubated (30 min, 38°C, 5% CO2) in capacitation medium (37 mM NaHCO3, 2.25 mM CaCl2, 2 mM caffeine, 0.5% BSA, 310 mM lactose). Sperm pellets (5000 *g*, 5 min, RT) from control (not incubated, CTL) and capacitated (CAP) were stored at −80°C until analysis for PRM1 and PRM2 mRNA relative abundance (RNA extraction and qPCR; GAPDH as reference gene). PRM1 mRNA abundance did not change in CAP (1.56 ± 0.35 vs. CTL, 1.00 ± 0.60), whereas PRM2 mRNA and PRM2/PRM1 expression ratio decreased (p = .046; CAP, 0.56 ± 0.06 vs. CTL, 1.00 ± 0.18; p = .035, CAP 0.24 ± 0.16 vs. CTL 1.00 ± 0.34, respectively). Although boar spermatozoa lack PRM2 protein (transcribed but not translated), mRNA presence and the changes caused by in vitro capacitation could lead to larger accumulation. Whether PRM2 mRNA changes relate to sperm fertility require further studies of boar cohorts. Funded by FORMAS (2019-00288), Sweden; IJCI-2015-24380 (MCIN/AEI/10.13039/501100011033), RTI2018-095183-B-I00, ID2019-108320RJ-I00 (MINECO-MCIN/AEI), LE023P20 (Cons. Educación JCyL), Spain, and FEDER/EU.

OC 2.3 | Refrigerated storage alters boar sperm DNA compaction as determined by the reduction of disulphide bonds and chromatin decondensation

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The early detection of subfertile boars is essential to minimize the economic losses in farming. Subfertility has been linked to a certain grade of DNA instability, protamine deficiency or incomplete compaction (deficiency in inter-protamine disulphide bridges) that usually goes unnoticed in field conditions. This work evaluated the chromatin integrity in boar spermatozoa from artificial insemination (AI) doses stored for 11 days at 17°C. Free thiols were labelled with the monobromobimane (mBBr) fluorochrome and the chromatin decondensation by the fluorescent chromomycin A3 (CMA3). AI doses from boars ($n = 18$) were analysed by flow cytometry for mBBr (500 μM, 10 min, 37°C) and CMA3 (50 μM, 20 min, RT) at day 0 (D0) and 11 (D11) at 17°C. The relative abundance of disulphide bonds (DB) and the mean fluorescence intensity of CMA3 (MFI-CMA3) were determined and analysed by linear mixed-effects models and Pearson correlation. After the storage, DNA stability and compaction were significantly ($p < .001$) reduced since DB decreased (D0: 32.76 ± 2.67; D11: 22.63 ± 3.32) and MFI-CMA3 increased (D0: 1.07 ± 0.03; D11: 1.09 ± 0.03). DB negatively correlated with MFI-CMA3 ($r = -.32$, $p = .007$). Alterations of sperm DNA integrity are evidenced by these novel techniques, potentially useful for the early detection of subfertility and AI underperformance. Future work will aim at assessing these parameters in boars of differing fertility, which would be useful to improve the reproductive efficiency in the pig industry. Funding: RTI2018-095183-B-I00 (MINECO/AEI/FEDER, EU), LE023P20 (Junta de Castilla y León/FEDER, EU). Thanks to Guillermo Rivas and Topigs-Norsvin (Spain).

OC 2.4 | Polymers as a promising alternative to antibiotics in canine semen extender

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Antibiotics are added to semen extenders to prevent bacterial growth, as this might compromise the quality of semen during storage and be a potential source of contamination for the female after artificial insemination. However, alternatives to antibiotics are at present under investigation since antibiotics are potentially toxic for spermatozoa and may induce antimicrobial resistance [1]. This study evaluated the effect of a new product, PolySE (Biodesiv, Strasbourg, France), composed of polymers bearing antimicrobial moieties on dog semen freezability. Semen from 10 dogs (one ejaculate per dog) was collected, divided into two aliquots and cryopreserved either in presence of antibiotics (gentamycin 1 g/L) or polymers (PolySE 10 mg/L). After thawing, semen motility, velocity parameters, morphology, plasma membrane integrity, mitochondrial membrane potential, and acrosome integrity were assessed in both groups. Results were analysed using a linear mixed effect model, and dog was taken as a random factor. Total motility was significantly higher when spermatozoa were cryopreserved in presence of PolySE in comparison to gentamycin, although progressive motility and velocity parameters did not differ between the two groups. Similarly, the other parameters investigated were not affected by the treatment. In conclusion, PolySE appears to be a potential alternative to antibiotics in the cryopreservation of canine semen, although further research is needed to evaluate its effect on semen fertility and on microbial contamination of the ejaculate. [1] Morrell, J. M., 2014, Pathogens, 3, 934–946.

OC 3.1 | Uterine fluid contains factors inhibiting myeloperoxidase enzymatic activity in mares

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Myeloperoxidase (MPO) is a marker of neutrophil activation and a potent microbicidal enzyme. However, it can potentially lead to undesirable damage of host cells by the same processes involved in the destruction of pathogens. In the uterine lumen of estrus mares, MPO is constantly detected at variable concentrations even in the absence of neutrophils. The aim of this study was to investigate whether MPO is enzymatically active (capacity to catalyse the peroxidase reaction), to better understand the consequence of its presence in the equine endometrium. Low-volume lavages from mares in

estrus with a histological detection in endometrial biopsies of three or less neutrophils per field (400×) were included (n = 26). MPO and its enzymatic activity were determined by a commercial equine ELISA kit and specific immuno-extraction followed by enzymatic detection (SIEFED), respectively. MPO was detected in all samples at variable concentrations (830.5–55,350 ng/ml) but the active form was marginally represented (0–7.7 ng/ml) and not correlated with total MPO concentration. Our results confirm a constitutive presence of MPO in the uterine lumen of mares during estrus in absence of inflammation, concordant with a possible physiologic role. However, the inactivity of MPO suggests the presence of inhibitory factors in uterine fluid, probably to prevent its harmful actions on the endometrium. Further studies are necessary to confirm the inhibitory potential of the uterine fluid and to understand the role of MPO in equine reproduction.

OC 3.2 | Metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) epigenetic signature in mare endometrosis

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Endometrial fibrosis, as the main feature of endometrosis, might result from the imbalance between metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs); and between fibrolysis and fibrogenesis. Since epigenetics are involved in human fibrotic disorders, we hypothesized that epigenetic mechanisms may modulate equine endometrosis. Thus, we assessed if transcription of some genes involved in equine endometrosis were epigenetically modulated, through DNA methylation. Endometrial biopsies (n = 24) from cyclic mares were graded by Kenney and Doig's classification (n = 6/category). Transcripts and DNA methylation of MMP2, MMP9, TIMP1 and TIMP2 were evaluated by qPCR and bisulphite pyrosequencing, respectively. Both MMP2 and MMP9 transcripts decreased with fibrosis with respect to healthy endometrium ($p < .05$) and TIMP1 transcripts raised in category III, compared to category I ($p < .05$). Methylation levels of MMP2 were higher in category III, with respect to category I ($p < .001$) and IIA ($p < .05$), and higher in MMP9 in category III compared to category I and IIA ($p < .05$). Between endometrial categories TIMP2 mRNA levels or TIMP1 and TIMP2 methylation levels did not differ. For both MMP2 and MMP9 genes, there was a negative correlation ($p < .05$) between their methylation and transcription levels with higher methylation percentage observed together with decreased transcription of those genes, with endometrial fibrosis progression. Our results show an epigenetic modulation of equine endometrosis that might occur through the inhibition of MMP2 and MMP9 genes rather than TIMP1 and TIMP2 genes. Therefore, these might be promising targets for therapeutic use by reversal of epigenetic changes.

OC 3.3 | Prevalence of *Taylorella equigenitalis*-positive samples in Icelandic stallions

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Contagious Equine Metritis (CEM) is a venereal disease caused by *Taylorella equigenitalis* (TE) with subclinical infections and the potential to cause short-term infertility in the mare. Transmission occurs commonly venereal and poses a risk for horses in natural mating programmes. The aim of this study was to investigate the prevalence of TE in intact males of Icelandic breed compared to a control group. We hypothesized that there is a significantly higher prevalence of TE in the Icelandic stallions. Therefore, 162 intact males from 41 randomly selected premises in southern Germany and Austria were examined. Swabs from recommended localizations of the male genital tract were collected and TE was determined using quantitative PCR. Samples of Icelandic intact males (n = 76) were compared with the control group (n = 86) consisting of 35 Haflinger and 51 Draft intact male horses (logistic regression). There was a significant higher prevalence of TE-positive samples in Icelandic intact males (n = 23) compared to the control group (n = 4; p = .0001). Both groups were further differentiated regarding their use for breeding. Pairwise comparison of both groups (Benjamini Hochberg correction) resulted in a significantly lower number of TE-positive intact males used for breeding compared to the horses without active breeding (control group: p = .019; Islandic group: p = .006). The hypothesis could be confirmed that in the Icelandic breed, a higher prevalence of TE-positive intact males exists. The spread of CEM is of economic interest and should be considered even in horses never used for breeding. Further studies in mares and probably also geldings might help to understand further ways of transmission.

OC 3.4 | Recombinant production of equine chorionic gonadotropin

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Equine chorionic gonadotropin (eCG) or pregnant mare serum gonadotropin (PMSG) is a glycoprotein hormone that displays both follicle stimulating hormone (FSH) and luteinizing hormone (LH) activities in non-equids. It is extensively used in livestock reproduction. Commercial eCG production depends on its extraction from the blood of pregnant mares with disadvantages including potential co-contaminants, animal welfare issues and inter-batch variations. Glycosylation, particularly sialylation, is important for the half-life and, consequently, the efficacy of the hormone. This study aims to produce a recombinant eCG (reCG) to substitute PMSG. The open reading frames (ORFs) encoding the alpha- and beta subunits of eCG were used to construct a tethered eCG expression vector in which the 5′ end of the alpha-subunit was fused to the 3′ end of the beta-subunit. The construct was transfected into CHO-K1 cells for expression. An indirect immunofluorescence assay (IFA) targeting the C-terminal 8xHis-tag of the tethered eCG protein as well as an eCG ELISA were used to assess expression. Successful transient and stable expression was observed using the two methods. Recombinant eCG was secreted into the medium. Purification was done using a 1 ml HisTrap column and western blot analysis was performed to verify the size of the recombinant protein. The C-terminal, 8xHis-tagged, tethered eCG could be detected via western blot and the apparent molecular weight was approximately 50 kDa. The results obtained demonstrate that the recombinant eCG represents a potential PMSG substitute, which will eliminate issues around PMSG extraction from blood.

OC 4.1 | Associations between heat stress, periparturient disorders and reproductive performance in dairy cows

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Dairy cows experience heat stress (HS), which dramatically decreases fertility. This study was designed to assess the effect of heat stress under central European conditions (i) on the occurrence of periparturient diseases and (ii) on reproductive performance. A total of 1825 visually healthy Holstein-Friesian cows were enrolled on a commercial dairy farm. Production-related diseases and fertility parameters were recorded for each animal. Cows were diagnosed with puerperal metritis (PM) and subclinical ketosis (SK) on day 5 post-partum (pp), and examined for signs of clinical and subclinical endometritis (CE and SE) on day 28 pp. Ambient temperature and relative humidity were recorded every 30 min by calibrated data loggers (Tinytag-Gemini Datalogers Ltd., Chichester, UK) placed in the barns. The time and amplitude of the temperature humidity index (THI) exceeding the threshold of 68 were calculated. For the analyses, animals were merged into three groups based on THI values: 'no-HS' (without HS, $n = 491$), 'low-HS' (below median, $n = 665$) and 'moderate-HS' (above median, $n = 669$). HS increased the risk for SK (Odds Ratio (OR): 1.51; CI: 1.26–1.80; $p < .05$) but was not related to increased risk of PM, CE and SE. The occurrence of CE was associated with a prolonged calving to conception interval ($p < 0.05$). Cows under HS had a decreased chance for pregnancy after first artificial insemination (AI; OR: 0.83; CI: 0.72–0.96; $p < 0.05$). HS was not associated with calving to conception interval and time to first AI. In conclusion, exposure to HS increased the risk for SK and had a negative effect on first service conception rate. Further research is needed to better understand the effect of HS on the establishment of pregnancy and embryonic mechanisms in European climate zone.

OC 4.2 | Study of influential factors on the expression of seasonal subfertility in pigs

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Seasonal subfertility in pigs is triggered by climate conditions such as high temperature and long photoperiod and leads to reduced reproductive performance due to reduced litter size (LS), delayed or no onset of oestrus in gilts and sows, as well as low boar fertility. Seasonal subfertility can occur from June to November. In order to investigate the extent in which influential factors affect the reproductive output of a sow herd, 3525 matings were observed over a period of 5 years in a 260-sow commercial sow unit. The factors examined were month, season (defined as calendar quarter) and year of insemination, temperature, boar (8 boars, 3 groups) and parity number. The reproductive output was assessed as farrowing rate (FR) and LS. Crosstabulations, chi-square and ANOVA were used adequately for statistical analysis. The results showed that there was a statistically significant difference in FR between seasons, with the lowest results obtained in the 3rd and 4th quarters (82.5% and 81.8%, respectively).The month and year of the mating were also significant factors for FR, with the lowest FR in October (75.9%) and the year 2017 (78.1%). The latter coincided with the significant effect of the highest temperatures (40.3°C, July 2017) and the use of the boar with the lowest single-sire FR (80.7%). Month, season and year were the significant factors for LS, but their effect did not correlate with high temperatures. The results of the present study demonstrate that month, season, year of mating, as well as temperature can significantly affect FR in sows. Boar fertility may also be depressed, in which case, low FR can extend in the fourth quarter.

It is concluded that seasonal subfertility in pigs can be significantly dependent on the effect of single influential factors.

OC 4.3 | Using simulation-based methods to teach vaginal cytology techniques in Veterinary Medicine – a multicentre study

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Vaginal cytology (VC) is an essential technique in reproductive medicine. Learning this procedure usually requires animals (live or cadavers). Currently, the increased animal welfare awareness promotes animal-free teaching methodologies. This study aimed to test a teaching strategy with a VC simulation model and augmented reality tools. Students' perceptions were evaluated through questionnaires that assessed satisfaction, motivation, confidence, expectations and impact on learning. One hundred and sixty-two students from 4 universities were enrolled. Students watched short videos of owners reporting clinical reproductive stories. After watching a short video explaining the VC technique, students practiced the procedure in simulators, collecting material and obtaining slides. Then, slides were viewed on microscope through augmented reality. Students identified proestrus, estrus, diestrus, anestrus and vaginitis (based on contents of previous theory classes and books). Before the simulation activity, most students (>95%) had little to no experience on VC. Over 99% of students considered that training VC was essential for long term application and >70% reported that repeating the procedure was the most important parameter for efficient learning. Microscopy slide videos enhanced the ability to identify vaginal epithelium cell types of and estrus cycle stage. Overall, students considered the simulation as an essential/highly relevant tool for learning VC in the bitch. Simulation activity improved the students' skills to perform VC in a safe and controlled environment, allowing repetition until students felt comfortable with the technique.

OC 4.4 | Expression of GnRH and kisspeptin receptors in the ovary and uterus in deslorelin treated late-prepubertal bitches

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Kisspeptin (KISS1) and its G protein-coupled receptor (KISS1-R) play key roles in the control of reproduction. Although it is well known that GnRH secretion is regulated by KISS1, studies of this hormonal cascade during the onset of puberty in dogs are limited. Therefore, we aimed to investigate the mRNA expression of GnRH, GnRH-R KISS1 and KISS-R in the ovary and uterus after induction of puberty with the GnRH agonist deslorelin (De). Uterine and ovarian samples (on the day 35 after implant insertion) from 25 healthy, mixed breed bitches aged 7.8 ± 0.2 months were used. The experimental bitches received 4.7 mg De implants (Suprelorin®, Virbac, F; $n = 16$) and the controls, a placebo ($n = 9$, CONT). Six De-treated bitches showed estrus symptoms (E), while 10 had no clinical signs (NON-E). Statistical analysis was performed using a one-way analysis of variance (ANOVA), and the pairwise differences were evaluated using Tukey's range test. In the canine uterine samples, De treatment exclusively changed GnRH1 expression. In detail, GnRH1 was strongly decreased in E and NON-E, compared with CONT animals. Interestingly, in ovarian samples, KISS1 and KISS1R expression were affected by De treatment ($p < .05$). Both factors showed greater expression in NON-E and CONT animals, compared with E group. As a conclusion, De treatment may be a valuable alternative to induce oestrus in prepubertal female dogs. However, changes in the expression of KISS1 and KISS1R in the ovary suggest that the long-term effect of De on reproductive activity and ovarian functionality in prepubertal dogs could vary individually. Thus, future studies investigating induction of puberty in bitches should consider further different factors playing important roles in ovarian physiology and functionality.

OC 5.1 | How is immune status connected with arachidonic acid metabolism in the uterus of red deer females (*Cervus elaphus* L.) in different reproductive stages?

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The immune status depending on the reproductive stage has not been outlined so far in seasonally reproduced ruminants such as red deer females. The aim is determination of selected CD cells in T and B lymphocytes in blood (flow cytometry); concentration of cAMP and a metabolite of prostaglandin (PG)I2–6-keto-PGF1α in blood plasma (ELISA); and the protein expression of PG endoperoxide synthase 2, 5-lipoxygenase (5-LO), PGE2 synthase (PGES), PGF2α synthase (PGFS), PGI2 synthase (PGIS), leukotriene (LT) A4 hydrolase (LTA4H), LTC4 synthase (LTC4S) in uterine endometrium (Western blotting) on 4th ($n = 6$), 13th ($n = 8$) day of the estrous cycle, anestrus ($n = 5$) and pregnancy ($n = 8$). Results were analysed using two-way analysis of variance followed by a Tukey's test. An increase in CD4+ percentage of lymphocytes during the estrous cycle and anestrus comparing with pregnancy was observed, the opposite effect was received for CD8+ and CD21+ between the experimental groups ($p < .05$). cAMP concentration was elevated during the estrous cycle and pregnancy comparing with anestrus, whereas 6-keto-PGF1α concentration was the highest in pregnancy and the nearest in anestrus ($p < .05$). LTA4H, LTC4S, PGES, PGFS and PGIS protein expression was the highest in pregnancy compared to estrous cycle and anestrus ($p < .05$). 5-LO protein expression was higher during the cycle compared to other phases ($p < .05$). We showed interaction between activation of immune system and AA metabolites production in uterus throughout different reproductive stages in hinds. Measurement of cAMP and to 6-keto-PGF1α concentration reflects those fluctuations in the peripheral blood. The results have potential in recognition of reproductive pathologies both in farmed and wild red deer. Financed from NCN OPUS 2017/25/B/NZ9/0254.

OC 5.2 | Oestrus length and mounting and standing behaviour in Norwegian Red cattle

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The aim of the study was to describe mounting and standing behaviours through oestrous cycle in Norwegian Red cattle. An observational study was performed in a commercial herd of 89 Norwegian Red cows housed in free stalls on concrete, slatted floors. The cows were monitored by continuous video recordings for 21 days. All mounting and standing activities were recorded and the consecutive stages of mounting oestrus; pre-stand, stand and post-stand were determined. The cycle stages metoestrus, dioestrus and proestrus were estimated based on the starting time and ending time of mount oestrus. Ovarian cyclicity was confirmed in the final study group ($n = 18$) by milk progesterone concentration analysis. All cows in the final study sample group exhibited the primary oestrous sign, 'standing to be mounted' during oestrus. Two (11%), 11 (61%) and six (33%) cows exhibited the behaviour 'standing to be mounted' during metoestrus, dioestrus and proestrus, respectively. The number of mounts initiated by individual cows was higher during mounting and standing oestrus than during the rest of the oestrous cycle. This study reports a median duration of mounting oestrus and standing oestrus of 21.0 h (interquartile range [IQR] 15.0–27.3) and 14.3 h (IQR 12.0–18.8), respectively. The median counts per hour of all mounting behaviours were 8.6 (IQR 5.6–11.3), 1.51 (IQR 0.3–3.8) and 1.7 (IQR 0.8–6.0) for standing oestrus, pre-stand and post-stand, respectively. This study shows that under commercial conditions, the Norwegian Red cow displays a high number of oestrous behaviours when compared to other breeds in similar studies.

OC 5.3 | Effect of twinning on milk fat-to-protein ratio and survival in dairy cattle

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Twinning in dairy cattle is linked to increased risk of health problems. Cows in early lactation are in negative energy balance and twins place additional strain on the cows' energy demand, which is difficult to recover from. We studied the effect of twinning on cows' milk fat-to-protein ratio (FPR) and if FPR within the first 2 weeks of lactation is associated with subsequent risk of culling during the lactation. We analysed lactation records from Estonian Livestock Performance Recording Ltd from 304,079 Estonian Holstein cows over 741,135 lactations (252,479 primiparous, 488,656 multiparous) from 2011 to

2020. FPR was higher during the first 6–9 days in milk (DIM) for twin calved (TC) cows than for single calved (SC) cows at all lactations ($p < .001$; 1.41 vs. 1.35). Cox proportional hazard regression models revealed that primiparous and multiparous cows with FPR >2 on the 6–9 DIM had culling hazard (HR) 1.60, 95% CI 1.40–1.86, and HR 1.18, 95% CI 1.12–1.24, respectively, compared with respective cows with FPR 1–1.5 on the 6–9 DIM. Out of all, 5.8% of TC cows had FPR >2 on 6–9 DIM, compared to 3.0% of SC cows ($p < .001$). Highest number of cows were culled within the first 60 days after calving and for that period the survival rates of TC and SC cows were 0.81 and 0.91, respectively. In addition, time from calving to 1st insemination for SC cows with FPR >2 on 6–9 DIM was 89 and 85 days for reference group (FPR 1–1.5; $p < .001$), whereas the respective time intervals for TC cows were 93 and 85 days ($p < .001$). In conclusion, TC cows had higher culling risk than SC cows and monitoring FPR in early lactation can help to identify cows at most risk. Supported by Estonian Research Council grant (MOBJD678).

OC 5.4 | Neonatal weight and growth of purebred kittens

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The present longitudinal study focuses on weight of kittens from birth to 21 days of life. Kittens ($n = 153$) belonged to 38 litters of seven breeds (Maine Coon, Birman, British shorthair, Siamese, Bengal, Ragdoll, Abyssinian). Kittens were weighted daily for 21 days. Deaths were recorded ($n = 8/153$, 5.8%) and quartiles for weight at birth were calculated (Q1 49–93.4 g, Q2 93.5–105.9 g, Q3 106–116.9 g, Q4 117–148 g). Two-sample *t*-test and one-way ANOVA with Bonferroni's post hoc test were used to assess differences in weight and weight variations based on breed, sex, parity of the queens, neonatal mortality, and quartile. Breed influenced the weight at birth ($p < .05$), but not at day 21. Kittens of primiparous queens were smaller than those of pluriparous ones (102.1 g ± 18.5 and 109.1 g ± 18.5, $p = .0008$). Gender did not influence weight at any time point. Neonates that died were smaller than survivors (82.7 g ± 19.3 vs. 106.1 g ± 17.4, $p = .001$). No difference in weight variation was found in the first 48 h based on weight quartile at birth. However, weight gain was higher for kittens in Q1 at 7, 14, and 21 days compared to kittens in Q2, Q3 and Q4 ($p < .05$; variation in the first 21 days was 72.7% ± 4.5 Q1, 67.4% ± 4.4 Q2, 67% ± 4.8 Q3, and 65% ± 4.4 Q4). Significant differences in weight were found between all quartiles until 9 days ($p < .05$), but not between Q2 and Q3. At 21 days differences were found only between Q1 and Q3–Q4 and between Q2 and Q4 ($p < .05$). Smaller kittens grew faster and differences in weight tended to disappear in the first 3 weeks of life. Primiparous queens have smaller kittens compared to pluriparous ones. Breed differences in weight were found at birth, although this effect should be assessed on larger samples.

OC 6.1 | Virulence genes of *Escherichia coli* isolates from milk and vaginal swabs of sows associated with post-partum dysgalactia syndrome

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The aim of this study was to identify the presence of virulence genes of *Escherichia coli* (*E. coli*) isolates from vaginal and milk samples associated with post-partum dysgalactia syndrome (PDS) in farmed sows. Two hundred and two sows from five commercial pig farms were clinically inspected for PDS 12–24 h after farrowing. Sows were defined as PDS-affected (PDSA) if they showed pathological vulvar discharge or mastitis followed by one or more clinical signs such as fever, anorexia and altered piglet behaviour. Milk samples and vaginal swabs for bacteriological testing were taken from PDSA ($n = 47$) and PDS-unaffected (PDSU, $n = 155$). In total, 96 isolates of *E. coli* were tested by multiplex polymerase chain reaction (mPCR) for the presence of virulence genes related to specific pathogen strains. Virulence genes associated with extraintestinal pathogenic *E. coli* (ExPEC) were the most prevalent among all tested *E. coli* isolates (92.6%). The most dominant among all *E. coli* isolates was Type 1 fimbrial (fimC) gene (90.6%), with the prevalence of 92.38% in PDSA and 94.4% in PDSU sows. There was no significance in the prevalence of virulence genes in milk samples between sows. The increased serum survival (iss) gene was significantly more prevalent ($p < 0.05$) in vaginal swabs of PDSA sows compared to PDSU sows. The multivariable logistic regression model showed that lower parity sows and the presence of iss and heat-stable cytotoxin associated with enteroaggregative *E. coli* (astA) genes were correlated ($p < 0.001$) with the occurrence of PDS. Lower parity sows vaginaly infected with *E. coli* associated with certain ExPEC strains are at higher risk of developing PDS. Sows with positive vaginal swabs for *E. coli* and iss gene early after parturition were associated with PDS.

OC 6.2 | Increased number of high-quality oocytes in antral follicles can determine higher litter size in two outbred high-fertility mouse lines

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Dummerstorf fertility lines FL1 and FL2 represent two mouse models of enhanced fertility characterized by doubling of the litter size compared to an unselected control population (Dummerstorf ctrl line) and might serve as bona fide animal models for pigs. Both FLs managed to reach this goal by increasing the ovulation rate per cycle, even showing irregular estrous cycle and unusual levels of some metabolic hormones connected with GnRH secretion [1]. The aim of the present study was to

analyse oocytes in terms of quality and quantity by comparing the entire pool of oocytes per ovary with those from the antral follicles within the same animal. We performed Brilliant Cresyl Blue (BCB) staining as a noninvasive marker of oocyte quality in combination with additional morphological indicators, for example cytoplasm clarity, cumulus cell layers, nuclear anatomy and shape. Statistical significance was analysed using ANOVA and Tukey's multiple comparison test as a post-test. The increase in the ovulation rate of those super fertile mice is not associated with an increased number of oocytes per ovary (~171–175 in FLs vs. ~150 in ctrl and ~237 in another line with lower litter size) but rather with a higher number of antral follicles and high-quality oocytes per cycle before ovulation (~26.8 and 28.6 in FLs vs. 16.6 in ctrl and 19 in the 4th line), based on better morphology and status after staining. The most conspicuous method to acquire oocytes with the highest quality in our lines is to assess their morphology, rather than their status after BCB staining. All these discoveries together may be of fundamental importance for further in vitro maturation/fertilization processes in livestock farming to improve production while lowering costs. [1] Calanni-Pileri M., 2022, Reprod Domest Anim.

OC 6.3 | Associations between uterine bacterial infections at the time of artificial insemination and fertility

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Post-partum uterine infections and resulting uterine diseases have detrimental effects on productivity and fertility. Several recent studies have elucidated the role of the microbiota in the development of post-partum clinical or subclinical endometritis. These studies shed light on the pathogenesis of endometritis, but they could not explain the causal relationship between bacterial findings and subfertility. The objective of this study was to characterize the microbial composition at the time of artificial insemination (AI) in cows with and without clinical endometritis and to investigate associations between bacterial findings, uterine health status and fertility. At the day of AI, intrauterine cytobrush samples were taken from cows with clear mucus (Vaginal discharge score [VDS] 0, $n = 58$) and from cows with mild endometritis, that is flecks of pus (VDS 1, $n = 62$). Bacteria were cultivated aerobically and identified by MALDI-TOF MS. In total, 49 genera and 116 species were isolated. The most abundant genera were Bacillus, Staphylococcus, Corynebacterium and Streptococcus (19.6%, 14.2%, 10.1% and 8.1%, resp.). Differences in the prevalence of bacteria were found between cows with different VDS. *Bacillus licheniformis* and *Bacillus subtilis* were absent in VDS 1 cows, but found in 27.6% and 8.6% of animals with VDS 0. The insemination success was lower in VDS 1 than in VDS 0, and the total bacterial load lowered the chance of pregnancy. Bacterial findings, however, were not related to the insemination success, and uterine pathogens, such as Trueperella pyogenes and *E. coli*, were rarely found. The uterine microbial community was diverse and complex, presumably representing a part of the physiological microbiota, and not directly linked to impaired fertility.

OC 6.4 | Vascular changes regarding histopathological type and degree of equine endometrosis

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Endometrosis is degenerative fibrosis in mare endometrium. Apart from glandular changes, also blood vessels seem to undergo remodelling. The study aimed to compare selected vascular measures in equine endometrium concerning the degree of equine endometrosis and its histopathological type. Endometrial sections in luteal phase ($n = 24$) were fixed, cut and stained with haematoxylin–eosin (HE) and Masson's Trichrome (MT). Slides stained with HE were histologically classified into categories I, IIA, IIB and III. Fibroblast metabolic status and damage of endometrial glands were evaluated. MT-stained slides were scanned with a semiautomatic brightfield system (TissueFaxsPlus). Blood vessels with a diameter >40 μm in endometrium were measured for wall thickness (WT), perivascular fibrosis (PF) and lumen to thickness index (L/T). Results (mean ± SEM) were analysed with Kruskal-Wallis and Dunn's tests. Concerning active histopathological type, PF was higher ($p = .006$) in category III of endometrosis (2619 ± 531 μm) than in other endometrium categories (I: 1579 ± 517 μm; IIA: 1403 ± 405 μm; IIB: 1290 ± 308 μm). Both WT ($p = .60$) and L/T ($p = .50$) did not differ between degrees, with mean values 1154 ± 192 μm and 2.39 ± 0.22, respectively. Concerning inactive histopathological type, WT (3364 ± 1089 μm, $p < .0001$) and PF (5415 ± 1610 μm, $p < .0001$) were higher, and L/T was lower (1.00 ± 0.10, $p < .0001$) in category III of endometrosis, than in other endometrium categories, with no differences between category I (WT: 1476 ± 558 μm; PF: 1579 ± 517 μm; L/T: 2.25 ± 0.43), IIA (WT: 418 ± 93 μm; PF: 576 ± 172 μm; L/T: 2.43 ± 0.22), and IIB (WT: 574 ± 185 μm; PF: 806 ± 245 μm; L/T: 1.89 ± 0.21). The endometrosis-related vascular changes in mare's endometrium are more pronounced in inactive than in active histopathological type.

OC 7.1 | Onset of puberty in ewe-lambs treated prenatally with melatonin

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There is available evidence indicating the importance of prenatal and early postnatal factors in developmental programming of reproductive function [1]. For this reason, this study was designed to evaluate the time of onset of puberty in ewe-lambs born from melatonin treated ewes. Fourteen Karagouniko breed female lambs were divided into two groups, the group M ($n = 8$) and C ($n = 6$), based on whether their mothers were treated with melatonin implants or not, respectively, throughout pregnancy. All ewes were exposed to heat stress for the first 100 days of pregnancy [2]. Lambs of both groups were fed the same diet after weaning (day 40). The body weight of lambs was recorded at birth, on day 40 and then monthly from the 7th until the 12th month of age. Progesterone (P4) was assayed by RIA in blood samples collected weekly from the 8th to the 9th month and then biweekly until the 12th month of age. A teaser ram was introduced biweekly during the same time period for detecting ewe-lambs in heat. The lambs' weight was compared between groups by repeated measures ANOVA and the age of puberty by *t*-test. The results showed that body weight did not differ within time between groups ($p > .05$). Based on P4, puberty, defined as the onset of full length luteal phase, was initiated earlier in ewe-lambs of group M compared with C (mean ± SE: 314.9 ± 2.2 vs. 324.7 ± 7.6 days of age, respectively; $p = .02$). This study indicates that pubertal reproductive activation is initiated earlier in ewe-lambs treated with melatonin throughout their embryonic life. Further research is needed to elucidate if this programming effect is exerted in female foetuses at gonadal level and/or at hypothalamic–pituitary axis. [1] Rhid SM (2001). Reproduction, 122: 205–214; [2] Bouroutzika E (2020). Antioxidants, 9 (3), 26.

OC 7.2 | Kinomics profiling of dog uterine stromal (DUS) cells following decidualisation, and inhibition of progesterone signalling with antigestagens – a pilot study

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Maternal stroma-derived decidual cells are the only cellular population in the canine placenta expressing the nuclear progesterone (P4) receptor (PGR), crucial for the maintenance of pregnancy. Indeed, lower P4 levels at term, or pre-term blockage of PGR with antigestagens, lead to the termination of canine pregnancy. In vitro, 0.5 mM cAMP, involving the serine/threonine kinase (STK) PKA activation, induces the decidualisation of immortalized dog uterine stromal (DUS) cells after 72 h. Furthermore, antigestagens decrease PGR expression, and modulate the function and viability of decidualised DUS cells. However, the underlying regulatory mechanisms, for example, involving protein kinases other than PKA, remain veiled. Here, we investigated changes in cAMP-driven activities of STK kinases in DUS cells after decidualisation, and in response to antigestagens, using the PamChip assay (PamGene). Decidualisation led to a predicted increased activity of 85 STKs, including PKA, but also PKC, ERK1/2 and PKG. Interestingly, treatment of decidualised DUS cells with 1 μM aglepristone or mifepristone for 6 h was associated with decreased activity of virtually all of these kinases. Modulated kinases were associated with a wide-range of intra- and extracellular activities, for example MAPK, FoxO, NFκB or chemokine signalling, cell cycle and vascularization. The importance of these kinases in DUS cell physiology still needs to be assessed functionally. Nevertheless, new clues regarding the regulation of decidual cells of species-specific and translational value were obtained, and further highlighted the importance of P4 signalling in these cells.

OC 7.3 | Seasonality in kidding after transfer of in vitro produced Saanen goat embryos into recipients of local Ukrainian breed

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To increase the production and reduce the price of dairy products, it is necessary to effectively use reproductive management. One of the tools of reproductive management is the use of assisted reproductive technologies to increase the number of goat livestock. In vitro produced embryos of dairy breeds can be transferred into recipient goats of other breeds, such as the local Ukrainian breed. Therefore, the aim of the present study was to investigate the seasonality of pregnancy success after transfers of in vitro-produced embryos of Saanen goats into surrogate mothers of the local Ukrainian breed. All animal manipulations were carried out following ethical standards (Strasbourg, 1986). Six Saanen goats were selected as oocyte donors. After hormonal stimulation, oocytes were retrieved by laparoscopic ovum pick-up. In vitro produced embryos were transferred laparotomically into 24 hormonally prepared recipients of a local Ukrainian breed. Fifty days after embryo transfers, eventual pregnancies were diagnosed by ultrasound. In the breeding season, 70.5 ± 4.2% of all fertilized oocytes continued to develop till day 7. $N = 15$ embryos were transferred laparotomically to recipient goats. After 50 days, 5 pregnancies (33.3%) were diagnosed. Four pregnancies (26.7%) ended with the birth of kids. In the non-breeding season, 50.7 ± 4.5% of fertilized oocytes reached the blastocyst stage. Nine embryos were transferred into the uterine horn of recipient goats laparotomically. Three recipient goats (33.3%) were diagnosed pregnant. Two pregnancies (22.2%) resulted in the birth of kids. To conclude, the transfer of in vitro produced Saanen goat embryos

to recipients of a local Ukrainian breed gives the opportunity to achieve pregnancies and kidding regardless of the breeding season.

OC 7.4 | A comparison of omentin action on the granulosa cell proliferation in Large White and Meishan pigs

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Omentin, also called intelectin-1, is an adipokine mainly expressed in the adipose tissue and plays the crucial roles in the maintenance of body metabolism and insulin sensitivity. It has anti-inflammatory and cardiovascular protective effects via activation of protein kinase B (Akt) or mitogen-activated protein kinase (ERK1/2). Our last study documented that omentin plasma concentrations and gene expression in the adipose tissue and ovarian follicles are lower in fat Meishan (MS) versus to lean Large White (LW) pigs. The aim of this study was to investigate the effect of omentin on granulosa cells (Gc) proliferation in MS and LW pigs. Porcine Gc were isolated from ovarian follicles on days 10–12 of the estrous cycle and omentin at doses 10, 50, 100, 250 ng/ml was added for 24, 48, 72 h of the culture. Cell proliferation was measured by AlamarBlue Assay, while PCNA and cyclin E expression by Western blot. Moreover, Akt (LY294002) and ERK1/2 (PD98059) inhibitors were used to study involvement of the kinases in omentin action. Statistical analyses were performed using one-way ANOVA. Results of the present study showed that omentin increased in a dose- and time-dependent manner Gc proliferation in LW pigs, while has no effect in MS pigs ($n = 5$, $p < .05$). These results were confirmed by protein levels of PCNA and cyclin E. Interestingly, mitogenic effects of omentin on Gc of LW pigs were abolished in response to the inhibition of both Akt and ERK1/2. These results suggest that the action of omentin on Gc proliferation depends on the animal's fat mobilization. Omentin by stimulatory effect on Gc proliferation in LW pigs may participate in the regulation of ovarian follicles growth, development, or folliculogenesis. Supported by National Science Centre, Poland (2020/37/B/NZ9/01154).

OC 8.1 | Biochemical and bacteriological characterization of Dachshund semen: a correlation study

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Little is known about potential changes to the biochemical milieu of the seminal fluid as a result of bacteriospermia. This study aimed to assess the bacterial profile of dog semen and to detect associations between the bacterial load (colony forming units – CFU/ml), sperm motility and biochemical characteristics of seminal plasma. Fresh semen was collected from adult Dachshunds ($n = 15$). Progressive motility (PROG) was evaluated by computer-assisted sperm analysis. Seminal plasma was subjected to the quantification of calcium (Ca), magnesium (Mg), phosphorus (P), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), creatinine (CRT), uric acid (UA), urea (U), bilirubin (BIL), triglycerides (TG), cholesterol (CHOL), total proteins (TP) and albumin (ALB). Bacterial identification was carried out by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry revealed the predominance of the Staphylococcus and Bacillus species. The Pearson correlation analysis unravelled a significant negative ($p < .001$) correlation between CFU and PROG. Significant positive associations were observed amongst PROG and particularly Ca ($p < .01$), ALT ($p < .01$), ALP ($p < .01$), CRT ($p < .01$), UA ($p < .01$) and TP ($p < .01$). In the meantime, we observed significant negative correlations amongst CFU and Ca ($p < .05$), Mg ($p < .05$), ALP ($p < .01$), BIL ($p < .05$), TG ($p < .05$), CHOL ($p < .05$), TP ($p < .05$), and ALB ($p < .05$). Our results suggest that bacteria may have a negative impact not only on the sperm motility, but also in the biochemical composition of the seminal plasma in Dachshunds. More detailed studies are necessary to elucidate specific mechanisms of bacteriospermia in dogs. Supported by APVV-15-0544 and VEGA 1/0239/20.

OC 8.2 | ProAKAP4 correlation with motility descriptors and spermatozoa subpopulations in stallion

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ProAKAP4 is a good biomarker of sperm quality is some species specially correlated with progressive motility. The aim of this study was to evaluate proAKAP4 in thawed semen of stallion and correlate it with

kinetic parameters and its motility subpopulations. Between 1 and 5 ejaculates of 13 stallions were used; one straw of each ejaculate was used to analyse with CASA (ISASv.1.0; Proiser S.L., Valencia, Spain), viability (eosin-nigrosine) and concentration was performed. The second straw was used to dose proAKAP4 by ELISA method (Horse 4MID® kits, 4BioDx, France). Spermatic motility subpopulations were calculated (Martí, Aparicio, & García-Herreros, 2011 method). Statistical analysis (R Software; V4.0.3, R Core team, Austria) was carried out using Pearson correlation to obtain the correlation between proAKAP4 concentration with sperm concentration, with sperm motility parameters, and with subpopulations of motile spermatozoa from frozen–thawed horse ejaculates. Results showed a positive correlation with the percentage of total motile spermatozoa ($r = .31$; $p < .05$) and with the percentage of spermatozoa with progressive motility ($r = .31$; $p < .05$), as well as with the kinematic parameters of speed: VCL ($r = .29$; $p < .05$), VSL ($r = .34$; $p < .05$), and VAP ($r = .33$; $p < .05$). It is observed that the proAKAP4 concentration presents a negative correlation with subpopulation 3 ($r = -.37$; $p < .05$), which was the slowest, with intermediate progressivity and with the lowest ALH and BCF values. The conclusion of our study confirms that proAKAP4 is a good marker of progressive motility in stallion and ejaculates with slower spermatozoa are poor in proAKAP4.

OC 8.3 | Melatonin affects the ability of red deer spermatozoa to respond to ionophore and LPC challenges in capacitating and non-capacitating conditions

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Melatonin is a pleiotropic molecule, affecting sperm physiology through MT1 and MT2 membrane receptors. Using red deer as a model for seasonal ruminants, we tested its effects at low concentrations (1 pM, 100 pM, 10 nM, and 1 μM) on the physiology of epididymal sperm from 6 hunted stags (*Cervus elaphus hispanicus*). Sperm were incubated in TALP-HEPES for 4 h (37°C, 5% CO2) in non-capacitating or capacitating conditions (heparin 2 U/ml), challenged with Ca2+ ionophore (calcimycin, 3 μM) or lysophosphatidylcholine (LPC, 0.3 mg/ml). The viable subpopulation of spermatozoa (PI−) was evaluated for intracellular Ca2+ as MFI ([Ca2+]i, Fluo-4), acrosomal reaction (PNA) and membrane disorder (merocyanine 540). Linear mixed-effect models evaluated melatonin effects. In non-capacitated sperm, pM concentrations decreased ($p < .01$) reacted acrosomes and membrane disorder (1 pM). The ionophore increased [Ca2+]i, with small effects in acrosomal damage and membrane disorder. In non-capacitated samples, pM concentrations reduced acrosomal damage ($p < .01$) and increased membrane disorder (10 nM and 100 pM), but decreased in capacitated (nM–pM, $p < .05$). LPC increased membrane disorder, with a lower incidence for 1 pM in non-capacitated ($p < .01$). In capacitated conditions, melatonin increased acrosomal reaction ratio ($p < .05$ for 1 μM; $p < .01$ for pM). This study supports the relevance of melatonin on sperm physiology and could contribute to the application of reproductive technologies in wild ruminants. Thanks to MINECO (grant AGL2013-43328, Spain), J. Vicente (SaBio, IREC, CSIC-UCLM-JCMM, Spain), wardens at Riaño Reserve, I. Álvarez, and C. Arija.

OC 8.4 | Effect of filtration, simple centrifugation, and presence of antibiotics on bacterial load and sperm quality of equine chilled sperm

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Microbial load negatively affects sperm preservation and is a reproductive risk for the mare [1, 2]. The objective of this study was to evaluate the effect of two semen processing techniques on bacterial load and sperm quality of chilled equine semen. Twenty-two ejaculates from 4 stallions were used. Each ejaculate was diluted in extender with (INRA96®) or without antibiotics (skimmed milk) and subjected to filtration (SpermFilter®) or simple centrifugation (450 *g*, 7 min) before cooling, resulting in four treatment groups: Filtration with (FD+) and without (FD−) antibiotics and Simple Centrifugation with (SCD+) and without (SCD−) antibiotics. Sperm quality and bacterial load on three different culture media (Columbia 5% Sheep Blood Agar (COS), Sabouraud Dextrose Agar (SDA) and MacConkey Crystal Violet Agar (MCK)) were evaluated, both initially and after 24 h of cooling. Results were statistically evaluated by Kruskal-Wallis test ($p < .05$). No significant differences were found on progressive motility or vitality between raw and processed semen. Except for MCK, where SCD− and FD− were not significantly different from raw semen, all treatments reduced bacterial load, although not significantly for SCD− in COS and FD− in SDA. In general, lower values were obtained in those samples processed with antibiotics. Thus, the use of filtration or centrifugation before cooling reduces sperm bacterial load and allows to maintain sperm quality over 24 h, independently of the presence of antibiotics in the cooling extender. Although further studies are needed, different semen processing protocols may reduce the non-therapeutic use of antibiotics, decreasing antimicrobial resistance. [1] Ortega-Ferrusola. 2009. Reprod Dom Anim 44:518–522. [2] Bennett. 1986. J. Am. Vet. Med. Assoc., 188:1390–1392.