

YSC 3 | Cow uterine fluid extracellular vesicles show oestrous cycle phase dependant proteomic changes affecting embryo development in vitro

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Extracellular vesicles (EVs) in the uterine fluid (UF) are known to regulate early embryo development. Moreover, the changes in UF-EV proteome during the bovine oestrous cycle and the effects of these proteins on embryo development are yet to be discovered. We used mass-spectrometry based shotgun quantitative proteomics to compare UF-EV proteomes at day 0, 7 and 16 of the oestrous cycle ($n=4$ per group). Also, we supplemented follicular and luteal phase UF-EVs to group embryo cultures to evaluate their impact on embryo development. Proteomics data was analysed using LFQ-analyst platform, while the differences of blastocyst rates were evaluated with logistic regression analysis. Proteomic analysis revealed pathways which are important for early embryo development and its nutritional needs, such as antioxidant activity, cell morphology and cycle, cellular homeostasis, cell adhesion and carbohydrate metabolic process. Furthermore, 159 UF-EV proteins differentially enriched at different timepoints. These proteins were involved in pathways related to antioxidant activity, actin cytoskeleton organization, immune processes, gene expression regulation and metabolic functions. The luteal phase UF-EVs supplementation to the embryo culture media increased blastocyst rates from $25.0 \pm 5.9\%$ to $41.0 \pm 4.0\%$ ($p=.03$). Overall, our findings suggest that there are significant differences in bovine UF-EV proteome throughout the oestrous cycle and UF-EVs improve in vitro embryo production, however further studies are required to identify the exact UF-EV cargo for optimal embryo development.

YSC 4 | Effect of equilibration temperature and time on feline ovarian tissue vitrification

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The entire members of Felidae are currently classified as endangered except for the domestic cat (*Felis catus*) making it an excellent model for conservation studies. Vitrification of feline ovarian tissue

is an emerging conservation technique suitable in field conditions however, not yet standardized. Thus, the aim was to establish a suitable vitrification protocol for feline ovarian tissue in field conditions. Feline ovarian tissue fragments were punched with a biopsy punch (1.5 mm diameter) and divided into 4 groups. Group 1 was directly placed in culture (Fresh control – FC), while the other three were placed on 30G needles (4 fragments/needle) and vitrified using 3 protocols (A, B, C). Protocol A involved two step equilibrations for 10 min each at 4°C and then vitrification [1]. Protocol B involved three step equilibrations for 14 min in total at room temperature [2], while protocol C was the same with protocol B except the equilibration timings which were reduced by half. Fragments were warmed and placed in culture [1] for 6 days. Follicular morphology, cellular proliferation (expression of Ki-67, MCM-7) and apoptosis (expression of caspase 3) were evaluated. Data were analysed using Chi square test. Proportions of morphological intact follicles were higher in FC ($p=.0001$) and protocol C ($p=.0383$) in comparison to the other protocols at the sixth day of culture. Generally, most follicles remained at primordial state which was confirmed by the low expression of ki-67, MCM-7 markers. In conclusion, protocol C, which has lower equilibration time at room temperature, can be used for vitrification of feline ovarian tissue. [1] Mouttham, *Cryobiology* 2016;73:187; [2] Amorim, *Human Reproduction*, 2013;28: 2146.

YSC 5 | Stress during the transition period shortens telomere length in dairy cows

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Telomere length (TL) has long been recognized as a biomarker of ageing. In humans, physiological stress is known to impact health and longevity, manifested by an accelerated shortening of TL. In cattle, the transition period from pregnancy to lactation is considered a crucial stage, during which cows undergo significant metabolic and physiological changes. We hypothesize that telomeres shorten during this critical period, due to oxidative and metabolic stress. Seventy-one Holstein Friesian cows, on one farm, were followed up during the transition period and blood and milk samples were collected at regular time points. Average relative leukocyte TL was measured by a modified quantitative real-time PCR (qPCR) protocol at 7 days before and 21 days after parturition. Oxidative, inflammatory, and metabolic parameters were also determined. From 7 days prior to 21 days after parturition, a paired t-test showed a significant decrease in TL of 0.08 ± 0.243 ($p=.022$). Multiple linear models, built in R, were used to assess factors influencing TL shortening. Both oxidized glutathione in blood (GSSG, oxidative

parameter) and beta-hydroxybutyrate in milk (BHB, metabolic parameter) were associated with an increased TL shortening. For each percentage increase in GSSG and for each 0.01 mmol/L increase of BHB, TL shortening tended to be 0.016 greater ($p = .051$ and $.097$ respectively). The preliminary findings of this study revealed a significant shortening of TL during the transition period in dairy cattle. In addition, higher levels of oxidative and metabolic stress parameters were associated with a greater TL shortening.

YSC 6 | Adipokines expression profiles in pituitary gland and plasma levels during the estrous cycle of large white and Meishan sows

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The relationship between reproduction and energy metabolism of domestic animals is very close. Previously data showed differences in adipokines levels in white adipose tissue in normal weight Large White (LW) and obese Meishan (MS) pigs. However, comparison of central-pituitary levels of adipokines during the estrous between LW and MS is still unknown. The aim of the study was to compare plasma concentrations (ELISA) and gene expression (RT-qPCR) of adipokines: adipolin, adiponectin, chemerin, visfatin, apelin, omentin, vaspin and their receptors in the anterior pituitary (AP) in LW and MS ($n = 5$) during the estrous. Statistical analysis was performed by two-way ANOVA, Tukey's post-hoc test, and Pearson correlation coefficient, two-tailed $p \leq .05$. Our results showed increased levels of apelin and adiponectin while decreased chemerin with receptors, adipolin, visfatin, omentin in AP of LW vs. MS. There are no differences in adipolin and visfatin plasma concentration, but increased levels of chemerin, vaspin in MS and omentin in LW depending on the phase of the estrous. We observed correlations between apelin expression and plasma level on days 2–3 in MS and 10–12 in LW. The LH concentration in plasma was positively correlated with visfatin level in LW and negatively correlated with adiponectin in MS during late luteal phase. There was no correlation between plasma FSH and any adipokine. These results demonstrated the pattern of expression of selected adipokines and their receptors in AP and plasma concentrations depending on the animal metabolic status and day of the estrous suggesting adipokines' action on pituitary level. Funding: Supported by NCN 2020/37/B/NZ9/01154; we thank the team of porcine experimental unit and the slaughterhouse of INRAE Centre Val de Loire.

YSC 7 | The role of phoenixin-14 on endocrine function of porcine corpus luteum, an in vitro study

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Phoenixin (PNX-14) is a neuropeptide which modulates ovarian follicular cells steroidogenesis and oocytes maturation. Our previous study showed expression of PNX-14 and its receptor GPR173 in the porcine corpus luteum (CL). However, the role of PNX-14 in luteal cells physiology has never been studied, so the aim of the study was to determine the in vitro effect of PNX-14 on endocrine function of CL during the estrus cycle. Porcine luteal cells were isolated from CL on days 10–12 of the estrus cycle and cells were stimulated with PNX-14 (1–1000 nM) with or without LH (100 ng/mL). The concentration of steroid hormone: P4 and E2 and prostaglandins (PGE2 and PGF2 α) was determined by ELISA assays. The transcript and protein levels of StAR, CYP11A1, 3 β HSD, CYP19A1, PTGER2 and PTGFR as well as expression of GPR173 and kinases ERK1/2 and PKA were analysed by RT-qPCR and Western-blot. Moreover, the involvement of GPR173 (by siRNA) and ERK1/2 and PKA (by pharmacological inhibitors) was studied in PNX-14 effect on luteal endocrinology. The results showed a dose-dependent stimulatory effect of PNX-14 on the secretion of P4, E2, and PGE2 and inhibitory action on PGF2 α . PNX-14 induced modulatory effect on LH-induced steroid synthesis. Moreover, PNX-14 increased the expression of steroidogenic enzymes and exerted a modulatory effect on prostaglandin receptors. In addition, PNX-14 stimulated expression of GPR173 and activation of ERK1/2, while downregulating PKA. In conclusion, we demonstrated that PNX-14 improved steroidogenesis and inhibited PGF2 α via GPR173 and ERK1/2 pathways, suggesting a direct role of this neuropeptide on the porcine luteal endocrinology. Supported by NSC: 2020/37/N/NZ9/00981.

YSC 8 | Fertility in a randomized controlled study of customized voluntary waiting period in primiparous dairy cows

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Previous studies have shown that primiparous cows in general, but not all individuals, are suited for extended voluntary waiting period (VWP), however, cows with less persistent lactation risk a long dry period and high BCS at the end of lactation. Customizing the VWP may reduce the risk of undesirable effects of extended VWP. This