Improvement of canine spermatozoa vitrification

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Sperm vitrification is an interesting and useful alternative to sperm cryopreservation that avoids specific equipment and time consuming protocols. A successful method has been described in dog sperm by dropping sperm suspension (sperm diluted in HTF® with sucrose and BSA) directly into liquid nitrogen, but replicability of results could not be obtained in other laboratories. The objective of this study was to try replicating this method in a comparative fashion with other cryopreservation medium, and including a density gradient centrifugation (DGC) step for sperm selection before vitrification. Sixteen pooled sperm samples from beagle dogs were conventionally washed with a Tris, citric acid and glucose buffer (TCG) or by DGC (BoviPure®). Sperm vitrification was performed using two different extenders: TCG based medium with 20% egg yolk and 250 mM sucrose vs. an HTF® medium with 250 mM sucrose and 1% BSA. Samples were vitrified by dropping 30 µl of sperm suspension directly into liquid nitrogen. After vitrification, warming procedure was performed by incubating pellets at 5% CO_2 with maximum humidity at 37°C with either TCG+20% EY or HTF+1%BSA. Motility was assayed using a computer-aided sperm analysis (CASA) system; membrane integrity and acrosomal status were analyzed by fluorescence microscopy. Sperm quality was evaluated before and after vitrification. Statistical analysis was performed using a T-test. As expected, sperm quality after vitrification and warming was lower ($p < 0.05$) compared to fresh samples. We observed that performing density gradient before vitrification improved sperm motility and viability after warming ($p < 0.05$). HTF extender returned significantly ($p < 0.05$) higher straight line velocity and curvilinear velocity in the DGC samples after warming.

Non-esterified fatty acids (NEFAs) increase the production of IL-6 and IL-8 in bovine endometrial epithelial cells (bEECs) in culture

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High-milking dairy cows submitted to negative energy balance (NEB) have increased levels of circulating IL-6, IL-8. Oleic (OA), palmitic (PA) and stearic (SA) acid are major NEFAs found during NEB. NEFAs increased the above cytokines for human and animal somatic cells but data are scarce in the endometrium. We investigated here the effects of OA, PA and SA on IL-6 and IL-8 production by bEECs in culture. Following collection of genital tracts from 4 cows at slaughterhouse, passage 4 bEECs were cultured for 24 and 48 h in media supplemented with 150, 300 and 500 µM of PA, SA or OA, or with 40 µl of 95% ethanol (used as vehicle for NEFAs) as a control. Cell-free supernatant was collected and IL-6 and IL-8 concentrations measured with bovine ELISA kit (Thermo Fisher Scientific, Waltham, MA, USA). IL-6 and IL-8 ratio (as total concentration in media/total number of cells) was calculated and data analyzed with SAS 9.4 (Proc Mixed). IL-6 ratio was significantly increased when cells were exposed to all dosages of OA or PA when compared to control ($p < 0.05$ to $p < 0.0001$), whereas the results did not differ with SA. IL-8 ratios were significantly higher than in controls, only after 500 µM of OA, PA or SA treatment ($p < 0.05$ to $p < 0.0001$). These results suggest that PA and OA are the most potent inducers of cytokine production in endometrial epithelial cells. Further studies are needed to investigate if the increase of pro-inflammatory cytokines possibly induced by metabolic imbalance may be of a critical importance at time of implantation. (Work supported by the EU project "Prolific" (Grant no. 311776) and by RMUTSV.)

Search for a pleiotropic effect of the FecLL prolific mutation in Lacaune meat sheep

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The L prolific mutation in the FecL major gene is segregating in the meat strain of Lacaune sheep. The L mutation was shown to not only affect the prolificacy, but also the fertility after insemination and the growth and mortality of lambs born from L carrier ewes. In order to increase our knowledge about a potential pleiotropic effect of the L prolific mutation, we have observed 20 + + and 23 L+ Lacaune ewe lambs from birth to adulthood. We have recorded their growth, average daily gain (ADG), endocrine hormone profiles, onset of puberty and fertility after first natural mating. The L mutation did not affect the birth weight or the ADG among the analyzed period. In contrast, these two traits were significantly decreased by the twin or triplet mode of birth compared to singletons. Interestingly, by checking circulating progesterone level (>0.5 ng/ml), L+ ewe lambs have shown precocious ovulation events one month earlier on average than ++ (5 vs. 6 months, $p < 0.001$). This apparent precocity was not correlated to a precocious set up of a regular cyclicity, but pregnancy rate at first mating of L+ tended to be higher than ++ (95.6% vs. 75.0%, $p = 0.08$). Moreover, we have established the Anti-Müllerian Hormone (AMH) endocrine profile as a known marker of the ovarian follicular reserve and possibly fertility. Circulating AMH rises from birth to 2 months of age, decreases from 2 to 3 months and remains

stable thereafter. We have found no correlation between AMH profiles and the other recorded parameters. However, L+ ewes have 3 times less AMH than ++, particularly at the time of the concentration rise ($p < 0.001$, 28–70 days), without negative impact on fertility. In conclusion, the L mutation impacts the ovarian function more widely than simply regulating the ovulation rate.

Bovine viral diarrhoea virus infection interrupts the regulatory pathways for uterine interferon stimulated gene expression in cows

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Non-cytopathic bovine viral diarrhoea virus (ncpBVDV) infection in cows has been associated with early embryonic mortality. Our previous studies have shown that ncpBVDV infection inhibits interferon tau (IFNT) induced interferon stimulated gene (ISG) expression (Cheng et al. 2017, Biol Reprod 96:1142), which may affect maternal pregnancy recognition (PR). Production of uterine ISGs is regulated by interferon regulatory factors (IRFs) and signal transducer and activator of transcription (STAT) 1 and 2. The aims of our study were to investigate the effect of ncpBVDV infection on IFNT-induced activation of the above ISG regulatory pathways using an established endometrial PR model (Cheng et al. 2017, Biol Reprod 96:1142). Bovine endometrial cells from 10 apparently healthy cows were cultured and treated with 0 or 100 ng/ml IFNT for 24 h in the presence or absence of ncpBVDV infection. RNA from treated cells was extracted for gene expression quantification using qPCR. Statistical data analysis was carried out using ANOVA with repeated measurements. Our results illustrated that IFNT significantly stimulated the expression of STAT1, STAT2, IRF9, IRF7, TYK2 and IFNG mRNA, confirming their importance in IFNT induced PR and ISG transcription. However, in the presence of ncpBVDV infection, the IFNT induced expression of STAT1, STAT2, IRF9 and TYK2 was significantly suppressed. Treatment with ncpBVDV alone induced expression of IFNAR2, STAT1, STAT2, JAK1, TYK2, PIAS2 and IFNG and this suggests activation of antiviral pathways. Neither ncpBVDV nor IFNT treatment altered IFNAR1 expression. Our results suggest that ncpBVDV infection alters the ISG regulatory pathway of IRF-STAT1 and 2 to inhibit IFNT induced ISG expression which is at least in part responsible for PR failure.

Advanced age reduces the total number of oocytes per OPU session but does not influence the blastocyst rate and embryo viability after ICSI in the mare

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The population of donor mares in OPU-ICSI commercial programs is skewed towards advanced age. Old mares have a reduced fertility in regular ET programs, however, the effect of mare age on the efficiency of an OPU-ICSI program is unclear. The objectives of this study were to investigate the effect of age on OPU-ICSI parameters by a model of variance; and on post-transfer embryo viability by logistic regression. Overall 434 mares were submitted for one OPU-ICSI session, and classified according to age in 4 groups: 2–6 (A; n = 88), 7–12 (B; n = 143), 13–17 (C; n = 100) and 18–25 y (D; n = 103). In addition, 395 ICSI embryos from 172 mares were transferred into health recipients. The number of aspirated follicles (21.9 ± 9.1), oocytes (10.5 ± 5.2) and recovery rate (48.9 ± 16.1%) were lower ($p < 0.01$) in group D than in the rest (23.7–26.2 follicles, 13.4–14.7 oocytes, and recoveries of 54.9–56.6%, respectively). However, more oocytes matured to MII within 28 h in group D (63 ± 17.5%) than in the rest (54.6–56.7%). The blastocyst rate (14.1, 12.9, 12.8 and 12.3%, for groups A-D, respectively) did not differ ($p > 0.1$) among groups. Group D tended ($p = 0.1$) to have fewer embryos per OPU (0.81 ± 1.0) than the rest (1.1 ± 1.4), but the proportion of mares with at least one embryo per OPU session was similar ($p > 0.1$) amongst groups (57.9, 56.6, 53 and 51.4% for groups A-D, respectively). The pregnancy status and loss by 120d post-ET was 52.7, 53.5, 48.1, 45.5% and 22.2, 13.9, 18.8 and 20.0% for groups A-D, respectively ($p > 0.1$). In conclusion, the reduced follicle pool and probable aspiration of more follicle-alike cysts (reduced recovery) may account for the lower efficiency of the OPU program in older mares. However, the oocyte competence and viability of their ICSI embryos appears to be unaffected.

Generation of TPC2 knock out pig embryos by CRISPR-Cas technology

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Changes in calcium ions constitute a central intracellular signalling mechanism. Recently, two-pore channels (TPCs) have been identified as the endo-lysosomal Ca^{2+} channels and key components of the NAADP receptor. Subsequent studies with TPC2 KO mice have demonstrated a role in different organs and systems and the importance in pathologies such as diabetes and cancer.