

# Reproduction in Domestic Animals

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## P81

## Effect of SOD (superoxide dismutase) on chilled epididymal cat spermatozoa

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Preservation of gametes is an important tool in assisted reproduction programmes. New approaches are being developed to find new methods that ensure a longer storage of cooled semen. It is known that high concentrations of reactive oxygen species (ROS) cause sperm pathology. The metalloprotein superoxide dismutase (SOD) is responsible for H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub> production. The aim of this study was to assess the quality of chilled cat semen processed with extenders containing SOD as antioxidant additive. Epididymides were collected from 20 domestic cats during routine neutering procedures. The cauda epididymis was finely minced to release spermatozoa. Each sample was divided in two aliquots: spermatozoa diluted in Tris extender without (1) or with SOD (2). Each sample was analyzed for motility, viability and acrosome status, immediately after semen preparation (T0) and after storage at 5°C for 24 h, 48 h and 72 h (T1, T2 and T3 respectively). The acrosome integrity was evaluated by PNA-FITC conjugated staining. A proteomic approach of ERK quantification was also evaluated as an indicator of oxidative stress. Quality parameters of sperm were significantly higher in aliquots added with SOD. ERK phosphorylation was statistically higher in the aliquots without SOD. In conclusion, SOD addition in semen extenders improved the quality of chilled cat semen and reduced ERK activation.

## P82

## The epidermal growth factor stimulates ram sperm capacitation and protein tyrosine phosphorylation

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The presence of epidermal growth factor receptor (EGFR), a specific tyrosine kinase receptor, in ram spermatozoa was determined by incubating ram sperm with Alexa488-conjugated EGF. Results are shown as mean  $\pm$  SEM of the number of samples indicated in each case. ANOVA test was performed, and post hoc comparisons were made using the Student-Newman-Keuls Multiple Comparisons Test. After 2 h of incubation in capacitating conditions, the proportion of sperm binding EGF increased up to 39.3  $\pm$  7.4%, 57.1  $\pm$  6.0% and 52.0  $\pm$  4.5% with 25, 50 and 100 nM EGF, respectively (n = 6). Western-blot analysis of the presence of EGFR in ram sperm lysates revealed a band of approximately 170 kDa (predicted molecular weight for this receptor). Having identified EGFR in ram spermatozoa, we investigated the effect of the inclusion of EGF during incubation on capacitation. Chlorotetracycline staining showed that 100 nM EGF increased the proportion of capacitated sperm from 36.2  $\pm$  1.8% in control conditions to 49.9  $\pm$  2.0% after 3 h of incubation (p < 0.001). An increase in protein tyrosine phosphorylation of 14.5  $\pm$  0.6% (p < 0.01) was concomitantly achieved (n = 3). The addition of tyrphostin AG555, a specific inhibitor of EGFR kinase activity, accounted for a significant increase (p < 0.001) in the percentage of non capacitated sperm (32.0  $\pm$  2.3% vs. 59.1  $\pm$  4.5%, 56.9  $\pm$  3.8% or 62.0  $\pm$  3.4% when 25, 50 or 100  $\mu$ M tyrphostin (n = 4). However, protein tyrosine phosphorylation did not change. Although tyrphostin acts as a stress signal activating the MAP kinase pathway, it is not clear if the described effects are the result of inhibiting EGFR or another upstream tyrosine kinase.

## P83

Effects of PGF<sub>2A</sub> administration at the onset or the end of a short-term progestagen treatment in serrana goats

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In order to assess the reproductive effects of Prostaglandin F<sub>2 $\alpha$</sub>  (PGF<sub>2 $\alpha$</sub> ) administration at the onset or the end of a progestagen treatment in Portuguese Serrana goats, an initial group of 44 females (five were later rejected for several reasons) aged between 3 and 7 years was used. In May (beginning of the breeding season), all goats were treated with an intravaginal sponge impregnated with 20 mg of fluorogestone acetate (FGA) for 5 days and injected (i.m.) with 300 UI of eCG at the time of sponge removal. Half goats received an injection (i.m.) of 100  $\mu$ g of cloprostenol at sponge insertion (SI) and the other half at sponge removal time (SR). Blood samples were taken for progesterone determination and four intact bucks with harness marker were used to identify oestrus. Transrectal ultrasound scanning was performed for pregnancy diagnosis 41 days after eCG administration. PGF<sub>2 $\alpha$</sub>  injection at the onset of the FGA treatment had a positive effect in oestrus (SI - 100.0% vs. SR - 90.9%; r<sup>2</sup> = 9.424; p < 0.01), ovulation (SI - 100.0% vs. SR - 95.5%; r<sup>2</sup> = 4.082; p < 0.01), pregnancy (SI - 100.0% vs. SR - 90.9%; r<sup>2</sup> = 9.424; p < 0.01) and fertility (SI - 100.0% vs. SR - 72.7%; r<sup>2</sup> = 31.214; p < 0.001) rates. Time of PGF<sub>2 $\alpha$</sub>  injection had no significant effect in prolificacy (SI - 2.1  $\pm$  0.8 vs. SR - 2.2  $\pm$  0.8; p > 0.05). In conclusion, data indicate that PGF<sub>2 $\alpha$</sub>  should be administered at the onset of the FGA treatment.

## P84

## Efficacy of tuohy needle in oocytes collection from excised mare ovaries

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Oocyte aspiration from equine follicles gives a low recovery rate and yields oocytes largely denuded of cumulus cells. Follicle scraping is labour intensive and increases the time required for collection, extending the holding time of oocytes that delays their maturation. The aim of this work was to develop an effective method for collecting equine oocytes combining the feature of aspiration (fastness) with that of scraping (high recovery rate of cumulus-intact oocytes). Furthermore, we examined differences in cumulus morphology and maturation rates, comparing this technique to aspiration and scraping, with or without tunica albuginea removal. Collection by vacuum pump aspiration was performed using a 16 g needle while the combination of aspiration and scraping was performed using a Tuohy needle (16 g) that is usually employed for inserting an epidural catheter and its tip shape is similar to a small curette. In unpeeled ovaries, the recovery rates by the Tuohy needle was higher (p < 0.05) than in the 16 g needle aspiration and in the scraped ovaries (57% vs. 36% and 47%) while the rate of cumulus-intact oocytes was higher than aspiration (46.9% vs. 39.36%) but lower than scraping (46.97%) (p < 0.001). In unpeeled ovaries there was no difference in maturation rate of oocytes recovered by Tuohy needle in respect to scraping in peeled ovaries (58.54% vs. 58.24% respectively; p < 0.05). In conclusion, combination of aspiration and scraping by Tuohy needle allows a faster and reliable collection of oocytes suitable for horse IVF.