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Comparison of Two Procedures for Sperm Cryopreservation in the Stallion

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The aim of this study was to evaluate the effect of two different freezing procedures on stallion sperm motility. Semen samples (n = 18) were obtained from eight adult stallions by artificial vagina (at least two samples per animal). Semen was extended in a skimmed milk diluent and total (TM) and progressive motility (PM) was assessed by CASA system (SCA®, Microptic SL, Spain). Only samples with 70% TM and 30% PM were used. The remainder of each sample was then centrifuged (1125 g/20 min) and the sperm pellet resuspended with Gent commercial extender (Minitube, Germany) to a final sperm concentration of 200×10^6 PM-sperm/ml. The sperm suspension was divided in two aliquots; one aliquot followed a slow freezing protocol (SP), cooling for 3 h at 5°C. The second one followed a rapid protocol (RP) without cooling (only equilibrated with the freezing extender for 10 min at 23°C). After that, both sperm suspensions were loaded into 0.5 ml straws, frozen in liquid nitrogen vapours (20 min) and thawed (37°C/30 s). Post-thaw TM and PM were compared between treatments by ANOVA. Significant differences were found between cryopreservation procedures. Higher percentage of post-thaw sperm motility were found using the SP, as much for TM (60.6 ± 10.8a vs 46.9 ± 15.3^b; $p < 0.01$) as for PM (25.5 ± 7.4^a vs 11.2 ± 6.1^b; $p < 0.001$). The lower percentage of sperm motility in a thawed sample is correlated with higher sperm cryodamage. Therefore, the SP showed better cryoprotection. We concluded that post-thaw TM and PM were higher in semen samples cryopreserved with SP.

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Toxicity and Osmotic Stress of Ethylene Glycol on Pig Oocytes and Parthenogenetic Embryo Development

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In order to asses the effect of cryoprotectant toxicity and osmotic stress on porcine oocyte viability and parthenogenetic embryo development, two ethylene glycol (EG) vitrification solutions were tested. Metaphase II porcine oocytes were divided into threee groups, 30% EG, 40% EG and 0% EG (control group: without cryoprotectants) in base solution (BS:199 - hepes + 10%FCS). Oocytes were kept for 5 min in equilibration solutions (1 : 1 vitrification:BS) at room temperature, then for 1 min in different vitrification solutions on BS. For thawing treatment, both 30% and 40% EG groups were treated with 0.25 M, 0.125 M and 0 M sucrose solution in BS for 5 min each at 37°C. Oocytes were activated with ionomycin and 6-dimethylaminopurine, and cultured in NCSU23 medium for 7d. Cleavage and blastocyst rates after chemical activation were evaluated. Cleavage rate was lower for 40% EG Group than 30% EG group and Control group (61 vs 81 and 75% respectively; $p < 0.05$), but there were no differences between 30% EG group and control group. However, no differences were observed among groups for blastocyst rates (2, 7 and 8%–40% EG group, 30% EG group and control group respectively), althought the presence of 40% EG showed a tendency ($p = 0.26$) to decrease the blastocyst rate. High cryoprotectant concentrations reduced oocyte and embryo viability. Reducing cryoprotectant concentrations, toxicity and osmotic stress are lower, and we could increase the success of the procedure. Supported by Generalitat Valenciana, GV05/212.

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Breed-Cross Pregnancies Increased Plasmatic Pregnancy-Associated Glycoprotein (PAG) Concentration in Pregnant *Neospora*-Seropositive Dairy Cows

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The use of beef bull semen can reduce the risk of abortion in *Neospora caninum*-infected dairy cows. The aim of this study was to evaluate the influence of cross-breed pregnancies on plasma pregnancy-associated glycoprotein (PAG) concentrations throughout the gestation in *Neospora*-seropositive dairy cows developing gestation to term. Thirteen seropositive cows were inseminated with Friesian (n = 7) or Limousin semen (n = 6). Blood samples were collected from each cow on days 40, 90, 120, 150, 180, 210 of gestation, and at parturition. Sera were tested for antibodies against *N. caninum* and PAG concentrations were determined by RIA. Bull breed effect on PAG concentration was analysed by GLM repeated measures analysis of variance, with breed of bull as the between subject effect. Factors significantly affecting PAG concentration were; day of gestation, bull and day of gestation × bull interaction. PAG-concentration of pregnant cows inseminated with Limousin semen were significantly higher throughout the gestation than Friesian semen and increased progressively from day 40 until parturition for both groups. The results indicate that the use of Limousine semen in Friesian cows increased plasma PAG concentration vs Friesian semen, and suggest that *Neospora* infection does not affect placental function in chronically infected cows not suffering abortion.

P25

Apoptosis Rate in Pre-pubertal Goat Cumulus-Oocyte Complexes Depending on Oocyte Diameter and Cumulus-Oocyte Complex Morphology

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Many studies have related *in vitro* produced blastocysts to cumulus-oocyte complex (COC) morphology. Our aim was to study if COC morphology at collection time is really related to apoptosis rate in cumulus cells. Pre-pubertal goats COCs were recovered by slicing and classified as: Healthy (H: compact cumulus and homogeneous cytoplasm) and Early atretic (EA: heterogeneous cytoplasm and/or initial cumulus expansion). Each morphology group was also classified by oocyte diameter: (i) 110–125 μm; (ii) 125–135 μm and (iii) >135 μm. Apoptosis in COCs (n = 447) was detected by Annexin-V. We classified apoptosis grade in four categories depending on the percentage of apoptotic cumulus cells (ACC): >25, 25–50; 50–75 and >75%. Apoptosis was classified as: early and late stage. Healthy COCs presented less percentage of ACC (<25%) than EA group (H: 80.46, 82.52, 94.64% and EA: 40.74, 48.05, 60% for (i), (ii) and (iii) groups, respectively); moreover the largest COCs showed less apoptotic cells than the other diameter groups in both morphologies. ACC in the largest COCs were in an earlier stage of apoptosis than in the other diameter groups, as well as in the Healthy group in comparison with EA group (H: 18.36, 27.18, 51.78% and EA: 12.96, 12.99, 27.14% for (i), (ii) and (iii) groups, respectively). In conclusion, apoptotic rate in COCs was lower in the largest ones, which could explain the higher blastocyst rate found in this diameter group in previous studies. Moreover, COC morphology is related to a differential incidence of apoptosis in cumulus cells.