## P147

### Crocin improves frozen/thawed bovine sperm motility and viability

**V Sapanidou[1], I Taitzoglou[1], I Tsakmakidis[1], Z Abas[2], I Zervos[1], S Lavrentiadou[1], M Tsantarliotou[1]**

[1]*School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece;* [2]*School of Agricultural Development, Democritus University, Nea Orestiada, Greece*

Crocin, a carotenoid constituent of spice Crocus sativus L. (saffron), is known for its antioxidant activity both *in vivo* and *in vitro*. During assisted reproductive techniques, oxidative stress affects motility and viability of spermatozoa and reduces their fertilization capacity. The aim of the present study was to evaluate the effect of crocin on bovine sperm motility and viability, *in vitro*. Frozen/thawed spermatozoa of four different bulls were pooled and incubated with three different concentrations of crocin (0.5, 1 and 2 mM), at 37°C and evaluated in three different time points (0, 120, 240 min) by CASA (Computer Assisted Sperm Analyzer). The experiment was repeated six times. The parameters 'Rapid', 'Medium', 'Slow', 'Static', 'Progressive Motility', VCL, VSL and VAP were analyzed as repeated measurements with the evaluation of semen viability included. The results indicate an interaction between time and the 1 mM concentration of crocin. Specifically, the percentage of 'Rapid' spermatozoa showed statistical significant increase ($p < 0.05$) in the presence of 1 mM of crocin. Moreover the incubation of spermatozoa with 1 mM of crocin for 120 min showed the greater number of live spermatozoa with intact acrosome. The rest of the motility parameters ('Slow', 'Progressive motility', VCL, VSL and VAP) showed a similar trend to increase, while the percentage of static spermatozoa showed a trend to decrease. We suggest the beneficial role of crocin on bovine sperm viability and motility in the media of assisted reproductive techniques.

## P148

### Abstract withdrawn.

## P149

### Early pregnancy detection using ultrasound imaging of uterine and corpus luteum characteristics in dairy cows

**S Scully[1], M Mullen[2], M Diskin[2], A Evans[3], M Crowe[1]**

[1]*School of Veterinary Medicine, University College Dublin, Dublin, Ireland;* [2]*AGRIC, Teagasc, Galway, Ireland;* [3]*School of Agriculture and Food Science, University College Dublin, Ireland*

To assess the accuracy of early pregnancy diagnosis using uterine echotexture and CL characteristics, reproductive tracts of dairy cows ($n = 22$) were examined from days 18 to 21 following AI by transrectal ultrasound using a Volusoni scanner fitted with a 12 MHz probe. A pregnancy diagnosis and certainty score (1–3) was based on uterine echotexture (homogeneity and contrast), CL tissue area and Vascularity [blood flow area, BFA)] at each scan. Blood samples were collected for progesterone (P4) analysis. Cows were retrospectively allocated to either pregnant (P. $n = 13$) or non-pregnant (NP. $n = 9$) following a final ultrasound exam between days 30 and 40 after AI. Diagnostic data were analysed using the N2 statistic. The mixed procedure of SAS was used for image analysis data. Visual diagnostic specificity increased numerically ($p = 0.86$) from 62.5% on day 18 to 87.5% on day 21. Certainty scores ranged from 2.5 to 3 on all days for both P and NP cows. Sensitivity remained between 71.4% and 78.6% for all days ($p < 0.05$). Sensitivity and specificity for cows were 76.7% and 62.5%, respectively. Homogeneity and contrast of the uterus were not different ($p > 0.05$) between P and NP on any day. Both CL tissue area and blood flow area were higher ($p < 0.05$) in P cows on days 20 and 21 following AI. P4 was higher ($p < 0.001$) in P compared to NP on all days. Specificity was most accurate on day 21 while image analysis on day 21 showed that NP cows had lower ($p < 0.05$) values for CL area and BFA than P cows.

## P150

### Effects of addition of Omega-3,6,9 fatty acids to the semen extender on the characteristics of frozen-thawed bull Sperm

**M Sheikholeslami[1], J Arshami[1], A Abavisani[2]**

[1]*Department of Animal Sciences, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran;* [2]*Department of Basic Sciences, Veterinary Faculty & Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran*

It has been shown that the presence of unsaturated fatty acids in the sperm membrane increases membrane fluidity and its resistance to freezing. In this study, several levels of combination of omega-3,6,9 fatty acids were added to the semen diluents to investigate its effects on motility, viability and morphology of bull frozen-thawed sperm. To emulsify the oil in semen extender, polyethylene glycol (PEG) was added and solution was finally sonicated. For experiment: in treatment 1, PEG was added alone to the samples and in treatments 2, 3 and 4 three different concentrations of omega-3,6,9 fatty acids (1%, 2.5% and 5%) were added to the semen extender. Five proven bulls were randomly selected and parameters of collected semen were recorded. Then semen samples were packed into 0.5 ml straws and were typically frozen. Samples were measured in terms of Motility, viability and morphology after 1 month. Motility and other dynamic parameters were analyzed by computer aided sperm analyzer (CASA). The results were evaluated by repeated measure ANOVA and $p < 0.05$ was considered significant. Motility, Viability and normal morphology were 32.24%, 10.38%, 11.83%, 9.71% and 8.02%; 65.5%, 17.5%, 21.25%, 15% and 15.67%; 87%, 77.75%, 80%, 78.25% and 78.67% for control, treatment 1, 2, 3 and 4 respectively. Our results showed that PEG had some detrimental effects on sperm and the combination of omega-3,6,9 fatty acids could not significantly improve frozen-thawed sperm motility and viability. In conclusion, it seems that this fatty acid combination could not improve sperm membrane resistance to freezing *in vitro*.

## P151

### Derivation of pluripotent stem cell-like cells from nuclear transferred cloned bubaline (Bubalus bubalis) embryos

**B Singh[1], S Gautam[1], V Verma[2], S Singla[3]**

[1]*Indian Veterinary Research Insitute, Regional Station Palampur, Kurukshetra, India;* [2]*National Heart Centre, Singapore;* [3]*National Dairy Research Institute, Karnal, India*

Stem cell technology has undergone remarkable metamorphosis, and has been shown to have enormous significance in livestock assisted reproduction, conservation of animal genetic diversity, regenerative medicine and various veterinary health applications. This study reports derivation of pluripotent stem cell-like cells from the inner cell mass (ICM) cells of nuclear transfer cloned bubaline embryos. The cultured cumulus cells at passage 6, were trypsinized and vitrified (−196°C) for 30 days. The oocytes were aspirated from abattoir-derived fresh ovaries, and matured *in vitro*. The IVM oocytes were denuded and enucleated, and cytoplasts were electrofused with re-cultured cumulus cells for producing somatic cell nuclear transfer (SCNT) cloned embryos. The SCNT morulae and blastocysts were used as sources of obtaining ICM cells by micromanipulator-guided glass capillary holding- and suction-pipettes. The ICM cell colonies were dome