## Poster abstracts
## Poster Session I (Student Competition; odd numbered posters)

## Abstract P1

### Developmental competence and lipid droplet features of maturing bovine oocytes are affected by saturated and not by unsaturated fatty acids

**H Aardema, PLAM Vos, BAJ Roelen, HM Knijn and BM Gadella**

*Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands*

Fertility in high producing dairy cows has declined over the last decades. An increased serum and follicular fluid concentration of non-esterified fatty acids (NEFA), due to body fat mobilisation in the early post-partum period, has been postulated as a cause for this fertility decline (*Leroy 2005: Reproduction 130, 485*). Therefore effects of unsaturated (oleic acid) and saturated (palmitic acid) NEFA during maturation of cumulus oocyte complexes (COCs) on developmental competence and lipid droplet (LD) features in the oocyte were examined in this study. COCs from 3 to 8 mm follicles of slaughter-house ovaries were cultured in control maturation medium (TCM199) and medium containing oleic or palmitic acid (100 $\mu$M NEFA bound to 0.1% BSA), based on follicular fluid concentrations early post-partum. After 23 h of maturation, COCs were fertilized (500 per group) and cultured to score embryonic development, or fixed (60 per group) for LD staining with $C_1$-BODIPY® 500/510 $C_{12}$. Embryonic development was impaired after COCs were matured in presence of palmitic acid (18 ± 1.4% blastocysts) compared to the control (23 ± 1.6%) and oleic acid group (23 ± 1.6%). Moreover, the average LD size (in $\mu m^2$) in MII stage oocytes matured in the presence of palmitic acid (2.16 ± 0.24) was smaller compared to oleic acid (2.91 ± 0.30), but not different from standard medium (2.81 ± 0.37). Whether the effect of palmitic acid on lipid droplet features in the oocyte is correlated with reduced developmental capacity of COCs is under current research.

## Abstract P3

### Leptin mRNA is Present in Bovine Ejaculated Spermatozoa

**A Abavisani[1], A Baghbanzadeh[2], P Shayan[2], P Tajik[2], H Dehghani[3] and M Bahreini[4]**

[1]*Department of Animal Sciences, University of Tehran, Iran;* [2]*Veterinary Faculty, Tehran, Iran;* [3]*Veterinary Faculty, Mashhad, Iran;* [4]*Country Breeding Center, Karaj, Iran*

Leptin, hormonal product of the *ob* gene, is involved in energy metabolism and reproduction. Expression of leptin and its receptor in some reproductive organs suggest that in addition to endocrine effects of leptin, it has paracrine/autocrine effects on reproduction. Evidence, such as expression and secretion of leptin in human and pig spermatozoa, have pointed to a direct role of leptin in the physiologic functions of spermatozoa. The present study was the first to investigate the presence of the leptin mRNA transcript in ejaculated spermatozoa of Holstein cattle using RT-PCR analysis. Total RNA was extracted from sperm cells by TRizol procedure, and used to construct cDNA. The PCR with spermatozoal cDNA and outer leptin primer pairs resulted in amplification of the expected product. To confirm the first results, RT-PCR products were amplified with nested PCR using inner leptin primer pairs. Both outer and inner primer pairs, which were located in exon 2 and exon 3 RNA specific, gave the expected PCR amplified products of 441 bp and 384 bp respectively, and ruled out the possibility of DNA contamination. The presence of leptin mRNA in the ejaculated spermatozoa of bulls suggests that leptin might act on physiological processes of bovine spermatozoa through autocrine/paracrine mechanisms.

## Abstract P5

### DNA Fragmentation in Fresh and Frozen-thawed Stallion Spermatozoa

**O Anglada, E Taberner, A Peña and J Miró**

*Facultat Veterinària. Universitat Autònoma de Barcelona, Spain*

Sperm DNA fragmentation (sDF) seems to be a cause of infertility. The aim of this study was to evaluate the level of sDF in fresh semen of several stallions and its evolution in frozen-thawed semen. 28 ejaculates were collected from 5 stallions. Semen was immediately diluted 1 : 5 in pre-warmed (37°C) Kenney extender in 50-ml Corning tubes. Semen samples were centrifuged at 660 × ***g***, 15 min, 20°C. The supernatant was removed and the pellet resuspended in Gent diluent to obtain a final concentration of $200 \times 10^6$ viable sperm/ml. Semen was packaged in 0.5-ml straws and frozen in a programmable liquid nitrogen freezer. Frozen semen was thawed in a water bath at 37°C for 30 s. Aliquots of 25 $\mu$l of extended semen before centrifugation and frozen/thawed semen were used to evaluate DNA fragmentation using the Sperm-Halomax kit. The slides were stained by propidium iodide in Vectashield mounting medium H-1000 and 200 sperm cells/sample were evaluated under fluorescence microscopy. No significant differences in sDF were observed between fresh semen samples (14.6 ± 1.7%) and frozen-thawed semen samples (17.2 ± 1.5%). No inter-individual differences were observed. The only significant differences in sDF were observed, by the General Linear Model (SAS 9.1), between two stallions in fresh semen (23.1 ± 3.3% vs 9.7 ± 1.5%, $p < 0.05$). However, a high intra-individual variability coefficient was noted. These results suggest that the freezing process does not affect DNA fragmentation and that there is variability between ejaculates of the same stallion.

## Abstract P7

### Effect of Porcine Oviductal Fluid on Spermatozoa-*zona pellucida* Binding

**K Avilés-López, FA García-Vázquez and C Matás**

*Dpto de Fisiología, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain*

Porcine IVP has not been fully developed because of several problems including polyspermy after IVF. *In vivo* the sperm cell is probably modified by the oviduct epithelium that adsorbs proteins from the sperm surface and secretes glycoproteins with an unknown function in sperm-ZP binding. The aim of the study was to evaluate the effect of porcine oviductal fluid (POF) protein on binding spermatozoa-ZP using ejaculated (EJ) and epididymal (EP) sperm under different treatments. EJ and EP spermatozoa were divided into 3 groups:1) spermatozoa without any treatment (control = C); 2) washed through Percoll® gradient (P); 3) washed through Percoll® and incubated with POF protein (50 $\mu$g/ml) for 30 min (P-POF). IVM oocytes (n = 1135) were inseminated in TALP medium for 30 min using different sperm groups. Finally, the oocytes were fixed and Hoechst stained to evaluate the spermatozoa bound under epifluorescence microscope. Six assays were evaluated. The results were analysed by two-way ANOVA. Data showed that i) the binding to ZP is higher using epididymal sperm than ejaculated (32.98 vs 17.74 respectively, $p < 0.001$); ii) binding decreased significantly when epididymal sperm was incubated with POF (25.36) vs other groups (C = 37.14, p = 36.44, $p < 0.05$); and iii) the ejaculated sperm binding results were similar in all experimental groups (C = 22.87, p = 15.73 and P-FOP = 14.62, $p < 0.118$). In conclusion sperm source as well as sperm treatment affect the binding on ZP. Protein POF decreased the binding of sperm in our studied conditions. *(Supported by Seneca 08752/PI/08 and AGL2006-03495.)*