Abstract OC1.5

Negative Correlation of Follicular Fluid Lipid Peroxidation and the Developmental Competence of Bovine Oocytes *In Vitro*

JBP De Clercq[1], M Nichi[1,2], IGF Goovaerts[1] and PEJ Bols[1]

[1]*Lab of Veterinary Physiology, Univ Antwerp, Belgium,* [2]*Faculty of Veterinary Medicine and Zootechnia, Univ São Paulo, Brazil*

Follicular fluid environment is known to play a crucial role on *in vitro* fertilization and embryo development, while lipid peroxidation affects many biological systems. A prolonged time interval between ovary collection and the onset of *in vitro* oocyte maturation may therefore induce follicular peroxidation and affect oocyte developmental capacity and blastocyst rate. To investigate this possibility, ovaries were collected in the slaughterhouse and kept warm for one (n = 24), two (n = 22) or four (n = 22) hours. Subsequently, follicles were punctured and the oocytes as well as the follicular fluid were pooled per group (3 replicates). While the oocytes were processed in a routine IVF-IVC set up (24 hr maturation, SOF culture medium in 5% CO_2, 5% O_2 and 90% N_2), follicular fluid was tested on peroxidation by measuring tiobarbituric acid reactive substances levels (TBARs, modified from Paskowski *et al*, Human Reprod., 2002). Blastocyst rates were calculated 8 days post fertilization. The interval between collection and processing the oocytes had no effect on *in vitro* blastocysts rates. However, a negative correlation was found between follicular fluid TBARs levels ($r = -0.89$; $p = 0.002$) and the final blastocyst rates. TBARs levels of 145 ng/ml resulted in a blastocyst rate of 24% while levels of 176 ng/ml gave 16% of blastocyst. These findings suggest that lipid peroxidation in follicular fluid may be a determining factor on *in vitro* oocyte developmental competence.

Abstract OC1.6

Evidence for the Presence of Sialidase Enzyme in the Bovine Oocyte. Its Role in the Block to Polyspermy

JG Velasquez[1], P Barajas[1], J Marcos[2], J Ballesta[2], M Avilés[2] and P Coy[3]

[1]*CORPOICA and la Salle University, Colombia,* [2]*Cell Biology, Medicine Faculty,* [3]*Physiology, Veterinary Faculty, University of Murcia, Spain*

A significant decrease in the amount of acidic oligosaccharidie chains of the bovine zona pellucida (ZP) glycoproteins has been described after fertilization. This decrease has been attributed to an enzymatic digestion of sialic acid residues, suggesting that a sialidase enzyme released from the oocyte could be operating in this process. We have previously demonstrated the involvement of the sialic acid in the sperm-ZP interaction. However, no evidence has been presented demonstrating neither the presence of a sialidase in the bovine oocyte nor its physiological role in the block to polyspermy. By employing the IVF as a tool to investigate the role of this enzyme, we showed that the addition of a sialidase inhibitor to the IVF medium significantly decreased the percentage of monospermy. A significant increase in the number of penetrated spermatozoa per oocyte (1.33 vs. 1.07) and in the number of spermatozoa bound to the ZP (38.3 vs. 18.4) was observed. Treatment of the oocyte with sialidase before IVF significantly decreased the percentage of penetrated oocytes compared to control (33% vs. 80%). These results strongly suggest the presence of sialidase in the bovine oocyte and its involvement in the block to polyspermy. The hypothesized mechanism would include the removal of sialic acid residues of the ZP and, consequently, the decreasing in the number of spermatozoa bound to the ZP. Supported by AGL2003-03144, BFU2004-05568.

Abstract OC2.1

Heterogeneity of Boar Sperm Response to Ion Channel Blockers and Osmolality

AM Petrunkina, E Jebe, D Waberski and E Töpfer-Petersen

Institute for Reproductive Medicine, School of Veterinary Medicine of Hannover, Germany

Spermatozoa of many species initially respond to hypotonicity as perfect osmometers. Thereafter they undergo a regulatory process resulting in cell volume decrease (RVD). In this study we investigated whether sperm are able to regulate their volumes after hypertonic stress (regulatory volume increase, RVI) and whether this ability is maintained in preserved sperm. Cell volumes and membrane integrity were recorded using electronic cell sizing and flow cytometry. Ion channel blockers quinidine, tamoxifen, and dydeoxyforskolin were used to study possible mechanisms of RVI. Spermatozoa exposed to hypertonic stress initially responded with an abundant subpopulation according to the perfect osmometer model and recovered their volume from this shrinkage after 20 min. RVI was inhibited by quinidine and tamoxifen, which indicates the involvement of the cellular ions sodium and chloride in this process. Volume regulatory ability was essentially maintained during 48 h-storage of liquid semen. However, the sperm response was heterogeneous. A second population rised, containing spermatozoa with larger volumes. These sperm demonstrated irregular behaviour in response to osmotic challenge, ion channel blockers and storage, probably associated with functionality of ion channels. In conclusion, RVI is clearly very sensitive to functional heterogeneity of sperm populations. These properties may be useful for the establishment of a volume regulation test for sperm quality assessment by identifying RVD/RVI-lacking subpopulations and may have use in cryopreservation.

Abstract OC2.2

Boar Spermatozoa are still Capable of *In Vivo* Fertilisation after 12 h Incubation *In Utero*

P Langendijk, NE Teodoru and NM Soede

Wageningen University, The Netherlands

The *in vivo* fertilising ability of spermatozoa, after long term incubation in the uterus (*in utero*), has never been studied. Nevertheless, the general belief is that within a few hours after insemination of sows, spermatozoa that remain in the uterine horns are voided by reflux, fagocytosed, or die because of the destabilising uterine environment. In a small study, fertilisation (Day 5 embryos) was examined in sows that were inseminated with semen recovered from the uterine horns of donor-sows at 4 (n = 3), 8 (n = 1) or 12 (n = 2) hours after insemination. Donor and recipient sows were synchronized using Regumate, PMSG and hCG. Donors were inseminated with full ejaculates (40 to 100 billion spermatozoa). After a given incubation period, donors were slaughtered, and the uterine horn was flushed to recover spermatozoa. Recipient sows were inseminated IU at 1 to 9 h before expected ovulation. For the 5 sows in the study, the number of spermatozoa inseminated after recovery from the donors was 6×10^7, 20×10^7, 0×10^7, 29×10^7, 5×10^7, and 178×10^7, respectively. At Day 5, the five sows had 0, 8 and 6 embryos (4 h-incubation), 17 embryos (8 h-incubation), and 3 and 21 embryos (12 h-incubation). We conclude that even after long-term uterine incubation (12 h), spermatozoa are still able to reach the oviduct and fertilise.

Abstract OC2.3

Porcine Sperm Mediated Gene Transfer: Use of Flow Cytometry to Evaluate Binding of Exogenous DNA to Spermatozoa

F García-Vázquez[1], D Gumbao[1], A Gutiérrez-Adán[2] and J Gadea[1]

[1]*Department of Fisiología, Facultad de Veterinaria, Murcia,*
[2]*Department of Reproducción Animal, INIA, Madrid, Spain*

Sperm Mediated Gene Transfer (SMGT) is an interesting tool for animal transgenesis and biotechnology because spermatozoa may be used as a vector for transmitting exogenous DNA into eggs. The aim of this study was to evaluate the capacity of spermatozoa to bind exogenous DNA, previously marked with fluorescein using flow cytometry. Semen from five fertile boars was recovered and immediately diluted 1:10 in SFM (Swine Fertilize Medium) at 37ordm;C and later centrifuged (800 g 10 min, 25°C) discarding the seminal plasma to avoid detrimental effect on DNA binding to cells. Linealised plasmid DNA (5.4 kb), marked with random primed DNA labeling method with fluorescein-12-dUTP (Roche, Germany) was added (1×10^8 spermatozoa/ml + 5 μg DNA/ml) and incubated at 16°C.

DNA binding was measured by flow cytometry at 0, 15, 30, 60, 90, 120 min of incubation. The results showed that the main DNA binding to spermatozoa was achieved as soon as at 15 min (15.69 ± 0.93), increased thereafter significantly until 30 min (17.89 ± 0.63); thereafter no further significant increase in the binding was detected (17.79; 18.02 and 19.49; at 60, 90 and 120 min, respectively). Nevertheless, a significant boar effect was detected. The percentage of binding detected by cytometry was lower than obtained by microscopy, because only strong binding is registered. These data suggest that flow cytometry is very helpful in evaluating sperm DNA binding capacity by providing a specific, objective, accurate and reproducible method compared to traditional microscopy-based methods.

Abstract OC2.4

Status of Lipid Peroxidation Protection during the Maturation of Bovine Epididymal Sperm

M Nichi[1,2], JBP De Clercq[1], IGF Goovaerts[1], VH Barnabe[2] and PEJ Bols[1]

[1]*Lab of Veterinary Physiology, University of Antwerp, Belgium,* [2]*Faculty of Veternary Medicine and Zootechnia, University of São Paulo, Brazil*

Bovine spermatozoa are known to be extremely susceptible to the damages imposed by reactive oxigen species (ROS), and therefore being dependent on the extracelullar protection. In addition, the sperm membrane plays an important role in the protection against ROS, especially during ejaculation and the transit through the female reproductive tract. Since sperm maturation includes several modifications, many of them still unknown, the aim of the present study was to verify if the acquisition of antioxidant protection during maturation is one of these. Sperm cells of 12 epididymides (cauda and caput) were collected by aspiration and submitted to induced oxidative stress by co-incubation with ascorbic acid and ferrous sulfate by 1.5 hrs. Subsequently, levels of tiobarbituric acid reactive susbtances (TBARS), qs an index of oxidative stress, were measured using spectrophotometer (Breininger, 2004 Theriogenology). Results were analysed using SAS, by paired t-test. Semen samples of the cauda epididymidis were found to be more resistent to the oxidative stress when compared to samples from the caput (318.4 ± 44 and 452.5 ± 50 ng of TBARS/10^8 spermatozoa, respectively; $p = 0.001$). These results suggest that maturation modifications during epididymal transit may include the addition of antioxidant protection to the plasma membrane, so that sperm cell viability is not only depending on the epididymal fluid or seminal plasma when it comes to resistance against ROS induced effects.

Abstract OC2.5

A Simple Technique to study Sperm DNA Fragmentation in Pig Spermatozoa

M Enciso[1], J L Fernández[2], A Gosálbez[1], P García[3], J Gosálvez[1] and C López-Fernández[1]

[1]*Ud. Genética,Universidad Autónoma de Madrid,* [2]*Ud. Genética Hospital Juan Canalejo, A Coruña* [3]*Ud. Reproducción Animal INIA Madrid, Spain*

The Sperm DNA Integrity (SDI) is an important factor for pregnancy results and normal embryo development. SDI is being recognized as a new parameter of semen quality and a potential fertility predictor. SDI is not being assessed as a routine part of semen analysis in the clinical laboratory and of course, less known in species other than humans. A new procedure, the Sperm Chromatin Dispersion (SCD) test, is now available for the determination of SDI in human sperm cells. This method gives rise to sperm nucleoids with a central core and a peripheral halo of dispersed DNA loops. In pig spermatozoa, adaptation of this methodology reveals that nuclei with low DNA fragmentation produce very small or absence of halos in the head of the spermatozoon, whereas those sperm nuclei without with high levels of DNA fragmentation release their DNA loops forming large halos. DNA Breakage Detection-Fluorescence, *In Situ* Hybridization and In Situ Nick Translation confirmed the presence of DNA fragmentation. Using both methodologies, single-stranded DNA motifs generated from DNA breaks can be detected and quantified under fluorescence microscopy. Subsequently, SDI or DNA fragmentation in sperm nuclei, as reflected by halo size, can be accurately determined by assessing halo size. This technique is adapted to analyse porcine sperm samples under bright field or fluorescence microscopy.

Abstract OC2.6

Preservation of Tomcat Semen at Variable Temperatures

M Siemieniuch and W Bielas

Veterinary Faculty, Wrocław, Poland

The aim of this study was to estimate cat sperms quality before and after preservation at 4°C and −196°C. Spermatozoa were obtained from the epididymis after castration from 12 clinically healthy cats. The percentage of spermatozoa characterized by progressive motility was 70–80%. The smears of semen were stained using eosine/negrosine for the evaluation of their viability. The efficiency of this method was confirmed by flow cytometry technics. The sperms were conjugated with SYBR-14 and propidium iodide and presented 82% alive spermatozoa. Giemsa stain was used for morphology and intact acrosomes assessment. Normozoospermia was present in 72% spermatozoa flushed from epididymis. Spermatozoa were equilibrated with the dilution and stored at 4°C or loaded into 0,25 ml plastic straws and plunged in LN_2. Sixty percent of motility sperms were achieved after thawing. However, the percentage of the sperm with intact acrosome was decreased and the percentage of the cells with midpiece and tail defects was increased. The viability of sperms amounting to 30–40% was achieved on 3rd-4th, even 7th day of conservation in the liquid phase. The viability oscylated between 70–80% 1st and 2nd day of conservation. Concluding: cat spermatozoa are easily preserved frozen or in liquids. Freezing of tomcat semen gave acceptable results as regards cells viability, motility and morphology. Short storage at 5°C for use within 2–3 days from collection is yet better than cryopreservation.

Abstract OC3.1

Catheter Insertion into the Uterine Horns During Deep Intrauterine Insemination does not Compromise Future Fertility Potential of Sows

A Bolarín, M Hernández, JM Vázquez, EA Martínez and J Roca

Department of Medicine and Animal Surgery, University of Murcia, Spain

Deep intrauterine insemination (DUI) is a reliable procedure for artificial insemination (AI) in sows, providing good fertility rates with low numbers of spermatozoa, including frozen-thawed semen. However, it has been speculated that there may be potential damage from the DUI catheter to the uterine mucosa, compromising subsequent fertility in the sow. The present experiment evaluated this possibility. A total of 159 weaned sows (parity 2–8) were DUI-inseminated twice in a commercial farm throughout 10 months (October to July) with $1\text{-}2 \times 10^9$ frozen-thawed spermatozoa. A total of 99 sows (62.69%) were pregnant. Farrowing rate was 97 (61.01%), with a mean litter size of 9.58 ± 0.28. Reproductive data of previous and subsequent AIs to DUI were recorded and compared. No differences ($p > 0.05$) were found in pregnancy (87.42% vs 86.16%) and farrowing (84.91% vs 84.28%) rates or litter size (11.08 ± 0.17 vs 11.50 ± 0.15 piglets born) between the AI before and after DUI. Moreover, not in pig rates were also similar (2.88% vs 2.19%, $p > 0.05$). In addition, among those sows that were not pregnant with DUI, and showed a regular or irregular oestrus return, there were no significant differences ($p > 0.05$) in pregnancy loss before or after DUI (0.63% vs 2.52%). In conclusion, insertion of the DUI catheter into the uterine horn does not affect subsequent fertility potential of sows. *Supported by CICYT (AGL01-0471) and INIA (RZ01-019).*