Abstract OC1.5

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

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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

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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

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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*

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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

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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.