Abstract P17

Effect of Heparin on Pig *In vitro* Fertilization

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Participation of the glycosaminoglycan heparin on the pre-fertilization zona pellucida hardening mechanism has been shown to improve *in vitro* fertilization efficiency in pigs (*Coy* et al., *2008, PNAS 105:15809–15814*). On the other hand, it was suggested that heparin did not support *in vitro* penetration of pig oocytes (*Wang* et al., *1991, J Reprod fertil 93:491–496*) although it is an active component for capacitation in cattle (*Parrish* et al., *1989, Biol Reprod 40:1020–1025*). Since heparin-like glycosaminoglycans are present in the porcine oviduct (*Tienthai* et al., *2000, Reprod Dom Anim 35:167–170*), this study was designed to clarify the role of heparin on different steps during pig *in vitro* fertilization. *In vitro* matured pig oocytes (n = 221) were fertilized in TALP medium containing or lacking (control) 1 μg/ml heparin. A gamete co-incubation time of 15 min and a sperm concentration of 1 × 105 cells/ml were used. At 18-h post-insemination, mean number of sperm bound to ZP, mean number of sperm inside the oocytes, and percentages of penetration and monospermy were assessed. The results showed that heparin did not affect the ability of the sperm to bind zona pellucida (43.8 ± 3.6 vs 39.84 ± 2.7), but it decreased their ability to penetrate it (87.7 ± 3.1% vs 28.97 ± 4.40%, $p < 0.001$). Since hyperactivation of capacitated spermatozoa is necessary to penetrate the zona (*Suarez, 2008, Hum Reprod Update 14:647–657*), the possibility that heparin can induce capacitation but blocks hyperactivation in pig is proposed, although other hypotheses such as failures in acrosome reaction cannot be discarded. *(Granted by MEC/FEDER, AGL2006-03495/GAN.)*

Abstract P19

Freezability of Piétrain Boar Ejaculates Evaluated by Sperm Kinematics

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Piétrain is a well-known paternal line used as the terminal boar in many crossbreeding systems, mainly due to its excellent food conversion and low fat indexes. Semen banking conserves valuable genetic stock for breeding. The present study compares changes in kinematic parameters in refrigerated (17°C) and frozen/thawed sperm, with the objective of describing the freezability of the Piétrain breed. Sperm-rich fractions of 180 ejaculates collected from 44 mature Piétrain boars were diluted 1 : 5 (v:v), transported and stored at 17°C in our laboratory until cryopreservation using the Westendorf method. Eight kinematic parameters were analyzed in refrigerated semen and post-thawing (30 min, 37°C), using CASA software. Values were compared using one-way ANOVA and significance was set at $p < 0.01$. 37.2% of the cryopreserved ejaculates displayed over 40% of progressive motility 30 min post-thawing. Data analysis revealed that all motility parameters decreased in Piétrain spermatozoa after freezing/thawing: -22.6% total motility; -22.3% progressive motility; -9.1% circular trips; -18.1 μm/s VCL; -6.2 μm/s VSL; -14.5 μm/s VAP; -0.2 μm ALH, and -1.4 Hz BCF.

Abstract P21

Pro/acrosin Expression in Epididymal Spermatozoa from Fertile Boars

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Acrosin is a spermatic enzyme stored as a zymogen, proacrosin, in the acrosomal compartment. It plays an important role in spermatozoa binding and penetration through the zona pellucida. The aim of this experiment was to study the expression of pro/acrosin along epididymis in order to characterize the changes associated to its maturation. Epididymal samples were obtained by cannulation of six epididymal regions of post-pubertal fertile boars (proximal and distal caput; proximal and distal corpus; proximal and distal cauda). Undiluted samples collected from each region were divided into two Eppendorf tubes. One tube was centrifuged at 13000 × ***g*** for 30 min at 4°C, and supernatant was centrifuged again during 5 min to obtain the sperm-free epididymal fluid. The other tube was washed twice with PBS at 600 × ***g*** for 10 min at 4°C to obtain the sperm fraction. Epididymal fluid and sperm samples of each region were analyzed by SDS-PAGE and Western Blot, differentiating four pro/acrosin isoforms. Both spermatozoa and fluid samples showed a similar pattern: in the proximal caput only the proacrosin band (54–49 kDa) was detected; the α-acrosin band (47–42 kDa) appeared in distal caput, whereas ß- (37–32 kDa) and γ-acrosin bands (32–27 kDa) appeared in proximal corpus and their expression increased progressively throughout the cauda. These results indicated that in the boar the maturation of proacrosin into acrosin isoforms begins in the caput and becomes progressively more intense throughout the corpus and, specially, the cauda regions.

Abstract P23

The Effect of the Local Administration of FSH and/or Misoprostol on Cervical Penetration in the Ewe during the Peri-Ovulatory Period

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Trans-cervical intrauterine insemination in the sheep is limited by the convoluted anatomy of the cervix, making passage of an insemination catheter into the uterine lumen almost impossible. The aim of the study was to investigate the effect of intra-cervical administration of FSH, Misoprostol (a PGE1 analogue) or a combination of the two on cervical penetration 54 h after sponge removal. Forty ewes were synchronised using progestagen sponges and were randomly assigned to four groups of 10: control; Misoprostol; FSH; Misoprostol plus FSH. Misoprostol (1 mg) was administered twice at 46 and 50 h after sponge removal while FSH (2 mg) was administered once 24 h after sponge removal. The hormones were prepared in 0.5 ml of 50% gum acacia in saline and deposited into the cervix as far cranial as possible. The depth of cervical penetration (mm) was measured 54 h after sponge removal using a modified cattle artificial insemination pipette. The experiment was performed in two replicates and the data were analysed using one way ANOVA. No significant differences in the depth of cervical penetration were found between the control group (mean = 14.5 ± 3.3) and the FSH (mean = 17.7 ± 2.2), Misoprostol (mean = 21.3 ± 3.5), and the Misoprostol plus FSH (mean = 19.2 ± 3.0) treated groups ($p > 0.05$). Misoprostol, FSH, or the combination treatment were not effective in dilating the cervix 54 h after sponge removal, the time at which artificial insemination produces the highest fertility rates in sheep.

Abstract P25

The Expression of Oxytocin Receptor, cPhospolipase A_2 and Cyclooxygenase 2 in the Cervix of Ewes during the Oestrous Cycle

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In the cervix of the ewe at oestrus, there is a degree of natural relaxation mediated by locally synthesised cervical prostaglandins. Oxytocin, binding to its receptor (OTR), may stimulate the synthesis of prostaglandin E_2 (PGE_2) through the activation of cytosolic phospholipase A_2 ($cPLA_2$), mobilizing the substrate arachidonic acid (AA), that cyclooxygenase 2 (COX-2) converts into prostaglandins. The aim of the present work was to determine the amounts of OTR, $cPLA_2$ and