Therapy System) was added (6×10^6 cells and 250 ng DNA) and incubated at room temperature. Seminal quality was measured at 0, 1 and 2 h in both DNA and control group for the three sperm treatments. Neither the sperm treatments nor the presence of DNA affected seminal quality ($p > 0.05$). DNA binding was measured with microscope fluorescence at 2 and 24 h. Significant effects of boar ($p < 0.001$) and sperm treatments ($p = 0.008$) were observed, with a higher DNA binding in groups A and B (18.10 ± 2.80% and 14.59 ± 3.63%) than C (5.65 ± 0.80%). However, there were not differences in the evaluation at 2 or 24 h ($p = 0.583$). These data suggest a detrimental effect of seminal plasma components on sperm–DNA binding, and a marked boar effect.

Abstract P55

Porcine Embryo Fragmentation and Apoptotic Markers: A Confocal Microscopy Study

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The relationship between embryonic fragmentation and apoptosis has been the subject of some controversy. In order to investigate a possible link, *in vivo* produced, *in vitro* cultured porcine embryos (n = 132) were scored for fragmentation at 7 days post-insemination and processed for annexin V and TUNEL labelling. The degree of fragmentation was assessed by differential interference contrast microscopy at 40× using two different scoring systems: (1) degrees of fragmentation (in percentage) and (2) patterns of fragmentation as described by Alikani et al. (2000). Subsequently, embryos were analyzed for apoptosis by annexin V and TUNEL assay using a confocal laser scanning microscope. A cell was categorized as apoptotic if: (i) the cell had nuclear morphological characteristics of apoptosis such as fragmentation or condensation; (ii) the cell membrane was annexin V positive and (iii) the nucleus of the cell was TUNEL labelled. Based on this definition an apoptotic cell ratio (ACR) was determined as the percentage of apoptotic cells per embryo. None of the embryos without fragmentation had cells categorized as apoptotic, whereas 49 out of 55 embryos with fragmentation possessed apoptotic cells. The degree and percentage of fragmentation were both linked with the apoptotic cell ratio ($p < 0.001$). The correlations detected between the degree of fragmentation and ACR, and the pattern of fragmentation and ACR, were 0.867 and 0.864, respectively. In conclusion, a significant relationship between porcine embryo fragmentation and apoptosis was shown in this study.

Abstract P56

Non-surgical Transfer of Porcine Embryos with two Types of Catheters

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The aim of this study was to evaluate the use of two catheters for artificial insemination (catheter for intracervical insemination – IC, and catheter type Verona for deep cornual insemination –CI) for non-surgical transfer of porcine embryos. Post-mortem method was used for recovery embryos at 5.5 days of estrus cycle from discarded superovulated breeding gilts. Transfers of embryos were made synchronously into gilts synchronized with Regumate, PMSG, HCG and into sows on first and second parity synchronized by weaning of piglets. Twenty transfers were made in IC group, pregnancy was recognized in seven recipients (35%), in CI 17 ET, seven recipients were pregnant (41.2%, $p < 0.05$). More (high number) embryos were transferred (11.9 ± 4.02 vs. 18.44 ± 6.49, $p < 0.001$) in CI recipients' group. No significant differences ($p > 0.05$) were found in pregnancy parameters among groups, however rate of born total, alive and weaned piglets from transferred embryos were significantly different ($p < 0.001$). Number of born piglets varied from 4 to 12.

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Abstract P57

One-step Dilution of SOPS-vitrified Porcine Blastocysts

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The objective of this experiment was to compare the *in vitro* development of SOPS-vitrified porcine blastocysts obtained after conventional (three-step dilution) or direct (one-step dilution) warming procedures. Expanded blastocysts were collected by laparotomy from weaned crossbred sows (n = 7) on day 6 of the cycle and vitrified as described by Berthelot et al. (2000) using SOPS straws. Conventional warming was carried out by plunging straws containing embryos in TCM199 Hepes with 20% new born calf serum (TCMm) and 0.13 M sucrose (S) for 1 min. Embryos were then transferred to another well with the same medium for 5 min, washed in TCMm with 0.075 M S for 5 min and transferred to TCMm for 5 min. In one-step dilution, embryos were placed in TCMm containing 0.13 M S for 5 min. To evaluate *in vitro* development, embryos warmed by conventional (n = 59) or direct (n = 58) procedures were cultured for 96 h. Non-vitrified fresh embryos were used as controls (n = 20). No significant ($p > 0.05$) differences were observed in the *in vitro* development of vitrified and non-vitrified blastocysts. The survival and hatching rates obtained by three-step (84.8% and 71.2%, respectively) and one-step dilution (86.2% and 74.1%, respectively) procedures were not different ($p > 0.05$). This experiment shows that porcine embryo vitrification and one-step dilution are promising procedures to be used under field conditions.

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Abstract P58

Effect of Acrosome Reaction Induced with Ca^{2+} Ionophore (A23187) during Intracytoplasmic Sperm Injection in Pigs

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It has been suggested that the presence of the intact acrosome during intracytoplasmic sperm injection (ICSI) into the oocyte could be harmful (Tesarik and Mendoza, 1999). Due to the low ICSI performance in pigs, we hypothesized that the induction of the acrosome reaction before ICSI could be a pre-requisite to improve the results. Our goal was to determine the effect of using spermatozoa in which the acrosomal status and viability were modified by treatment with two Ca^{2+} ionophore concentrations, on the porcine ICSI. Oocytes were matured in NCSU-37 for 44 h. Sperm samples were washed on a Percoll® gradient. Pellet was incubated in TALP medium with 1 or 5 μM Ca^{2+} ionophore during 15 and 30 min, respectively. Concentration 0 μM was used as control. ICSI was basically conducted as described by Martin (2000). Injected oocytes were transferred to TALP until 18 h when they were either fixed and stained in acetic/orcein to check fertilization or transferred to NCSU-23 medium for further embryo culture. Results (analyzed by ANOVA) show that 60% of spermatozoa treated with 1 μM Ca^{2+} ionophore were live reacted, 57.8% of those treated with 5 μM were dead reacted, whereas 74% of them were live non-reacted for the control group. However, the sperm treatment did not affect the rates of activation (80.95 ± 4.99, 78.67 ± 4.76 and 85.11 ± 5.25), fertilization (74.51 ± 6.16, 84.75 ± 4.72 and 80.00 ± 3.49), cleavage (61.02 ± 6.40, 64.41 ± 6.29 and 65.08 ± 6.05) or blastocyst formation (2.78 ± 2.78, 13.16 ± 5.56 and 12.20 ± 5.17) for control, 1 and 5 μM Ca^{2+} ionophore groups, respectively.