Abstract P50

Establishing of Somatic Cell Lines from some Cervidae Species and from the European Bison (Wisent)

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Of the living 37 species and perhaps 200 subspecies of Cervidae as many as 29 are listed in the Red Data Book of the IUNC. The population of European wisent (*Bison bonasus* L.; over 2800 animals) is still considered vulnerable. The establisment of cell banks for preservation of the genome of endangered species has been postulated long before. The technique of preserving embryo- and fetal-derived cell lines of various livestock species has been used in our laboratory to establish fibroblast cell lines originating from the skin (ear and velvet) of adult animals of various rare Cervidae species including two extremely rare mutant strains of red deer (*C. elaphus*; St. Hubertus white deer and the white blazed, bald faced deer). At present, their overall population is slightly over 100 and 20 individuals, respectively. Somatic cell lines were also established *post-mortem* from the skin, ovary and follicular cells of adult wisent cows and from the skin of adult wisent bulls. All samples were frozen after one to three passages and are stored in liquid nitrogen. The cell lines will be used as a source of donor cells in our efforts to inter-species somatic cloning. The recent development of advanced reproduction techniques such as somatic cloning of wild mammals (for example, gaur, mouflon, banteng and white-tailed deer) suggests that these techniques could have clear benefits for conservation biology.

Abstract P51

Equine Oocyte Classification According to Follicular Size, Recovery Method and Ovary of Origin

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As oocyte recovery method, selection criteria or oocyte rejection to be used in *in vitro* reproduction techniques are not well defined in equines, oocytes recovered from the ovaries of slaughtered mares were morphologically classified in relation to the follicle, the ovary of origin and the recovery method. The recovery method used was follicular aspiration followed by ovary slicing in order to obtain the maximum number of oocytes. Recovery rate was 5.5 oocytes per ovary (5.9 with aspiration and 5.2 at slicing). A 38.11% of oocytes showed a more than three-layer granulosa cells compact cumulus, and 39.75% a less than three-layer compact cumulus. Follicular aspiration was observed to change the quality of the oocytes (53.61% with less than three layers, and 29.27% with more than three layers). When using follicular aspiration as recovery method, subsequent slicing of the ovary substantially increased the number of oocytes not obtained through aspiration (46.10%). Both follicles (≥20 and 5–20 mm) provided good quality oocytes (83.55% and 82.97%, respectively). A positive correlation was observed between the number of oocytes coming from the right (5.67%) and left (5.45%) ovaries. Nevertheless, no significant differences were observed when comparing the oocyte quality from right or left ovary.

Abstract P52

Effect of Recovery Method and Maturation Time on *In Vitro* Maturation of Equine Oocytes

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Two methods for oocyte revovery have been compared: Follicular aspiration followed by slicing (AS) or slicing only (S). Oocytes were matured *in vitro* for either 24 or 40 h. *In vitro* maturation medium was TCM 199 supplemented with 10% fetal bovine serum (FBS) and 1 IU hCG. The recovery rate after aspiration followed by slicing or slicing only was 5.1 oocytes and 6.3 ocytes per ovary, respectively. The method of revovery had a highly significant influence ($p \leq 0.001$) on the quality of oocytes. 60.9% of oocytes obtained by slicing had more than three layers of cumulus cells, whereas aspiration and slicing resulted in more oocytes with less than three layers of cumulus cells (47.57%). The percentages of oocytes reaching the MII stage in group S24h, A40h, AS24h and AS40h were 59.5%, 45.5%, 37.0% and 56.7% respectively. Neither the recovery method nor maturation time had a significant influence. Slicing of oocytes provides an acceptable number of oocytes with good quality. Moreover, this recovery method takes less time than follicular aspiration. Indistinctly, maturation time can be 24 or 40 h, so this factor can be adapted to the requirements of each laboratory's work.

Abstract P53

Glutathione Content in *In Vivo* and *In Vitro* Matured Equine Oocytes

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Cysteamine, a glutathione (GSH) synthesis enhancer, has been shown to increase intracellular GSH synthesis and to improve embryo development when added during *in vitro* maturation (IVM) of bovine, porcine and ovine oocytes. The aim of this study was to determine whether equine oocytes benefit from the addition of cysteamine during IVM and to compare the GSH content of horse oocytes after *in vivo* maturation and IVM. *In vivo* matured cumulus–oocyte complexes (COCs) were collected by transvaginal ultrasound-guided aspiration from pre-ovulatory follicles and oocyte GSH concentration was determined after cumulus removal and MII stage assessment. Immature COCs were collected *in vivo* or from slaughterhouse ovaries and matured in TCM199 + 20% fetal calf serum + 50 ng/ml EGF supplemented or not with 100 μM cysteamine for 30 h. After culture, nuclear stage was assessed and MII oocytes were analyzed for GSH content. Our data showed that the maturation rate was similar in both *in vivo* aspirated oocytes and in those isolated from slaughterhouse ovaries. GSH content was similar in *in vivo* and *in vitro* matured (MII) oocytes, and was significantly lower in immature (GV) oocytes. Moreover, the addition of cysteamine during IVM affected neither GSH content nor maturation rate. We hypothesize that factor(s) other than GSH are involved in the achievement of a full *in vitro* developmental capacity of equine oocyte.

Abstract P54

Porcine Sperm-mediated Gene Transfer: Effect of Sperm Treatment on DNA Uptake

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Sperm-mediated gene transfer (SMGT) is an interesting tool for animal transgenesis and biotechnology consisting in the use of sperm cells as a vector for transmitting exogenous DNA into eggs at the moment of fertilization. The aim of this study was to evaluate the effect of different sperm treatments on the capacity to bind with exogenous DNA and on the sperm quality (motility, progressive motility and membrane integrity). Semen from six boars was recovered and divided into three aliquots. Group A: sample was immediately centrifuged to eliminate the seminal plasma and diluted in a commercial extender (BTS), the other two were diluted in the extender 1:10 (maintaining 10% seminal plasma, Group B) and 1:1 (50% seminal plasma, Group C). After 1 h at room temperature samples were processed according to Lavitrano et al. (2002). Plasmid DNA marked with Rhodamine (pGeneGrip-Rhodamine/GFP Gene

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.