Abstract P130

Early Fertilization Events in Bovine IVF Employing OPU-Oocytes and Sex-Sorted Frozen/Thawed Sperm

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Epigenetic disturbances may occur in embryos if fertilization is delayed by asynchronous pronucleus development. The objective of this study was to investigate the effects of flow cytometrical sperm sorting on early fertilization events of OPU-oocytes after IVF with sex-sorted frozen/thawed sperm. 84 OPU sessions were carried out in 18 cyclic, dry and non-stimulated *Holstein Friesian* and *German black pied* cows. OPU oocytes were matured *in vitro* 24 h and fertilized with sex-sorted sperm, separated using the Beltsville Sperm Sexing Technology. Controls were fertilized with unsorted sperm from the same ejaculates. 18 and 24 h after IVF ova were fixed and stained to determine penetration (PEN), monospermy (MON), male pronucleus formation (MPF), performance (PERF, monospermic oocytes with 2 pronuclei from total matured oocytes), syngamy (SYNG) and synchrony of male and female pronuclei development (SYNC) (Xu & Greve, 1988, J Reprod Fert 82:127–34). PEN, MON, MPF, SYNG and SYNC did not differ between sperm treatment and hpi, the only statistical interaction was observed for PERF ($p < 0.05$). PEN ranged from 80–85.2% (sex-sorted) to 87.5–100% (unsorted) and SYNG ranged from 33.4–16.1% (sex-sorted) to 14.3–12.4% (unsorted) for 18–24 hpi, respectively. PEN and SYNG are the most significant parameters as a predictive value of the efficiency of bovine embryo production *in vitro*. Although previous studies have reported a reduction of *in vitro* fertility of sex-sorted sperm, we did not see differences in the early fertilization parameters among treatments.

Abstract P132

Effect of eCG on Steroid Hormone Concentrations and Plasminogen Activator Activity in the Genital Tract of Ewes

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Survival and growth of early mammalian embryos before implantation largely depend on uterine environment. This study investigates the effect of eCG used for estrus synchronization or superovulation on ovarian steroid concentrations and plasminogen activator activity (PAA) in the genital tract of ewes, before implantation. At the end of a 12-day progestagen treatment, 13 adult Chios ewes received either 500 IU (ES, $n = 6$) or 1000 IU (SOV, $n = 7$) eCG (Intergonan); 7 ewes served as controls. On day 5 (day of intrauterine artificial insemination = day 0), the embryos, a sample of each uterine horn flushing (UHF) and samples of caruncular endometrium (CE) and intercaruncular endometrium (ICE) were collected, after slaughter. Embryo quality was evaluated stereoscopically. Progesterone and estradiol-17β concentrations were determined using radioimmunoassay. PAA was determined spectrophotometrically. Compared to controls: progesterone concentration was higher ($p < 0.01$) in the UHF of SOV and ES ewes; estradiol-17β concentration was higher ($p < 0.05$) in the UHF and in the CE of SOV ewes and was lower in the ICE of SOV and ES ewes; PAA was higher in the UHF of SOV ewes. Positive linear relationships were noticed between PAA in the UHF and the number of corpora lutea, embryos or high quality embryos collected ($p < 0.01$). In conclusion, eCG used for estrus synchronization or superovulation affects ovarian steroid concentrations and PAA in the genital tract of ewes, before implantation.

Abstract P134

Effect of eCG on the Activity of Glucosidases in the Genital Tract of Ewes

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Survival and growth of early mammalian embryos before implantation largely depend on uterine environment. Glucosidases play a significant role as initiators of the adhesive phase of implantation and may be involved in early embryonic development by altering membrane permeability allowing the entry of important metabolites. This study investigated the effect of eCG used for estrus synchronization or superovulation on glucosidase activity in the genital tract of ewes, before implantation. At the end of a 12-day progestagen treatment, 13 adult Chios ewes received either 500 IU (ES, $n = 6$) or 1000 IU (SOV, $n = 7$) eCG (Intergonan); 7 ewes served as controls. On day 5 (day of intrauterine artificial insemination = day 0), the embryos, a sample of each uterine horn flushing (UHF) and samples of caruncular endometrium (CE) and intercaruncular endometrium (ICE) were collected, after slaughter. Embryo quality was evaluated stereoscopically. α-mannosidase (α-man) and β-*N*-acetyloglucosaminidase (β-NAGASE) activities were determined spectrophotometrically. Compared to controls: α-man activity was higher ($p < 0.01$) in UHF, CE and ICE of SOV ewes and in UHF and ICE of ES ewes; β-NAGASE activity was lower ($p < 0.01$) in UHF and ICE of SOV ewes. Positive linear relationships were noticed between α-man activity in UHF and the number of embryos or high quality embryos collected ($p < 0.001$). In conclusion, eCG used for estrus synchronization or superovulation affected glucosidase activity in the genital tract of ewes, before implantation.

Abstract P136

The Use of Flow Cytometrics for Simultaneous Measurement of Cell Cycle and Late Apoptosis in Lipofected Porcine Foetal Fibroblasts before Somatic Cell Cloning

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The objective was to investigate the *in vitro* developmental outcome of porcine nuclear transfer (NT) embryos reconstructed with non-apoptotic foetal fibroblast cells that had been lipofected with *pWAPhGH-GFPBsd* gene construct. The nuclear donor cells were derived from such cell line populations whose representative random samples had been analyzed for both cell cycle and late apoptotic cell death through the non-vital DNA fluorescent, i.e., propidium iodide-mediated staining and subsequent flow cytometrics (FACS). The FACS analysis revealed that out of all the fibroblast cells diagnosed (no less than 1×10^4 cells per each sample), 95.2% were cycling and up to 4.8% were late-apoptotic. In turn, from among the non-apoptotic cells, an average of 93.4% were at G1/G0 stages of the cell cycle, 3.9% were at S stage and 2.7% were at G2/M stages. In conclusion, the FACS analysis for mitotic cycle of 100%-confluent lipofected foetal fibroblasts confirmed that the cell cycle synchronization at G1/G0 phases was highly efficient, while the frequency of late-apoptotic cells was relatively low. Furthermore, the relatively high percentages of morulae (238/387; 61.5%) and blastocysts (132/387; 34.1%) developed *in vitro* from NT embryos reconstructed with foetal fibroblast cells undergoing lipofection. Porcine cloned morulae and blastocysts exhibited approximately 100% index of xenogeneic eGFP gene transcriptional activity, which revealed the live diagnostics of emission intensity for enhanced green fluorescent protein-derived biochemiluminescence.