

PW1259 - Postnatal productive performance in bovine offspring derived from assisted reproductive technologies

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Maternal environment during early pregnancy programs embryonic development in a manner that modifies postnatal phenotype. Abnormal fetuses, neonates, and adult offspring from assisted reproductive technologies (ART) have been reported. Here, we tested the hypothesis that bovine females derived by ART have altered postnatal growth and adult performance as compared to ones derived by artificial insemination (AI). A retrospective study evaluated performance of offspring derived from in vitro embryo production (IVP, n=637), multiple ovulation and embryo transfer (MOET, n=170), and AI (n=1629) in a large dairy herd that uses all three reproductive technologies, performs genomic testing, and selects genetically-superior females as embryo donors. IVP embryos were produced by a commercial laboratory. Data were analyzed by ANOVA using the GLM procedure of SAS for effects of technique (AI, IVP, MOET), dam parity while pregnant, and birth month; offspring's predicted transmitting ability of milk production (PTAM) was used as a covariate for lactation data. Results are least-squares means \pm SEM. Genetic estimates were higher for IVP than AI and MOET for net merit dollars (338.1 \pm 3.2, 459.4 \pm 5.2, and 418.9 \pm 9.9; AI, IVP, and MOET, respectively; $P < 0.0001$) and PTAM (487.3 \pm 13.1, 629.5 \pm 21.3, and 509.9 \pm 41.1; AI vs IVP; $P < 0.0001$ and IVP vs MOET; $P = 0.0269$). Birth (39.2 \pm 0.1 vs 39.8 \pm 0.2 kg; $P = 0.0315$) and weaning weights (88.7 \pm 0.5 vs 91.2 \pm 0.9 kg; $P = 0.0164$) were greater for IVP than AI. Weights for MOET were intermediate and not different from either group (birth, 39.3 \pm 0.4 kg and weaning, 88.9 \pm 1.5 kg). Daily gain from birth to weaning, age at first conception and first calving, and days open for first lactation did not differ among groups ($P > 0.05$). Lactation records were available for 2,292 first and 675 second lactations. Adjusted for PTAM, IVP-derived cows had projected 305 d (proj305) actual milk yield lower than AI in first lactation (11,105.6 \pm 38.7 vs 10,884.7 \pm 70.9 kg; AI and IVP, respectively; $P = 0.0065$) but similar in the second (10,913.4 \pm 266.6 vs 10,709.3 \pm 313.3 kg; $P = 0.34$). Proj305 actual fat yield was higher in IVP than AI in first lactation (386.3 \pm 1.7 vs 393.0 \pm 3.1 kg; $P = 0.0597$), but not in the second (398.3 \pm 11.3 vs 390.7 \pm 13.2 kg; $P = 0.4061$). For MOET-derived cows, milk yield in first lactation (10,994.8 \pm 138.2 kg) was intermediate and not different from AI ($P = 0.44$) or IVP ($P = 0.47$). Fat yield (400.5 \pm 6.0 kg), however, was greater than AI ($P = 0.024$) but similar to IVP ($P = 0.27$). Milk and fat yields for second lactation MOET cows did not differ from AI or IVP. Somatic cell score, proj305 actual protein, fat-corrected milk, and energy-corrected milk yields did not differ in either lactation. Results suggest alteration of programming in the embryo produced by IVP, with effects that persist into later life, including a slight reduction in milk yield. The mechanisms involved remain to be determined. (Support: Southeast Milk Inc. Milk Check-off Program).

PW1260 - Pre-maturation with cAMP modulators: results on bovine oocyte and in vitro produced embryo quality and production rates

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Recent studies had shown the use of cAMP modulators to minimize asynchrony between nuclear and cytoplasmic maturation in in vitro production (IVP) systems. This study aimed to evaluate IBMX and forskolin in two different periods of pre-maturation to improve bovine oocyte and IVP embryo quality. First, COCs obtained from slaughterhouse ovaries (n=85) and by OPU (n=46) with two or more layers of cumulus cells and homogeneous cytoplasm (grade I and II) were selected and randomly distributed in control group (Con, no pre-IVM, n=37), Pre-IVM 2 group [Pm2, n=41; pre-IVM 2h in a pre-IVM medium (TCM-199 Hepes, 1.6mg/ml BSA, 100mM sodium pyruvate, ITS 100x, 10.000UI penicillin, streptomycin 10mg/ml, 100µM forskolin and



500µM IBMX), and Pre-IVM 4 group (Pm4, n=38; pre-IVM 4h in pre-IVM medium). For gap junctional activity assessment, groups underwent IVM for 6h in commercial medium (Bioklone® Animal Reproduction, Brazil) and were stained with calcein-AM 1:100 and evaluated in a fluorescence microscope. Immature oocytes (n=15) were used as a calibrator group. Fluorescence intensity was measured using ImageJ software. For cleavage and blastocyst rates assessment, grade I and II COCs from slaughterhouse ovaries obtained in three replicates were randomly among groups (Con, Pm2 and Pm4), followed by 24h standard IVM, IVF (Talp medium) and IVC (SOF medium) procedures. For apoptosis and total cell number analysis, blastocysts (Con=25; Pm2=28; Pm4=33) were fixed in 4% PFA and stained for caspase 3 immunofluorescence and HOECHST 33342. Gap junctional activity was compared among groups by ANOVA and Dunnett test using Minitab14 software, production rates (cleavage, blastocyst) was compared by Fisher's Exact Test, mean cell number was compared by ANOVA with Kruskal-Wallis correction, and apoptotic cells number and apoptosis rate by ANOVA using GraphPad InStat software, at 5% significance level. Pre-IVM significantly ($p<0.05$) increased oocyte gap junctional activity in Pm2 and Pm4 (141.49b and 149.13b, respectively) when compared to Con (85.66a). Cleavage rate was lower ($p<0.05$) in Pm4 compared to Con and Pm2 (51.61b% vs 79.07a% and 73.01a% respectively), and no difference was observed in blastocyst rate among cleaved embryos (Con=35.20a%; Pm2=41.32a%; Pm4=38.28a%). No difference between groups was observed in mean number of cells (Con=108.76a; Pm2=119.71a; Pm4=122.61a), mean number of apoptotic cells (Con=9.44a; Pm2=12.96a; Pm4=10.12a) and average rate of apoptosis (Con=9.06a; Pm2=5.11a; Pm4=8.55a). We concluded that pre-maturation with forskolin and IBMX before conventional IVM improved oocyte gap junctional activity, but no beneficial effect over quality (based on apoptosis and cell number) and production rates of bovine IVP embryos was detected in this experiment.

Key words: bovine – forskolin – IBMX - IVP

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PW1261 - Constant administration of PGF2 α during the periovulatory period in superovulated buffalo

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The use of superovulation (SOV) and embryo transfer (ET) biotechnologies in buffaloes still shows inconsistent results, particularly in terms of low embryos recovery rate. It may be related to failures in the collection and/or transportation of oocytes in the fallopian tube after SOV treatment. Evidences showed that the administration of sequential doses of PGF2 α during the periovulatory period stimulated the tubal tissues to commence contractile activity, and allowed the fimbrial portions of the tubal ampullae actively to capture oocytes in rabbits [1]. Thus, the aim of this study was to evaluate the effect of constant administration of PGF2 α during the periovulatory period on the embryo recovery rate of superovulated buffaloes. Buffaloes were synchronized with an intravaginal P4 device plus 2.0 mg i.m. of estradiol benzoate at random stage of the estrous cycle (D0). From D4 to D7, all buffaloes received 200 mg i.m. of FSH twice-daily, in 8 decreasing



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