ABSTRACTS FOR POSTER PRESENTATION

Embryo Manipulation

164 BOVINE PLACENTAL LACTOGEN (bPL) AND BOVINE PREGNANCY-SPECIFIC PROTEIN B (bPSPB) AS INDIRECT MEASURES OF PLACENTAL FUNCTION IN IN VITRO-DERIVED BOVINE PREGNANCIES

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Associations between abnormal placental and fetal development after in vitro embryo manipulations have been suggested to play a key role in the occurrence of high birth weights. This study was designed to investigate bovine conceptus development in in vivo- (controls) and in vitro-produced (IVP) concepti and newborn calves, and its association with specific placental proteins usually present in maternal, fetal and neonatal plasma and fetal (amniotic and allantoic) fluids. Females were superovulated to obtain control embryos, whereas IVP embryos were derived from established in vitro embryo production procedures (Bertolini *et al.*, 2002 Theriogenology 58,973). Pregnant animals from each group were slaughtered on Days 90 and 180 of gestation ($n = 4$ control, $n = 5$ IVP pregnancies/day), or allowed to develop to term ($n = 6$/group). Conceptus and neonatal physical traits were recorded after slaughter or delivery (Bertolini *et al.*, 2001 Theriogenology 55, 347; 2002 57, 361; 2002 58, 973). Maternal and fetal plasma and fluids were sampled after slaughter; maternal blood was sampled weekly from Day 30 of pregnancy to term. Neonatal blood samples were collected at 10 min, and at 1, 6, 12 and 24 h after birth. Bovine placental lactogen (bPL) and pregnancy-specific protein B (bPSPB) concentrations were determined in plasma and fluid samples, according to Wallace (1993 Dom. Anim. Endocrinol. 10, 67) for bPL, and by a commercial service for bPSPB (BioTracking, Moscow, ID, USA.). Data were compared by Proc GLM of SAS and Pearson's correlation test (SAS Institute, Cary, NC, USA.). No major physical differences in conceptus traits were observed between groups on Day 90, but concentrations of bPL and bPSPB were higher in fetal plasma (8.1 ± 0.5 v. 10.7 ± 0.5 ng mL^{-1} for bPL, and 4.4 ± 8.2 v. 32.5 ± 5.8 ng mL^{-1} for bPSPB) and allantoic fluid (bPL only; 3.6 ± 1.1 v. 7.8 ± 1.0 ng mL^{-1}) of IVP concepti ($P < 0.05$). Day-180 IVP pregnancies displayed larger uterine and conceptus traits ($P < 0.05$), and bPSPB concentrations were 2.9-fold lower (84.3 ± 22.4 v. 20.5 ± 22.4 ng mL^{-1}) in the allantoic fluid ($P < 0.05$) of IVP pregnancies, for a 2- to 3-fold larger allantoic fluid volume than controls ($P < 0.07$). Concentrations of bPL in fetal plasma and fluids were higher than in maternal plasma, but no differences in bPSPB concentrations were observed across fluid types. Newborn IVP calves and fetal membranes were larger, displaying 3- to 4-fold higher concentrations of plasma bPL ($P < 0.05$) and bPSPB ($P < 0.08$) than controls (10 and 60 min after birth) and maternal plasma (at delivery). Maternal concentrations of bPL in IVP pregnancies were lower than controls during the last 8 weeks of gestation ($P < 0.05$), to become similar as parturition approached. Generally, concentrations of bPL and bPSPB in plasma were correlated with physical traits ($0.750 > r > 0.958$, $P < 0.001$) and with one another in plasma and fluids ($0.715 > r > 0.938$, $P < 0.001$). Our results indicated that differential patterns of secretion of bPL and bPSPB into the maternal and fetal systems occurred at distinct stages of gestation, which were associated with altered conceptus development after in vitro embryo manipulations, indirectly demonstrating deviations in placental function in IVP pregnancies.

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165 RE-EXPANSION AND QUALITY OF SPLIT BOVINE EMBRYOS IN VITRO

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Transferring split bovine embryos results in a higher number of calves born per embryo. In addition to generating genetically identical progeny, biopsies can be made for molecular biological analyses. We aimed to determine the effect of splitting ratio on the in vitro development of Day 7 (164 to 168 h post insemination) IVP bovine embryos. The inner cell mass (ICM) and trophoblast cells were split in three ratios (50:50, 60:40 and 70:30) with a Beaver microblade (Becton Dickinson, N.J., USA.) fixed to a micromanipulator under an inverse microscope at 100 X (Leica, Bensheim, Germany). Split blastocysts were cultured singly in 50 μL drops of CR1aa medium at 39°C under 5% CO_2 in a moisture-saturated atmosphere. After 1 and 2h culture, the morphology was assessed by judging the shape of the embryos and re-development of the blastocoel. On Day 8 (after 22 h culture), the shape of the blastocysts, development of the ICM, blastocoel, proportion of degenerated cells and embryos and re-expanded blastocysts were recorded. Embryos were stained with propidium iodide and Hoechst 33258 for cell counting. The re-expansion status of Group A (50, 60 and 70%) and B (50, 40 and 30%) embryos after 1 and 2 h and their quality after 22 h culture (1: excellent = <10% degenerated cells, well-defined ICM; 2: fair = <20% degenerated cells) are shown in Table 1. With regards to Group A split blastocysts, a higher ($P < 0.05$) percentage of embryos that re-expanded after 1 and 2 h and which yielded Quality 1 and 2 embryos suitable for embryo transfer was observed with the 60% and 70% than with demi-embryos. There were significant differences ($P < 0.05$) between all split blastocysts in Group B after 1 h culture. The 30% split embryos showed the lowest re-expansion rate and quality of embryos after 2 h and 22 h culture, respectively. No differences ($P < 0.05$) were seen in the ratio of the ICM to the total number of cells in both Group A and B. This study showed that the ratio in which blastocysts were split had a significant effect on re-expansion and quality but not on the number of cells.

Table 1.

Splitting ratio (*n*)	Group A				Group B			
	Re-exp 1 h (%)	Re-exp 2 h (%)	Quality 1 + 2* 22 h (%)	Cell number ICM/Total (%)	Re-exp 1 h (%)	Re-exp 2 h (%)	Quality 1 + 2* 22 h (%)	Cell number ICM/Total (%)
50 : 50 (59)	69[a]	81[a]	66[a]	20/53 (38)[a]	68[a]	81[a]	68[a]	20/40 (41)[a]
60 : 40 (38)	95[b]	97[b]	95[b]	29/73 (38)[a]	87[b]	84[a]	68[a]	15/46 (33)[a]
70 : 30 (57)	96[b]	100[b]	89[b]	>32/82 (39)[a]	44[c]	65[b]	25[b]	10/36 (28)[a]

*1: excellent, 2: fair; Re-exp.: re-expansion; [a,b,c]: $P < 0.05$ (chi-square test).

166 MOUSE EMBRYONIC DEVELOPMENT FROM ONE-CELL STAGE TO BLASTOCYST, UTILIZING KSOM SUPPLEMENTED WITH AMINO ACIDS IN A MICROCHANNEL DEVICE

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Great efforts have been made to improve in vitro culture for enhancement of embryonic development. However, in vitro development of mammalian embryos still remains a challenge for the scientific community. Recently, the use of microfluidic culture devices, as an alternative technique compared to the standard drop, has allowed mammalian embryos to develop to the hatched blastocyst stage. With the use of a different medium, mouse strain, and microchannel device than previously reported (Raty S *et al.*, 2001 Theriogenology 55, 241 abst), this study was undertaken to determine if a microchannel device fabricated from borosilicate and poly-dimethylsiloxane would support development of mouse embryos from one-cell to blastocyst, as an alternative to standard microdrop culture. Mice (F1 inbred C57BL/6CRL X SJL) from 3 to 8 weeks old were superovulated with 5 IU of PMSG and 5 IU of hCG. The female SJL strain of the mice has demonstrated low reproductive performance. One-cell embryos were collected in M2 medium (Sigma, St. Louis, MO, USA.). For each treatment, 240 embryos in 24 replicates were cultured. Groups of 10 embryos were cultured in the microchannel device using 500 μL of KSOM with amino acids (MR-106-D, Speciality Media, Phillipsburg, NJ, USA.); no additional supplements were added. Groups of 10 embryos were cultured in standard microdrops (control) using 30 μL of the same medium covered with mineral oil. Embryos were cultured in a 100% humidified, 5% CO_2 in air atmosphere at 37°C for 96 h. Embryos were allocated to the control treatment or the microchannel device treatment using a randomized block design. The percentage of embryos at each stage of development was evaluated at 24-h intervals. The stage of embryo development at each observation was analyzed by ANOVA using the general linear model in SAS (PROC GLM, type I sum of squares). Blastocyst development in the microchannel device was not different when compared to results obtained in the standard drop. The percentage of blastocysts developing, when analyzed from one-cell stage, was 29 ± 5% for the control and 26 ± 6% for the microchannel. The percentage of blastocysts, when analyzed from cleavage, was 35 ± 5% for the standard drop and 31 ± 7% for the microchannel device. The results obtained are encouraging, when considering the non-optimized medium and mouse strain utilized in this experiment. In conclusion, the results show the microchannel device may be considered an alternative technique for use in embryo culture as it supports development of mouse embryos from one-cell stage to blastocyst.

167 THE USE OF BULL ROUND SPERMATIDS FOR PRODUCING RECONSTRUCTED EMBRYOS

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Recently, sperm has been used as a vector to carry exogenous genes for the production of transgenic animals. However, the success in cattle is low, due to deficiencies in oocyte activation and sperm decondensation caused by high disulphide bond (S=S) content in mature sperm. This study was carried out to develop an effective method for producing transgenic animals with round spermatids (RS). Two methods of embryo production—electric fusion (EC) or intracytoplasmic injection (IC)—and three activation treatments were compared. RS were isolated from bull testes by Percoll density gradients (20, 35, 40, 45 and 90%). Fusion between ooplast and RS was performed with a single DC electric pulse delivered by BTX 200 (1.0 KV cm^{-1}, 45 μs) in 0.28 M mannitol solution supplemented with 100 μM $CaCl_2$ and 100 μM $MgCl_2$. MII oocytes were divided into three activation groups for EC and IC methods, respectively. In Group 1, oocytes were activated with ionomycin (5 μM, 5 min) prior to EC/IC. In Group 2, oocytes were activated with ionomycin (5 μM, 5 min) after EC/IC. In Group 3, oocytes were activated twice with ionomycin, before and after EC/IC. All eggs were then incubated in cycloheximide (10 μg mL^{-1}) for 5 h and cultured in CR1aa medium to Day 8. The rates of cleavage and blastocyst development were evaluated on Days 2 and 8, respectively. All experiments were performed as 5 replicates, and statistical differences were analyzed with one-way ANOVA ($P < 0.05$). In IC, cleavage rates were significantly higher ($P < 0.05$) in Group 3 (99/123, 80.5%) compared to Groups 1 and 2 (31/68, 45.6% and 45/70, 64.3%, respectively). Blastocyst development rates were also significantly higher ($P < 0.05$) in Group 3 (15/123, 12.2%) compared to Group 2 (1/70, 1.4%), but did not differ compared to Group 1 (4/68, 5.9%). In EC, the cleavage rates in all three activation treatment groups remained low (43–45%), and no blastocysts developed. These results suggest that intracytoplasmic RS injection combined with repeated ionomycin activation is more efficient than electric fusion for producing developmentally competent embryos. [Supported by High Technology Development Project for Agriculture and Forestry Korea, MAF-SGRP, 300012-05-3-SB010 and Cho-a Pharm. Co. LTD.]