65 Improvement of bovine early embryo development *in vitro* by coculture with endometrial epithelial cells

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We optimized a bovine endometrial epithelial cell (BEEC) line as a valuable research model for the study of very early embryo-maternal interactions *in vitro*. In this study, we aimed to (1) characterise the BEEC monolayers along the primary culture and first passages with respect to the expression of epithelial and mesenchymal cell markers and abundance of functional key transcripts; (2) to test whether direct or indirect contact with endometrial cells alter the quality of the embryos *in vitro;* and (3) to test the specificity of the effect. In Exp. 1, after isolation from slaughterhouse uteri at the early luteal phase, BEEC were cultured in DMEM/F12 phenol red-free medium supplemented with 10% fetal bovine serum (FBS) from primary culture until subculture 3. Fixed samples were immunostained for cytokeratin and vimentin. Transcript abundances for cellular lineage markers (*KRT18* and *VIM*), oestrogen receptor (*ESR1*), interferon α/beta receptor 1 (*IFNAR1*), and prostaglandin G/H synthase 1 (*PTGS1*) and 2 (*PTGS2*) were evaluated by real-time quantitative PCR. Statistical analyses were carried out by ANOVA and Tukey test. Immunofluorescence data revealed that the BEEC line co-expresses cytokeratin together with a mesenchymal marker (Vimentin). This indicates that these epithelial cells underwent an epithelial-mesenchymal transition *in vitro*. Gene expression data showed a 6-fold increased ($P < 0.001$) abundance of *VIM* mRNA from the primary culture to the subculture 1, which remained constant until subculture 3; however, *KRT18*, *ESR1*, *IFNAR1*, *PTGS1*, and *PTGS2* were similar between the passages, suggesting that the cells conserved their functional characteristics. In Exp. 2, groups of 15 morulas (Day 5.5) were cultured in SOF medium supplemented with 5% FBS in the absence (control) or in the presence (co-culture) of BEEC at passage 2, for 48 h. Embryos were placed on direct or indirect contact with a BEEC monolayer using a 96-well insert containing 8 µm pores. Developmental rates were compared by chi-square test and *P*-values were adjusted by Tukey's test. The percentage of embryos that had developed from morula into blastocyst stage on Day 7.5 was significantly higher in the direct and indirect contact co-culture (65%; $P < 0.05$) groups compared with the control (53%) group. Moreover, 63% of the blastocysts were expanded, hatching, or hatched in the co-culture groups, whereas a rate of 46% was found in the control counterparts ($P < 0.05$). In Exp. 3, the same experimental conditions from Exp. 2 were used, but groups of 15 Day 5.5 morulas were cultured in control, or conditioned medium from BEEC (CondBEEC) or bovine fibroblasts (CondFib). Blastocyst development rate on Day 7.5 was higher in the CondBEEC group (71%; $P < 0.001$) compared with the control (54%) and CondFib (50%) groups. In conclusion, based on the markers studied, BEEC monolayers undergo epithelial-mesenchymal transition *in vitro* but preserve functional characteristics after few passages. The co-culture system improves bovine embryonic development from morula into blastocyst stage. This support is BEEC specific and does not rely on a direct cell-to-embryo contact.

66 Bovine corpus luteum affects embryo developmental competence and production

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This study was conducted with the aim to determine the effect of the bovine corpus luteum on *in vitro* embryo production. Immature cumulus-oocyte complexes (COC) were aspirated from abattoir ovaries from cows (mainly Holstein and dairy crossbred cows and heifers) with (ipsilateral; CL+) and without (contralateral; CL−) corpus luteum (CL), and from cows without CL in any of the ovaries. The average weight of the ovaries from each group was 10.4 ± 0.25, 5.7 ± 0.25, and 6.1 ± 0.25 g for CL+, CL−, and C, respectively. The experiment was completed within 12 replicates (100 ovaries per group). The COC were cultured in maturation medium (TCM-199 supplemented with 10% fetal bovine serum, 100 µg mL^{-1} sodium pyruvate, 0.75 mg mL^{-1} of L-glutamine, 4 µg mL^{-1} of FSH-p, 100 µM cysteamine, and 250 µg mL^{-1} of gentamicin) followed by IVF (synthetic oviducal fluid medium supplemented with 10 µg mL^{-1} heparin) and *in vitro* culture (citrate synthetic oviducal fluid medium). On Day 7 after IVF, the embryos were evaluated and classified into morulae (M), early blastocysts (EB), regular blastocysts (RB), expanded blastocysts (ExB), and hatched blastocysts (HB), and the embryos with quality 1 (according to IETS criteria) were recorded. Data were analysed by logistic regression and general linear model of SAS (SAS Institute Inc., Cary, NC, USA), and means were compared by the least squares means method. Results of cleavage, embryo rate at Day 7, and rates of M + EB, RB, ExB, and HB are shown in Table 1. The number of embryos per ovary was greater ($P < 0.01$) in CL+ (1.16 ± 0.11) than in CL− (0.45 ± 0.15) and C (0.55 ± 0.15). Also, the number of embryos per cultured oocyte was significantly greater in CL+ than in CL− and C (0.27 ± 0.02 *v.* 0.14 ± 0.03 and 0.15 ± 0.03, respectively; $P < 0.01$). The results of this study reveal that the presence of the corpus luteum in the ovary at the time of the oocyte recovery affects the developmental capacity of the bovine embryos, and such influence probably occur through intraovarian interactions.

Table 1. Effect of bovine corpus luteum on embryo production

Ovary	No. of oocytes	Cleavage (n) %	Total embryos on Day 7 (n) %	Developmental stage of blastocyst on Day 7 (n) %			High-quality embryos (n) %
				M + EB	RB	ExB + HB	
CL+	(492)	(313) 63.6[a]	(122) 39.0[b]	(51) 41.8[c]	(36) 29.1[a]	(35) 28.7[b]	(82) 67.2[a]
CL−	(395)	(164) 41.5[b]	(48) 29.3[c]	(34) 70.8[d,e]	(9) 18.7[a]	(5) 10.4[c]	(15) 31.2[b]
C	(427)	(199) 46.6[b]	(62) 31.1[c]	(40) 64.5[c]	(16) 25.8[a]	(6) 9.7[c]	(24) 38.7[b]

Values with different letters in the same column differ: [a,b]$P < 0.01$; [b,c]$P < 0.05$; [c,d]$P < 0.01$; [c,e]$P < 0.05$.