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Embryology, developmental biology, and physiology of reproduction

Bovine embryos lacking progesterone receptor (PGR) develop normally through early elongation

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Endogenous maternal progesterone levels and exogenous progesterone supplementation have been positively linked with conceptus length and embryo survival in cattle. However, the mechanism by which progesterone enhances conceptus development is unknown. Progesterone may act directly on the embryo or indirectly, by promoting changes in uterine fluid composition favoring conceptus growth. To discriminate between both possibilities, we have analyzed the development of bovine embryos lacking progesterone receptor (PGR) generated by CRISPR technology. To that aim, we have microinjected *in vitro* matured oocytes with mRNA encoding for Cas9 alone (control group, C, solely composed by WT embryos) or combined with sgRNA against *PGR* (C+G group, partially composed by KO embryos). Following fertilization and culture up to Day 7 (D7) blastocyst, subsequent development was analyzed *in vitro* and *in vivo*, assessing lineage development by immunostaining for CDX2 (trophectoderm), SOX17 (hypoblast) and SOX2 (epiblast), and conducting genotyping by miSeq. *In vitro* development from D7 to D12 was similar between groups (85.4±5.7 vs. 81.3±6.3, mean±s.e.m. for C and C+G, respectively, t-test p>0.05). In C+G group, 22/45 D12 embryos analyzed were KO (i.e., contained only KO alleles). Embryo diameter at D12 was not affected by embryo genotype (772±74 vs. 648±64 vs. 731±44 µm, mean±s.e.m. for WT, edited non-KO and KO, respectively, ANOVA p>0.05), and the proportion of embryos attaining complete hypoblast migration was similar in WT (23/32, 72%), edited non-KO (12/20, 60%) and KO (19/22, 86%) embryos (Chi-square p>0.05). No differences were noted either on embryonic disc (ED) formation rate (20/32 63% vs. 6/20 30% vs. 10/22 45% for WT, edited non-KO and KO, respectively Chi-square p>0.05). To assess *in vivo* development, 40 blastocysts from C+G group were transferred to two synchronized recipient ewes. Pregnancy was supported by exogenous progesterone (CIDR-Ovis) and conceptuses were recovered 9 days after embryo transfer at a developmental stage equivalent to day 14 (E14). All intact conceptuses recovered from recipient ewes were edited by CRISPR (23/23) and 10/23 were KO. Conceptus growth was not affected by PGR ablation (1.3±0.3 vs. 3.7±1.3 cm, mean±s.e.m. for edited non-KO and KO, respectively, Two-Way ANOVA p>0.05) and all conceptuses showed hypoblast migration. Embryonic disc was present in 7/10 (70%) KO and 12/13 (92%) edited non-KO conceptuses and embryonic disc size was not affected by the ablation (333±72 vs. 234±43 µm, mean±s.e.m. for KO and edited non-KO, respectively, Two-Way ANOVA p>0.05). In conclusion, the ablation of PGR does not impair embryo development up to E14, suggesting that progesterone-mediated conceptus growth enhancement is indirectly mediated by triggering changes in the uterus. Supported by StG-757886 from ERC and PID2020-117501RB-I00 from MINECO.

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