Animal Reproduction

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EMBRYOLOGY, DEVELOPMENTAL BIOLOGY, AND PHYSIOLOGY OF REPRODUCTION

Gene targeting of NANOG changes gene expression of bovine embryos

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The second event of cell differentiation in mammals consists of the separation between the epiblast (EPI) and the primitive endoderm (PE). This occurs shortly after the segregation of the inner cell mass (MCI) and the trophectoderm (TE). It is known in mice that NANOG drives EPI differentiation, although it leads to FGFsecretion that acts on neighbor cells, allowing GATA6 and subsequent SOX17 expression in the PE. Overall, we aim to test the hypothesis that NANOG is necessary for the specification of EPI in bovine embryos. The specific objective of this study was to assess expression of genes related to this second cell differentiation event after gene editing of NANOG using CRISPR/Cas9. The experimental group consisted of IVP-derived embryos microinjected at 16 hours post fertilization with 80 ng/µl of two different guide RNAs (gRNA) targeting NANOG homeobox domain and 70 ng/µl of TrueCut™ Cas9 Protein v2 (ThermoFisher), while the control group was not microinjected. Embryos were cultured until 216 hours post fertilization (hpi) and harvested individually for genotyping by PCR or gene expression analysis of *NANOG, GATA6* and *SOX17* by absolute q-RT-PCR, using 11 injected and 5 control embryos. Cleavage (at 90hpi), blastocyst (number of blastocysts/number of zygotes) and development (blastocysts/cleaved embryos) rates were recorded in five replicates. Data was analyzed by ANOVA followed by Tukey's comparison of means. The level of significance was considered 5% or less. We observed a significant decrease in cleavage (44.40 ± 2.93%) and blastocyst (7.95 ± 2.92%) rates in the injected group compared to the control group (73.87 ± 2.93% and 19.63 ± 2.92%, respectively); however, no significant changes were observed in development rates (17.71 ± 4.84% vs. 26.88 ± 4.84%). Genotyping by PCR and agarose gel electrophoresis revealed that a minority (1/6 tested) of injected embryos presented expected gene deletion caused by editing from both gRNA. Q-RT-PCR analysis revealed that *NANOG* expression was significantly reduced, but not extinguished in injected embryos. Interestingly, expression of GATA6 was not different between groups, while expression of another PE marker, *SOX17*, was significantly reduced in injected embryos. In conclusion, targeting of *NANOG* in bovine embryos using CRISPR allowed blastocyst formation while reducing *SOX17* gene expression.

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