Animal Reproduction

ABSTRACTS: 34TH ANNUAL MEETING OF THE BRAZILIAN EMBRYO TECHNOLOGY SOCIETY (SBTE)

Embryology, developmental biology, and physiology of reproduction

Use of CRISPR/Cas9 for deletion of the LATS2 gene in bovine embryos - preliminary results

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The first cellular differentiation event in mammals consists in the separation between the internal cellular mass (ICM) and the trophectoderm (TE). It is known in mice that the HIPPO signaling pathway, via LATS2 protein kinase, plays an important role in controlling the expression of genes that define the segregation of ICM and TE. In bovine, it is suggested that the process of differentiation may be regulated differently, but HIPPO pathway may still be involved in this biological event. In order to test the hypothesis that LATS2 activity is necessary for differentiation of ICM and TE in bovine embryos, we performed microinjections in bovine zygotes to induce genetic deletion of LATS2, using CRISPR/Cas9 system. CRISPR/Cas9 system design was performed in silico using the specific sequence of the LATS2 gene (Gen Bank accession number XM_025000092.1). Two RNA guide sequences (gRNA) were then designed using CRISPR RGEN Tools online software. The forward and reverse oligonucleotide sequences were commercially synthesized, then in vitro annealed, phosphorylated, and cloned in pX330-U6-Chimeric_BBh-hSpCas9-mSA (modified from Addgene) plasmid. DNA sequences corresponding to each gRNA (LATS2 gRNA) were amplified from the generated vector and in vitro gRNA synthesis was performed using the MEGAshortscript™ T7 Transcription Kit (Thermo Fisher). Bovine oocytes were then aspirated from commercial slaughterhouse ovaries, in vitro matured, fertilized and randomly distributed among three groups: Control group without microinjection, Cas9 group, in which zygotes were microinjected with only 70ng/ml of Cas9 enzyme (Sigma), and LATS2 group, in which zygotes were microinjected with 12.5ng/ml of each LATS2 gRNA and 70ng/ml of Cas9 enzyme. Microinjection into groups Cas9 and LATS2 occurred 10 hours after fertilization. Embryos were evaluated at day 4 of culture (D4) for assessment of cleavage rates (cleaved/total oocytes) and at D8, for assessment of blastocyst (blastocysts/total oocytes) and development (blastocysts/cleaved) rates. Embryos injected with LATS2 gRNA were stained with 1mg/ml Hoechst 33342 (Sigma) to verify if they have surpassed the 16-cell stage. Only mean rates and respective standard deviations are reported due to lack of degree of freedom to perform statistical analyses yet. The results of three replicates show that for cleavage, blastocyst and development rates control group presented 58 ± 3.90%; 30 ± 1.95% and 52 ± 5.57%, the Cas9 group presented 36 ± 5%; 10 ± 7% and 31 ± 25%, while the LATS2 group presented 50 ± 6.88%, 6 ± 1.12% and 12 ± 2.9%, respectively. Embryos in LATS2 group that did not develop to the blastocyst stage showed more than 16 cells after nuclear staining. These preliminary results suggest that injection of CRISPR/Cas9 along with LATS2 gRNA inhibited the formation of blastocysts without blocking embryonic development at earlier stages. Financial support by FAPESP, grants no. 2017/09576-3, 2017 / 25574-0 and 2018/18924-8.