CDX2 is not essential for bovine blastocyst formation but plays a role on first lineage specification

Emel Tütem Sevím E[1,2], Beatriz Galiano-Cogolludo B[1], Alba Pérez-Gómez[1], Inés Flores-Borobia I[1], Julieta G Hamze JG[1,3], Priscila Ramos-Ibeas P[1], Pablo Bermejo-Álvarez P[1]

[1]Animal Reproduction Department, INIA, CSIC, Spain

[2]Akdeniz University, Turkey

[3]Department of Cell Biology and Histology, Universidad de Murcia. International Excellence Campus for Higher Education and Research (Campus Mare Nostrum), Spain.

First cell lineage differentiation results in the formation of the trophectoderm (TE) and inner cell mass (ICM) populations that characterizes the early blastocyst stage. CDX2 is a transcription factor essential for proper trophectoderm (TE) differentiation in mice, where its ablation prevents blastocoel maintenance. Its role in other mammals is unknown, as whereas its TE-specific expression is conserved, the CDX2-mediated transcriptional repression of the ICM-specific transcription factor POU5F1 reported in mice is not applicable to other species, where neither CDX2 binds to POU5F1 promoter nor POU5F1 expression is ICM-specific. To gain insight on how first lineage differentiation occurs in bovine, we have analyzed the developmental ability of *CDX2* knock-out (KO) bovine embryos. *In vitro* matured oocytes were allocated into two groups: one (n=168) was microinjected with messenger RNA (mRNA) encoding for cytosine base editor (CBE) and guide RNA (gRNA) designed to generate a premature stop codon on *CDX2* (group C+G, containing KO embryos) and other (n=91) was injected with mRNA encoding for CBE alone (group C, composed entirely by unedited WT embryos). Following microinjection, *in vitro* fertilization (IVF) was performed and embryos were cultured in SOF. Immunohistochemistry (IHC) analysis was performed on 46 blastocysts from C+G group and 31 from C group collected at Day (D) 8 to detect CDX2, and TE (GATA3+) and ICM (SOX2+) cells. Blastocyst rate was similar between microinjection groups and all blastocysts analyzed in C+G group were KO, as confirmed by Sanger sequencing and the absence of CDX2 expression. CDX2 KO blastocyst displayed a similar total cell number (118±8 vs. 105±6, mean±s.e.m. for WT vs. KO), but a reduced number of TE cells (90±7 vs. 64±6, mean±s.e.m. for WT vs. KO, Mann-Whitney $p<0.05$) and an increased number of ICM cells (29±2 vs. 44±3 mean±s.e.m., for WT vs. KO, Mann-Whitney $p<0.05$). To determine if the trophectoderm could proliferate beyond blastocyst hatching, D7 blastocyst were cultured in a post-hatching system based on N2B27 medium up to D12. Survival rate from D7 to D12 was unaffected by CDX2 ablation and IHC was conducted to detect TE (GATA3+), hypoblast (SOX17+) and epiblast (SOX2+) cells in 23 WT and 38 KO embryos. D12 CDX2 KO embryos displayed similar hypoblast migration and epiblast survival rates, but displayed a reduced diameter (852±65 vs. 610±41 µm, mean±s.e.m. for WT and KO, Mann-Whitney $p<0.05$) and number of epiblast cells (82±1 vs. 24±5, mean±s.e.m. for WT vs. KO, Mann-Whitney $p<0.05$). In conclusion, TE differentiates and develops in the absence of CDX2, but CDX2 ablation increases significantly the ICM:TE proportion leading to larger embryonic discs and smaller extra-embryonic membranes. Supported by projects StG-757886 from ERC and PID2020-117501RB-I00 from MINECO.