Conceptus derived PGs are not essential for early bovine post-hatching development

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Prostaglandins (PGs) are lipid signaling molecules playing critical roles on gestation whose concentrations in bovine uterine fluid increase as conceptus elongation progresses. Previous studies have observed that pharmacological inhibition of PGs impairs conceptus length, but it is unclear whether conceptus-derived PGs play an autocrine role on their own development. To solve that question, we have assessed the developmental ability of bovine embryos lacking the rate limiting enzyme for PG synthesis: PTGS2. *In vitro* matured oocytes were allocated into two groups: one (n=396) was microinjected with messenger RNA (mRNA) encoding for cytosine base editor (CBE) and guide RNA (gRNA) designed to generate a premature stop codon on *PTGS2* (group C+G, containing KO embryos) and other (n=234) 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 medium up to Day (D) 7 and subsequently transferred to a post-hatching system based on N2B27 medium to allow further development up to D12, when they were fixed for lineage analysis and subsequently genotyped by Sanger sequencing. A subset of D7 embryos were individually cultured from D9 to D12 to determine total PGs concentration in the spent medium by ELISA. Similar developmental rates to D7 blastocyst (25±2 vs 21±3 %, mean±s.e.m. for C vs. C+G) and from D7 to D12 (88±5 vs 88±4, %, mean±s.e.m. for C vs. C+G, respectively) were observed in both microinjection groups. 65/68 of the D12 embryos analyzed in C+G group were KO for *PTGS2* (i.e., contained only KO alleles). PGs concentration in the spent media was analyzed in 16 embryos per group, being significantly reduced by *PTGS2* ablation (~500-fold reduction: 32707 ± 8493 vs 65,08 ± 26.63, pg/mL, mean±s.e.m. for WT and KO, Mann-Whitney test $p<0.05$). Lineage development was analyzed by immunohistochemistry for trophectoderm (CDX2), hypoblast (SOX17) and epiblast (SOX2) markers in 56 WT embryos and 65 KO. *PTGS2* ablation did not affect embryo growth (800±50 vs 790±40 µm, mean±s.e.m. for WT vs. KO), hypoblast migration rate (24/56 (43 %) vs. 24/65 (37 %), WT and KO), embryonic disc formation rate (40/56 (71 %) vs. 42/65 (65 %), WT vs. KO) or the number of epiblast cells (47±6 vs. 60±7, mean±s.e.m. for WT vs. KO). In conclusion, PGs synthesis inhibition by *PTGS2* ablation does not affect bovine embryo development up to early post-hatching stages. Supported by projects StG-757886 from ERC and PID2020-117501RB-I00 from MINECO and 21985/JLI/22 from Fundación Séneca.