Oviduct Epithelial Spheroids during in vitro Culture Enhance Bovine Embryo Development, Mitigate the Negative Impact of Oxidative Stress and Induce Changes in Embryonic Transcriptome

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The mammalian oviduct provides an optimal environment for early embryo development. On the contrary, in vitro embryo production exposes the embryos to oxidative stress with deleterious effects on blastocyst development and quality. Considering the antioxidant capacity of the oviduct epithelium, we developed an in vitro model co-culturing bovine oviduct epithelial spheroids (OES) and embryos. We hypothesized that co-culture of OES with in vitro produced embryos up to the time of embryonic genome activation (5 days) or blastocyst stage (7-8 days) could mitigate the effects of oxidative stress conditions.

Oocytes were collected from bovine ovaries from a slaughterhouse and *in vitro* matured for 22 h. Then, oocytes were fertilized using frozen-thawed Percoll-washed bull semen at 38.8°C. After 24h (day 0), groups of 25 presumptive zygotes were *in vitro* cultured in 25 µL droplets of SOF medium with 5% fetal calf serum, at 38.8°C, 5% CO_2, and under 5% or 20% O_2, with or without OES and during two co-culture times (5 or 8 days), as follows: 1) controls-5%; 2) controls-20%; 3) 5dOES-5%; 4) 8dOES-5%; 5) 5dOES-20%; and 6) 8dOES-20%). Cleavage rates were evaluated on day 2 and blastocyst rates on days 7 and 8. Expanded blastocysts on days 7-8 were either evaluated for total cell numbers (Hoechst staining) or for gene expression analysis by Illumina RNA-sequencing. Embryo development rates were compared by ANOVA, Tukey's post-tests. RNA-seq data was processed to remove adapter sequences and low-quality bases. Differentially expressed genes (DEGs) were identified with EdgeR (FDR≤0.05). Functional analysis of DEGs was performed using Metascape.

No differences were found on cleavage rates among the different culture conditions (73-81%, 8 replicates). Under 5% O_2, the presence of OES for 5 or 8 days did not affect blastocyst rates compared to controls without OES (27-32% vs. 30% on day 8) but increased the number of cells per blastocyst when OES were co-cultured for 8 days (137.6 ± 10.8 vs. 102.6 ± 8.4 cells; $P<0.05$). Under 20% O_2, blastocyst rates on days 7 and 8 were significantly higher in the presence of OES compared to controls without OES (30.7% and 31.8% in 5dOES-20% and 8dOES-20% vs. 19.8% in controls-20%; $P<0.05$). Furthermore, cell numbers per blastocyst were significantly increased compared to controls with no difference between 5 and 8 days of co-culture (112.7 ± 7.8. and 138.1 ± 10.5 cells, respectively, vs. 82.1 ± 4.5 cells in controls; $P<0.0001$). Under 5% O_2, 545 and 992 DEGs were identified in blastocysts co-culture with OES for 5 and 8 days, respectively, compared to controls. Under 20% O_2, 627 and 1580 DEGs were identified in blastocysts co-cultured with OES for 5 and 8 days, respectively, compared to

controls. The most enriched cellular components of DEGs were mitochondrial membrane, endosome membrane and intercellular bridge. The most enriched pathways involved chromatin organization, cell-cell adhesion, cell proliferation, membrane trafficking, and lipid metabolism.

In conclusion, OES co-culture improved embryo quality in all groups and mitigated the negative impact of high oxidative stress conditions on in vitro embryo development. OES co-culture up to the time of embryo genome activation (5 days) was enough to induce this beneficial effect. Finally, the OES altered the embryonic transcriptome with highest impact under oxidative stress conditions, evidencing for the first time a modulation of the embryo-OES dialog according to the environment.