Bovine Oviductal Organoid-Derived Secretions Increase Blastocyst Yield at Day 7 and Day 8 of In Vitro Culture.

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With the aim of mimicking the physiological environment in which fertilization and early embryo development take place, reproductive fluids (RFs) have been used as supplements to in vitro culture media by various research groups over the last decade (Coy et al., 2022). In cattle, embryos produced with RFs showed improved developmental kinetics, a higher number of blastomeres and higher post-vitrification survival rates (Lopera-Vasquez et al., 2017).

Due to inter-individual variability in the effect of the RFs, it is necessary to collect, process and test large batches of abattoir material before including them in the IVP media. Additionally, RFs are not immune to sanitary risks, so the detection of bluetongue, BVD or IBR viruses by PCR in each fluid batch must be done before use.

The recent development of organoids derivation could contribute to overcome these problems and maintain the high efficiency of bovine culture media with standardized biological additives. As organoids have already been derived from human fallopian tubes and endometrium (Turco et al., 2017), we proposed to generate organoids resembling the bovine oviduct, and derived them to produce fluids similar to those surrounding the embryo during the early developmental stages in the oviduct.

Reproductive tracts were collected from a local abattoir, dissected on ice and the epithelial tissue was minced, centrifuged, and cryopreserved. From this stage, the derivation of oviductal organoids and the collection of organoid-derived secretions (ODS) was performed as described in Turco et al., (2018) and Simintiras et al., (2021).

Since organoids respond to hormonal stimulation, we generated 3 different types of organoids: without hormonal stimulation (CODS); stimulated with estradiol resembling the estrus cycle preovulatory phase (E2); and stimulated first with estradiol and then with progesterone, resembling the early postovulatory phase (P4). The protein concentration in the ODS ranged between 2.5 and 3.1 mg/mL. All ODS were resuspended in SOF-BSA medium and added at a final concentration of 5 or 10% (v/v) to the embryo culture media from d1 to d3 post-insemination, when cleavage rate was assessed (D3CL), and embryos were washed in fresh medium and transferred to new droplets until d7 and d8 of culture, when blastocyst yield was assessed (D7BL and D8BL, respectively).

Data are expressed as Mean ± SE. Only statistically significant differences ($p<0.05$) are described. D3CL was reduced in E2-10% and P4-10% groups (66.7±4.6% and 68.0±4.6%, respectively) compared to CODS-5%, P4-5% and CODS-10% (87.1±3.5%, 87.4±3.4% and 87.9±3.2%, respectively). D7BL was higher in P4-5% group (20.0±4.1%) than in Control, E2-5%, CODS-10% and E2-10% groups (7.1±2.4%, 5.2±2.3%, 7.5±2.6% and 3.8±1.9%; respectively). D8BL was higher in P4-5% (26.3±4.5%) than in all groups (11.6±3.0, 14.0±3.6, 9.3±3.0, 13.1±3.3, 4.8±2.1 and 11.7±3.2; for Control, CODS-5%, E2-5%, CODS-10%, E2-10% and P4-10%, respectively).

These preliminary results show that ODS, which mimic the early luteal phase of the estrous cycle, can be used as additives in embryo culture media when stimulated first with estrogens and later with progesterone. The 5% concentration was found to be sufficient in increasing the IVP blastocyst yield. To the authors' knowledge this is the first study to report the use of this standardized biological additives in bovine embryo culture. However, to complement this work, it is necessary to add ODS from uterine tissues that resemble the mid-luteal phase of the estrous cycle to the embryo culture media from d3 to d8.

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