

Innovations in 3D Culture Systems for the Study of Sperm-Oviduct Interactions

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The oviducts play a crucial role in reproduction by connecting the ovary to the uterus, facilitating the fertilization of the oocyte, and initiating embryonic development. Understanding the cellular processes that occur during the transport of gametes through the oviduct is essential for improving the reproductive efficiency of these animals. In this context, the present study proposes a magnetic 3D culture system to understand the interaction between the isthmus and bovine sperm, aiming to enhance the reproductive efficiency of these animals. For this purpose, epithelial and stromal cells from the bovine oviduct were separately cultured in a monolayer system and magnetized by centrifugation using nanoshuttle™-PL and the magnetic 3D system [Greiner Bio-One CELLSTAR®, Germany] for spheroid formation. In experiment 1, our goal was to determine the spheroids responsiveness to steroid hormones mimmetizing the estrous cycle. For that, the spheroids were divided into two experimental groups with different hormonal treatments [Control (no hormone) Progesterone-100 ng/mL 14 days or Progesterone-100 ng/mL 11 days followed by Estradiol-300 pg/mL for 3 days] and subjected to cell viability analysis using Hoescht and Propidium Iodide staining (Sigma-Aldrich). In experiment 2, our goal was to verify if the method of semen processing would affect the ability of sperm cells to adhere to the spheroids. For that, frozen semen from one bull were submitted to two different seminal processing methods (Swim-up in TALP medium, supplemented with Hyaluronic Acid (10 µg/mL HA), or Percoll® discontinuous gradient 90/45%) and stained with Mitotracker™ Red CMXRos (ThermoFisher) to stain the middle piece of the sperm cell and allow its identification after attachment to the spheroid. Sperm cells (3×10^6) were co-cultured with one spheroid for 12 hours and the number of red dots were assessed by fluorescence microscopy. For both experiments, images were analyzed in ImageJ software and groups were compared by ANOVA followed by LSD's test (experiment 1) and Student's t test (experiment 2). In experiment 1, both hormonal treatments showed a significant response of the spheroids, and the treatment using Progesterone and Estradiol together demonstrated a lower proportion of necrotic cells compared to Progesterone alone [$P < 0.0008$]. In experiment 2, both sperm processing resulted in the same

adherence capability to the spheroid [p=0.14]. This finding suggests that spheroids generated by our system present responsiveness to hormonal treatments and allow sperm adherence *in vitro*. The development of this study model may bring significant advances in the reproductive efficiency of cattle.

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