Maturation of bovine cumulus-oocytes complex in microfluidic device: Effects on lipid accumulation and blastocyst rates

Franciele Flores Vit[1]; Juliano Rodrigues Sangalli[1]; Mariane Farias Fiorenza[1]; Helena Fabiana Reis de Almeida Saraiva[1]; Juliana Germano Ferst[1]; Felipe Perecin[1], Flávio Vieira Meirelles[1]; Lucimara Gaziola de la Torre[2] and Juliano Coelho da Silveira[1]

[1] Departament of Veterinary Medicine, University of São Paulo, Pirassununga, São Paulo, Brazil.

[2] Department of Material and Bioprocess, State University of Campinas, Campinas, São Paulo, Brazil.

The study of oocyte *in vitro* maturation (IVM) is significant as it can still improve oocyte quality, which is the ability to be fertilized and to develop a healthy embryo. Based on that, new techniques applied to the *in vitro* maturation can help to increase the *in vitro* production rates. The conventional IVM method consists of culturing COCs in plates and in restricted media volume. One of the limitations of this type of culture can be the access to nutrients, hormones, and other molecules as well as the accumulation of metabolic waste, which can impact the maturation process. Compared with the *in vivo* situation we believe that the dynamic exchange of the microenvironment within the follicle could impact the maturation success. In this case, we constructed a reversible and reusable microfluidics device that enables cumulus-oocyte complex (COC) culture while media exchange in real-time. We used a reversible sealing and reusable microdevice to perform IVM and evaluated lipid accumulation at the end of the maturation period. Initially, we developed and validated a reusable and reversible sealing microfluidic device in laminated polydimethylsiloxane ($PDMS_{LAM}$), glass, and Polymethyl methacrylate (PMMA-acrylic). The microdevice validation steps included flow rate, media recovery, media evaporation, and PH changes. After that cumulus-oocyte complex (COC) from bovine ovaries were aspirated and COCs with one or two layers of cumulus cells and homogeneous cytoplasm were selected to continue in the experiment. The COCs were matured in TCM119 B supplemented with 10µg/ml sodium pyruvate, 25µg/ml gentamicin, 0.4% BSA, 5 ng/ml 10−2 IU/ml human recombinant FSH and 50 ng/ml human recombinant luteinizing hormone (LH). The COCs cultured in plates were cultured with 30µL of medium, and the oocytes cultured in microfluidic were cultured with 6µL of medium with a constant flow rate of 1µL. min^{-1} for 22 h. We performed 5 routines with a pool of 60 COCs per routine (30 in plates-control and 30 in microfluidic device). After 21h in maturation media COCs were stripped and only the matured COCs were stained with BODIPY, and the lipid droplet profile was evaluated. The matured COCs were also parthenogenetic activated to verify whether microfluidics affects the blastocyst rate. The data were expressed as means ±SEM, and $p < 0.05$ was considered a significant difference. We observed that lipid droplets in COCs matured on microdevices were two folds smaller than in COCs matured on plates. Furthermore, we did not observe statistical differences in maturation and blastocyst rates. However, the blastocysts from COC that were subjected to in vitro maturation in microdevices showed larger diameter and an increased number of cells than oocyte matured in culture plates ($P<0,0002$). The results suggest that microdevice does not interfere with the COC maturation process but improves lipid accumulation and blastocyst cell numbers. The microfluidic device can be a good alternative for improving in vitro embryo production.

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