Animal Reproduction

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Innovation and technology

# Liquid marbles micro-bioreactor as a new system for in vitro bovine oocyte maturation and embryo production

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## Resumo

Liquid Marble (LM) is a small drop of liquid encapsulated by hydrophobic powder particles allowing cells to be cultivated in a three-dimensional manner in a small liquid system. This study aimed to evaluate the use of LM during cumulus-oocyte complexes (COCs) in vitro maturation (IVM) and embryo in vitro culture (IVC) system. For the IVM experiment, COCs aspirated from slaughterhouse ovaries were divided into two different groups for IVM: 1) control (bi-dimensional culture using conventional drops) and; 2) LM group. Seven replicates were performed with n=20 oocytes per replicate per group. After 21 hours of IVM, the COCs were denuded to remove cumulus cells (CC) and the IVM-rates were evaluated by the second polar body extrusion. CC and denuded oocytes were collected, snap-frozen, and stored at -80ºC for expression analysis. For the IVC experiment, COCs from slaughterhouse ovaries were aspirated, selected, conventionally in vitro matured, and fertilized. After 18 hours of in vitro fertilization, 120 presumptive zygotes per replicate, were denuded to remove CC and divided into two groups for IVC: 1) control; and 2) LM group. For IVC experiment, eight replicates were performed and 3 pools of 5 blastocysts (each pool containing 2 blastocysts and 3 expanded blastocyst) per group were obtained on Day 7 for further 380 miRNA profile assessment, which are responsible for regulating ~60% of bovine genes. RNA was extracted using QIAzol, converted in cDNA and qPCR for the genes EIF4b, EIF4e, BAX, BCL2, CDK6, HAS2, GAPDH, and FOXO3a in CC and GDF9, BMP15, PI3K, PTEN, FOXO3a, BAX, and BCL2 in oocytes. Cycle threshold for each gene were normalized by the geometric mean of PPIA and ACTB. MiRNA reverse transcription was performed using miScript II RT Kit (HiSpec) in Day 7 blastocysts. Relative expression bovine miRNAs were determined by RT-qPCR with SYBR Green PCR kit (QIAGEN) and the miR-99b was used as housekeeping. Statistical analyses were performed by Student's t-test, a $P<0.05$ was considered for statistical difference. We found that the IVM rate did not differ between groups ($P=0.5633$). In CC, EIF4e (involved in the initiation of gene translation in eukaryotes), BCL2 (encodes an anti-apoptotic protein) and GAPDH (enzyme that breaks down glucose for energy and carbon molecules) had their expression reduced in LM compared with control. In IVC experiment, a total of 32 miRNAs were identified, and the miRNA miR-615 was downregulated in the LM, and the bta-let-7f was exclusively expressed in the control. These results suggested that a three-dimensional culture based on LM was capable of modulate the cumulus cells and the blastocysts at molecular levels. However, more analyses investigating the use of LM on IVM and IVC are necessary to generate a solid knowledge of the biological pathways affected in CC and blastocysts.

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