Cell-free DNA in spent culture media: possible marker for embryo development and quality in cattle

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Oocyte and embryo quality is the central factor influencing the outcome of in vitro embryo production systems. However, quality evaluation is mainly limited to an assessment of morphological criteria. Therefore, there is a need to identify non-invasive markers to improve the accuracy of selection. This study aimed to evaluate if quantification of cell-free DNA (cfDNA) in maturation (IVM) and culture medium(ICV) can be used as a marker for oocyte competence and embryo quality. To do that, two experiments were carried out. Experiment 1 evaluated of the amount of cfDNA in maturation containing oocytes with high (EMB) and low (NEMB) capacity to produce embryos in vitro. The oocytes were matured, fertilized, and cultured individually until day 8 (D8) of development, and the maturation medium was stored for later analysis. In experiment 2, the cfDNA was quantified in spent culture medium from expanded blastocysts that that did (P) or did not (NP) establish pregnancy after transfer. In vitro produced embryos that were at morula stage at D5 were cultured individually until D7, when culture medium was collected and the blastocyst were transferred to recipient cows. Pregnancy was confirmed 60 days after embryo transfer. cfDNA was extracted from 15 µL of both media as reported by Kussano et al.,2024 (10.1371/journal.pone.0298316). Then, the number of copies of ART2 and Bov-tA, were used to quantify cfDNA in IVM and ICM by qPCR. Those sequences are short interspersed nuclear elements (SINEs) found in ruminants that are equivalent to ALU used in humans. A total of 30 IVM and IVC medium samples obtained from each group EMB/NEMB and P/NP were analyzed by qPCR. Data of the cfDNA quantification were compared using Mann–Whitney U test , GraphPad Prism 9 (GraphPad Software, San Diego, California USA). Initially, the level of cfDNA quantified by ART2 gene, was higher in the IVM medium in the NEMB than in the EMB group. While for Bov-tA, the levels of cfDNA were similar between groups

(P>0.1). In the second experiment, when we compared de quantification of cfDNA from spent culture medium (D5-D7), from P and NP groups medium no differences (P>0.01) were observed in the level of ART2 genes (P>0.1). Conversely, Bov-tA genes did not amplify in any of the samples. It can be concluded that lower levels of the ART2 in IVM medium may indicate a greater potential of the oocytes to develop into an embryo. Financial Support: FAP-DF, Embrapa, CAPES and CNPq.