Presence of Colony stimulating factor 2 from day 1 to day 7 of culture does not affect bovine embryo development and quality

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Colony stimulating factor 2 (CSF2), recognized as an embryokine, has been extensively studied for its role in embryonic development. Most studies report the use of CSF2 between day 5 and day 7 of embryo culture. However, its use is not practical for the routine of a commercial laboratory. Therefore, we tested the possibility of using it from day 4, on the day of feeding, and throughout the culture period of bovine embryos. Cumulus oocyte complexes were recovered from slaughterhouse ovaries and subsequently matured and fertilized in vitro. After 18 hours of co-incubation with spermatozoa, the presumptive zygotes were distributed into three treatment groups, based on the culture medium used: control – cultured in SOF supplemented with 2.5% bovine fetal serum (BFS), CSF2D1 - SOF supplemented with 2.5% BFS and 10 ng/mL of CSF2 from day 1 to day 7 of culture, CSF2D4 - SOF supplemented with 2.5% BFS and 10 ng/mL of CSF2 from day 4 to day 7 of culture. In all groups, feeding was performed on day 4 of culture, employing the respective treatment-established medium. Cleavage rates on day 4 and blastocyst rates on day 6 and day 7, along with developmental stage, were evaluated. On day 7, embryos reaching the expanded blastocyst stage were stored at -80°C for genes expression analyses. The genes PDRX3, HSPA5, OCT4, INTau, PLAC8, KRT8, SLC2A1, SLC2A3, CASP3, NANOG and HASP70, genes related to embryo quality, were quantified by real-time PCR (RT-qPCR) using GAPDH as the constitutive gene. Total RNA was isolated from four pools of 16 expanded blastocysts from each of the three treatment groups. The results of embryo development were analyzed using the chi-square test ($P < 0.05$). Genes that were normally distributed were analyzed using ANOVA and Tukey's test, while those that were not normally distributed were evaluated using the Kruskal-Wallis and Mann-Whitney tests. All analyses were performed using GraphPad Prism 9 (GraphPad Software, San Diego, California USA). Cleavage and blastocyst rate at day 7 were similar ($P > 0.05$) among treatments, being 82.9 and 37.0 %, 82.0 and 40.1% and 85.5 and 40.7% for control (n=598), CSF2D1 (n=594) and CSF2D4 (n=605) treatments, respectively. However, percentage of embryos that reached expanded blastocyst stage and hatched on day 7 was higher on the presence of CFS2 than on the control ($P < 0.05$). Of the 11 genes evaluated, only PLAC8 gene presented a lower amount of transcripts in embryos cultured with CFS2 from day 4. In conclusion, CSF2 can be used throughout the entire culture period without affecting embryo production and

developmental kinetics compared to CSF2 added on day 4. However, the addition of CSF2 on day 4 of culture affected the expression of PLAC8 in expanded blastocysts. Financial Support: FAP-DF, Embrapa, Capes.