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Association of two epigenetic modulators on the initial development of female bovine embryos

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Female embryos are more sensitive to embryo culture *in vitro* and X-chromosome inactivation seems to be one of the epigenetic events related to this fragility. The supplementation of the culture medium with agents that aim to align this process is presented as an alternative to stimulate the embryonic development. The present study aimed to identify the influence of the association of two epigenetic modulators (Tricostatin A and Folic Acid - TSA + AF, at concentrations of 10μM and 5 nM, respectively) in the initial development of female bovine embryos, since the use of these separately showed no influence on embryonic development and blastocyst rate (unpublished data). Oocytes were obtained from slaughtered ovaries, matured, fertilized *in vitro* (d0) with sexed semen (X chromosome) and cultivated (d1) in embryo culture system in pools, allowing individual monitoring of structures during development (*homemade chamber*). Two groups according to the time of development (4 days -Gd4 and 5 days -Gd5) were used for the time of supplementation. The percentages of cleavage and blastocysts were compared using Fisher's Exact Test, and the embryonic development analyzes were submitted to the normality test and evaluated using the Mann-Whitney U Test (5%, Graphpad Instat Demo). The number of cells estimated for analysis was established based on previous results produced in our laboratory, in which embryos smaller than 16 cells were categorized containing 14.3 cells, 30 morulae and d10 blastocysts 101.1. In Gd4, the cleavage rate did not differ (TSA + AF: 74% -n = 47; C: 63% -n = 48) between groups. The blastocyst rate was significantly higher (p = 0.048) in the control group (TSA + AF: 17% -n = 13; C: 31% -n = 20 **), and the modulators did not influence in the number of cells blastocysts recovered at d7 (TSA + AF: 21.14 ± 4.69; C: 41.22 ± 6.70). At time d5, the cleavage rate did not differ (TSA + AF: 59% -n = 82; C: 59% -n = 66). The blastocyst rate was significantly higher (p = 0.0125) in the control group (TSA + AF: 18% -n = 26; C: 33% -n = 37 **), and the supplement showed (p = 0496) a negative influence on the number of blastocyst cells recovered at d7 (TSA + AF: 18.61 ± 5.77; C: 29.99 ± 8.617 **). Thus, we concluded that in the concentrations used, the association between folic acid and Trichostatin A was not beneficial for delayed female embryos, and the time of treatment did not seem to influence the effects caused by supplementation in the medium. Acknowledgments: FAPEMIG, EMBRAPA, CAPES, CNPq.