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Theophylline associated or not to heparin as a capacitation inducing agent to the *in vitro* production of bovine embryos**B.C. Carvalho¹, L.P. Silva², F.C. Varago², J.R. Ribeiro², C.A.C. Fernandes², M.M. Gioso², M. Gimenez³, J.P. Neves²**¹EMBRAPA Gado de Leite; ²UNIFENAS; ³BIOTRAN.**Keywords:** bulls, cleavage, fertilization.

The theophylline mechanism of action is similar to the caffeine mechanism. However theophylline is more efficient to increase the intracytoplasmic levels of adenosine 3',5'-cyclic monophosphate (cAMP), a nucleotide involved in the sperm capacitation process. The aim of this study was to evaluate theophylline as a capacitation inducer agent in replacement or in combination with heparin in the development of bovine embryos produced *in vitro*. The culture media used to produce the embryos were provided by Biodux® company (Campinas, SP, Brazil). Theophylline (T1633 - Sigma-Aldrich®) was added to the fertilization medium. The experiment was carried out with 4 Gir breed bulls and 3 treatments, in a total of 12 experimental groups. Each bull was evaluated according to the following treatments: Treatment 1 (T1): Heparin - 10mg / mL; Treatment 2 (T2): Theophylline - 5 mM; Treatment 3 (T3): Heparin (10mg / ml) + Theophylline (5mM). The cumulus-oocyte complexes (COCs) recovered from slaughtered cow ovaries were incubated in the maturation medium at 38.8°C in atmosphere of 5% CO₂ for 24 hours. In IVF step, the capacitating agents were added to the medium, composing the treatments T1, T2 and T3. The fertilization day was considered as day 0 (D0). The cleavage rate, embryo production and hatching were evaluated after 2, 7 and 10 days of fertilization, respectively. The data were submitted to variance analysis using generalized linear models. The averages were compared by Tukey Kramer test ($p < 0.05$). The embryo production rate was higher ($p < 0.05$) for the T1 (37.97 ± 13) relative to the T2 and T3 (10 ± 28.55 , 27.60 ± 11.0 , respectively). The same was observed in the hatching rate ($p < 0.05$) in the T1 (33.50 ± 14) compared to T2 and T3 (22.81 ± 11 ; 23.08 ± 10 , respectively). Bull did not influence cleavage rates ($p > 0.05$) in the cleavage rates, produced embryos and hatching. Apparently, theophylline alone or associated with heparin did not induce a good sperm capacitation, which could be the reason of reduced fertilization rate. However, this fact was not evidenced by the cleavage rate, which was similar between treatments. This provides evidence that, although the rate of cleavage did not differ between treatments, not all of cleaved structures were competent to sustain the embryo development. These findings may be due the increased incidence of parthenogenesis or polyspermy and consequently blocking of embryonic development (Ramos et al., Brazilian Journal of Veterinary Medicine and Animal Science, V.52, 2000, online). Further investigations are necessary in order to explain the possible factors that led to theophylline alone or associated with heparin to reduce embryo production rates in *in vitro* fertilization.

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