Proceedings of the 29th Annual Meeting of the Brazilian Embryo Technology Society (SBTE); Gramado, RS, Brazil, August 20th to 23rd, 2015. Abstracts.

A180 OPU-IVP and ET

Acrosome reaction induced by theophylline associated or not to heparin in bovine semen

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Keywords: capacitation, fertilization, sperm.

The sperm capacitation consists in biochemical and physiological changes during which the sperm becomes hyperactivated and able to undergo the acrosome reaction to penetrate the zona pellucida of the mature oocytes. The aim of this study was to evaluate theophylline as a capacitation inducing agent in replacement or in combination with heparin for sperm cells acrosome reaction. Theophylline (T1633 - Sigma-Aldrich®) was added to fertilization medium provided by Biodux® company (Campinas, SP, Brazil). The experiment was performed with 4 Gir breed bulls and 3 treatments, in a total of 12 experimental groups. Each bull was evaluated using the following treatments: Treatment 1 (T1): Heparin - 10µg/mL; Treatment 2 (T2): Theophylline – 5 mM; Treatment 3 (T3): Heparin (10µg/mL) + Theophylline (5mM). Heparin is used as capacitation agent in most of embryo production laboratories, and for this reason was chosen as the control group. The acrosome integrity was analyzed by double staining technique (Trypan blue/Giemsa – TBG) described by Didionet al. (Gamete Res, v.22, p. 51-57, 1989). The semen was thawed and submitted to Percoll gradient separation (45 and 90%). Tubes containing fertilization T1, T2 and T3 medium were inseminated with 2 x 10^6 sperm/mL and kept on incubator at 38.8°C and 5% CO_2, in the absence of oocytes. The semen was incubated in capacitation medium for 0, 6, 12 and 18h then stained with Trypan blue / Giemsa and analyzed by bright field microscopy with 1000x magnification to evaluate the acrosome reaction. The analyzed characteristics in sperm cells were true acrosome reaction - Acrosome and post-acrosome region unstained; False acrosome reaction - Acrosome unstained and post-acrosome region stained; Dead - Stained post-acrosomal region and acrosome staineds. The data were submitted to analysis of variance followed by Tukey Kramer test ($p < 0.05$). Treatment did not affect the acrossome reaction analysis. The same was observed to bulls. However, the true acrosome reaction rate was higher ($p < 0.05$) at time 0h (61.50 ± 6.78) compared to 6h (19.63 ± 12.71), 12h (7.21 ± 4 40) and 18h (7.04 ± 2.66). For the dead sperm, we observed a higher rate ($p < 0.05$) at time of 12h (84.46 ± 5.82) and 18h (86.75 ± 4.19). It was observed that the incidence of true acrosome reaction was relatively lower than the dead sperm rate, which may be due to the total incubation time (18 hours). This suggests that the incubation conditions in the absence of COCs and essential growth factors for the acrosome reaction stimulation impaired sperm viability in vitro. However, theophylline was as effective as heparin in the induction of acrosome reaction.

Acknowledgments: to FAPEMIG (CVZ - APQ-00798-12), CAPES, CNP by financial support, UNIFENAS and Biotran.