A018 Physiology of Reproduction in Male and Semen Technology

The influence of different methods of frozen-thawed ovine spermatozoa selection on sperm capacitation and viability after incubation

C.C.S. Olivares[1], J.M.G. Souza-Fabjan[1], A.B.V. Peneiras[1], M.F.A. Balaro[1], V.J.F. Freitas[2], J.F. Fonseca[3], F.Z. Brandão[1]

[1]Universidade Federal Fluminense; [2]Universidade Estadual do Ceara; [3]EMBRAPA Caprinos e Ovinos.

Keywords: ram, sperm longevity, sperm selection.

Sperm capacitation is an essential event for fertilization; however, it decreases the sperm lifespan and viability. The aim of this study was to evaluate the effects of four sperm selection techniques on sperm capacitation and viability after incubation. A pool of frozen-thawed sperm from 10 Santa Inês rams was used. The samples were submitted to one of the following sperm selection techniques: sperm washing, Percoll gradient, mini-Percoll gradient, Swim-up and control group. At mini-Percoll technique, was used 400 microliters of 90% and 45% gradients and a centrifugation at 500 xg for 5 minutes. In Percoll, was used 1 mL of each gradient and a centrifugation at 700 xg for 10 minutos. During Swim-up, the sperm was incubated in 1 ml of SPERM-TALP for 45 minutos in humidified atmosphere at 37.5^{o}C. Finaly, at sperm washing the sample suffered centrifugation at 300 xg for 8 minutes, using SPERM-TALP. At the end of each treatment, the selected spermatozoa were incubated at 37^{o}C for 1 h, 2 h, and 3 h. Viability was assessed using acridine orange-propidium iodide combination by computer-assisted sperm analysis. Capacitation status was evaluated using chlortetracycline staining and observed under epifluorescence microscopy. Data were analyzed by ANOVA, followed by Tukey test ($P<0.05$). After 3 h of incubation, the capacitated sperm was decreased ($P<0.05$) in all treatments. The capacitated sperm rate was similar ($P>0.05$) among Percoll (36%), mini-Percoll (34%) and Swim-up (30%), and were lower ($P<0.05$) than control group (47%) and sperm washing (41%), regardless of the time of incubation. The non-capacitated sperm percentage was higher ($P<0.05$) at 0 h (12%) and decreased after 3 h (1.5%), in all treatments. Regarding to acrosome reacted cells, there was an interaction ($P<0.05$) between incubation and sperm selection treatment. The acrosome reacted spermatozoa showed a lower percentage ($P<0.05$) at 0 h (50%) and 1 h (53%) and higher after 3 h (64%). Percoll and mini-Percoll were higher about acrosome reacted spermatozoa ($P<0.05$; 60% vs. 61%), whereas control group was the lowest (49%). There was an interaction ($P<0.05$) between incubation and treatment in sperm viability. Viability assays revealed that 0 h resulted in a higher rate (17.5%; $P<0.05$) of membrane integrity, after all treatments. Swim-up treatment showed a higher membrane integrity rate (17.4%; $P<0.05$), regardless of time of incubation. In conclusion, the incubation affects the capacitation status and viability of frozen-thawed ovine sperm. Sperm selection increases the acrosome reacted cells rate and Swim-up allows better viability during incubation.

Financial support: Faperj (E-26/111.694/2013).