Mini-Percoll processing of domestic ruminant frozen-thawed semen dispenses the use of heparin in capacitating medium

Processamento pela técnica de mini-Percoll em sêmen congelado/descongelado de ruminantes domésticos dispensa o uso da heparina em meio capacitante

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Sperm capacitation is a prerequisite for mammal successful fertilization. Although usually a capacitating substance such as heparin is used during sheep *in vitro* fertilization, evidences suggest that the cryopreservation process and Percoll technique could induce spontaneous capacitation. This study aimed to compare ovine, caprine and bovine frozen-thawed sperm after mini-Percoll processing on sperm parameters, receiving or not heparin supplementation. To evaluate the sperm motility and capacitation parameters in caprine (n = 3; Alpine), ovine (n = 3; Santa Inês) and bovine (n = 3; Holstein) species, commercial frozen- thawed semen were used. For each replicate (n = 6), two straws of each animal were thawed at 37 °C for 30 s, totaling six straws for each species. From this pool, samples (after thawing) were obtained to determine sperm concentration, motility, plasma membrane integrity and capacitation status. The remainder pool was submitted to sperm selection with Percoll gradient. After selection, the samples were evaluated and received or not 5 µg/mL heparin (Sigma Chemical, USA) supplementation in incubation medium. Finally, these samples were submitted to 1.5 h, 3 h, 6 h and 18 h incubation. Sperm parameters were assessed in all intervals. ANOVA was performed with Tukey or Fisher-LSD tests for means comparisons. The non-normal variables were subjected to Kruskal-Wallis test followed by Dunn´s test. After mini-Percoll, there was a reduction ($P<0.05$) in total motility in ovine and bovine when compared with after thawing values, respectively (ovine: 14 ± 2 *vs*. 46 ± 6; bovine: 28 ± 2 *vs*. 46 ± 5%). Similarly, there was a decrease ($P<0.05$) in progressive motility after mini-Percoll in ovine and bovine (ovine: 3 ± 1 *vs*. 23 ± 3; bovine: 8 ± 1 *vs*. 20 ± 3%). Conversely, there was an increase ($P<0.05$) in capacitation rates after mini-Percoll in all species (ovine: 28 ± 7 *vs*. 17 ± 3; caprine: 41 ± 1 *vs*. 26 ± 3; bovine: 41 ± 3 *vs.* 32 ± 2%) and an increase ($P<0.05$) in acrosome-reacted cells after mini-Percoll when compared with after thawing moment (ovine: 61 ± 2 *vs*. 55 ± 4; caprine: 48 ± 2 *vs*. 46 ± 4; bovine: 45 ± 5 *vs.* 40 ± 1%). Ovine presented higher acrosome-reacted cells after thawing and after mini-Percoll than the other species. Heparin supplementation did not affect ($P>0.05$) the parameters evaluated. However, a lower ($P<0.05$) rate of sperm agglutination was observed under heparin presence than heparin abscence in ovine in all moments assessed. Additionally, ovine showed a lower ($P<0.05$) rate of intact cells and a higher ($P<0.05$) agglutination rate along the incubation compared to the other species. However, capacitation status was similar ($P>0.05$) among all species throughout the incubation, regardless the presence of heparin. In conclusion, frozen-thawed ovine, caprine and bovine spermatozoa processed with mini-Percoll behave similarly regarding to capacitation status and does not require heparin supplementation during *in vitro* incubation to achieve capacitation.

Keywords: sperm capacitation, ovine, caprine, bovine.
***Palavras-chave**: capacitação espermática, ovinos, caprinos, bovinos.*

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