



A178 Physiology of Reproduction in Male and Semen Technology

Influence of mini-Percoll techniques in sperm capacitation and plasma membrane integrity of ram frozen-thawed sperm

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The success of IVF and subsequent development of embryos are directly related to sperm selection and quality. This study aimed to evaluate the effect of different forces and time of centrifugation in mini-Percoll (MP) techniques for sperm selection on capacitation status and plasma membrane (PM) integrity of ram frozen-thawed sperm. Commercial semen from 10 rams of Santa Inês breed, aging 2-5 years old, were used. At post-thawing (PT), the PM integrity was evaluated by CASA using the SCA® system (Sperm Class Analyzer – Microptic Automatic Diagnostic Systems, Barcelona, Spain). For traditional Percoll (Sigma Chemical, St. Louis, USA), a 2 mL-gradient (90/45% density) was subjected to a 700 x g centrifugation for 10 min followed by 200 x g for 5 min. The MP techniques consisted in an 800 µL-gradient (90/45% density) subjected to either: I) two centrifugations of 5000 x g for 5 min; II) two centrifugations of 2500 x g for 5 min; III) two centrifugations of 1250 x g for 5 min; IV) 700 x g for 10 min, followed by 200 x g for 5 min. At the end of all treatments, aliquots (post-protocols = 0 h) were taken for evaluation of PM integrity and capacitation status by chlortetracycline stain (Sigma Chemical, St. Louis, USA). Later on, samples of all treatments were submitted to incubation at 37°C, 1 h, 2 h and 3 h and the same parameters were assessed. The variables were subjected to either ANOVA or Kruskal-Wallis tests depending on normality, Tukey and Fisher-LSD analysis ($P < 0.05$). There was no difference ($P > 0.05$) among all treatments for capacitation status and PM integrity. The capacitated rate was higher ($P < 0.05$) at 3 h ($28.6 \pm 2.5\%$) when compared with 0 h ($23.3 \pm 1.7\%$) and 1 h ($23.8 \pm 1.4\%$) of incubation, regardless of the treatment. There was no difference ($P > 0.05$) for PM integrity among the protocols at 0h. Regardless of the treatment, the intact and damaged PM rate, respectively, were similar ($P > 0.05$) for PT ($16.4 \pm 2.0\%$ vs. $84.0 \pm 2.0\%$) and 0 h ($19.0 \pm 2.9\%$ vs. $81.1 \pm 2.9\%$; average of all treatments). When analyzing just intact cells, the PT values were greater ($P < 0.05$) than any incubation interval, whereas the latter were lower than 0 h values ($P < 0.05$). In conclusion, MP may be used as an ideal alternative to the traditional Percoll, decreasing costs and time of sperm handling, without cell damages in ram sperm.