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Sperm Morphology and Preparation Method Affect Bovine Embryonic Development

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This study was conducted to evaluate the effect of sperm separation methods of semen samples collected from bulls subjected to scrotal insulation on embryonic development after in vitro fertilization (IVF) and to determine whether IVF results would be affected by various heparin concentrations. Morphologically abnormal semen samples were obtained and cryopreserved from Holstein bulls following scrotal insulation for 48 hours. Standard protocols using the Percoll gradient (90%/45%) method and the swim-up method were used to separate spermatozoa fractions in experiment I. The pellet (A_p) and the 45% layer (B_p) were isolated from the Percoll separation, while for the swim-up separation, the supernatant (A_s) and the interphase (B_s) were isolated. The overall blastocyst rate for our laboratory control semen was $23.1 \pm 2.1\%$ for Percoll separations (A_p and B_p) and $18.2 \pm 2.0\%$ for swim-up (A_s and B_s) separations. This rate was higher ($P < .01$) than the rate observed for the semen from the bull that had the greatest response to scrotal insult 5 days prior to the insult, when it was $9.2 \pm 2.1\%$ for the Percoll separation and $20.7 \pm 2.3\%$ for the swim-up separation, while semen from 27 days after scrotal insulation (D +27) resulted in no blastocyst formation for the Percoll separation and a $4.2 \pm 2.1\%$ rate for the swim-up separation. In experiment II, semen was sampled from the bulls that responded in the greatest and least degrees to scrotal insult 5 days before scrotal insulation (D -5) and on days 23 (D +23) and 34 (D +34) after scrotal insulation. These samples were exposed to IVF mediums with 3 different heparin concentrations (0.1, 1.0, and 10 $\mu\text{g}/\text{mL}$). There was a significant difference ($P < .05$) in developmental scores between the D -5 (1.08 ± 0.08), D +23 (0.9 ± 0.08), and D +34 (0.8 ± 0.08) samples, but no differences were observed in blastocyst formation based on the number of cleaved embryos. Increasing the heparin concentration resulted in higher ($P < .01$) embryonic developmental scores. In conclusion, when semen samples with high percentages of abnormal spermatozoa are used for IVF, semen separation preparation methods affect results. Our results show that the separation methods used under these conditions were inadequate in their ability to provide potentially competent sperm for IVF. However, selecting appropriate sperm separation procedures could improve in the IVF embryonic development of semen from bulls used in artificial insemination. Also, an increase in the heparin concentration was able to partially overcome deficiencies, which suggests that morphologically abnormal spermatozoa undergo capacitation despite possible

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structural changes to the plasma membrane.

Key words: Percoll, swim-up, abnormal spermatozoa, thermal insult, in vitro fertilization

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