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Collection, Cryopreservation, and Fertilizing Potential of Bovine Spermatozoa Collected from the Epididymis Evaluated by Conventional Techniques and by Flow Cytometry

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Abstract : In the present study, the fertilizing capacity of bovine spermatozoa was evaluated before and after its cryopreservation. For this, the testicles of 100 bulls slaughtered on Terceira Island were dissected, the epididymal tails were separated, and semen was recovered by the flotation method and then evaluated by phase contrast microscopy and by flow cytometry. For phase contrast microscopy, a drop of semen was used to evaluate the percentage of motile spermatozoa (from 0 to 100%) and motility (from 0 to 5). After determining the concentration and the abnormal forms, semen was diluted to a final concentration of 50 x 106 spz/ml and evaluated by flow cytometer for membrane and acrosome integrity using the conjugation of fluorescent probes propidium iodide (PI) and Arachis hypogea agglutinin (FITC-PNA). Freezing was carried out in a programmable semen freezer, using 0.25 ml straws, in a total of 20 x 106 viable sperm per straw with glycerol as a cryoprotectant in a final concentration of 0.58 M. It was observed that, on average, a total of 7.25 ml of semen was collected from each bull. The viability and vitality rates were respectively 83.22 ± 7.52% and 3.8 ± 0.4 before freezing, decreasing to 58.81 ± 11.99% and 3.6 ± 0.6, respectively, after thawing. Regarding cytoplasmic droplets, it was observed that a high percentage of spermatozoa had medial cytoplasmic droplets (38.47%), with only 3.32% and 0.15% presenting proximal and distal cytoplasmic drops, respectively. By flow cytometry, it was observed that before freezing, the percentage of sperm with the damaged plasma membrane and intact acrosome was 3.61 ± 0.99%, increasing slightly to 4.21 ± 1.86% after cryopreservation ($p<0.05$). Regarding spermatozoa with damaged plasma membrane and acrosome, the percentage before freezing was 3.37±1.87%, increasing to 4.34 ±1.16% after thawing, and no significant differences were observed between these two values. For the percentage of sperm with the intact plasma membrane and damaged acrosome, this value was 2.04 ± 2.34% before freezing, decreasing to 0.89 ± 0.48% after thawing ($p<0.05$). The percentage of sperm with the intact plasma membrane and acrosome before freezing was 90.99±2.75%, with a slight decrease to 90.57±3.15% after thawing ($p<0.05$). From this study, it can be clearly concluded that, after the slaughtering of bulls, the spermatozoa can be recovered from the epididymis and cryopreserved, maintaining an excellent rate of sperm viability and quality after thawing.

Keywords : bovine semen, epididymis, cryopreservation, fertility assessment
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