

The effect of different dilution rates with bioxcell extender on frozen-thawed ram semen quality

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Glycerol- based diluents, to which egg yolk (with or without milk) is added, are commonly used for the cryopreservation of ram semen. But in the last years new diluents with lecithin based cryoprotective components were introduced into practise. Such an extender is commercially available for bull semen (Bioxcell[®], IMV, L' Aigle, France), and it has previously been tested in vitro and in vivo for freezing bull semen, with satisfactory results. The objective of the present study was to evaluate the effect of sperm dilution rates (200, 400 and 800 x 10⁶ sp/ml) with Bioxcell extender on the post-thaw ram semen quality. During the nonbreeding season, semen was collected from two adult Karayaka rams (Anatolian breed) by artificial vagina. Ejaculates were collected from each male twice a week for 5 weeks. Semen extended in Bioxcell diluent to final concentration of 200, 400, 800 x 10⁶ sp/ml were loaded into 0.25 ml straws and equilibrated at 5°C for 2 hours . Straws were frozen in liquid nitrogen vapour and then plunged in liquid nitrogen (-196°C) for storage. After thawing (at 37°C for 30 sec), sperm motility, acrosome intact and membrane integrity (HOS test) were assessed. Prefreezing sperm concentration influenced (P<0.001) freezability of spermatozoa and affected negatively the in vitro parameters at 400 x 10⁶ and 800 x 10⁶ sp/ml. Semen frozen at 200 x 10⁶ sp/ml, the percentage of motility and HOS test (40 %, 29 %) were significantly (p<0.001) higher than frozen at 400 x 10⁶ (20 %, 18 %) and 800 x 10⁶ (13 %, 14 %) sp/ml. The lowest percentage of abnormal acrosome was seen in the semen frozen at 200 x 10⁶ sp/ml (29 %) with significant differences (P<0.05) compared to 400 and 800 x 10⁶ sp/ml (40 %, 48 %). Our results indicate that significant differences exist between dilution rates, and that the low concentrations better protects sperm from damage incurred during cryopreservation. These preliminary findings suggest that different dilution rates and sperm concentrations within the ranges tested may affect the post-thaw sperm characteristics.