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Flow cytometric evaluation of ram semen freezability treated with α-tocopherol

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Abstract

The aim of this study was evaluation of ram semen freezability treated with different rate of α-tocopherol in non-breeding season. In this study, ejaculates were collected from four Merino rams (2-3 ages were used and belonging to the special sheep farm in Bademli, Burdur/Turkey). The ejaculates containing spermatozoa with >80% motility and concentrations higher than 1.5x109 spermatozoa/ml (normozoospermic) were mixed and used in the study. The mixed ejaculates were divided into five equal aliquots and samples were extended with tris base medium (tris, citric acid, glucose) containing 800 µM group, 400 µM group, 200 µM group, 100 µM group or 0 µM group (control) α-tocopherol and they were equilibrated to 4°C for 2 hours, frozen in mini straws then stored in liquid nitrogen. Straws were thawed at 37°C for 30s in water bath for plasma membrane acrosome integrity (PMAI %) and high mitochondrial membrane potential (HMMP %) with flow cytometric analyses. Flow cytometry analyses were performed with a Cytoflex Flow Cytometer (Beckman Coulter, Fullerton, CA, USA). Assessments of semen samples were made with a laser beam at 488 nm (50 mW laser output), with 525 ± 40, and 585 ± 42 and 610 ± 20 nm emission filters. Data were collected from 10.000 events. The highest PMAI (24.39±2.11 %) and HMMP (39.90±3.13 %) were in 100 µM compared to other groups ($p<0.05$). Also, the lowest PMAI and HMMP values were obtained in 800 µM and 400 µM groups respectively ($p<0.05$). In conclusion, 100 µM α-tocopherol should be supplemented to ram semen freezing extender for membrane & acrosome integrity and high mitochondrial membrane potential.

Keywords: α-tocopherol, flow cytometry, ram semen freezing, plasma membrane & acrosome integrity, mitochondrial membrane potential.

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