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DETERMINING THE IN-VITRO FERTILIZING ABILITY OF RAM SEMEN FROZEN WITH DIFFERENT EXTENDERS

Ramazan Arici*, Ahmet Eser, Selin Yağcioğlu, Kamber Demir, Serhat Pabuccuoğlu, Kemal Ak, Serhat Alkan

Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, 34320, Avcilar, Istanbul, Turkey

The required investment for the organization of artificial insemination in small ruminants is often uneconomical and the success in the frozen semen techniques is not at the desired level. In this study, different combinations of cholesterol and iodixanol, the chemical substances were used in freezing ram semen. The TRIS base extender and 5% glycerol were used for semen dilution and freezing. Semen samples were collected from 5 rams and pooled. The pooled semen were divided into 4 groups, CLC (Cyclodextrin Loaded Cholesterol), iodixanol and CLC – iodixanol and control respectively. Total and progressive motility were observed by CASA and motility value by using phase contrast microscope. Spermatological properties were evaluated by HOST and fluorescent staining. After incubating at 37°C for 4 hours and at 46°C for 15 minutes for Thermal Stress Test (TST), spermatological traits were observed by CASA. With semen samples from each group, IVF was performed. There were no differences in the motility values between the groups at pre-dilution, incubation and after cooling. After equilibration, cholesterol containing groups (1. and 3.) had worse results in motility ($p<0,05$). After thawing, these groups showed better results by evaluated features (motility, membrane integrity, viability, $p<0,05$). However, the progressive motility results were lower in these groups($p<0,05$). The lowest cleavage embryo rates in IVF studies were found in groups containing 60,70% and 58,45% CLC ($p <0,05$). The cleavage rates of iodixanol and control groups were 75.13% and 76.12%, respectively. The highest blastocyst rates were determined in the iodixanol group compared to the control and CLC-iodixanol combinations ($p <0,05$). As conclusion, it is clear that iodixanol is the most successful group according to the IVF results. Iodixanol significantly influenced in vitro cleavage and blastocyst rates positively from spermatological properties; CLC iodoxanol combination is needed to perform more IVF or insemination studies to demonstrate that it can be used as an extender in the freezing of ram semen.

Key words: ram semen, cryopreservation, cholesterol, iodixanol, IVF

This study was funded by Istanbul University with the project number: 43724