

A Study on The Freezing of Dallagh Ram Semen Using Various Levels of Glycerol

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Sperm cryopreservation induces the formation of intracellular ice crystals, osmotic and chilling injury that causes sperm cell damage (Isachenko 2003). Glycerol is often poly-hydroxylated, capable of hydrogen bonding with water and capable of permeating across the cell membrane. When glycerol is added during cryopreservation of spermatozoa, it replaces intracellular water and maintains necessary cellular volume, interacts with ions and macromolecules and depresses the freezing point of water. This lowers the electrolyte concentrations in the unfrozen fraction so that less ice forms at any given temperature (Holt, 2000). The aim of this experiment was to study the freezing of Dallagh ram semen using various levels of glycerol.

Six mature, healthy and fertile 2-3 year old rams with an average liveweight of 45 ± 5 kg were chosen from a herd and kept apart from ewes at University farm, Gorgan, Iran. Semen samples were collected by an electro-ejaculator once a week from each ram during October to December 2009. Collected samples were pooled and extended in tris buffer containing fructose, citric acid, egg yolk as described by Evans and Maxwell (1987). The levels of glycerol in tris buffer were 4, 6 and 8 % (v/v) for three treatments. Extended semen samples were cooled to 5°C for two hours and then 0.5 ml straws were filled. Straws containing chilled extended semen were horizontally placed in a rack inside a box at 4–6 cm above the liquid nitrogen surface to freeze in seven minutes. Straws containing frozen semen samples were transferred into a liquid nitrogen tank and stored for ten days. Semen samples were thawed in water bath at 37°C for 30 seconds and evaluated for percentages of live spermatozoa, progressive motility (Evans and Maxwell, 1987), total defects and normal spermatozoa. Total spermatozoa defect is the sum of major and minor defected cells as described by Blom (1983). This experiment was conducted in a completely randomized design and data were analyzed using general linear model at 0.05 probability level (SAS 2001).

The effects of various levels of glycerol on post-thawing spermatozoa viability and motility were significant ($P < 0.05$). The highest percentages of live and progressive motile spermatozoa were observed in tris buffer with 4% glycerol respectively. Higher levels of glycerol decreased live and progressive motile spermatozoa, but did not affect percentages of defected and normal spermatozoa ($P > 0.05$) (Table1).

Table1. Mean and SEM of post-thawing ram spermatozoa characteristics (%) with various levels of glycerol

Treatments	Live spermatozoa (%)	Progressive motile spermatozoa (%)	Total spermatozoa Defects (%)	Normal spermatozoa (%)
4% Glycerol	$26.66^a \pm 1.56$	$21.66^a \pm 1.46$	$7.66^a \pm 1.37$	$92.33^a \pm 1.37$
6% Glycerol	$19^b \pm 1.56$	$13.88^b \pm 1.46$	$7.66^a \pm 1.37$	$92.33^a \pm 1.37$
8% Glycerol	$14.33^c \pm 1.56$	$9.44^c \pm 1.46$	$8^a \pm 1.37$	$92^a \pm 1.37$

Means with different superscript in each row are significantly different ($p < 0.05$).

In conclusion, the addition of 4% glycerol in tris buffer for extending and freezing ram semen of Dallagh rams is suitable and would be recommended.

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