

The effect of dilution rates and freezing methods on post-thawing motility of Baluchi ram spermatozoa

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Introduction For the effective use of Artificial insemination technique in sheep industry, investigation on the methods of ram semen dilution and freezing is necessary. Ahangari (1992) showed that various rates of dilution from 1:1 to 1:4 did not affect $p > 0.05$ post thawing survival of Cambridge ram spermatozoa. Fiser et al (1987) achieved a 73% c.f. 67% pregnancy rate using thawed semen, previously frozen to -100 and -79 respectively. The objective of this study was to investigate the effect of two rates of dilution of semen and two methods of freezing on post-thawing motility of ram spermatozoa.

Materials and methods Three Baluchi rams were trained to ejaculate semen into an artificial vagina at Baluchi Sheep Breeding Station, Abbas-abbad, Mashhad, Iran. Baluchi is meat and carpet wool type sheep. They are raised extensively throughout the North to East of country. Semen was collected and assessed. Semen samples at a concentration of 4000 millions per ml with a good wave motion > 3 were mixed. Pooled semen was diluted with tris buffer containing egg-yolk (15% v/v) at two rates of 1:1 or 1:2 (semen:diluent). Glycerol was added in two steps at +35 and +5 reaching to a final concentration of 4% (v/v). Diluted semen samples were cooled to +5 and then 0.5 ml straws were filled. Semen was frozen manually by placing straws in liquid nitrogen vapor using two methods. Straws were held at -120 in liquid nitrogen vapor for 6 minutes and then being plunged into a liquid nitrogen tank (fast freezing). Straws were gradually lowered in liquid nitrogen vapor at -70 to freeze within ten minutes and then being plunged into a liquid nitrogen tank (slow freezing). Post-thawing progress linear motility of ram semen samples were assessed and data was corrected using the following formula (Gill, 1978). $Y = \text{Arcsin}(X/100)^{0.5}$, Y=corrected motility percentage and X=motility percentage. The experiment was planned on 2x2 factorial in a completely randomized design to examine two rates of dilution of semen and two methods of freezing with three replications. Mean comparison was carried out using Duncan multiple range tests at 0.01 probability level.

Results The corrected means of post-thawing motility of spermatozoa for treatments are shown in Table 1. The differences between rates of dilution, 1:2 or 1:1 on post-thawing motility of spermatozoa (27.56 Vs 12.9) was significant ($p < 0.01$). This is in agreement with Mathur (1991) who suggested a similar rate of dilution for obtaining an optimum cryosurvival of ram spermatozoa. The differences between methods of fast freezing and slow freezing on post thawing motility of spermatozoa (23.46 Vs 16.32) was significant ($p < 0.01$). This confirmed previous report of Ahangari (1992) that semen in straws, frozen to -100 and -120 before being plunged into liquid nitrogen survived better than semen frozen to -60 or -80.

Table 1 The corrected mean (s.e.) of post-thawing motility of spermatozoa for each treatment ($p < 0.01$)

Methods of dilution and freezing	Corrected means (s.e.) of post-thawing motility
1:1 and Fast	13.97 (0.87)c
1:1 and Slow	9.90 (1.79)c
1:2 and Fast	33.17 (1.28)a
1:2 and Slow	22.65 (1.66)b

Conclusion The dilution rate of 1:2 (semen:diluent) and using a manual method of fast freezing to -120 before being plunged into liquid nitrogen tank can be suggested for a long term storage of Baluchi ram semen in Iran.

References

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