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# Effect of Season on Freezability of Semen from Two Breed-Types of Desert Sheep in the Sudan

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**Abstract:** The objective of this study, was to investigate the freezability of frozen semen collected from two breed-types of Desert sheep (Hamari and kabashi). Nine rams (6 Hamari and 3 kabashi) aged 2-4 years were used in this study to prepare a total of 293 semen ejaculates collected by means of an artificial vagina. The samples were extended in Tris-based diluent, frozen and stored in liquid nitrogen at -196°C. The date obtained revealed that, both breed-types responded equally to the effect of season. Higher percentages of sperm motility were recorded before freezing in autumn (67.07±14.40% vs. 65.42±11.17% for Hamari and Kabashi rams , respectively), while the lowest values were observed in semen samples collected in summer (55.36±7.68% vs. 59.00±5.83). However, no significant difference ($p>0.0.5$) was found between breeds or seasons on post-thawing sperm motility, although better records were obtained in the Kabashi rams (59.00±5.83%) during Summer, compared to Hamari rams (56.96±6.48%) in the same season. A significant difference ($p<0.05$) was observed between the two breed-types and season of the year on the percentage of rejected samples after freezing (15.38 vs 6.35%) with better results obtained in the Kabashi rams. However, post-freezing rejection percentage decreased during autumn and winter (5.56 and 14.29% for Hamari rams with no rejected samples for Kabashi rams). From this study, it could be concluded that, semen collected, throughout the year, from the two breed-types was suitable for freezing. The best semen quality after freezing was obtained during Autumn and Winter.

**Key words:** Sheep, semen, season, freezability, breed-types, desert sheep

## INTRODUCTION

Sheep population in the Sudan is estimated as 47 million heads (CBS, 2003). According to their phenotypic features and ecological distribution Sudan sheep were classified into five major breeds (Mcleroy, 1961). These breeds include the Desert, the Nilotic, the Mountain, the Dry Equatorain and the West African.

The Desert sheep constitute 65% of sheep population, in the Sudan and dominate the export market of live sheep and mutton and this is attributed to their marketable features and their outstanding performance in feedlot trials (Elamin and Suleiman, 1983; Babiker and Mohamed, 1990; Arabi, 1995). Desert sheep are raised by nomadic and seminomadic tribes, in the area located between latitudes 13 and 17° North and longitudes 25 and 37° East. Many ecotypes evolved and they are usually named after the tribes or localities where they exist (Mcleroy, 1961). Hamari and Kabashi breed-types derived their names from Hamar and Kababish tribes, resident in western Sudan, where Desert sheep are dominant.

Previous studies on the measurements of the reproductive capacity, seasonality and fertility of Sudan Desert sheep are scarce (Galil and Galil, 1982; Alsayed, 2001; Makawi *et al*., 2005; Makawi and Manahil, 2007). Cryopreservation and Artificial Insemination (AI) are prerequisites for effective breeding programmes aiming at genetic improvement as well as for the long-term storage of genetic materials (Gil *et al*., 2000). Freezing of semen is done in gradual steps to avoid damaging the delicate sperm cells. This can be done by utilizing cryogenic nitrogen, dry ice or alcohol bath (Herman *et al*., 1994).

The temperature variation involved in freezing and thawing of semen, however, reduces the proportion of motile spermatozoa and causes ultrastuctural, biochemical and functional damage (Söderquist *et al*., 1999). Sperm survival after freezing-thawing is dependent on freezing technique, diluents, equilibration interval and thawing method (Prathalingam *et al*., 2006).

Sheep and goats show large seasonal variations in semen quality (Lebouef *et al*., 2000). In temperate zone, a clear influence of photoperiod on seminal quality is well

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known and improvement in the percentage of motile sperms occurs during the decreasing photoperiod (Karagiannidis *et al*., 2000; D'Alessandro and Martemucci, 2003). However, in the tropics high ambient temperature may limit reproductive ability (Parkinson, 1987; Roca *et al*., 1992). It adversely affects quality of semen with a decrease in the motility of sperms and appearance of high abnormalities and dead cells.

The present study aimed at examining the potentials of producing frozen ram semen to adopt AI. for sheep breeding for the first time in the Sudan.

## MATERIALS AND METHODS

**Location and climate:** This study was conducted at the National Artificial Insemination Centre, Khartoum North, Sudan, located at a latitude of 15° 36′ N, a longitude of 32° 33' E and an altitude of 380 m above sea level. Maximum air temperature (42.8°C) was recorded during summer, while the minimum temperature (14.7°C) was observed during winter. The highest relative humidity (43%) was recorded during autumn, while the lowest (12%) was observed during summer. The longest daylight hours (13.1) were observed during summer, while the shortest (11.2) were recorded during winter.

**Animals:** Nine rams (6 Hamari and 3 Kabashi) were allotted to this study. The rams were 2-4 years old and with average body weight of 73.60±6.85 and 62.13±5.42 kg for Hamari and Kabashi rams, respectively.

**Husbandry and management:** The rams were housed in individual shaded pens (2×1.5 m). Each animal was fed daily on 0.5 kg of ready-made concentrates (Khartoum Feed Mill) and ground-nut hay was offered *ad-libitum*. The rams were allowed free access to drinking water and mineral licks.

**Semen collection and evaluation:** Semen was collected from each ram twice a week by means of an artificial vagina (41°C) into a prewarmed tube at 30°C and was maintained at this temperature until processed. A teaser ewe on heat was used as a mount animal for semen collection. The tubes with the freshly collected semen were immediately transferred to the laboratory and immersed in a water bath at 30°C.

The percentage of motile spermatozoa was assessed by diluting a drop of semen (1:5; semen to diluent), transferring it into a warm slide (37°C), covered with a cover slip and examined under high magnification of the microscope (400x). An average visual estimation of the percentage of moving sperms from successive observations of more than 5 different areas of the field was made (Chemineau and Cagnie, 1991). A Tris-based extender was used in this study (Evans and Maxwell, 1987). The semen was diluted following a two-step method, were by, the extender was divided equally into 2 parts, the first without glycerol, while the second with 14% glycerol, resulting in a final concentration of 7% (v/v). The flask of partially extended semen was cooled down slowly and progressively to +5°C. The second (pre-cooled) part of the diluent containing the cryoprotectant was then added in three steps, 10 min apart, to prevent osmotic shock to the spermatozoa. The extended semen was packed in plastic medium straws of 0.5 mL capacity (I.M.V, France) and sealed with polyvinyl chloride powder. The straws were then immersed for two hours in the ice-water bath to allow equilibration of the spermatozoa with the diluent. After the outer surface had been dried, the straws were transferred to a pre-cooled rack, suspended horizontally in liquid nitrogen vapour (4-5 cm) above $LN_2$ surface level (-110-120°C) for 8-9 min. Finally, the straws were plunged into liquid nitrogen at -196°C. After storage in liquid nitrogen for 48 h, random straws were thawed in warm water (37°C) for 15 sec. Immediately after thawing, a drop of semen was placed on a warm glass slide, covered with a cover-slip and examined at 400×magnification for sperm motility. The freezability of spermatozoa was expressed as the percentage of motile sperms surviving the deep-freezing process.

**Statistical analysis:** Data were treated and analyzed statistically using Statistical Package for Social Science (SPSS). Mean comparisons were made using Fisher's least significant test.

## RESULTS

Pre-and post-freezing sperm motility was shown in Table 1. Season of the year had a significant ($p<0.05$) effect on individual sperm motility. The highest mean values (67.07±14.40 and 65.42±11.7% for Hamari and Kabashi, respectively) were recorded during autumn, while the lowest percentages of sperm motility were observed during summer in the two breed-types (55.36±27.86 and 59.00±5.83, respectively). Mean values of post-thawing sperm motility obtained in the present study were 56.39±5.95 and 54.19±5.32% for Hamari and Kabashi, respectively. No significant difference ($p<0.05$) was observed between breed and season on post-thawing sperm motility. However, rejected semen samples recorded significant differences ($p<0.05$) between breed and season of the year (Table 2). Lesser rejected semen samples

Table 1: The effect of breed and season on the pre- and post-freezing sperm motility of Kabashi and Hamari rams

| | Pre-freezing motility (%) | | Post-thawing motility (%) | |
|---|---|---|---|---|
| Season | Hamari | Kabashi | Hamari | Kabashi |
| Summer | 55.36±27.68[a] | 59.00±5.83[a] | 56.96±6.48[a] | 53.18±10.72[a] |
| Autumn | 67.07±14.40[b] | 65.42±11.17[a] | 55.00±6.32[a] | 55.45±4.98[a] |
| Winter | 64.05±10.76[b] | 64.39±8.96[b] | 57.22±4.15[a] | 55.00±4.47[a] |

[a,b,c]Different superscripts in the same column indicate significant differences ($p<0.05$)

Table 2: The effect of breed and season on pre- and post-freezing rejection rate of Kabashi and Hamari rams

| | Pre-freezing rejection (%) | | Post-freezing rejection (%) | |
|---|---|---|---|---|
| | Hamari | Kabashi | Hamari | Kabashi |
| Summer | 32.60[a] | 31.00[a] | 19.57[a] | 19.00[a] |
| Autumn | 33.33[a] | 50.00[b] | 5.56[b] | 00.00[b] |
| Winter | 21.43[b] | 16.67[c] | 14.29[a] | 00.00[b] |

[a,b,c]Different superscripts in the same column indicate significant differences ($p<0.05$)

before freezing (21.43 and 16.67% for Hamari and Kabashi rams, respectively) were recorded during winter. However, post-thawing rejection percentages for Hamari rams decreased during autumn and winter (5.56 and 14.29, respectively), while in Kabashi rams no rejected samples were detected in the same period.

## DISCUSSION

The results obtained in this study revealed that, season of the year influenced semen quality of Hamari and Kabashi rams (Individual sperm motility and rejected semen samples). Whereas, Post-thawing sperm motility as a parameter for semen quality, was not affected by season or breed. Greater values of individual sperm motility were observed during autumn, while lower values were recorded during summer. These results support similar findings of other authors (Dufour *et al*., 1984; Daader *et al*., 1987; Sackmann and Schone, 1990; Alsayed, 2001). Low values of individual sperm motility during summer in the tropics, might be attributed to high ambient temperature that adversely affected semen quality (Parkinson, 1997; Roca *et al*., 1992). Post-thawing sperm motility obtained in the present study, falls within the normal range reported by other authors (Söderquist *et al*., 1999; Gil, 1999; D'Alessandro and Martemucci, 2003; Purdy, 2006). The results indicated that, semen collected from Hamari and Kabashi rams and frozen throughout the year was suitable for artificial insemination programmes. Similar results were recorded by Alsayed (2001) working on Desert rams.

In contrast, other authors (Roca *et al*., 1997; Alsayed, 2001; D'Alessandro and Martemucci, 2003) suggested that capacity of spermatozoa to withstand dilution and storage could be influenced by season of semen collection. Sperm survival after freezing-thawing is dependent on freezing technique, diluents, equilibration interval and thawing method (Gil *et al*., 2003; Prathalingam *et al*., 2006). The percentage of rejected semen samples (post-freezing) indicated significant ($p>0.05$) seasonal and breed effects. More rejected samples were observed during summer, while the best semen was collected during autumn and winter where Kabashi rams recorded zero rejection.

## CONCLUSION

From this study, it can be concluded that semen collected from Hamari and Kabashi rams was suitable for freezing throughout the year although autumn and winter are favoured for harvest of frozen semen.

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