Influence of Season on Cryopreservation of Semen from Kenana Bulls

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SUMMARY

The objective of this study was to explore the possibilities of cryopreservation of semen from Kenana bulls and the effect of season on the post-thawing sperm motility.

Four mature Kenana bulls (3 years of age) were used in this study to prepare a total of 242 ejaculates of semen. The samples were extended using two diluents (Tris-based and Sodium citrate-based extenders), frozen and stored in liquid nitrogen at -196 ^{0}C. After 48 hours randomly picked straws were thawed in water at 37 ^{0}C for 15 seconds before sperm evaluation.

The results obtained revealed better post-thawing sperm motility when the samples were diluted in Tris-based extender (68.42±2.97%) than those diluted in Sodium citrate extender (52.19±6.45%).

The influence of season on post-thawing sperm motility was significant ($P< 0.05$). The best values were observed during autumn and winter (69.06±2.31% and 69.50±3.07%, respectively) using Tris-based extender, while the lowest mean value was observed during summer (41.17±8.02%) using Sodium citrate extender.

In conclusion, freezability of Kenana semen throughout the year in the two diluents was within the acceptable range, whereas semen frozen in Tris-based extender showed the best results.

INTRODUCTION

Kenana cattle are known as potentially promising dairy Zebu breed in Sudan. They are dominant in the Blue Nile, White Nile and Gezira States. Although their number is estimated to be about 3 million heads (M.A.F.N.R., 1983), it is believed to be declining due to extensive crossbreeding with other indigenous and exotic breeds in the country.

The aim of the storage of semen is to prolong the fertilizing capacity of spermatozoa by reducing or arresting their motility and metabolic reaction (Noakes *et al.*, 2001). The dilution and storage of semen particularly in frozen state, causes damage and deterioration to the spermatozoa resulting in a reduction of motility, viability, impaired transport and fertility (Watson, 1995; Lessard, 2000; Amirat *et al.*, 2005). Therefore, a suitable diluent is a basic necessity for successful preservation of spermatozoa. Cryopreservation of farm animals semen is routinely done for artificial insemination using different extenders containing egg yolk or milk (Hafez and Hafez, 2000). Singh *et al.*, (1998) working on Jersey, Sahiwal and their half-bred bulls, observed a reduction in the percentage of motile spermatozoa from 79.8 to 55.2 due to freezing. The average post-thawing sperm motility of Swedish dairy bulls recorded by Januskaskas (1999) was > 50%.

Makawi (1994), studying post-thawing sperm motility of Butana and Kenana bulls in Sudan, found mean values of 56.14% and 55.59%, for the two breeds respectively.

However, Makawi *et al.* (2000), reported 47.39% post-thawing sperm motility for Friesian bulls in the same country.

Breed differences in response to seasonal effects were noticed between indigenous and exotic bull breeds (Kumi-Diaka *et al*., 1981, Haugan *et al.*, 2005). Makawi *et al.*, (2000), working on Friesian bulls in Sudan found a significant seasonal effect on post-thawing sperm motility. The highest sperm motility recorded for Holstein, Bulgarian Brown and Rodopi bulls were observed during autumn, while the lowest values were found during spring (Panayotova *et al*., 1996). In contrast, Elsharif (1989), observed no significant difference in semen quality collected from crossbred bulls in Sudan during summer and winter.

Although information is available on semen characteristics of indigenous cattle breeds, little is known about the effect of season on cryopreservation of Kenana bulls semen under climatic conditions prevailing in Sudan. Thus the aim of this study was to explore the possibilities of semen processing and freezing for artificial insemination purposes and for genetic resources preservation.

MATERIALS AND METHODS

Geography and Climate:

This study took place in the National Artificial Insemination Centre, Khartoum North, Sudan located at a latitude of 15^0 36' N, a longitude of 32^0 33' E and an altitude of 380 m above sea level. Maximum air temperature (43.9 ^{0}C) was recorded during summer, while the minimum temperature (12.4 ^{0}C) was observed during winter. The highest relative humidity (58%) and average rain fall was recorded during autumn. To investigate the seasonal effect, the year was divided into 3 seasons:

a: Autumn: July to October.

B: Winter: November to February.

C: Summer: March to June.

Animals:

Four mature Kenana bulls (3 years of age) were used in this study to prepare 242 ejaculates of semen. Each animal was fed daily on 5 kg of concentrate mixture containing sorghum grains (25%), wheat bran (25%), cotton seed cakes (18%), wheat (20%), molasses (10%) and salt (2%). Sorghum grass (Abu 70) was fed *ad-libitum*. Water and mineral licks were available, throughout the day.

Semen collection:

Semen was collected from each bull twice a week by means of an artificial vagina (41 ^{0}C). A teaser bull 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 ^{0}C.

Semen evaluation:

The percentage of motile spermatozoa was assessed by diluting a drop of semen (1:5; semen: diluent), transferring it into a warm slide (37 ^{0}C), covered with a cover slip and examined under high magnification of the microscope (400x). Individual motility of sperms was estimated from 0 – 100 according to the percentage of sperms moving straight forward over the field of the microscope (Noakes *et al*., 2001). Semen samples were diluted in Tris-egg yolk-glycerol extender and Sodium citrate-egg yolk- glycerol extender (Makawi, 1994). A two-step method for semen extension was used (Makawi *et al.*, 2000). The extenders used were divided equally into two 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 ^{0}C. The second (pre-cooled) part of the diluent containing the cryoprotectant was then added in three steps, 10 minutes apart, to prevent osmotic shock to the spermatozoa.. The extended semen was prepared 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 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 (LN2) vapour 4 – 5 (cm) above LN2 surface level (-110 to -120 ^{0}C) for 8 – 9 minutes. Finally, the straws were immersed into liquid nitrogen at –196 ^{0}C. Each straw contained 40 x 10^6 sperms. Assessment of spermatozoa viability was expressed as the percentage of motile sperms surviving the deep-freezing process.

Statistical analysis:

The statistical analysis of the data was done according to complete randomized design, factorial arrangement. Analysis of variance was performed using Statistical Package for Social Science (SPSS) Programme.

RESULTS AND DISCUSSION

The seasonal variations in the freezability of Kenana bulls semen are shown in Table (1). Pre-and post-freezing motility differed significantly ($P<0.05$) among seasons. Higher initial percentage of sperm motility (77.41±9.32) was reported during winter, while the lowest mean value (64.11±8.99%) was found during summer. The highest values of post-thawing sperm motility (69.06±2.31% and 69.503.07%) were obtained with Tris-diluted semen during autumn and winter respectively, while the lowest mean value (41.17±8.02%) was observed with Sodium citrate-diluted semen during summer. The recovery of spermatozoa after thawing was better for semen diluted with Tris-based extender than semen diluted with Sodium citrate-based extender.

The average percentage of individual sperm motility studied, falls within the normal range reported by Makawi (1994), for Zebu, Temperate and Crossbred bulls in Sudan and Panayotova and Karabaliev (1996) for Holstein, Bulgarian Brown and Rodopi bulls. Higher mean value of sperm motility (89%) was reported by Mathew *et al.*, (1982) for Crossbred-Brown bulls. The results obtained in this study revealed that season of the year

influenced both the pre-and post-freezing progressive motility in Kenana bulls semen in Sudan. Greater values of individual sperm motility were obtained during winter and autumn, while the lowest values were observed during summer. These findings are in agreement with the findings of Panayotova and Karabaliev (1996) who recorded higher sperm motility during autumn. Low values of sperm motility during summer might be attributed to high ambient temperature that adversely affects semen quality (Parkinson, 1987). The results obtained for post-thawing sperm motility in this study, are in agreement with values reported by other authors (Makawi, 1994; Januskaskas, (1999). These results indicated a significant variation due to season. Higher values were recorded during autumn and winter, while low values were observed during summer. These findings support similar results of Makawi *et al.,* (2000) working on Friesian bulls in Sudan. High ambient temperature during summer is assumed to be responsible for the low values of post-thawing sperm motility, where temperature

variations involved in the freezing and thawing of semen reduced the proportion of motile spermatozoa by causing ultrastuctural, biochemical and functional damage (Soderquist *et al*., 1999, Amirat *et al.,* 2005).

Table (1): Influence of season on pre-and post-freezing sperm motility of Kenana bulls diluted with Tris and sodium citrate based extenders.

Season	Extender	Initial motility (%) (%) Mean ± SD	Post-thawing motility (%) Mean ± SD
Autumn	Tris	72.92±6.85[a]	69.06±2.31[a]
	Sod. citrate	68.72±3.33[b]	57.75±4.21[b]
Winter	Tris	77.41±9.32[a]	69.50±3.07[a]
	Sod. citrate	71.39±8.38[a]	55.12±7.12[b]
Summer	Tris	67.11±8.99[b]	64.28±3.54[a]
	Sod. citrate	64.25±7.99[b]	41.17±8.02[c]

a, b, c = Means with different superscripts in the same column differ significantly (P<0.05).

The average incidence of post-thawing sperm motility in this study using Tris and Sodium citrate extenders, were found to be 68.42±2.97% and 52.19±0.45% respectively. The best post-thawing sperm motility was observed in Tris-based extender. Similar results

were obtained by Naidu *et al*., (1981) who stated that, Tris extender might have given better results if the samples were preserved at frozen form than liquid form.

From this study, it can be concluded that seasonality is a factor in the successful semen freezing of Kenana bulls in Sudan. The best semen was produced and processed during winter and autumn. The feasibility of long-term storage of spermatozoa, makes possibility of banking genetic resources in cattle breeding in Sudan.

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أثر الموسم على تجميد السائل المنوي للثيران من سلالة الكنانة

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الملخص:

كان الهدف من هذه الدراسة البحث في إمكانية حفظ السائل المنوي لثيران الكنانة بالتجميد العميق وأثر الموسم على حركة الحيوانات المنوية بعد التجميد والإحماء.

استخدم في هذه الدراسة أربعة من ثيران الكنانة البالغة (متوسط العمر ثلاث سنوات). جمعت من هذه الثيران خلال عام كامل 242 عينة سائل منوي تم تمديدها في مخففين أحدهما يعتمد على مادة الترس كمنظم للأس الهيدروجيني (دارئ) والآخر على سترات الصوديوم ، أخضعت هذه العينات للتبريد والتجميد العميق في النتروجين السائل (−196 درجة مئوية) بعد 48 ساعة تم اختيار عينات عشوائية وإسالتها بلاحماء في درجة حرارة 37 درجة مئوية لمدة 15 ثانية بغرض فحصها مجهريا لتحديد نسبة الحيوانات المنوية الحية. النتائج أثبتت أن حركة الحيوانات المنوية بعد الإحماء كانت أفضل بدرجة معنوية في العينات المخففة بمادة الترس مقارنة بالعينات المخففة بسترات الصوديوم. أثر الموسم كان واضحا على حركة الحيوانات المنوية بعد الإحماء ، حيث سجلت أفضل النتائج خلال فصل الخريف والشتاء في العينات المخففة بمادة الترس وسجلت أدني النتائج خلال الصيف في العينات المخففة في سترات الصوديوم. من هذه الدراسة يتضح أن تجميد السائل المنوي لثيران الكنانة المخفف بالترس أو سترات الصوديوم خلال عام كامل كانت في الحدود المقبولة. كما أن نتائج العينات المخففة في الترس كانت أفضل من تلك التي كانت في الحدود المقبولة. كما أن نتائج العينات المخففة في الترس كانت أفضل من تلك التي كانت في مخفف سترات البوتاسيوم.