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Factors affecting the quality of indigenous ram semen in Bangladesh

Md. Faruk Ahmmed*, Arup Ratan Chaki, Md. Zinnu Rine, Farida Yeasmin Bari and Md. Golam Shahi Alam

Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

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Corresponding Author

MF Ahmmed
E-mail: farukdvm07@gmail.com

ABSTRACT

The aims of the present study were to determine the influence of age, body weight, collection interval and temperature of environment on the quality of indigenous ram semen. Eight indigenous rams were used in this study. The rams were divided into two groups considering age (24-36 and 37-48 months), body weight (12-18 kg and 18-24 kg), collection interval (3 days and 7 days interval) and environmental temperature (25-29°C and 29-32°C temperature). Semen was collected by artificial vagina method and ejaculates were collected from each ram. After collection the semen was evaluated for macroscopic characteristics by standard procedures. The volume and sperm concentration were significantly (P<0.05) higher at 37-48 months of age (1.4±0.1 vs. 1.3 ±0.1, 4.8±1.0 vs. 4.4±0.9), whereas, the percentages of live and normal spermatozoa was significantly (P<0.05) higher at 24-36 months of age (90.0±1.8 vs. 88.9±2.8, 92.1±1.4 vs. 91.2±1.7) compared with 37-48 months of age. The percentages of normal spermatozoa was significantly (P<0.05) higher (92.0±1.4 vs. 91.2±1.8) in 12- 18 kg body weight group. The volume was significantly (P<0.05) higher at 7 days interval (1.4±0.1 vs. 1.3 ±0.2). Whereas the percentages of live and normal spermatozoa were significantly (P<0.05) higher at 3 days interval group (91.0±1.0 vs. 90.0±1.8, 93.1±1.0 vs. 92.2±1.5). The volume of ejaculate, sperm motility and percentages of live and normal spermatozoa were significantly (P<0.05) higher at 25-29°C temperature group (1.4±0.1 vs. 1.3±0.2, 84.8±3 vs. 83.1±2.5, 89.9±1.6 vs. 88.8±2.4, 92.0±1.5 vs. 91.2±1.6). In conclusions, better quality semen may be obtained in indigenous rams at 37-48 months age, weighing 18-24 kg, at 3 days collection intervals in 25-29° C temperature in Bangladesh. Further study is required to draw a final conclusion.

Introduction

To increase and diversify the production of livestock sector in Bangladesh sheep farming might be the best choice as the indigenous sheep are noted to be eminent for their adaptability, survivability and disease resistance power. They are comparatively more resistant to stress, environmental (climate) changes and some diseases like parasitic, coldness and other infective disease like PPR (Flowers, 1997). These animals are mostly raised by landless, marginal farmers and the baperies. However, the disadvantages of Bangladeshi sheep are that they are smaller in body size and low genetic merit. There is also scarcity of breeding ram. Therefore to increase and improve the sheep production in addition with ewe management, it is urgent to preserve the ram semen and to do Artificial insemination (AI) of ewe through preserved semen.

Fertility depends on male factor (Anel et al. 2005). Quality of indigenous ram semen is greatly depends on age, body weight, season, scrotal circumference and frequency of semen collection (Parvez et al., 2009). Age is one of the major contributing factors to semen characteristics (Toe et al., 1994), with testicular size being closely related to total sperm output (Oldham et al., 1978; Ahmad and Noakes, 1995). David et al. (2007) found that motility; concentration and number of spermatozoa were higher up to the age between 2-3 years and then declined chronologically. Sperm concentrations and proportion of live spermatozoa decreased significantly with the advancing of age, whereas the proportion of abnormal spermatozoa increased significantly (Bujarbaruah et al., 1982). Level of nutrition and body weight of rams was reported to affect reproductive performance (Thwaites, 1995). Mamabolo (1999) found higher volume of semen, percentages of live and normal spermatozoa, sperm concentration in goat less than 18 kg body eight. Long abstinence periods (Pascual, 1993) and successive ejaculations (Ollero et al., 1994) have been associated with membrane alterations of spermatozoa. A decrease in semen volume and sperm concentration with successive ejaculations has been reported in several studies (Ollero et al., 1996; Kaya et al., 2002). Seasonality in breeding is not a problem in the tropics, seasonal differences in fertility have been observed and have been associated with nutrition (Schoeman and Combrink, 1987; Mukasa-Mugerwa and Ezaz, 1992). Sevi et al. (2002) and Johnston and Branton (1993) reported that high temperature than 29°C affects the body growth, the biological functions and semen quality of ram. Exposure of the bull to heat stress (>29°C) tends to damage the primary spermatocytes, while the spermatids and spermatozoa are also sensitive to heat stress (King, 1993; Bearden and Fuquay, 1997).Exposure of the testes to cold seems to be less damaging than high temperature. In addition with semen preservation it is also necessary to looking for the factors affecting

the quality of semen for increasing and improving the sheep production in Bangladesh. No detailed observed work has been conducted on the factors affecting quality of ram semen in indigenous rams Bangladesh (if any). Therefore, the present work was designed to determine the factors affecting the quality of local ram semen.

Materials and Methods

Study area

The work was conducted at the Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202 during the period from May to August 2012.

Selection and Management of rams

Nine indigenous rams bought from the local market were used for this study. They were purchased by observing the age, body weight and body appearance. The age of the rams ranged from 24 to 48 months. The body weight ranged from 12 to 22 kg. All rams were apparently healthy with absence of any clinical signs of abnormality. They were acclimatized for 21 days and dewormed once before starting of the work. The rams were provided feed at the rate of 1.5 to 2.0% of the total body weight on dry matter basis. Total required feed included 25% concentrates and 75% roughages. The concentrate feed included wheat bran, rice polish, maize bran, dry fish meal, DCP powder and table salt. The rams were provided with green grass as a source of roughages. They were allowed to free grazing 7-8 hours daily. Water was provided *ad libitum.*

Determining the factors affecting quality of local ram semen

Age

Two different age groups of rams, Group I: 24 to 36 months (n=4) and Group II: 37 to 48 months (n=4) months were categorized to observe the effects on ram semen with respect to volume, color, density, mass activity, fresh motility, sperm concentration and morphology of spermatozoa. Semen was collected at 7 days interval for evaluating of eight ejaculate from each ram.

Body weight

The body weight was categorized into two groups, Group I: 12 to 18 kg (n=4) and Group II: 18 to 24 kg(n=4) to evaluate the effects of body weight on indigenous ram semen with respect to volume, color, density, mass activity, fresh motility, sperm concentration and morphology of spermatozoa. All rams were collected at 7 days interval for evaluation of semen for eight ejaculates from each ram.

Semen collection interval

To determine the effects of collection interval on semen quality two collection interval groups were designed, Group I (n=4): Once in a week and Group II (n=4): Twice in a week. The parameters were evaluated on eight ejaculates from each ram.

Temperature

To determine the influence of temperature on semen quality with respect to volume, density, mass activity, fresh motility, sperm concentration and morphology all rams were divided into two groups on the basis of temperature on the day of collection. Group I: 25 to 29°C and Group II: 29 to 32°C temperature. All rams were collected at 7 days interval for evaluation of semen for eight ejaculates from each ram.

Semen collection

Semen was collected once in a week during the first period of study (May- June, 2012), followed by twice in a week during next period of study (July- August, 2012). Two ejaculates were collected from each ram during each collection time. Collection was always performed in the morning between 7- 9 AM. Semen was collected aseptically. After collection, semen was kept at 37°C in water-bath until the media were added with it. Each collected sample diluted with semen extender at 1:6/8 ratios depending upon the concentration. Then the sample was evaluated. The semen extender was prepared with Tris 3.603 gm, fructose 1.488 gm, citric acid 2.024 gm, penicillin 1 lac, and streptomycin 500 mg in a 100 ml volume. Final diluents were made by mixing stock solution with 10% egg yolk.

Preparation of artificial vagina

Semen was collected by Artificial Vagina (AV) method (Miller, 1986). The AV consists of outer rubber cylinder, inner rubber line, rubber band, cone and collecting tube. Semen was collected with the help of a teaser ram. All the apparatus used for semen collection was sterilized before collection using autoclave machine. The inner liner temperature of AV was maintained at 42-43°C temperature by loading two-third area of jacket with water of 52-54°C temperature. The rest of one-third area of water jacket was filled with air. Before collection of semen some sterile non spermicidal Vaseline was given into the inner side of artificial vagina by a glass rod. The AV was attached to the rubber cone attached with graduated collection tube.

Evaluation of collected semen

Individual fresh ejaculates were evaluated for volume, color, density, mass activity, sperm concentration, motility and morphology according to factors included in the study. The volume of fresh semen was recorded from the graduated mark of the semen collecting tube. The color was recorded with necked eye as slightly creamy-milky white. The density of the fresh ejaculate was recorded

and scored in 5 scales, 1=watery, 2=milky, 3 =thin creamy, 4= creamy, 5= creamy to grainy (Coulter, 1992). A small drop of fresh undiluted semen was placed on pre-warmed (37°C) geese free slide and observed under microscope at 10X without cover slip and mass activity (0-5) score was recorded following the criteria; 1= no perceptible motion, 2= few spermatozoa were moving without forming any waves, 3= small slow moving waves, 4= various movement with moderately rapid waves and eddies and 5= dense vary rapidly moving waves and eddies. Sperm concentration was determined by using haemocytometer under a 40X magnification. For sperm motility, a small drop (5µl) of diluted semen was placed on a clean pre-warmed glass slide and covered with a cover slip. Then motility was determined by eye estimation observing the proportion of spermatozoa actively moving forward at medium magnification (40X) and expressed as percentage. The proportion of normal spermatozoa with respect to Head, midpiece and tail was evaluated in buffered formol saline-fixed semen.

Morphological evaluation

The morphology of spermatozoa is used as one of the important criterion in the evaluation of semen quality in domestic animals. The spermatozoa having no abnormalities with respect to acrosome, midpiece and tail were considered as normal spermatozoa. The buffered formol saline was prepared according to the procedure described by (Perera, 2005). A drop (10µl) of formol saline-fixed semen was placed on a clean glass slide with a cover slip and the edges were soaked with tissue paper to remove excess fluid. The slide was then held for five minutes to allow spermatozoa to settle down and then examined under a microscope. At least 200 spermatozoa were evaluated to determine the abnormalities with respect to Head, midpiece and tail.

Statistical analysis

All data were entered in Excel program. They were expressed as mean ± standard deviation. Significant difference between the two groups of each factor was determined by using t-test. The difference between groups was regarded as significant when the P value was less than at least 0.05 ($P<0.05$).

Results and Discussion

Effect of age on quality of semen

The effect of age of rams on semen quality is presented in Table 1. The volume, density, mass activity, sperm concentration and motility were higher in 37-48 months age group compared with 24-36 months of age group rams, however significant ($P<0.05$) difference was existed only on volume and concentration (1.4±0.1 vs. 1.3±0.1 and 4.8±1.0 vs. 4.4±0.9). These findings correlate with the findings of other researchers with the similar age group (Foote 1986; Everret and Bean 1982; Shannon and Vishwanath 1995; Garner et al., 1996; Mathevon et al., 1998). They reported that the volume of ejaculate, sperm concentration and semen motility improve with the advance of age of the ram more than 3 years. But the present finding is inconsistent with David et al. (2007). They reported that motility, concentration and number of spermatozoa were higher up to the age between 2-3 years then declined chronologically. According to them, semen production traits tend to decrease for rams older than 3 years. This inconsistent with David et al. (2007) could be due to breed and environmental variation, no. of sample size and skills in doing work and purity of the chemicals.

Table 1: Influence of age of rams on semen parameters.

Semen parameters		Age (Months)	
		Group I (24-36) (n=4)	Group II (37 - 48) (n=4)
Ejaculate volume(ml)		1.3±0.1a	1.4±0.2b
Density (1-5)		3.8±0.4	4.0±0.5
Mass activity (1-5+)		3.8±0.4	4.1±0.4
Sperm concentration (n × 10^9)/mL		4.4±0.9a	4.8±1.0b
Sperm motility (%)		84.2±3.1	85.3±2.5
Live (%)		90.0±1.8a	88.9±2.78b
Normal spermatozoa (%)		92.1±1.4a	91.2±1.7b
Abnormal spermatozoa (%)	Head	4.4	4.9
	Midpiece	0.7	0.8
	Tail	2.1	2.2
	Others	0.8	1.1
	Total	7.9	8.9

***The mean values within same row with different superscript letter denote significantly difference between the groups at ($P<0.05$).

In the present study, the effects of age on percentages of live and normal spermatozoa were opposite to the effect on volume, density, mass activity, concentration and motility. The significantly ($P<0.05$) higher percentages live and normal spermatozoa (90.0±1.8 vs. 88.9±2.8, 92.1±1.4 vs. 91.2±1.7, respectively) was present in the 24-36 months compared with 37-48 months of age. This is difficult to explain why the percentages of live and normal spermatozoa are higher in 24-36 months compared with 36-48 months. It could be real effect as it correlates with the findings of other workers (Collins et al., 1962; Halm et al., 1969 and David, et al., 2007). They stated that percentages of live and normal spermatozoa decline as the ram gets older than 3 years. The percentages of abnormal head was higher followed by tail, others and mid piece in both the age groups. Theses abnormality percentages was also higher in 37-48 months age groups compared with 24-36 months, indicating that increasing ages of indigenous rams results increased production of abnormal spermatozoa.

Effects of body weight on quality of semen

The effects of body weight of rams on semen quality are shown in Table 2. The volume, sperm concentration and percentages of live spermatozoa were higher in 12-18 kg body weight group compared with 18-24 kg body weight. On the

contrary, the higher sperm motility was observed in 18-24 kg compared with 12- 18kg body weight group (85.6±2.8 vs. 84.2±3.1). However, these parameters did not vary significantly between the body weight groups. The percentages of normal spermatozoa was significantly ($P<0.05$) higher (92.0±1.4 vs. 91.2±1.8) in 12- 18 kg body weight group compared with 18-24 age group. In the morphological study (Figure 1), the percentages of abnormal head was higher followed by tail, others and mid piece in both the age groups. This abnormality percentage was also higher in group 12-18 kg body weight group compared with 12-18kg body weight group. This result indicates that increased body weight as with advancing age increases the abnormal percentages of spermatozoa production in indigenous rams. The random selection of rams in body weight groups may reflect the opposite body weight effect compared with age on these parameters. However, the higher motility percentages were higher in higher body weight, similar to higher age group effect. These body weight effect correlates with Thwaites (1995) and Mamabolo (1999). Thwaites (1995) claimed that the body weight of rams affect reproductive performance. His report showed that decreased volume of semen, sperm concentration, percentages of live and normal spermatozoa noticed with the advancing of body weight than normal. Mamabolo (1999) also found higher volume of semen, percentages of live and normal spermatozoa, sperm concentration in goat less than 18 kg body eight. However, the present finding on body weight is inconsistent with the Tabbaa et al. (2006). His report claimed that, BCS or body weight did not influence any semen characteristics. This inconsistency with Tabbaa et al. (2006) could be due to same causes as stated above.

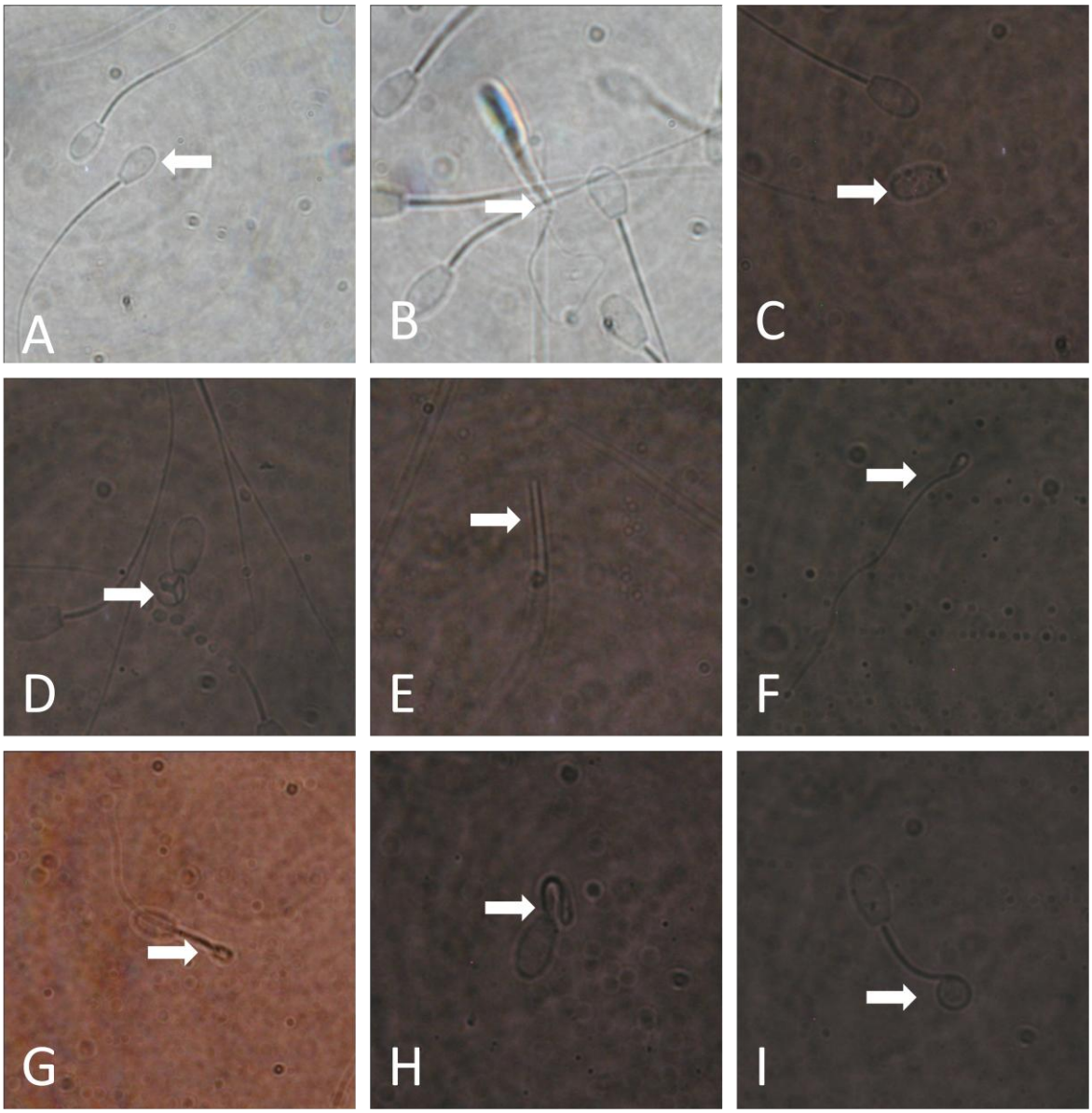

Figure 1: The normal spermatozoa (A), Excessively narrowed tail (B), Detached head (C), Dag defect (D), Detached tail (E), Excessively elongated with narrow body (F), Bent tail (G), Excessively Short and bent tail (H), Coiled mainpiece (I)

Table 2. Influence of body weight of rams on semen parameters

Semen parameters		Body weight (kgs)	
		Group I: (12-18) (n=4)	Group II: (18 24) (n=4)
Ejaculate volume(ml)		1.4 ±0.1	1.3 ±0.8
Density (1-5)		3.9±0.4	3.9±0.5
Mass activity (1-5+		3.9±0.5	3.9±0.4
Sperm concentration (n × 10^9)/mL		4.7±1.0	4.6±1.0
Sperm motility (%)		84.2±3.1	85.6±2.8
Live (%)		89.2±1.9	89.1±2.4
Normal spermatozoa (%)		92.0±1.4[a]	91.2±1.8[b]
Abnormal spermatozoa (%)	Head	4.6	4.7
	Midpiece	0.6	0.8
	Tail	2.1	2.1
	Others	0.7	1.1
	Total	8.0	8.8

***The mean values within same row with different superscript letter denote Significantly difference between the groups at (P<0.05)

Effects of collection interval on quality

The higher volume of semen, density, mass activity and sperm concentration, sperm motility were obtained from collection at 7 days interval (1.4±0.1, 3.6±0.4, 3.8±0.4, 4.7±0.9, 85.6±2.8, respectively (Table 3). However, the difference in volume was significantly (P<0.05) higher at 7 days interval (1.4±0.1 vs. 1.3±0.08). Whereas the percentages of live and normal spermatozoa were significantly (P<0.05) higher at 3 days interval group compared with 7 days interval group (91.0±1.0 vs. 90.0±1.8, 93.1±1.0 vs. 92.2±1.5). In the morphological study, the percentages of abnormal head was higher followed by tail, others and mid piece in both the age groups. These abnormality percentages were also higher at 7 days collection interval compared with collection at 3 days.

Table 3. Influence of collection interval on semen parameters.

Semen parameters		Collection interval (days)	
		Group I: (7 days) (n=4)	Group II: (3 days) (n=4)
Ejaculate volume(ml)		1.4 ±0.1[a]	1.3 ±0.2[b]
Density (1-5)		3.6±0.4	3.6±0.5
Mass activity (1-5+)		3.8±0.4	3.6±0.4
Sperm concentration (n × 10^9)/mL		4.7±0.9	4.7±1.0
Sperm motility (%)		84.2±3.1	85.6±2.7
Live (%)		90.0±1.8[a]	91.0±1.0[b]
Normal spermatozoa (%)		92.2±1.5[a]	93.1±1.0[b]
Abnormal spermatozoa (%)	Head	4.4	3.8
	Midpiece	0.6	0.5
	Tail	2.1	1.6
	Others	0.8	1.0
	Total	7.8	6.9

***The mean values within same row with different superscript letter denote significantly difference between the groups at (P<0.05).

The higher volume, sperm concentration and motility at 7 days interval and higher percentages of live and normal spermatozoa at 3 days interval is consistent with several other studies. (Ollero et al., 1996 and Kaya et al., 2002). They reported a decrease in semen volume and sperm concentration with successive ejaculations. Salamon and Maxwell (1995) reported that the proportion of normal spermatozoa was higher at 3 days interval group compared with collection at 7 days interval in ram (Amiri, 1997). The effect of collection interval on semen quality has also been established in other species. The volume, concentration and mass activity was lower in bull semen when the collection interval was less than 4 days (Everret et al., 1978; Everret and Bean, 1982; Mathevon et al., 1998).

Effects of temperature on semen quality

The effect of temperature of rams on semen quality is presented in Table 4. The difference in volume, motility and percentages of live and normal spermatozoa between 25-29 and 29-32°C temperature groups were significantly (P<0.05) different. The significantly (P<0.05) higher value (1.4±0.1 vs. 1.3±0.2, 84.8±3.0 vs. 83.1±2.5, 89.9±1.6 vs. 88.8±2.4, 92.0±1.5 vs. 91.2±1.6) of these parameters were found at 25-29°C compared with 29-32°C temperature. In the morphological study, the percentages of abnormal head was higher followed by tail, others and mid piece in both the temperature groups. Theses abnormality percentages were also higher in 29-32°C compared with 25-29°C.

Table 4. Influence of temperature on semen parameters

Semen parameters		Temperature (°C)	
		Group I: (25-29°C) (n=4)	Group II: (29-32°C) (n=4)
Ejaculate volume(ml)		1.4±0.1[a]	1.3±0.2[b]
Density (1-5)		3.9±0.5	3.9±0.4
Mass activity (1-5+)		3.9±0.5	3.9±0.4
Sperm concentration (n × 10^9)/mL		4.6±1.0	4.7±1.0
Sperm motility (%)		84.8±3.0[a]	83.1±2.5[b]
Live (%)		89.9±1.6[a]	88.8±2.4[b]
Normal spermatozoa (%)		92.0±1.5[a]	91.2±1.6[b]
Abnormal spermatozoa (%)	Head	4.8	4.5
	Midpiece	0.8	0.8
	Tail	1.8	2.5
	Others	0.6	1.1
	Total	8.0	8.8

***The mean values within same row with different superscript letter denote significantly difference between the groups at (P<0.05)

The result is correlated with the study of Sevi et al. (2002) and Johnston and Branton (1993). They reported that high temperature than 29°C affects the body growth, the biological functions and semen quality of ram. Exposure of the bull to heat stress (>29°C) tends to damage the primary spermatocytes, while the spermatids and spermatozoa are also sensitive to heat stress (King, 1993; Bearden and Fuquay, 1997). They observed higher volume of ejaculate, percentages of live and normal spermatozoa less than 28°C. Casady et al.

(1953) and McDowell (1972) reported the critical temperature for the inhibition of spermatogeesis is 29.4 °C under continuous exposure. Higher temperature alters the scrotal thermo-regulatory mechanism (defined as the mechanism by which testes combat high and low temperature). Johnston and Branton (1993) showed a highly significant correlation between fertility and daily temperature (r=0.46). The frequency of abnormal spermatozoa in ejaculates depends on consistency in environmental temperature, normality of spermatogenesis and epidiymal functions (Rathore, 1970; Gustafsson et al., 1972).The increases the scrotal temperature leading to the damage of primary spermatocytes, spermatids and spermatozoa (King, 1993; Bearden and Fuquay, 1997). Exposure of the testes to cold seems to be less damaging than high temperature.

Conclusions

The results of the present study lead to the following conclusions:

1. The indigenous rams of ages between 37-48 months and body weight between 18- 24kg body weight may be used for obtaining better quality semen in respect of volume, density, mass activity, sperm concentration and motility.

2. Collection intervals at 3 days may be the choice of semen collection for obtaining good quality semen in respect of motility, percentages of live and normal spermatozoa.
3. The climatic temperature between 25-29°C may be the choice for collection of semen in terms of volume, motility, percentages of live and normal spermatozoa.

The present work was conducted on 4 rams in each comparison groups, having 8 ejaculations. Further study is required to draw a final conclusion.

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