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CHARACTERIZATION OF NATIVE RAM SEMEN IN BANGLADESH

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The experiment was conducted to know the quality of ram semen in respect of volume, color, density and mass activity as well as effect of chilled preservation of semen exhibited by motility count of spermatozoa. Six ejaculates were collected from nine native ram breeds at 7 days intervals. After collection the semen was evaluated for macroscopic characteristics. Motility was observed at 0, 24 and 48h of collection. The mean volume of semen collected from native rams was 1.62 ± 0.16 ml. The range of volume varied from 1.3 ml to 2.0 ml. The differences in semen volume among the rams were not significantly different (P>0.05). The color of ram semen was milky to creamy color. The density of semen collected from rams was 2.79 ± 0.28 score, where the density varied from 2 to 3 score. The differences in semen density among the rams were not significantly different (P>0.05). The average mass activity of the collected semen was 3.88 ± 0.91. The range varied from 2.5 + to 5 +. The difference in semen mass activity among the rams was significantly different (P<0.01). The motility percentages immediately after collection were 85.37 ± 4.19. The range varied from 80- 92 %. This differences in semen motility among the rams were not significantly different (P>0.05). The motility of the semen 24h after collection was 68.48 ± 5.39%. The range 24 h after collection varied from 60-80 %. The differences in semen motility among the rams were significantly different (P<0.001). The motility of semen 48h after collection from rams were 54.71 ± 4.61%. The sperm motility 48h after collection varied from 50-64%. The differences in semen motility among most of the rams were significantly different (P<0.05). This is the preliminary results involving the native ram. Work is going on in the same laboratory to establish the results of the present study involving quality of ram semen.

Key words: Ram, semen quality, chilling of semen

In the livestock sector, sheep is one of the important livestock animals. The total sheep population is twenty three lakhs one thousand five hundred in our country. Bangladeshi sheep are noted to be eminent for their adaptability, survivality and disease resistance power. Comparatively sheep mortality is less than goat and highly sustainable in winter season, flood and others inclement weather. In our socio-economic aspect, due to lack of knowledge meat habited sheep owner castrates their male lambs at early age resulting severe shortage of breeding rams. Moreover, there is scarcity of high quality ram. So, it is important to improve meat quality progeny for increasing the profitable sheep farming as well as playing role in GDP (Gross Domestic Product) in our country.

Selected best quality rams could only be exploited through using artificial insemination (AI) technique. Preservation of fresh semen is a major innovation in AI technology in all over the world. AI in sheep with fresh-semen is not yet popularized in our country. Also fresh-semen is not available in our country. Preservation of ram

fresh semen may allow keeping it for short time thereby the problem of lacking good quality ram may be solved. Therefore, introducing AI with selected ram's semen could be the easiest and cheapest source of popularizing desirable germplasm within short period. The use of fresh-semen has resulted in greater conception and pregnancy rates when deposited at the cervix (Salamon and Maxwell, 1995). The availability of an efficient sheep AI service would greatly enhance the scope for pedigree and commercial breeders to respond positively and effectively to consumer demands.

In modern sheep breeding, where AI is the most widely applied tool facilitating extensive utilization of frozen semen from genetically superior rams, cryopreservation has been an invaluable technique. In order to extend the time span of the viability of spermatozoa, their metabolic rate has to be slowed down thereby reducing the rate at which substrates are used and toxins are produced. As a general rule cooling of spermatozoa is the simplest method that can successfully depress spermatozoal metabolic rate and therefore, prolong sperm survival (Curry *et at.*, 2000; Colenbrander *et at*., 2003; Cremades *et al*., 2005). The use of chilled-stored semen is limited by its relatively short time fertilizing capacity. Oxidative damage of spermatozoa during storage is a potential cause of the decline in motility and fertility during hypothermic storage of liquid semen (Ball *et al.*, 2001). The survival of ejaculated spermatozoa in seminal plasma alone is limited to few hours. To maintain spermatozoa for longer periods and to chill semen, dilution with protective solution is necessary (Ax *et al.*, 2000). However ram semen fertility in egg yolk-buffer diluents which presently used as extenders, maintain only for 12-24 h (Arthur *et al.*, 1996). However, to our knowledge, there is no work has been conducted in preservation of native ram semen in Bangladesh. Therefore, the present research work was designed to know the quality of ram semen in respect of volume, color, density and mass activity and motility of spermatozoa on Day 0, 24h and 48h of preservation.

MATERIALS AND METHODS

The research was conducted at the Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh during the period from November to December, 2012.

Selection of breeding rams and management

Nine rams of 4-8 months old were selected from the Department stock bought from the local market in Mymensingh district. The rams were kept in a single flock. Routine deworming and vaccination available in our country have been performed to the experimental animals. They were allowed to free grazing 7-8 hours daily. In addition to grazing, rams were provided add libitum water and concentrate supplementation @ 300gm/ram/day. The concentrate supplementation included; wheat bran, rice polish, maize bran, dry fish meal, DCP powder and salt.

Preparation of semen extender

TRIS based diluent was used to dilute the collected semen. All the chemicals and ingredients used in diluents were purchased from Roopganj Scientific Company Limited. The ingredients used in TRIS based diluents were TRIS 0.378 gm, Fructose 0.214 gm, citric acid 0.160 gm, egg yolk 2.0 ml penicillin 0.125 i.u., and streptomycin 0.1 mg. A stock solution was prepared by mixing the above ingredients except egg yolk which was added to make final diluents.

Preparation of artificial vagina

Semen was collected by Artificial Vagina (AV) method. 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 animal. All the apparatus used for semen collection was sterilized before collection of semen using autoclave machine. The inner liner temperature of AV was maintained at 42-43°C water by loading at 52-54°C two-third of its volume. The rest of one-third area of water jacket was filled with air. Before collection of semen some sterile non spermicidal gel was applied into the inner side of artificial vagina by glass rod.

Collection and processing of semen

Semen was collected once in a week as described by. Heterosexual animal was used as a teaser for collection of semen. Before collection, the prepuce of the ram was wiped clean to prevent semen contamination. Donor rams were allowed usually at least 1-2 false mounts before collection of ejaculation.

During collection, the AV was held in right hand along the ram's flank. The open end was facing towards the penis and downwards at an angle of 45°. When ram mounted, the erected penis was directed to the open end of the AV to permit vigorous upward and forward thrusts. The ram was allowed to withdraw its penis immediately after ejaculation in the AV. The graduated collecting tube was separated from the cone and its mouth was closed with a plastic cap and labeled. After collection, semen was kept at 37°C in water-bath until the media were added with it. Each collected sample was diluted with semen extender at 1:6/8 ratios depending upon the density score. Then the sample was divided into two equal parts loaded in aliquots with labelling, one for 24 h and another for 48 h of observation. All the samples were preserved in a refrigerator at 5°C temperature.

Fig. 1. Collection of semen by AV and color of semen

Semen evaluation

After collecting the semen, macroscopic (volume, color, density) and microscopic (mass activity and motility) evaluation were done. Volume, color and density were estimated by eye estimation. The volume of semen was recorded by reading the graduated mark of the collection tube. The color was recorded with necked eye as slightly creamy to 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). Phase contrast microscope was used to evaluate the microscopic evaluation. A small drop of fresh undiluted semen was placed on prewarmed (37°C) greese free slide and observed under microscope at 100× without cover slip. Then 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. The % progressive forward (PM) motility was recorded on 0h of preservation immediate after dilution of semen. PM motility of semen samples was also recorded on 24 h and 48 h of preservation time. For recording motility, a small drop of diluted semen was placed on prewarmed (37°C) glass slide, covered with a cover slip and then observed under microscope at 40×.

Statistical analysis

The data were entered in Microsoft Office Excel program, sorted and descriptive statistical was done to get the mean±SD value. One way ANOVA done by using SPSS® (version 17.0) package to find out the significant difference between the parameters.

RESULTS

Evaluation of macroscopic characteristics of fresh ram semen:

The macroscopic characteristics (volume, color, and density), mass activity and motility of fresh ram semen are shown in Table 1. The volume of semen collected from ram #16 to 24 were 1.68 ±0.17, 1.78±0.07, 1.60±0.14, 1.53±0.13, 1.63±0.15,

Table 1: Effects of individual ram on sperm quality (mean ±SD)

Ram ID (n=6)	Parameters					
	Volume (ml)	Density (unit)	Mass Activity (+)	Motility (%) at		
				0 h	24 h	48 h
16	1.68±0.17	2.91±0.20	4.25±0.61	86.67±4.08	70.83±3.76	59.33±3.61
17	1.78±0.07	2.83±0.26	4.33±0.52	89.00±3.22	74.67±4.08	57.33±4.36
18	1.60±0.14	2.83±0.25	3.92±0.66	87.50±2.74	70.50±4.18	56.34±3.82
19	1.53±0.13	2.75±0.27	2.75±0.27	83.33±2.58	67.50±5.24	55.83±4.62
20	1.63±0.15	2.66±0.25	3.83±0.98	82.50±2.74	69.17±3.76	52.84±4.49
21	1.70±0.18	2.83±0.26	2.75±0.27	83.67±4.76	63.67±3.83	51.01±1.09
22	1.53±0.08	2.75±0.27	4.25±0.88	85.83±4.92	67.50±5.39	53.32±5.16
23	1.58±0.11	2.75±0.25	4.83±0.41	86.50±4.68	64.17±3.76	52.00±2.44
24	1.60±0.14	2.79±0.42	4.08±0.80	83.33±4.08	68.33±5.16	54.71±4.76
P value	0.056	0.905	0.000	0.064	0.003	0.015
Level of sig.			***		**	*

n= number of ejaculation, The mean values within the same column differ significantly at least $p<0.05$

1.70±0.18, 1.53±0.08, 1.58 ±0.11 and 1.60± 0.14 ml, respectively (Table 1). However, the volume of semen in different rams varied from 1.3 to 2 ml. The volume of semen was highest in ram # 17 (1.78±0.07) compared with the lowest one in ram # 22 (1.53±0.08). However, the differences in semen volume among the rams were not significant (P>0.05). The color of ram semen was creamy white (Fig.1). The density of semen collected from ram #16 to 24 were 2.91±0.20, 2.83±0.26, 2.83±0.25, 2.75±0.27, 2.66±0.25, 2.83±0.26, 2.75±0.27, 2.75±0.25 and 2.79±0.42 score, respectively (Table 1). However, the density of semen varied from 2 to 3 score. The density of semen was the highest in ram # 16 (2.91±0.20) and the lowest in ram #20 (2.66 ±0.25). The differences in semen density among the rams were not significant (P>0.05).

The mass activity of semen collected from rams #16 to 24 were 4.25±0.61, 4.33±0.52, 3.92±0.66, 2.75±0.27, 3.83±0.98, 2.75±0.27, 4.25±0.88, 4.83±0.41 and 4.08±0.80, respectively (Table 1). The mass activity ranged from + to +++++. The mass activity of semen was the highest in ram # 23 (4.83±0.41) compared with the lowest mass activity (2.75±0.27) observed in ram # 19 and # 21. The differences in semen mass activity among the rams were significantly different (P<0.01), where the activity was significantly lower in ram #19, 21 compared with others.

Motility

The motility of semen immediately after collected (0 h) from ram #16 to 24 was 86.67±4.08, 89.00±3.22, 87.50±2.74, 83.33±2.58, 82.50±2.74, 83.67±4.76, 85.83±4.92, 86.50±4.68 and 83.33±4.08%, respectively (Table 1). The percentages ranged from 80 to 92. The motility of semen was the highest in ram # 17 (89.00±3.22%) and lowest percentages in ram #20 (82.50±2.74 %). The differences in semen motility among the rams were not significant (P>0.05). The motility of semen observed at 24 h after collection from ram #16 to 24 were 70.83±3.76, 74.67±4.08, 70.50±4.18, 67.50±5.24, 69.17±3.76, 63.67±3.83, 67.50±5.39, 64.17±3.76 and 68.33±5.16 %, respectively (Table 1). The motility ranged

from 60 to 80 after 24 h of collection. The motility of semen was the highest in ram # 17 (74.67±4.08%) compared with the lowest value in ram #21 (63.67±3.83%). The differences in semen motility among the rams were significant (P<0.001).

The motility of semen observed at 48 h of collection from #16-24 were 59.33±3.61, 57.33±4.36, 56.34±3.82, 55.83±4.62, 52.84 ±4.49, 51.01±1.09, 53.32±5.16 , 52.00±2.44 and 54.71±5.76%, respectively (Table 1). The motility varied from 50 to 64 at 48 h of collection. The highest motility was observed in ram # 16 (59.33±3.61%) compared with the lowest motility in ram #21 (51.01±1.09%). The differences in semen motility among most of the rams were significantly different (P<0.05).

Evaluation of sperm quality in pooled data

The volume, density, mass activity and motility of native ram semen have shown in Table 2. The mean volume of semen collected from native rams were 1.62 ± 0.16 ml. The range of volume varied from 1.3 ml to 2.0 ml (Table 2). The density of semen collected from rams were 2.79 ± 0.28 score, where the density varied from 2 to 3score (Table 2). The average mass activity of the collected semen were 3.88 ± 0.91. The minimum mass activity was 2.5 + where the maximum mass activity was 5 + (Table 2).

The motility of the ram semen immediately, 24 h and 48 h after collection is placed in Table 4.2. The motility percentages immediately after collection were 85.37 ±4.19. The range varied from 80- 92 %. The motility of the semen 24 h after collection was 68.48 ± 5.39%. The range 24 h after collection varied from 60-80 %. The motility of semen 48hrs after collection from rams were 54.71 ± 4.61%. The sperm motility 48hrs after collection varied from 50-64%. There was significant difference among the time of preservation on the motility of the ram semen. The motility were significantly higher immediately after collection compared with 24 h (P<0.001) and 48 h (P<0.05), respectively.

Effect of time of preservation on motility of chilled semen

The mean value ±SD at 0 h, 24 h and 48 h was 85.37 ±4.19, 68.48 ± 5.39 and 54.71 ± 4.61 respectively is shown in figure 2 and the difference in sperm motility among the rams was significant (p<0.05).

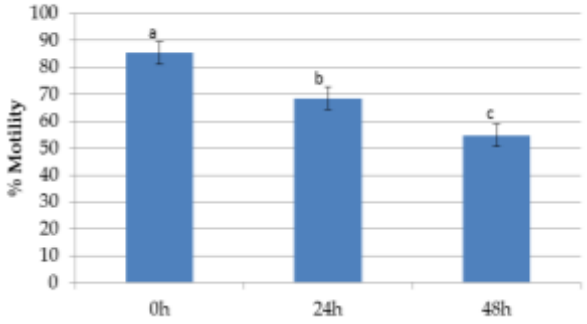

Fig. 2. Effects of time of preservation on motility of chilled semen. a, b, c mean significant at p<0.05 level

DISCUSSION

The history of artificial insemination (AI) goes back all the way to 1949 when a scientist in England discovered that a ram's spermatozoa can be chilled. The success of Al programme mostly depends on the spermatozoa which has good fertilizing capacity. The fertilizing capacity of preserved ram semen depends on the number of functionally and structurally normal spermatozoa finally deposited in the female

Table 2: Mean ± SD and range (min -max) value of native ram semen parameters at 5˚C

Parameters	Mean ±SD	Range	
		Minimum	Maximum
Volume (ml)	1.62 ± 0.16	1.3	2
Density (score)	2.79 ± 0.28	2	3
Mass Activity (+)	3.88 ± 0.91	2.5	5
Motility % (0 h)	85.37 ±4.19	80	92
Motility % (24 h)	68.48 ± 5.39	60	80
Motility % (48 h)	54.71 ± 4.61	50	64

genital tract. So, the best quality chilled semen must be used to maintain successful Al in rural areas of Bangladesh.

The present study was conducted to observe the quality of ram semen native breed. Nine rams were used in the present study. Six ejaculates were collected from each ram. The color of native ram semen was creamy white.

The volume of semen in different rams varied from 1.3 to 2ml. The volume of semen was the highest in ram # 17 (1.78±0.07) compared with the lowest one in ram # 22(1.53±0.08). However, the differences in semen volume among the rams were not significantly different (P>0.05). In pooled data the mean volume of semen were 1.62 ± 0.16 ml. The volume of ram semen of the present study is similar with the volume in other published works in aboard involving other breeds of ram (Salamon, 1962; Perry, 1989; Carter *et al.*, 1990). They observed the ram semen volume between 1.2 to 2.5 ml in suffolk, white merino. This result indicates the absence of ram semen volume between the breed.

The density of semen in different rams varied from 2 to 5 score. The density of semen was the highest in ram #16 (2.91±0.20) compared with the lowest one in ram # 20(2.66±0.25). However, the differences in semen density among the rams were not significantly different (P>0.05). In pooled data the mean density of semen were 2.79± 0.28. The density of the native ram semen of the present study is not similar with the density in other published work in aboard involving other breeds of ram (Salamon, 1962; Dukelow *et al.*, 1960; Singh *et al.*, 1985; Ahmed *et al.*, 1993). They observed the density between 4 to 5 score. This difference of results regarding density could be due to geographical, environmental and nutritional difference.

The mass activity of semen in different rams varied from 2.5 to 5. The mass activity of semen was significantly higher (p<0.01) in ram # 23 (4.83±0.41) compared with ram # 19 and 21 (2.75±0.27). In pooled data the mean mass activity of semen were 3.88± 0.9. The higher body condition score of # 23 ram may be responsible for this difference between ewes. The mass activity of the native ram semen of the present study is similar with the mass activity of semen of other published work in aboard involving other breeds of ram (Sharma *et al.*, 1999). They observed the mass activity between 3 to 5+.

The motility of semen immediately after collection in different rams varied from 80 to 92 %. This motility percentage of the fresh native ram's semen is excellent. The motility of semen was highest in ram # 17 (89.00±3.22) compared with the lowest one in ram # 20 (82.50±2.74). However, the differences in fresh semen motility among the rams were not significantly different (P>0.05). In pooled data the mean motility of semen were 85.37± 4.19. The motility of the native ram semen of the present study is similar with the motility in other published work in aboard involving other breeds of ram (Dukelow *et al.*, 1960; Singh *et al.*, 1985). They used the white merino ram and the percentages of motility were 83 to 93. The motility of semen at 24h of collection in different rams varied from 60 to 80 %. The motility of semen was significantly higher in ram # 17 (74.67±4.08) compared with the ram # 21 (63.67±3.83) (P<0.001). Normally, the motility of chilled semen declined with the progress of time of collection. The highest motility of fresh semen of the ram #17may reflect the highest motility of chilled semen at 24 h of collection compared with others. In pooled data the mean motility of semen were 68.48±5.39. The motility of the native ram semen of the present study is similar with the motility in other published works involving other breeds (Singh, 1985, Salisbury *et al.*, 1978, Dukelow *et al.*, 1960). They used American merino, suffolk and Australian merino breed and observed 62 to 85 motility 24h of collection.

The motility of semen at 48h of collection in different rams varied from 50 to 64 %. The motility percentages of semen was significantly higher in ram # 16 (59.33±3.61) compared with # 23 (52.00±2.44) (P<0.05). The higher motility percentages of ram #16 at 0 and 24h of collection compared with ram #23, may be the explanation for higher survivability of

spermatozoa at 48hrs of collection. In pooled data the mean motility percentages of semen were 54.71±4. The motility of the native ram's semen at 48h of collection is not similar with the motility in other published works (Dukelow *et al.*, 1960 and Salisbury *et al.*, 1978). This difference of the survival percentages of semen at 48h of collection could be due to differences in the preciseness of the work especially due to quality of reagent used for making diluents.

CONCLUSIONS

It might be concluded that the volume of native semen varies from 1.3 to 2.00 ml, color is creamy white, density is 2 to 3 score, mass activity ranged from 2.5 to 5+, motility immediately after collection, at 24 and 48 h of collection vary from 80 to 92, 60 to 80 and 50 to 64%, respectively. This is the preliminary results of semen quality of native ram. Work is going on in the same laboratory to establish the results of the present study.

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