

## **Selection of breeding rams by evaluating semen quality**

**Pankaj Kumar Jha<sup>\*1,2</sup> Md. Golam Shahi Alam<sup>2</sup> Md. Abdullah Al Mansur<sup>2</sup>  
Md. Taohidul Islam<sup>3</sup> Farida Yeasmin Bari<sup>2</sup>**

<sup>1</sup>Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur, Kathmandu, P. O. Box: 1950, Nepal.

<sup>2</sup>Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

<sup>3</sup>Department of Medicine, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

\*Corresponding author, E-mail address: drpankaj.np@gmail.com

### **Abstract**

The best Bangladeshi indigenous breeding rams (Wera breed) were selected by evaluating semen characteristics for using in future semen production and artificial insemination (AI) program. Of total rams (n=16), 12.5% (2/16) failed to show better performance and 87.5% (14/16) trained for semen collection. A total of 172 ejaculates were collected and evaluated during a period of 3 months. The mean frequency of semen collection was  $12.3 \pm 7$  and varied from 4 to 24. The quality of semen varied significantly among the rams ( $p < 0.05$ ). The selected 62.5% (10/16) best ram had the semen parameters  $\geq 0.5$  ml volume,  $\geq 3$  color (milky white),  $\geq 4$  mass activity (vigorous movement with moderately rapid wave and eddies),  $\geq 80\%$  sperm motility,  $\geq 90\%$  sperm viability,  $\geq 2500 \times 10^6$  sperm/ml of concentration,  $\geq 85\%$  sperm plasma membrane integrity,  $\geq 90\%$  sperm acrosome integrity and  $\geq 80\%$  normal sperm morphology. It was suggested that twice the number of ram may be selected before actual semen production and preservation.

**Keywords:** Bangladeshi ram, semen evaluation, ram selection

## Introduction

A ram is "half the flock". One ejaculate from a ram can be used to inseminate several ewes. Selection of breeding rams would be the prerequisite for optimum herd productivity. The selected ram must be of high genetic potential, good libido and produce quality semen in routine collection (Poulton and Robinson, 1987). It is important to assess the potential fertility of ram before it is intended to use for semen production and artificial insemination (AI). This is usually performed by evaluating the semen quality. Evaluation of semen such as semen volume, sperm concentration, sperm motility and morphology, allows the detection and elimination of clear cut cases of male infertility or subfertility (Verstegen et al., 2002; Madhuri et al., 2012). Semen volume and color are the indicators of sperm concentrations. Color can be an evidence of injury or infections in the tract. Sperm motility, viability and plasma membrane integrity are the strong indicator of sperm function (Pena et al., 2005). It also predict the fertilizing capacity of frozen-thawed than fresh semen (Santiago-Moreno et al., 2009). To facilitate fertilization, spermatozoa with normal acrosomal integrity only ensure acrosome reaction, ability to penetrate the egg's zona pellucida and ability to fuse with the egg plasma membrane (Esteves et al., 2007). Cited information regarding selection of breeding rams evaluated by semen quality is absent in our country. Therefore, the aim of the present study was to select best Bangladeshi breeding rams by evaluating semen for future semen production and AI program.

## Materials and Methods

The study was conducted between September 2015 to March 2016 at the Department of Surgery and Obstetrics, Bangladesh Agricultural University (BAU), Bangladesh. The university is located at N 24.73 latitude and E 90.44 longitude, and 9 m above sea level. The area receives on average 174 mm of rainfall with the mean annual minimum and maximum temperatures ranges are 16.46 to 29.13°C, respectively.

### Ram and management

All animal procedures were approved by Animal Experimental Ethics Committee (AEEC) of BAU, Mymensingh, Bangladesh (Ref. no. AEEC/ DSO-BAU/ 02/ 2015). Apparently mature healthy Bangladeshi rams (n=16), aged 8-14 months, body weight 10.5-16 kg, scrotal circumference (SC) 16.4-21 cm, body condition score (BCS) 3.5-4.0 were selected. Rams were dewormed against internal (Endex®; Novartis, Bangladesh), external parasites (A-Mectin Plus Vet®; The ACME Laboratories Ltd., Bangladesh) and vaccinated against Tetanus (Tetanus Vaccine®, Dano Vaccine & Biologicals Pvt Ltd., India), Foot and Mouth disease (FMD; Raksha-Ovac Trivalent®, Indian Immunologicals Ltd., India) and *Peste des Petits Ruminants* (PPR-Vac®, Livestock Research Unit, Mohakhali, Dhaka, Bangladesh). The rams were managed under semi-intensive system. They were provided 150-200 gm hand formulated concentrates (50% wheat bran, 25% crushed maize, 20% soybean meal, 1% fish meal, 2% dicalcium phosphate (DCP® Plus, Oponin Pharma Ltd., Agrovvet Division, Barisal, Bangladesh) powder, 0.5% vitamin mineral premix (Megavit®, Oponin Pharma Ltd., Agrovvet Division, Barisal, Bangladesh) and 1.5% common salt with the provision of 8-10 hours natural grazing and free access to drinking water daily.

### **Semen collection**

After 2 months of acclimatization, rams were started training for semen collection twice a week for a period of 3 months. Semen was collected using an artificial vagina (AV; Minitube, Germany) following two successive false mount as described by Mishra et al. (2010). Briefly, Rams were allowed to mount homosexually for ejaculation into AV filled with warm water (50-52°C). Immediately after collection, semen containing tube was maintained at 35°C in water bath for evaluation.

### **Semen evaluation**

All chemicals used in this study were from Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

### **Semen volume, color and pH**

Semen volume was measured directly reading the graduated collection vial. The semen color was scored by visual appearance (1-4 grades; Jha et al., 2013). The semen pH was estimated with pH-indicator paper (phenolphthalein paper) by matching with color scale.

### **Sperm concentration**

Sperm concentration ( $\times 10^6$  sperm/ml) was calculated as described by Azizunnesa et al. (2014) using haemocytometer (Neubauer counting chamber). Semen sample was diluted with buffered formol saline at 1:200. Briefly, 10  $\mu$ l of formol saline fixed semen was counted onto Neubauer counting chamber under microscope (400x).

### **Sperm mass activity and motility**

Sperm mass activity was estimated according to Ferdinand et al. (2012). Briefly, 5  $\mu$ l of semen was

assessed for wave motion under microscope (40x) without using coverslip and scored into 1-5 grades. Sperm motility was estimated by placing a drop (5  $\mu$ l) of fresh semen and observed under microscope (100x) using cover slip.

### **Sperm viability**

The sperm viability was estimated by means of the nigrosine-eosin staining as described by Uysal and Buck (2007). A semen smear was prepared by mixing 2  $\mu$ l semen and 10  $\mu$ l eosin-nigrosin stain (10 gm of Nigrosin, 1.7 gm Eosin and 2.9 gm of sodium citrate in 100 ml of distilled water). Sperm cells counted as alive that exclude strict exclusion of the stain and dead that stain eosin against nigrosin background (400x).

### **Sperm plasma membrane integrity**

The sperm plasma membrane integrity was estimated by means of hypo-osmotic swelling (HOS) test as described by Emamverdi et al. (2013) with some modifications. Briefly, 10  $\mu$ l semen was diluted with 100  $\mu$ l hypo-osmotic solution (4.9 gm sodium citrate, 9 gm fructose in 1000 ml distilled water) in Eppendorf tube and incubated at 37°C for 30-45 minutes. After incubation, smear was prepared and counted under microscope (400x). Sperm with swollen and coiled tails were recorded as intact plasma membrane integrity.

### **Sperm tail and mid-piece morphology**

Sperm tail and mid-piece abnormalities were evaluated by buffered formol saline wet mount technique (Jha et al., 2013). Briefly, 10  $\mu$ l semen was diluted with 1000  $\mu$ l buffered formol saline (6.2 gm disodium hydrogen phosphate, 2.5 gm potassium dihydrogen phos-

phate, 5.4 gm sodium chloride and 175 ml concentrated formaldehyde in 1000 ml of distilled water). A drop (10  $\mu$ l) of diluted semen was examined under microscope (1000x).

### **Sperm head morphology**

Sperm head morphology was evaluated by Williams staining technique (Jha et al., 2013). The stain was prepared as: Stock solution-I by dissolving 10 gm of basic fuchsin in 100 ml of 95% alcohol. Stock solution-II was prepared by dissolving a saturated solution of bluish eosin in 95% alcohol. Stock solution-III was prepared by mixing 10 ml of stock solution-I with 170 ml of 5% phenol solution. The final working solution contained 25 ml of stock solution-II and 50 ml of stock solution-III. A thin semen smear was prepared, treated with absolute alcohol, chlormine and stained with carbol fuchsin for 8 minutes and examined under microscope (1000x).

### **Acrosome integrity**

Acrosomal integrity was evaluated according to Soderquist et al. (1997) with some modifications. A dry smear was prepared from diluted semen (10  $\mu$ l semen and 100  $\mu$ l buffered formol saline). The presence or absence of a normal apical ridge (NAR) of the sperm cells were examined (1000x).

At least 200 spermatozoa were examined from each smear. Photograph were captured by digital eyepiece camera (MEM1300 Digital Eyepiece, Future Optics Sci. & Tech. Co., Ltd, China) and Differential Interference Contrast (DIC) optics (Olympus®, Bx51 Olympus Optical Co. Ltd., Tokyo, Japan) equipped with the microscope.

### **Data analysis**

The data were analyzed using SPSS (20 Version) software package. One-way analysis of variance (ANOVA) followed by Duncan's new multiple range test (DMRT) was performed to find out the significant differences in semen parameters among the rams. Significance was assigned at  $p < 0.05$ .

## **Results**

Of the total rams ( $n=16$ ), 37.5% (6/16), 43.75% (7/16) and 87.5% (14/16) were trained for semen collection in 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> month of training period (Fig. 1). Whereas, 12.5% (2/16) were failed for semen collection. The mean frequency of semen collection was  $12.3 \pm 7.0$  and varied from 4 to 24 times (Fig. 2). The selected 62.5% (10/16) best ram had the semen parameters  $\geq 0.5$  ml volume,  $\geq 3$  color,  $\geq 4$  mass activity,  $\geq 80\%$  sperm motility,  $\geq 90\%$  sperm viability,  $\geq 2500 \times 10^6$  sperm/ml of concentration,  $\geq 85\%$  plasma membrane integrity,  $\geq 90\%$  acrosome integrity and  $\geq 80\%$  normal sperm morphology (Table 1).

### **Semen evaluation**

The semen volume, color, pH, sperm mass activity, sperm motility, sperm concentrations, sperm viability, sperm plasma membrane integrity, sperm acrosome integrity and normal morphology were studied for the evaluation of fresh ram semen. The Mean $\pm$ SD values of semen parameters and level of significance is shown in Table 1.

### **Semen volume, color, pH and sperm concentration**

The semen volume ranged from  $0.2 \pm 0.1$  to  $0.9 \pm 0.3$  ml. There were no significant difference ( $p > 0.05$ ) in semen volume among the rams except Ram #09,

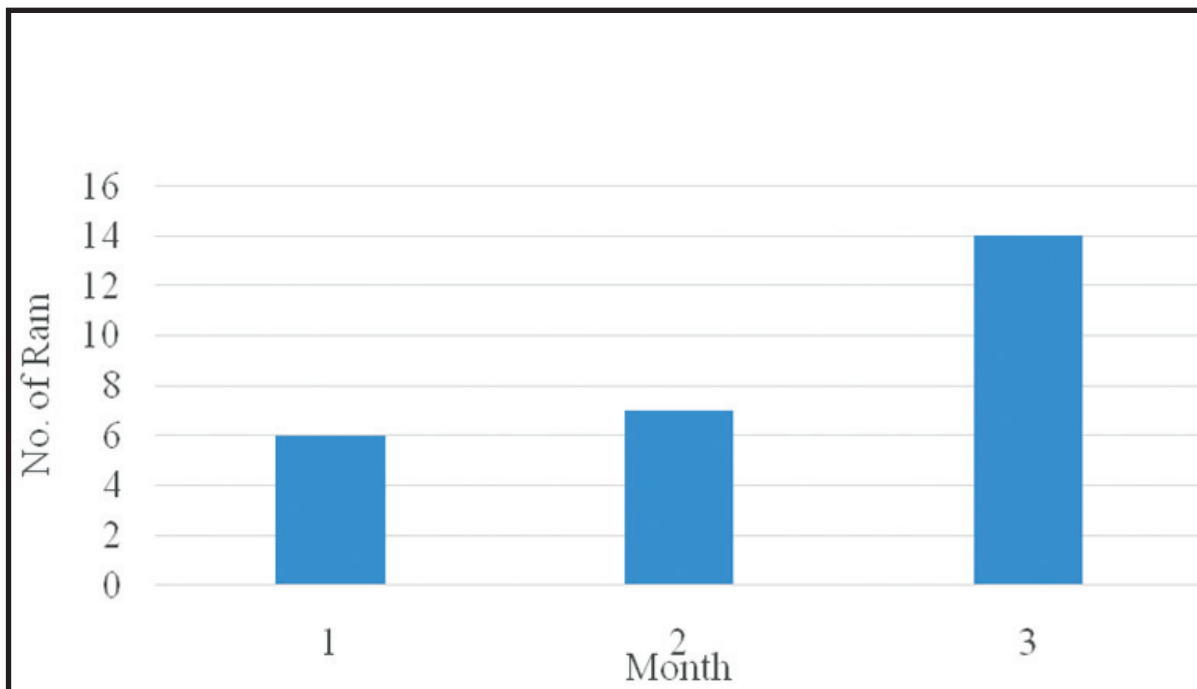


Figure 1 Number of ram trained for semen collection during 3 month period

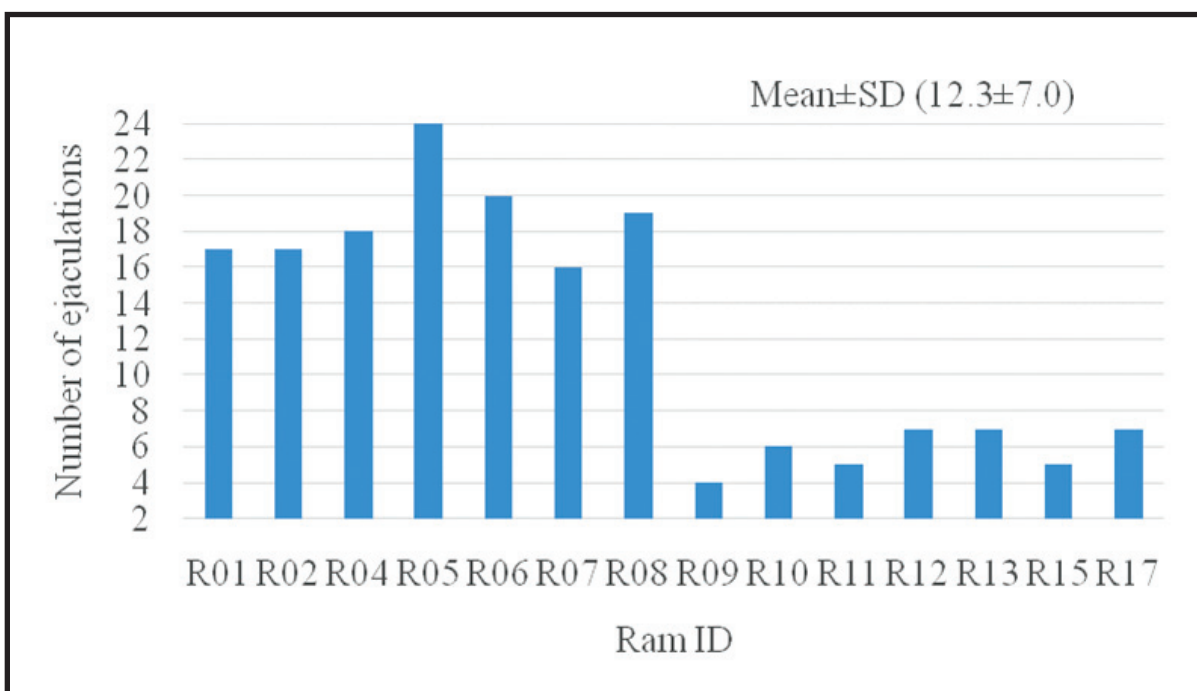


Figure 2 Frequency of semen collection during 3 month period (n=14)

**Table 1** Evaluation of fresh ram semen (Mean±SD)

Ram ID	Volume (ml)	Colour (1-5 grades)	pH	Mass activity (1-5 grades)	Motility (%)	Concentration (x10 <sup>6</sup> sperm/ml)	Viability (%)	HOST (%)	Acrosome integrity (%)	Normal sperm (%)
R01	0.7±0.2 <sup>a</sup>	3.8±0.5 <sup>a</sup>	7.2±0.3 <sup>a</sup>	4.4±0.5 <sup>ab</sup>	86.2±5.5 <sup>a</sup>	2827.2±798.0 <sup>abc</sup>	93.1±2.1 <sup>a</sup>	85.8±4.4 <sup>a</sup>	92.5±1.8 <sup>a</sup>	86.1±1.4 <sup>a</sup>
R02	0.8±0.3 <sup>a</sup>	3.8±0.7 <sup>a</sup>	7.1±0.2 <sup>a</sup>	4.5±0.5 <sup>ab</sup>	85.6±4.3 <sup>a</sup>	2741.9±907.4 <sup>abc</sup>	93.4±2.0 <sup>a</sup>	87.9±3.4 <sup>a</sup>	92.2±1.9 <sup>a</sup>	86.2±1.5 <sup>a</sup>
R04	0.7±0.3 <sup>a</sup>	3.5±0.8 <sup>a</sup>	7.1±0.2 <sup>a</sup>	4.2±0.5 <sup>b</sup>	85.3±7.4 <sup>a</sup>	3025.6±1035.6 <sup>abc</sup>	92.8±2.4 <sup>a</sup>	86.1±4.0 <sup>a</sup>	93.2±2.0 <sup>a</sup>	86.4±2.0 <sup>a</sup>
R05	0.8±0.3 <sup>a</sup>	3.7±0.5 <sup>a</sup>	7.1±0.4 <sup>a</sup>	4.4±0.5 <sup>ab</sup>	85.8±5.0 <sup>a</sup>	3492.8±800.8 <sup>abc</sup>	93.1±4.3 <sup>a</sup>	84.8±18.5 <sup>a</sup>	92.8±2.9 <sup>a</sup>	85.0±3.2 <sup>ab</sup>
R06	0.8±0.3 <sup>a</sup>	3.7±0.5 <sup>a</sup>	7.0±0.3 <sup>a</sup>	4.7±0.4 <sup>ab</sup>	86.3±4.6 <sup>a</sup>	3628.1±654.1 <sup>a</sup>	94.3±1.5 <sup>a</sup>	86.2±6.5 <sup>a</sup>	93.8±2.6 <sup>a</sup>	85.1±1.9 <sup>ab</sup>
R07	0.8±0.4 <sup>a</sup>	1.9±1.0 <sup>b</sup>	7.3±0.4 <sup>a</sup>	2.6±0.5 <sup>c</sup>	75.3±3.9 <sup>b</sup>	2644.4±555.9 <sup>c</sup>	83.3±3.8 <sup>b</sup>	71.2±4.4 <sup>b</sup>	88.3±4.3 <sup>b</sup>	77.3±2.9 <sup>c</sup>
R08	0.7±0.3 <sup>a</sup>	1.9±1.0 <sup>b</sup>	7.3±0.4 <sup>a</sup>	2.5±0.6 <sup>c</sup>	74.7±3.9 <sup>b</sup>	2712.4±531.3 <sup>c</sup>	83.2±3.0 <sup>b</sup>	73.4±3.6 <sup>b</sup>	88.6±3.2 <sup>b</sup>	76.7±3.7 <sup>c</sup>
R09	0.2±0.1 <sup>b</sup>	2.0±0.0 <sup>b</sup>	7.0±0.0 <sup>a</sup>	2.8±0.5 <sup>c</sup>	66.3±4.8 <sup>c</sup>	2647.9±748.5 <sup>c</sup>	80.0±2.8 <sup>c</sup>	74.3±4.1 <sup>b</sup>	85.3±3.1 <sup>c</sup>	72.8±3.9 <sup>d</sup>
R10	0.6±0.3 <sup>a</sup>	4.0±0.0 <sup>a</sup>	7.3±0.4 <sup>a</sup>	4.7±0.5 <sup>ab</sup>	83.3±4.1 <sup>a</sup>	3199.9±681.3 <sup>abc</sup>	93.7±2.6 <sup>a</sup>	88.5±5.6 <sup>a</sup>	92.0±3.0 <sup>a</sup>	83.0±2.4 <sup>b</sup>
R11	0.7±0.2 <sup>a</sup>	4.0±0.0 <sup>a</sup>	7.0±0.0 <sup>a</sup>	4.8±0.4 <sup>a</sup>	87.0±4.5 <sup>a</sup>	3561.7±596.8 <sup>ab</sup>	93.2±1.8 <sup>a</sup>	85.6±1.1 <sup>a</sup>	92.6±1.5 <sup>a</sup>	85.6±1.1 <sup>ab</sup>
R12	0.9±0.2 <sup>a</sup>	4.0±0.0 <sup>a</sup>	7.1±0.2 <sup>a</sup>	4.6±0.5 <sup>ab</sup>	86.4±3.8 <sup>a</sup>	3137.0±582.3 <sup>abc</sup>	94.3±2.7 <sup>a</sup>	86.3±1.9 <sup>a</sup>	92.3±2.0 <sup>a</sup>	85.1±1.6 <sup>ab</sup>
R13	0.9±0.3 <sup>a</sup>	4.0±0.0 <sup>a</sup>	7.0±0.0 <sup>a</sup>	4.6±0.5 <sup>ab</sup>	85.7±4.5 <sup>a</sup>	3081.0±768.9 <sup>abc</sup>	94.7±1.0 <sup>a</sup>	86.7±2.3 <sup>a</sup>	91.7±1.8 <sup>a</sup>	84.9±1.7 <sup>ab</sup>
R15	0.8±0.2 <sup>a</sup>	4.0±0.0 <sup>a</sup>	7.0±0.0 <sup>a</sup>	4.6±0.5 <sup>ab</sup>	87.0±2.7 <sup>a</sup>	3352.7±628.3 <sup>abc</sup>	93.8±1.5 <sup>a</sup>	85.8±2.2 <sup>a</sup>	91.2±1.5 <sup>a</sup>	85.4±2.4 <sup>ab</sup>
R17	0.6±0.2 <sup>a</sup>	0.0±0.0 <sup>c</sup>	7.1±0.7 <sup>a</sup>	2.4±0.5 <sup>c</sup>	60.0±9.6 <sup>d</sup>	2898.4±472.6 <sup>bc</sup>	79.7±3.6 <sup>c</sup>	72.1±5.4 <sup>b</sup>	84.7±3.0 <sup>c</sup>	73.4±3.3 <sup>d</sup>

<sup>abcd</sup>Different superscripts within columns are significantly differ (p < 0.05).

Color (1-4 grades): 1 = watery, 2 = yellowish white, 3 = milky white, 4 = creamy white; 0 indicates abnormal color (presence of blood)

Mass activity (1-5 grades): 1= no perceptible motion, 2 = weak motion without forming any waves, 3 = small, slow moving waves, 4 = vigorous movement with moderately rapid waves and eddies, 5 = dense, very rapidly moving waves and eddies.

which had significantly lower ( $p<0.05$ ) semen volume ( $0.2\pm 0.0$  ml). The semen color varied significantly ( $p<0.05$ ) among the rams with a range of  $1.9\pm 1.0$  to  $4.0\pm 0.0$  (Yellowish to creamy). Ram #01, #02, #04, #05, #06, #10, #11, #12, #13 and #15 showed significantly ( $p<0.05$ ) good quality semen color (milky to creamy white) except Ram #17, which showed significantly ( $p<0.05$ ) abnormal color (presence of blood). The pH of semen ranged from  $7.0\pm 0.0$  to  $7.3\pm 0.4$  and showed no significant difference ( $p<0.05$ ) among the rams. The sperm concentration ( $\times 10^6$  sperm/ml) varied significantly ( $p<0.05$ ) with a range of  $2644.4\pm 555.9$  to  $3628.1\pm 654.1$ . Ram #07, #08 and #09 had significantly lower ( $p<0.05$ ) sperm concentration, whereas Ram #06 had significantly higher ( $p<0.05$ ) sperm concentration.

#### **Sperm mass activity and motility**

The sperm mass activity varied significantly ( $p<0.05$ ) among the rams with a range of  $2.4\pm 0.5$  to  $4.8\pm 0.4$  (weak motion without forming any waves to dense, very rapidly moving waves and eddies formation). Ram #01, #02, #4, #05, #06, #10, #11, #12, #13 and #15 had significantly higher ( $p<0.05$ ) sperm mass activity (4-5; vigorous movement with moderately rapid waves and eddies to dense, very rapidly moving waves and eddies formation). Ram #11 was the best one and Ram #17 was the worst one. The sperm motility varied significantly ( $p<0.05$ ) among the rams with a range of  $60.0\pm 9.6\%$  to  $87.0\pm 2.7\%$ . Ram #01, #02, #04, #05, #06, #10, #11, #12, #13 and #15 had significantly higher ( $p<0.05$ ) sperm motility with a range of  $83.3\pm 4.1\%$  to  $87.0\pm 2.7\%$ , whereas Ram #07, #08, #09 and #017 had significantly lower ( $p<0.05$ ) sperm motility with a range of  $60.0\pm 9.6\%$  to  $75.3\pm 3.9\%$ . Ram #17 had significantly lower ( $p<0.05$ ) sperm motility of  $60.0\pm 9.6\%$ .

#### **Sperm viability and plasma membrane integrity**

The sperm viability among the rams varied significantly ( $p<0.05$ ) with a range of  $79.7\pm 3.6\%$  to  $94.7\pm 1.0\%$ . Ram #01, #02, #04, #05, #06, #10, #11, #12, #13 and #15 had significantly higher ( $p<0.05$ ) sperm viability with a range of  $92.8\pm 2.4\%$  to  $94.7\pm 1.0\%$ , whereas Ram #09 and #17 had significantly lower ( $p<0.05$ ) viability  $80.0\pm 2.8\%$  and  $79.7\pm 3.6\%$ . The sperm plasma membrane integrity varied significantly with a range of  $71.2\pm 4.4\%$  to  $88.5\pm 5.6\%$ . Ram #01, #02, #04, #05, #06, #10, #11, #12, #13 and #15 had significantly higher ( $p<0.05$ ) sperm plasma membrane integrity with a range of  $84.8\pm 18.5\%$  to  $88.5\pm 5.6\%$ , whereas Ram #9 and #17 had significantly lower ( $p<0.05$ ) sperm plasma membrane integrity  $74.3\pm 4.1\%$  and  $72.1\pm 5.4\%$ .

#### **Sperm morphology and acrosomal integrity**

The normal sperm morphology varied significantly ( $p<0.05$ ) with a range of  $72.8\pm 3.9\%$  to  $86.4\pm 2.0\%$ . Ram #01, #02 and #04 had significantly higher ( $p<0.05$ ) normal sperm morphology with a range of  $86.1\pm 1.4\%$  to  $86.4\pm 2.0\%$ , whereas Ram #7, #8, #9 and #17 showed significantly lower ( $p<0.05$ ) normal sperm morphology with a range of  $72.8\pm 3.9\%$  to  $77.3\pm 2.9\%$ . The acrosome integrity varied significantly ( $p<0.05$ ) with a range of  $84.7\pm 3.0\%$  to  $93.8\pm 2.6\%$ . Ram #01, #02, #04, #05, #06, #10, #11, #12, #13 and #15 had significantly higher acrosome integrity with a range of  $91.2\pm 1.5\%$  to  $93.8\pm 2.6\%$ , whereas Ram #09 and #17 showed significantly lower ( $p<0.05$ ) acrosome integrity  $85.3\pm 3.1\%$  and  $84.7\pm 3.0\%$ .



## Discussion

Subjective assessment of semen quality have been employed to ensure the selection of superior breeding rams for optimum herd productivity (Verstegen et al., 2002; Madhuri et al., 2012). In the present study, selected best Bangladeshi rams met the standard range of seminal parameters that could be used for semen production, preservation and AI program (Alvarez et al., 2012; Roostaei-Ali Mehr et al., 2013).

### Semen volume, color, pH and sperm concentration

Semen volume is one of the important factors in semen evaluation and reproductive performance in males (Ax et al., 2000). The semen volume in our study was in agreement with Malama et al. (2013) who reported 0.59 to 0.99 ml. In contrast, Azizunnesa et al. (2013) reported higher semen volume 1.05 to 1.6 ml in Bangladeshi ram aged  $\geq 2$  years. Some breeds like Suffolk, Walachian and Sumava sheep produce  $1.8 \pm 0.5$ ,  $1.6 \pm 0.6$  and  $1.9 \pm 0.7$  ml semen, respectively (Hernandez et al., 2012). The standard age of ram is 1.5 years or older to produce normal ejaculatory volume 0.8 to 1.2 ml with a mean of 1 ml (Foote, 1974). The ejaculatory volume is also affected by methods of semen collection. Semen collection by electro ejaculation results in larger ejaculate volume than artificial vagina. This might be due to electrical stimulation on accessory glands that provokes addition secretion of seminal plasma (Evans and Maxwell, 1987). The semen volume decreased (by 25-53%) with frequency and interval of collections (Thwaites, 1995). The semen color in our study (milky to creamy white) was consistent with Azizunnesa et al. (2013). The normal color of semen is milky-white or pale cream (Evans and Maxwell, 1987). Some rams can produce yellowish semen color, which is normal and due to

presence of riboflavin pigment in the ejaculate. Color may also an indicator of injury or infection in reproductive tract. The presence of blood or pus flakes may indicate affection in reproductive tract (Nabil et al., 2006). The pH of semen in our study was within the range 6.9-7.2 reported by Al-Samarrae (2009). Good quality of semen is always slightly acidic (Madhuri et al., 2012). The sperm concentration in our study was comparable with Foote (1978) and Khalifa et al. (2013) who reported 2000-3500  $\times 10^6$  sperm/ml. However, our results disagree with Marti et al. (2011) and Azizunnesa et al. (2014) who reported  $4.8 \pm 1.8$  to  $5.4 \pm 1.9 \times 10^9$  sperm/ml. Normal concentration of ram spermatozoa per ml varying from  $1.6 \times 10^9$  to  $6.0 \times 10^9$  sperm/ml with an average of  $3.6 \times 10^9$  sperm/ml (Moss et al., 1988) which suggested our findings were within the normal range. The sperm concentration increased with increasing age (Alexopoulos et al., 1991) and decreased with successive frequent ejaculations by 19-55% (Thwaites, 1995; Kaya et al., 2002). Higher the number of sperm/ml allow to produce higher number of insemination doses ultimately create opportunity to inseminate larger number of females to inseminate (Robinson et al., 2006). The quality of semen is influenced by age, body weight and size of the testicles. The size of the testicles increases with advancement of age (Toe et al., 2000). The body weight is more important than age in determining testicular growth and development, its influence on semen quality (Notter et al., 1985).

### Mass activity and motility

The mass activity and motility of spermatozoa provides strong evidence for sperm maturation. Sperm motility is a fairly reliable indication of the sperm viability (Grahman et al., 1980). The sperm mass activity in our study was comparable with Azizunnesa et al.



(2013) and Khalifa et al. (2013) who reported between 3 to 5 in 1-5 scale. However, some studies have reported comparatively higher sperm mass activity (FAO, 1991; Cunha et al., 2012). Similarly, sperm motility in our study was within the range to that of Khalifa et al. (2013) and Azizunnesa et al. (2014) who reported 60-85%. The minimal value of sperm motility for the ram is 60% (Garner and Hafez, 1982). The sperm mass activity and motility is affected by frequency of semen collection and nature of diet. The sperm motility decreases by 19-36% with successive and frequent ejaculation (Thwaites, 1995; Kaya et al., 2002). It is indicated that there is decrease in sperm individual motility in feed restricted rams which is due to low seminal plasma fructose concentration and depressed activity of the pituitary gland (Chandrasekhar et al., 1986).

#### **Sperm viability and plasma membrane integrity**

The sperm viability in our study was within the range to that of Fernandez et al. (2004) and Malama et al. (2013). The sperm plasma membrane integrity in our study was comparable with Akourki et al. (2004) and Juyena (2011) but disagree with Marti et al. (2012) who reported 63.33 to 73.33% in different season. There is increase chances of sperm membrane alterations and increase percentage of dead and morphologically abnormal spermatozoa (Alexopoulos et al., 1991) in poor fed ram. The HOS test is used to evaluate the functional integrity of the sperm membrane. Live spermatozoa with normal membranes show swelling of the tail due to water influx when exposed to hypo-osmotic conditions (Liu and Baker, 1992). An intact sperm cell membrane reflects semen fertility more closely than sperm motility (Perez et al., 1998).

#### **Sperm morphology and acrosomal integrity**

The normal sperm morphology in our study was comparable with Malama et al. (2013) who found 78.48% normal spermatozoa. In contrast, Azizunnesa et al. (2014) reported 86-98% which is higher than our study. Breeding ram should have more than 70% morphologically normal spermatozoa (Kasimanickam et al., 2007). The acrosome integrity in our result was higher than Malama et al. (2013) but lower than Akourki et al. (2004). Percentage of morphologically normal spermatozoa was affected by diet. Prolonged feeding of poor quality diets hinder the function of the epididymis that results subnormal levels of testosterone production and increase proportion of cell abnormalities (David et al., 2007; Tufarelli et al., 2011). Semen from most males contains some abnormal spermatozoa. Sperm quality improves with age in the adult ram (Wiemer and Ruttle, 1987). It has been indicated that ejaculates from younger rams contains a greater number of abnormal cells, which indicate incomplete spermatogenic activity and incomplete epididymal maturation (Colas, 1983). It also has been demonstrated that specific ingredients in diet, such as Vitamin E, may have a positive effect in increasing semen quality and quantity (Yue, et al., 2010).

#### **Conclusion**

It was suggested that double the number of ram may be initially introduced for semen evaluation to select the best rams for semen production and breeding program.

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## References

- Akourki A, Gil L, Echegaray A, Espinosa E, Josa A, de Blas I, et al. Effect of the extender supplement Equex-STM on cryopreserved semen in the Assaf sheep. *Cryo Letters*. 2004; 25(2): 147-54.
- Alexopoulos K, Karagiannidis A, Tsakalof P. Development of macroscopic and microscopic characteristics of ejaculates from Chios, Serres and Karaguniki breed lambs. *Theriogenology*. 1991; 36(4): 667-80.
- Al-Samarrae SH. Semen quality of arrabi and karradi iraqi rams. *Diyala Agric. Sci. J.* 2009; 1(2): 30-6.
- Alvarez M, Tamayo-Canul J, Anel E, Boixo JC, Mata-Campuzano M, Martinez-Pastor F, et al. Sperm concentration at freezing affects post-thaw quality and fertility of ram semen. *Theriogenology*. 2012; 77(6): 1111-8.
- Ax RL, Dally MR, Didon BA, Lenz RW, Love CC, Varner DD, et al. Artificial Insemination. In: Hafez B, Hafez ESE. *Reproduction in farm animals*. 7<sup>th</sup> ed. Philadelphia: Lea and Febinger. 2000. p. 376-89.
- Azizunnesa, Zohara BF, Bari FY, Alam MGS. Baseline study of reproductive performances of indigenous rams in Bangladesh. *IOSR J. Agric. Vet. Sci.* 2014; 7(6): 83-9.
- Azizunnesa, Zohara BF, Bari FY, Alam MGS. Effects of concentrate supplementation on reproductive performances and semen quality of indigenous rams in Bangladesh. *J. Embryo Transf.* 2013; 28(4): 325-35.
- Chandrasekhar YD, DiOocchio MJ, Setchell BP. Reproductive hormone secretion and spermatogenesis function in thyroidectomized rams receiving graded doses of exogenous thyroxine. *J. Endocrinol.* 1986; 111(2): 245-53.
- Colas G. Factors affecting the quality of ram semen. In: Haresign, W. (Ed.), *Sheep Production*. Butterworths, London. 1983. p. 453-65.
- Cunha MGG, Gonzalez CIM, Carvalho FFR, Soares AT. Effect of diets containing whole cottonseed on the quality of sheep semen. *Acta. Sci. Anim. Sci.* 2012; 34(3): 305-11.
- David I, Druart X, Lagriffoul G, Manfredi E, Robert-Granié C, Bodin L. Genetic and environmental effects on semen traits in Lacaune and Manech tête rousse AI rams. *Genet. Sel. Evol.* 2007; 39(4): 405-19.
- Emamverdi M, Zhandi M, Zare Shahneh A, Sharafi M, Akbari-Sharif A. Optimization of ram semen cryopreservation using a chemically defined soybean lecithin-based extender. *Reprod. Domest. Anim. Zuchthygiene*. 2013; 48(6): 899-904.
- Esteves SC, Sharma RK, Thomas AJ, Agarwal A. Evaluation of acrosomal status and sperm viability in fresh and cryopreserved specimens by the use of fluorescent peanut agglutinin lectin in conjunction with hypo-osmotic swelling test. *Int. Braz. J. Urol.* 2007; 33(3): 364-74.
- Evans G, Maxwell WMC. Collection of semen; Handling and examination of semen; Dilution of semen; Frozen storage of semen; Insemination. In: *Salmon's artificial insemination of sheep and goats*. Butterworths, Sydney. 1987. p. 85-166.
- FAO (Food and Agriculture Organization of the United Nations). *Asian Livestock. Monthly Technical Magazine of the FAO. Animal Production and Health Commission for Asia and the Pacific (APHCA)*. 1991; 8: 85-7.
- Ferdinand N, Thomas TT, Augustave K, Henry DF, Fernand T, Etienne PT. Effects of buck age, storage duration, storage temperature and diluent on fresh west african dwarf buck semen. *J. Reprod. Infertil.* 2012; 3(3): 58-66.

- Fernández M, Giráldez FJ, Frutos P, Lavín P, Mantecón AR. Effect of undegradable protein supply on testicular size, spermogram parameters and sexual behavior of mature Assaf rams. *Theriogenology*. 2004; 62(1-2): 299-310.
- Foote RH. Artificial insemination. In: *Reproduction in Farm animals*. Hafez E.S. E. (ed.). Lea and Febiger, Philadelphia. 1974. p. 409-31.
- Foote RH. Factors influencing the quantity and quality of semen harvested from bulls, rams, boars and stallions. *J. Anim. Sci.* 1978; 47(II): 1-11.
- Garner DL, Hafez ESE. Spermatozoa and seminal plasma. In: *Reproduction in farm animals*. 7<sup>th</sup> ed. Philadelphia. 1982. p. 12-7.
- Graham EF, Schmehl MKL, Nelson DS. Problem with laboratory assays. *Proc. 8<sup>th</sup> Tech. Con. Arti. Ins. Reprod. NAAB*, 1980. p. 1-8.
- Hernández PJE, Fernández RF, Rodríguez SJL, Juárez RE, Soto MYG, García RAD. Effect of cryopreservation of sheep semen related to its viability and acrosomal status. *Rev. Salud. Anim.* 2012; 34(2): 78-83.
- Jha PK, Paul AK, Rahman MB, Tanjim M, Bari FY, Alam MGS. Improvement of preservation quality of chilled bull semen using  $\alpha$ -tocopherol as an antioxidant. *J. Embryo Transf.* 2013; 28(1): 31-9.
- Juyena NS. Protein profiles and biochemical characteristics of semen: Influence on frozen-thawed spermatozoa quality in rams (*Ovis Aries*) and Alpacas (*VicugnaAacos*). Ph.D. Thesis. University of Padova, Italy. 2011. p. 123-73.
- Kasimanickam R, Kasimanickam V, Pelzer KD, Dascanio JJ. Effect of breed and sperm concentration on the changes in structural, functional and motility parameters of ram-lamb spermatozoa during storage at 4 degrees C. *Anim. Reprod. Sci.* 2007; 101(1-2): 60-73.
- Kaya A, Aksoy M, Tekeli T. Influence of ejaculation frequency on sperm characteristics, ionic composition and enzymatic activity of seminal plasma in rams. *Small Rumin. Res.* 2002; 44(2): 153-8.
- Khalifa T, Lymberopoulos A, Theodosiadou E. Association of soybean-based extenders with field fertility of stored ram (*Ovis aries*) semen: a randomized double-blind parallel group design. *Theriogenology*. 2013; 79(3): 517-27.
- Liu DY, Baker HWG. Evaluation and assessment of semen for IVF/ICSI. *Asian J. Androl.* 2002; 4: 281-5.
- Madhuri D, Gupta V, Nema S, Patidar A, Shivhare M, Singh N, et al. Modern semen evaluation techniques in domestic animals: A review. *DHR Int. J. Biomed. Life Sci.* 2012; 3(1): 62-83.
- Malama E, Bollwein H, Taitzoglou IA, Theodosiou T, Boscós CM, Kiossis E. Chromatin integrity of ram spermatozoa. Relationships to annual fluctuations of scrotal surface temperature and temperature-humidity index. *Theriogenology*. 2013; 80(5): 533-41.
- Martí JI, Aparicio IM, Leal CLV, García-Herreros M. Seasonal dynamics of sperm morphometric subpopulations and its association with sperm quality parameters in ram ejaculates. *Theriogenology*. 2012; 78(3): 528-41.
- Martí JI, Aparicio IM, García-Herreros M. Head morphometric changes in cryopreserved ram spermatozoa are related to sexual maturity. *Theriogenology*. 2011; 75(3): 473-81.
- Mishra B, Alam MGS, Khandokar M, Mazumder S, Munsu M. Qualities of goat semen in Tris-Citrate-Glucose extender containing glutathione. *Bangladesh Vet.* 2010; 27(2): 46-55.

- Moss JA, Melrose DR, Reed HCB, Vendeplassche M. Spermatozoa semen and artificial insemination. In: Laing JA, Brinley Morgan WJ. Fertility and infertility in domestic animals. 4<sup>th</sup> ed. Balliere Tindall, London. 1988. p. 132-54.
- Nabil AH, Sayed TI, Marzook M A. Artificial Insemination. In: Artificial Insemination and Embryo Transfer in Farm Animals. 1st ed. King Faisal University: Publisher King Fahd National Library, 2006. p. 55-113.
- Notter, DR, Lucas JR, McLaugherty FS, Copenhaver JS. Breed group differences in testicular growth patterns in spring-born lambs. *J. Anim. Sci.* 1985; 60(3): 622-31.
- Peña FJ, Saravia F, Johannisson A, Walgren M, Rodríguez-Martínez H. A new and simple method to evaluate early membrane changes in frozen-thawed boar spermatozoa. *Int. J. Androl.* 2005; 28(2): 107-14.
- Perez-Claring R, Bermudez J, Anderssen H, Burguena J. Influence of nutrition on testicular growth in corriedale rams during spring. *Reprod. Nutr. Dev.* 1998; 38: 529-38.
- Poulton AL, Robinson TJ. The response of rams and ewes of three breeds to artificial photoperiod. *J. Reprod. Fertil.* 1987; 79: 609-26.
- Robinson JJ, Ashworth CJ, Rooke JA, Mitchell LM, Mc Evoy TG. Nutrition and fertility in ruminant livestock. *Anim. F. Sci. Tech.* 2006; 126(3-4): 259-76.
- Roostaei-Ali Mehr M, Chambary B, Ghavi Hossein-Zadeh N. Effect of different diluents and storage time on field fertility of cooled ram semen after vaginal insemination. *Small Rumin. Res.* 2013; 115(1-3): 82-5.
- Santiago-Moreno J, Castaño C, Coloma MA, Gómez-Brunet A, Toledano-Díaz A, López-Sebastián A, et al. Use of the hypo-osmotic swelling test and aniline blue staining to improve the evaluation of seasonal sperm variation in native Spanish free-range poultry. *Poult. Sci.* 2009; 88(12): 2661-9.
- Söderquist L, Madrid-Bury N, Rodríguez-Martínez H. Assessment of ram sperm membrane integrity following different thawing procedures. *Theriogenology.* 1997; 48(7): 1115-25.
- Thwaites, CJ. The comparative effects of under nutrition, exercise and frequency of ejaculation on the size and tone of testes and on semen quality in the ram. *Anim. Reprod. Sci.* 1995; 37(3-4): 299 -309.
- Toe F, Rege JEO, Mukasa-Mugerwa E, Tembely S, Anindo D, Baker RL, et al. Reproductive characteristics of Ethiopian highland sheep I: genetic parameters of testicular measurements in ram lambs and relationship with age at puberty in ewe lambs. *Small Rumin. Res.* 2000; 36(3): 227-240.
- Tufarelli V, Lacalandra GM, Aiudi G, Binetti F, Laudadio V. Influence of feeding level on live body weight and semen characteristics of Sardinian rams reared under intensive conditions. *Trop. Anim. Health. Prod.* 2011; 43(2): 339-45.
- Uysal O, Bucak MN. Effects of oxidized glutathione, bovine serum albumin, cysteine and lycopene on the quality of frozen-thawed ram semen. *Acta Vet. Brno.* 2007; 76: 383-90.
- Verstegen J, Iguer-Ouada M, Onclin K. Computer assisted semen analyzers in andrology research and veterinary practice. *Theriogenology.* 2002; 57(1): 149-79.
- Wiemer KE, Ruttle JL. Semen characteristics, scrotal circumference and bacterial isolates of fine wool range rams. *Theriogenology.* 1987; 28(5): 625-37.
- Yue D, Yan L, Luo H, Xu X, Jin X. Effect of Vitamin E supplementation on semen quality and the testicular cell membranal and mitochondrial antioxidant abilities in Aohan fine-wool sheep. *Anim. Reprod. Sci.* 2010; 118(2-4): 217-22.