

Baseline Study of Reproductive Performances of Indigenous Rams in Bangladesh

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Abstract: Selection of breeding rams would be the prerequisite for getting better fertility can be accomplished through selecting some important reproductive traits. Cited information regarding reproductive performances of ram necessary for breeding programme is absent in our country (if available). The objective of the present study was to measure the reproductive performances of indigenous rams. Ram lambs were selected on the basis of indigenous characteristics. They were maintained on natural grazing with lump some amount of concentrate. Body weight and growth rate, scrotal circumference and its growth rate, BCS and libido index were measured weekly. Age, body weight, BCS and scrotal circumference at puberty were recorded. Semen was collected once a week using artificial vagina and subjected for evaluation of volume, color, density, mass motility, concentration, motility, functional integrity and normal morphology of spermatozoa. The body weight, BCS, scrotal circumference, growth rate of body weight and scrotal circumference were 20.8±0.6(kg), 3.6±0.1, 22.8±0.2(cm), 17.0±0.6(g/d), 1.6±0.2(mm/15d), respectively. Age, body weight, BCS and scrotal circumferences at puberty were 6.8±0.1(months), 10.0±0.4(kg), 3.2±0.1, 18.6±0.7(cm), respectively. The volume, density, mass motility, concentration were 1.2±0.0ml, 2.9±0.0, 4.3±0.0 and 4.1±0.7x10⁹, respectively. The rate of motility: 89.0±0.2, 72.4±0.2, 62.0±0.6, viability: 91.8±0.1, 75.6±0.2, 64.8±0.6, functional integrity: 87.3±0.2, 69.1±0.2, 50.2±0.5 and normal spermatozoa were 94.0±0.1, 77.3±0.1 and 75.0±0.2 in fresh, chilled and frozen semen, respectively.

Keywords: Indigenous ram, reproductive characters, semen quality

I. Introduction

Sheep may contribute a significant amount in the protein content and income generation to the people of Bangladesh since they are easy to rear and more resistance to infectious diseases compared with goat. There are 3.12 million sheep in Bangladesh [1]. The small non-descript indigenous sheep probably originated from south-eastern sub-tropical region's. In our country sheep is generally managed under rural condition. They are sparsely distributed throughout some specific areas within the country [2]. Recently the farmers are aware of their benefits and have great interest to increase the sheep production through farming. However, there is scarcity of breeding rams and absence of cited information regarding reproductive performances required for breeding purpose. The genetic potential and productivity of goats and sheep are deteriorating due to indiscriminate type of breeding. This may be minimized by providing sound breeding knowledge to the breeder [3]. The reproductive performances are influenced by several factors [4]. Selection of young rams for fertility can be accomplished through selecting some important reproductive traits such as age at puberty, body condition score, body growth rate, scrotal circumference, scrotal growth rate and semen quality [5]. There was a positive relationship between body weight, scrotal circumference and testicular weight [6] and in general increased testicular weight lead to produce good quality semen [7].

As mentioned above there is paucity of information regarding reproductive performances, quality of semen for breeding and AI. Hence, the sheep is producing without any improvement in any economic trait resulting in significant economic loss of the country. Limited studies were undertaken to observe the productive and reproductive performances of ewes in Bangladesh [8]. However, the same attention was not offered to indigenous ram. When the ewes are in estrus the farmers desperately looks for the ram for service and become disappointed both by scarcity and high quality ram. It is urgent to select sound rams for breeding programme to speed up the production of economic traits with high genetic merit. Therefore, a comprehensive study was designed to observe the baseline study of reproductive performances of indigenous ram.

II. Materials and Methods

2.1 Study area and period

The study was conducted at the Department of surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh-2202 during the period from May 2011 to April 2013. The research units are located on N 24.73 and E 90.44 latitude and longitude, respectively and elevated 9 m above sea level. The area receives on average 174 mm of rainfall. Mean annual minimum and maximum temperatures experienced at the site are 16.46 and 29.13°C, respectively.

2.2. Experimental animals and management

Fourteen ram lambs 4-5 months old, selected and purchased from the local market on the basis of indigenous or local characters and kept under semi-intensive condition at the Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh. After acclimatization, rams were dewormed and vaccinated against rabies and tetanus. Rams were maintained on natural grazing. Lump some amount of concentrates consisted of wheat bran (50%), crushed maize (25%), soy bean meal (20%), fish meal (1%), dicalcium phosphate (DCP) powder (2%), vitamin mineral premix (0.5%), and salt (1.5%) was supplied to the rams along with grazing. Rams were supplied *ad libitum* drinking water.

2.3. Experimental design

Body weight (kg), scrotal circumference (cm) and their growth rate were measured weekly. Body condition score (BCS) was measured by palpating lumber region as like other small ruminants and recorded in a score (1-5 with 0.5 increment) [9]. Scrotal circumference (cm) was measured by passing a flexible tape around the scrotum (both testes at the same level) at the point of maximum circumference. Age (months), BCS, body weight (kg) and scrotal circumference (cm) at puberty were calculated. Puberty was determined as the age when the ejaculate contained $>50 \times 10^6$ spermatozoa, sufficient to accomplish fertilization [10]. Libido index was graded from 0 to 3; 0 no desire to move towards a teaser ewe. 1 very reluctant to reach the teaser. 2 willingly moved towards the teaser. 3 moved towards the ewe in an uncontrolled manner. Other sexual behaviours were recorded during the whole experimental period.

2.4 Semen Collection, preservation and evaluation

Semen was collected by artificial vagina once a week. All glassware for collection and handling were cleaned and sterilized using high-pressure steam, dried and warmed at 35°C. After ejaculation, the tube was immediately placed in a bath at 37°C. The volume of semen was measured directly from the graduated collecting tube. Colour was estimated by visual inspection and density was scored by making the tubes slant with score range 0-5. Microscopic examination was performed under phase contrast microscope (Gallenham, No. 82TT8, Cat No. M/6-200-H HZ 60, England). Mass motility was estimated by assessment of wave motion of fresh undiluted semen under microscope 10x with 0-5 score. The concentration of spermatozoa was counted by placing a drop of diluted (1:400) semen on haemocytometer. To evaluate sperm motility, 5 μ l of diluted semen was placed on a warmed (37°C) slide, covered by a cover slip and examined (400x). Semen was diluted (1:10) with tris based diluents and was stored at chilling (5-8°C) for up to 48h and freezing method accordingly.

2.5. Morphological evaluation

Eosin-nigrosin stain was used to determine the viability of spermatozoa. Small drop of semen and one drop of eosin-nigrosin stain were placed on a clean slide and mixed with a clean stick, a thin smear was made, dried in air and examined under microscope (400x). At least 200 spermatozoa were examined from each smear to calculate the percentages of live spermatozoa. Hypo-osmotic swelling (HOST) test was used to measure the proportion of spermatozoa that swelled, giving an estimate of the proportion with functional integrated spermatozoa. Normal (acrosome, midpiece and tail) rate of spermatozoa were evaluated using Spermac stain® (Minitube, Box 152, Wellington, 7654, South Africa) (400-1000x). Before evaluation, semen was taken to warm up and thawed at 39 to 40 for 14 second for chilled and frozen, respectively.

2.6. Statistical analysis

Data generated and statistical analyses were carried out for mean \pm SEM in each particular parameter. Data were analyzed using SPSS 17.0 computer program package (SPSS, USA).

III. Results

3.1. Reproductive characters

The body weight, scrotal circumference and body condition score of selected ram lambs were 8.4 ± 0.4 (kg), 14.8 ± 0.8 (cm) and 3.1 ± 0.0 . The same parameters for mature rams were 20.8 ± 0.6 (kg), 22.8 ± 0.2 (cm) and 3.6 ± 0.1 , respectively. The growth rate of body weight and scrotal circumference was 17.0 ± 0.6 (g/d) and

1.6±0.2(mm/15d), respectively. Age, BCS, body weight and scrotal circumference at puberty were 6.8±0.1(months), 3.2±0.3, 10.0±0.4(kg), and 18.6±0.7(cm), respectively. The mean libido index was 2.6±0.1 (Table 1).

Table 1. Reproductive parameters of indigenous rams

Parameters	Mean ±SEM	Range
Selected ram lambs body weight (kg)	8.4±0.4	6.5-11.5
Mature body weight (kg)	20.8±0.6	17.5-25
Body growth rate (g/d)	17.0±0.6	15.1-21.9
Selected ram lambs body condition score (1-5)	3.1±0.0	3-3.5
Mature body condition score (1-5)	3.6±0.1	3-4
Selected ram lambs scrotal circumference (cm)	14.8± 0.8	10.5-18.5
Mature scrotal circumference (cm)	22.8±0.2	21-24
Scrotal growth rate (mm/15d)	1.6±0.2	0.8-2.5
Age at puberty (months)	6.8±0.1	6-7.5
Body condition score (1-5) at puberty	3.2±0.3	3-3.5
Body weight at puberty (kg)	10.0±0.4	8-12.7
Scrotal circumference (cm) at puberty	18.6±0.7	13-21.7
Libido index (0-3)	2.6±0.1	2-3

Values are presented as mean±SEM

The investigating others sexual behaviours in rams are presented in Table 2

Table 2. Others sexual behaviours

Parameters	Characters
Libido characteristics	Neck outstretched and head held horizontally (searching female), flehmen, sniffing and licking of ewe's vulva, nudging ewe, repeated dorsal elevation of scrotum, penile protrusion with no dribbling of seminal fluid.
Duration of copulation	Very short period.
Ejaculatory behavior	Before ejaculation several times of mount have been observed however sometimes ejaculate take place by 1 st mount, homosexual behaviours was observed.
Mating characters	Serious fighting with other male to mate female, dominance effects and gargling, rams normally display agonistic behavior when introduced into the ewe flock.

3.2 Indigenous ram semen characters

The colour of fresh semen was creamy white immediately after collection. The volume, density, mass motility and concentration of semen were 1.2±0.0ml, 2.9±0.0, 4.3±0.0 and 4.5±0.1x10⁹/ml, respectively. The rate of motility, viability, functional integrity and normal morphology was 89.0±0.2, 91.8±0.1, 87.3±0.2 and 94.0±0.1 in fresh semen while those of chilled semen were 72.4±0.2, 75.6±0.2, 69.1±0.1 and 77.3±0.1, respectively. However, the rate of motility, viability, functional integrity and normal morphology of post thawed semen were 62.0±0.6, 64.8±0.6, 50.2±0.5 and 75.0±0.2, respectively (Table 3).

Table 2. Quality of indigenous ram semen

Parameters	Mean ±SEM	Range
Colour	Creamy white	-
Volume (ml)	1.2 ±0.0	0.2-1.8
Density (0-5)	2.9±0.0	2-4
Concentration (x10 ⁹)	4.5±0.1	2-8.9
Mass motility (0-5)	4.1±0.0	2.5-5
Motility	Fresh	89.0±0.2
	Chilled after 48h	72.4±0.2
	Post thawed	62.0±0.6
Viability	Fresh	91.8±0.1
	Chilled after 48h	75.6±0.2
	Post thawed	64.8±0.6
HOST test	Fresh	87.3±0.2
	Chilled after 48h	69.1±0.2
	Post thawed	50.2±0.5
Normal sperm	Fresh	94.0±0.1
	Chilled after 48h	87.2±0.1
	Post thawed	75.0±0.2

values are presented as mean±SEM

IV. Discussion

Reproductive characters

Body weight, scrotal circumference, growth rate, BCS

The information is available regarding reproductive performances of rams [11]. The present study observed the concerning reproductive performance of native rams in Bangladesh. The mean body weight and scrotal circumference of the present study were 20.8 ± 0.6 kg and 22.8 ± 0.2 cm, respectively which was lower than other breeds in the cited references [12]. They reported the body weight and scrotal circumference of adult Awassi rams were about 79.1 ± 1.0 kg and 33.5 ± 0.8 cm, respectively. It was also showed that the body weight of other exotic sheep breeds like Dorper sheep have live weight of 40 kg. [13]. Live weight of a mature Border Leicester ram would be in the range of 140–175 kg. However, the live weight of sheep in subcontinent countries comparatively lower than others. There was a report on adult ewes body weight was 25.6 ± 2.9 - 47.1 ± 4.4 kg in Pakistan [14]. The overall mean scrotal circumference was recorded 32.0 ± 1.2 cm [3]. The mean growth rate of live weight and scrotal circumference of our indigenous rams were also lower than reported by other [15]. This discrimination of body weight [11] and scrotal circumference may be differed depending on different breeds [16], Feeding practice affect the scrotal circumference and scrotal growth rate in ram lambs ($p < 0.001$) [17]. Our experimental rams showed the mean BCS 3.6 ± 0.1 which is similar to the findings of Tabbaa et al [12] in adult rams. In most cases, healthy sheep and goats should have a BCS of 2.0 to 3.5 and breeding ram should have BCS 3-4 [18].

Age, weight, BCS and scrotal circumference at puberty

For maintenance of better reproductive management it is need to be known the information about the onset of puberty and sexual maturity [19]. The study revealed that the age at onset of puberty in our indigenous rams is similar to other studied [20]. The mean age and body weight at puberty is comparatively lower but BCS is higher than the findings of Mukasa-Mugerwa and Ezaz [15]. However, the similar condition score (3.3 ± 0.4) was observed [14] for yearling rams. Although the satisfactory scrotal circumference was 30-36 cm during the period of 8-14 months age [21], however, our indigenous ram showed 18.6 ± 0.7 cm scrotal circumference at pubertal age which was also lower than reported (29.6 ± 1.3 cm) in yearling rams [12].

Libido Index

Our present results on the libido score (2.6 ± 0.1) of indigenous rams showed to be normal. Snowden et al. [22] reported that the overall mean libido score was 3.5 ± 0.02 when the score ranged from 1-6. Rams may exhibit a wide range of libido levels [23]. Day length has an effect on reproduction in male and female. The highest libido, fertility, and semen volume with good quality were observed in late summer and autumn, when the females will allow breeding [24]. Even though the present study was not related to feeding practice however, it was reported that diet has effect on libido. Carbohydrate or protein deficiency may impair libido in males [25]. It was also reported that the supplemented diet had tendency to increased sex drive compared to control though the difference was insignificant ($p > 0.05$) [26].

Other sexual behaviours

The recorded some other sexual characters in indigenous rams during the experimental period were similar to the findings of Perkins and Roselli [27]. Mounting behavior in rams is accompanied by a series of shallow pelvic thrusts. The rams in this study mounted several times before ejaculation however, it was not exceptional that the rams exhibited ejaculation by 1st mount within very short period: 1-2 seconds. This type of sexual behavior also showed by Bermant et al. [28] in rams. Rams failed to jump estrous ewes and ejaculates those were transferred from their mother and sisters by 90 days of age and raised in a group with all male. However they were homosexual. A similar result was described by Perkins and Roselli [27]. They noted that 20 to 35 percent of sexually inexperienced yearling rams reared in all male groups were sexually inactive when they were first exposed to estrous females. Fighting was a serious problem among rams. Mature and larger rams were almost always dominated over smaller rams. Sometimes smaller rams were more aggressive and bred more ewes.

Semen characters of indigenous rams

Volume, density, mass motility and concentration

Semen volume is one of the important factors in semen evaluation and reproductive performance in males [29]. The present result regarding the volume of semen in our rams is similar to the finding of others [3] but dissimilar result was also observed [30]. They reported the overall mean volume of semen from Pirlak rams was 1.5 ± 0.1 ml. The quality of semen in native rams was not studied. The typical ram ejaculate was 1 to 2 ml and contains 1 to 5 billion sperm cells [21]. The volume of semen could be varying with the breed, age, and the collection interval. The Suffolk, Walachian and Sumava sheep produce 1.8 ± 0.5 , 1.6 ± 0.6 and 1.9 ± 0.7 ml semen,

respectively [31]. The mean density was 2.9 ± 0.0 and mass motility of semen from this study was 4.3 ± 0.0 which was comparatively higher than that reported by others [3, 32]. The finding of concentration of spermatozoa ($4.1 \pm 0.7 \times 10^9$) per ml was found to be similar with the other result [30]. He recorded that the concentration of spermatozoa was 4.2×10^9 /ml of Pirlak rams. On the other hand, Ibrahim [33] showed the higher sperm concentration ($4.9.7 \times 10^9$ /ml) in rams. Similar observation was also observed [3]. Normal concentration of ram spermatozoa per ml varying from 1.6×10^9 to 6.0×10^9 with an average of 3.6×10^9 [34]. Our result was within the normal range of sperm concentration. 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 [35]. Sperm production, as well as total number of spermatozoa per ml, can be affected by breed [36] and nature of diet [37].

Motility, viability, functional integrity and normal morphology of spermatozoa

The proportion of motile spermatozoa for fresh, chilled and post thawed frozen semen was within a standard range as the minimal value of sperm motility for the ram is 60% [38]. The motile spermatozoa provide strong evidence for sperm maturation. Sperm motility is a fairly reliable indication of the viability of semen [39]. After chilling or freezing the percentages of sperm motility was reduced. The results confirmed the other finding [30] for chilled and [40] for frozen semen.

Determination of % viable sperm is important in assessing semen for AI. The percent viable spermatozoa in our result comparatively higher than other [30]. He reported the mean proportion of live spermatozoa in Pirlak rams was 92.9% at 0h of observation. Although the preservation decreased the rate of sperm viability from 91.8 ± 0.1 to 75.6 ± 0.2 when chilled and 64.8 ± 0.6 when frozen, however, the rate of viability was within the standard ranged accordingly [41]. The time of preservation damaged the sperm membrane and ultimately cell death [42].

In fresh semen the values for HOST positive spermatozoa in this study is higher than the finding of Juyena [43] reported 76% HOST positive sperm cell in Padovana rams. In chilled semen, the present result for hypo-osmotic swelling test value is higher than in Pirlak rams semen after chilling. The similar observation was observed in preservation time decrease in the hypo osmotic resistance of spermatozoa [30]. From above discussion it was observed that the rate of functional integrity of spermatozoa varies depending breed and species and preservation methods.

Before preservation the morphologically normal spermatozoa is similar to Danaa et al. [44]. They recorded 95% normal spermatozoa in rams before preservation. After chilling and freezing the rate of normal morphology of spermatozoa was decreased. Generally semen from most males contains some abnormally formed spermatozoa [45]. Beside this, breeding potential of a ram should have more than 70% morphologically normal sperm. In the present study the proportion of abnormal spermatozoa did not exceed 30% both for fresh and after chilling. The morphology of post thawed ram spermatozoa was also within the standard value [46]. Different factors may affect the morphology of spermatozoa e.g methods of preservation [47], age of the animal [48]. They recorded good quality sperm was found in 2-3 years rams after that decline gradually.

V. Conclusions

It would be concluded that the productive and reproductive performances of indigenous rams are satisfactory even though the body weight and scrotal circumference comparatively smaller than other breeds in the world. They attained to become puberty early with good libido scoring. The quality regarding volume, density and mass motility, concentration, motility, viability, functional integrity and normal morphology of semen were within the standard ranged published by others. The quality of semen from indigenous ram was also satisfactory after chilling up to 48h and freezing. The others sexual behaviours were normal. Therefore, emphasis should be given to introduce AI in ewes using semen from indigenous rams to observe the success of fresh as well as preserved semen and increase sheep population with high genetic merit.

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