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Review article

The current and advanced situation of ram semen quality in Bangladesh

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Abstract

This study aimed to survey the literature on Bangladeshi rams' semen characteristics, quality evaluation, and production required for sheep breeding and production strategy programs. The ram potentiality must be assessed to optimize production performance and maximize stakeholders' use of high genetic values. This is usually accomplished through an andrological examination of the male, which evaluates the characteristics and quality of the sperm produced. Microscopic semen evaluations, such as sperm motility, viability, normal morphology, plasma membrane integrity, and acrosome integrity, and macroscopic semen evaluations, such as semen volume, color, and pH, enable the identification and removal of clear-cut cases of male infertility or subfertility. Therefore, the current paper reviewed, discussed, summarized, and compared all the research performed on Bangladeshi rams' semen characteristics, quality evaluation, and production.

Keywords: Advance, Current, Quality, Ram, Semen, Situation

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INTRODUCTION

The updated report provides a status of a total of 35.37 million sheep in Bangladesh, and sheep are the third after the cattle and goat population (DLS, 2019). They seem to be primarily native animals sparsely dispersed across the country, with a more significant proportion in several agro-ecological regions, including the coastal region, barind tract, north-eastern wetlands, Sundarbans-delta regions, and Jamuna River basin areas (Pervage et al., 2009). Farmers in Bangladesh's western regions, such as Meherpur, Choadanga, and Chapainawabganj, produce the Muzaffarnagari cross, an Indian exotic faster-growing with medium to large sheep (Sardar, 2016). The Bangladeshi subtropical climate is favorable for sheep breeding and production. Since sheep are resistant to parasitic and infectious diseases compared with goats and cost-effective rearing on natural grass with less care (Ahmed et al., 2018), sheep can influence livestock production and income generation.

Low fertility, inbreeding, and poor genetic qualities of the breeding herd of sheep have impacted livestock productivity in Bangladesh (Rahman, 2005). For the socio-economical context and lack of knowledge of the superior breeding ram, most sheep owners castrate or sell their male lambs early (Rahman et al., 2015). Moreover, village farmers are reluctant to exchange their breeding males if they have any. Consequently, improving meat quality progeny is essential for developing successful sheep farming while also contributing to our country's Gross Domestic Product.

The researchers postulated that a ram is "half the flock" (Jha et al., 2018) and that reproduction is crucial in the livestock economy. A selective breeding program could exploit the potentiality of a higher-performance male. They applied assisted reproductive technologies in an increased and intensive production system. In the field, mating capacity, testicular traits, physical soundness, and semen quality assessment can all be used to estimate the potential fertility of breeding males. Semen analysis has been employed to predict ram fertility, particularly in artificial insemination operations. Considering the increased sheep productivity, many researchers afforded Bangladeshi ram's fertility test, semen characteristics, quality test, and frozen production and qualities. Any compilation report is yet not available. Therefore, an updated database on Bangladeshi rams' semen quality, evaluation, and production is required in sheep breeding and production strategy programs.

SHEEP BREEDS OF BANGLADESH

Bangladeshi sheep are most often indigenous. This indigenous sheep *(Ovis aries)* is descended from Asia's wild Urial sheep *(Ovis Orientalis vignei)*. Individuals have a pretty mixed coat color (45% white, 26% brown, 24% white-brown, and 3% black-brown) (Hassan and Talukder, 2011) with an average mature live weight, scrotal length, and circumference of a ram ranging from 16.19 to 19.19 kg, 10.50 to 11.35 cm, and 15.43 to 16.77 cm, respectively (Islam et al., 2018). Apart, an exotic crossbreed sheep, the Muzaffarnagari, is very popularly adopted in the country's western parts. This crossbreed sheep originated from the Indian districts of Muzaffarnagar, Bulandshahar, Meerut, and Bijnor. Pure-breed rams typically have a lengthy body size and polled heads while crossbreed rams may have curving horns. The ram has an average mature body weight, scrotal length, and circumference ranging from 41.89 to 66.83 kg, 13 to 21 cm, and 23 to 31cm, respectively (Asaduzzaman et al., 2020). (Figure 1).

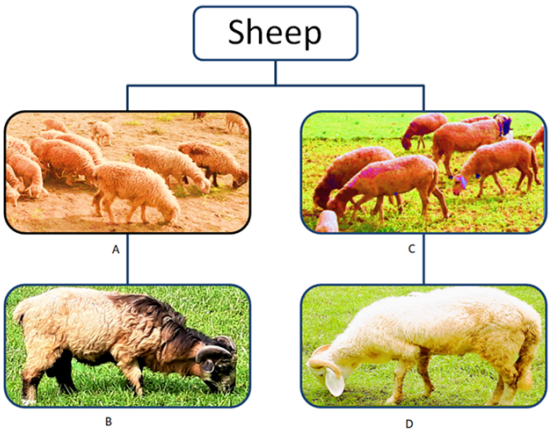

Figure 1 Bangladeshi sheep: A. Native/ Indigenous sheep, B. Native/ Indigenous ram, C. Exotic cross Muzaffarnagar sheep, D. Muzaffarnagar ram

SEMEN AND SPERMATOZOA IN RAM

Semen is the liquid of the reproductive tract, constituted of sperm cells, and fluid with seminal plasma (Hafez and Hafez, 2000). Sperm cells are produced through the spermatogenesis process in the seminiferous tubules of the testis. They are shifted via rete testis and vasa efferentia to the epididymis until the final maturation stage, making capable sperms fertilize the ovum (Hafez and Hafez, 2000). Seminal plasma is a thick, yellowish-white, viscous complex fluid released by the rete testis, epididymis, and accessory glands (seminal vesicle, prostate, and bulbourethral glands) to enhance the chemical changes of the semen (Mann and Lutwak-Mann, 1981; Juyena and Stelletta, 2012). Semen is a transparent or opaque fluid in rams that is usually isotonic, neutral, and, has a pH of around 7.0 (Evans and Maxwell, 1987). Sperm cells (spermatozoa) have three parts: a head, a midpiece, and a tail (Söderquist et al., 1991). The head contains genetic materials (haploid chromosomes) with an acrosome covering that contains an enzyme to facilitate oocyte penetration by lysing the oocyte membrane during the fertilization process. The acrosome is a cap-like structure that covers the first two-thirds of the sperm head (Taloni et al., 2017). Mitochondria are found in the mid-piece and serve as an energy source for the tail's movement (Trout, 2013). Seminal plasma lipids, proteins, enzymes, sugars, and electrolytes are linked to sperm metabolism, motility, and surface characteristics (Fernández-Juan et al., 2006). The shape, metabolism, capacitation, and gamete fertilization of sperm cells is influenced by seminal plasma lipids, particularly phospholipids, and cholesterol (Hafez, 2000; Juyena and Stelletta, 2012). The seminal plasma protein binds to the sperm surface (Dominguez, et al., 2008).

SEMEN QUALITY EVALUATION

At an earlier time, semen was evaluated to know about sperm/ spermatozoa fertility and the potentiality of the male individual (Juyena, 2011). Spermatozoa's freeze-ability is currently examined during cryopreservation. There is a link between the properties of sperm and cryopreservation (Patel and Siddiquee, 2013). Nonetheless, the quality of a valued sire's sperm is linked to his fertility and litter size (Amann and Hammerstedt, 1993). As a result, animal production's primary concern is semen quality and its relationship to fertility.

The semen quality is evaluated grossly with necked eyes (macroscopic evaluation) and with a microscope (microscopic evaluation) (Boshoff, 2014). To get

the best estimate of male fertility, several factors are considered, including sperm motility, concentration, and morphology (Correa and Zavos, 1994). Besides, the sperm functional tests- including the hypo-osmotic swelling test (HOST test) and permeability test are also done to evaluate the semen quality (Moussa, 1999). Furthermore, before using the above tests, the vitality of sperm and the percentage of motile sperm cells must be assessed (Rowe et al., 1993). Within an hour of collection, the semen sample is either moved to the lab next door or brought there in a thermos flask at 37 °C. Semen evaluations, both macroscopic and microscopic, are performed in the lab (Matshaba, 2010).

Macroscopic semen quality

Semen color

The first sight of semen color predicts semen quality before all other macroscopic and microscopic evaluations. The collected fresh semen appears milky-white or pale cream (Evans and Maxwell, 1987). Colors range from milky white to pale creaminess in ram semen (Bag et al., 2002). Sometimes, rams may have a very yellowish creamy color ejaculation indicating the presence of high levels of harmless riboflavin pigments in the semen (Mansour et al., 2006). Contaminated ejaculates negatively influence male fertility; therefore, they should be discarded. The presence of blood or pus flakes in semen indicates an infection in the male genitalia. Other aberrant colors, odors, or foreign elements like hair, feces, bedding, and dirt can indicate a lack of hygienic quality (Mansour et al., 2006). Semen color may also change while contaminated with urine during collection using the electro-ejaculator method (Watson, 1990). Semen color may vary with physical factors such as breed type, age, and season. A matured ram may produce thick, creamy consistent semen samples during the breeding season and cloudy water or thin milk-type semen by a sub-normal ram (Elsharif, 2010). According to Hafez and Hafez (Hafez and Hafez, 2000), the color of the semen ejaculate correlates with sperm concentration. The literature on the semen color of Bangladeshi rams is presented in Table 1.

Ejaculate volume

The volume of semen obtained by an artificial vagina or electro-ejaculator is measured using 15 mL graded Falcon tubes. Then the semen sample is labeled and placed in a warm water thermos flask at 37 °C immediately after shifting or transporting to the laboratory (Matshaba, 2010). The volume of ejaculate in mature rams is between 0.5 and 2 ml, whereas in young rams it is between 0.5 and 0.7 ml (Mafolo, 2018). Factors including age, environment, health status, semen collection, process, technician skill, time of year, frequency of the collection, breed, and individual animal traits might be contributing factors to these disparities (Hafez and Hafez, 2000; Hafez and Hafez, 2013). When semen is collected three times or more a day or a long time, the volume of ejaculation is decreased (Gil et al., 2003). The ejaculate volumes of Bangladeshi ram, as stated in the literature are presented in Table 1.

Mass motility

The most influential parameter in semen analysis is sperm mass motility, representing the collective movement that produces a wave of spermatozoa in ejaculated semen (Dhurvey et al., 2012). The mass activity is scored from 0 to 5 using the following criteria: 1 = no detectable motion, 2 = without forming any waves, 3 = small slow-moving waves, 4 = various movements with relatively fast waves and eddies, and 5 = thick very rapidly moving waves and eddies (Rahman et al., 2015; Benia et al., 2018). This score also indicates the percentage of living and motile spermatozoa; for example, a mass activity level of four suggests 70 to 80 percent of live sperm (Baril et al., 1993) which is indicative of good quality semen, often rendering further motility evaluation usually unnecessary. According to Benia et al., (2018), the mass activity is not affected by seasonal variation but

shows a significant difference between age groups: 4.42 ± 0.14 for adults and 3.80 ± 0.21 for young. Feed supplementation affects mass activity (Panin and Mahabile, 1997). The mass motility of Bangladeshi ram, as presented in the literature, is shown in Table 1.

Table 1 Macroscopic qualities of Bangladeshi ram semen

Ram breed type	Semen characteristics and qualities				References
	Ejaculate volume (ml)	Color/grade (1-4)	Mass activity (0-5 grade)	Sperm concentration	
Native	0.864 ± 0.23	--	--	3.52 ± 0.90 x10^9/ml	Pervage et al., 2009
Indigenous	1.2 ± 0.0	Creamy white	4.1 ± 0.0	4.5 ± 0.1 x10^9/ml	Zohara et al., 2014
Indigenous	0.8 ± 0.2 (0.6 ± 1.0-0.8 ± 0.2)	--	2.75 ± 0.27 - 4.25 ± 0.61	3.9 ± 0.7 x10^9/ml (3.1 ± 0.4 - 4.2 ± 0.7)	Mahmuda et al., 2015
Native	1.53 ± 0.08 - 1.78 ± 0.07	--	4.0±0.1 (3.8 ± 0.5 - 4.1 ± 0.6)	--	Rahman et al., 2015
Indigenous	1.4 ± 0.1	--	4.1 ± 0.1	5.1 ± 0.1 x 10^9/ml	Chaki et al., 2015
Native	1.0 ± 0.1 (0.9 ± 0.2-1.2 ± 0.2)	Milky and creamy white	4.0±0.1 (3.8 ± 0.5 - 4.1 ± 0.6)	4.1 ± 0.2 x10^9/ml (3.9 ± 0.3 - 4.3 ± 0.4)	Hossain et al., 2016
Indigenous	0.42 (0.35-0.50)	--	3.27 (3.00-3.70)	1.60 (1.40-1.80) ×10^9/ml	Rahman et al., 2018
Indigenous	0.2 ± 0.1 - 0.9 ± 0.3	--	2.4 ± 0.5 - 4.8 ± 0.4	(2644.4 ± 555.9 -3628.1 ± 654.1) ×10^6/ml	Jha et al., 2018
Indigenous	0.81	--	--	4.09 x10^9/ml	Khan et al., 2019
Indigenous	0.77 ± 0.04	3.73 ± 0.1	3.6 ± 0.11	364.47 ± 7.363×10^6/ml	Asaduzzaman et al., 2021
Muzaffarnagari cross	0.7 ± 0.04	3.87 ± 0.10	3.73 ± 0.12	392.60 ± 5.707×10^6/ml	Asaduzzaman et al., 2021
Indigenous	0.4 ± 0.0 - 1.2 ± 0.3	--	2.8 ± 0.4 - 4.3 ± 0.3	1614.1 ± 230.6 - 4686.7 ± 139.7 x 10^6/ml	Sharmin et al., 2021
Muzaffarnagar cross	0.79 ± 0.02	3.6±0.09	3.78 ± 0.06	3308 ± 54.80 x10^6/ml	Saha et al., 2021

Sperm concentration

The sperm concentration is defined as the number of spermatozoa per volume unit of ejaculated semen (Benia et al., 2018). This term denotes the sperm cells per milliliter of sperm (Hafez, 1993). It is done primarily to determine the amount of dilution during semen processing to ensure that each insemination dose has a sufficient number of sperm cells. When determining male fertility for breeding purposes, the concentration of sperm is among the most crucial semen parameters to evaluate (Graffer et al., 1988). The concentration can be measured with the aid of a hemocytometer, spectrophotometer, trophotometer, and colorimeter (Evans and Maxwell, 1987). Although the hemocytometer method is a little more tedious to the technician, this is the economical, simplest, and fundamental approach to estimating sperm concentration. The semen of a high-quality ram typically contains 3.5 to 6.0 billion (×10^9) spermatozoa per milliliter (Evans and Maxwell, 1987). According to Mitchell et al., (2004), the average ram ejaculate contains between 1-3 billion (×10^9) sperm per milliliter while Cameron et al. (1987) found 140-1050×10^6/ml sperm in an individual ram's ejaculate. The concentration of ejaculates can be affected by various circumstances, including the regularity with which they are collected, their diet and health, breed diversity (Mann, 1964; Verma et al., 1999), and the breeding season (Dabas et al., 1997). The sperm concentration in the semen of Bangladeshi rams, as represented in the literature, is shown in Table 1.

Microscopic semen quality

Total sperm motility and progressive motility

Sperm motility evaluation yields information about the current condition of semen, the percentage of living, motile spermatozoa in the ejaculated semen, a criterion for choosing the ejaculate for processing and insemination, and information related to animal breeding (Baril et al., 1993). Semen should be evaluated for successful fertilization by looking for progressively motile sperm, or sperm that travel in a straight line (Perumal et al., 2014). Sperm motility, the simplest

trait of semen fertility and quality, is an important property of spermatozoa linked to the capacity of the semen to fertilize as noted by Verstegen et al. (2002). According to Tsakmakidis (2016), spermatozoa must pass through the female reproductive tract's cervical mucus to reach the oviduct and fertilize the oocytes. Generally, sperm motility is measured on a scale of 0 to 5 (0 = no motility, 1 = 20%, 2 = 40%, 3 = 60%, 4 = 80%, and 5 = 100% motility) (Karatzas et al., 1997). Evaluators normally prefer to express sperm motility in percentage only. The ram's sperm motility must be at least 60% to be considered viable. Sperm motility and fertility of ram are negatively affected by heat, cold, pH, extender types, and urine (O'Hara et al., 2010). Mandiki et al. (1990) reported that sperm motility increases and the abnormal sperm decreases with age. While the age of the ram increases, the percentages of sperm motility and viability increase (Hassan et al., 2009). A phase-contrast microscope's assessment of sperm motility and its outcomes rely on the technicians' steadiness (Rodriguez-Martinez, 2003; Kumar et al., 2016). In light microscopy evaluation, the motility can range from 30 to 60% depending on the evaluator's expertise (Amann, 1989). Reference to Hafez and Hafez (2000), highlights that sperm motility assessment involves determining the viability of sperm cells and the quality of sperm motility. The sperm motility in the semen of Bangladeshi rams as revealed in the literature is shown in Table 2.

Sperm viability

Sperm viability is an indicator of how many living spermatozoa are present in a sample of semen. It is an essential part of modern fundamental semen analysis. Determining semen quality is necessary for distinguishing between immotile dead sperm and immotile living sperm, a key factor for successful reproduction. Nilani et al. (2012) reported that having >70% viable spermatozoa is required for a successful conception rate. Eosin-nigrosin staining is utilized to discriminate between viable and non-viable spermatozoa (Hafez and Hafez, 2013). This technique has been used as a conventional staining approach to test sperm viability (WHO, 1999; Rodriguez-Martinez, 2003). Rodriguez- Martínez (2003) and Brito et al. (2003) stated that eosin-nigrosin (Eo-Nig) staining is the most common method for determining sperm viability in field-level assessment. This technique offers several advantages, such as its rapid and straightforward application to stain spermatozoa clearly and facilitate easy counting. Eosin-nigrosin stain is highly reliable in contrast to other methods of motility study. Normal and pathologic cells can also be distinguished. Moreover, this is the sole method by which a permanent record of motile sperm can be secured; however, Hancock (1956) reported that the use of eosin-nigrosin stain sometimes results in partial staining of spermatozoa.

The underlying principle behind this staining is that eosin penetrates non-viable cells, turning them red. At the same time, nigrosin forms a dark background that makes it easier to detect viable, unstained cells. Felipe-Pérez et al. (2008) found that live sperm appeared colorless or transparent. The viable sperm cells with intact cell membranes are not stained, while dead sperm with disintegrating cell membranes absorb the stain and expose pinkish (Jha et al., 2013). The sperm viability of Bangladeshi ram's semen as in the studied literature is presented in Table 2.

Table 2 Microscopic qualities of Bangladeshi ram semen

Ram breed	Stages of semen quality			References
	Fresh semen	Pre-freeze semen	Freeze /Frozen semen	
		Sperm motility (%)		
Native	78.45 ± 3.26	--	--	Pervage et al., 2009
Indigenous	83.3 ± 4.3 (79.8 ± 3.4 - 84.9 ± 4.0)	74.7 ± 2.3 (71.3 ± 2.1-76.1 ± 3.4)	40.1 ± 3.1 (35.5 ± 0.7-42.3 ± 1.0)	Mahmuda et al., 2015
Indigenous	89.2 ± 4.3	--	35.3±7.8(5% glycerol) 15.6±3.9 (7% glycerol)	Chaki et al., 2015
Native	82. 50 ± 2.74 - 89.00 ± 3.22	--	--	Rahman et al., 2015
Native	83.4 ± 3.3 - 86.3 ± 3.5	--	--	Hossain et al., 2016
Indigenous	78.46 (75-82)	--	20.00 ± 2.1 (skim milk) 14.83 ± 1.01 (tris)	Rahman et al., 2018
Indigenous	60.0 ± 9.6 - 87.0 ± 4.5	--	--	Jha et al., 2018
Indigenous	--	80.6±1.5(7% glycerol) 80.0±1.3(5% glycerol)	41.7±2.6 (7% glycerol) 52.1±2.6 (5% glycerol)	Jha et al., 2019
Indigenous	80 ± 1.09	74.67 ± 1.333	--	Asaduzzaman et al., 2021
Muzaffarnagari cross	81.67 ± 0.93	77.33 ± 0.826	44 ± 2.45	Asaduzzaman et al., 2021
Indigenous	75.5 ± 2.5 - 92.75 ± 1.0 %	--	--	Sharmin et al., 2021
Muzaffarnagari cross	80.07 ± 0.77	--	--	Saha et al., 2021
		Sperm viability (%)		
Native	93.05 ± 3.16	--	--	Pervage et al., 2009
Indigenous	91.8 ± 0.1 (80-95)		64.8 ± 0.6 (57-75)	Zohara et al., 2014
Indigenous	88.2 ± 4.4 (84.5 ± 3.7 - 90.0 ± 4.0)	78.8 ± 4.9 (74.9 ±2.6-81.0 ± 4.1)	44.6 ± 2.8 (40.5±0.6 - 46.4 ± 0.5)	Mahmuda et al., 2015
Native	87.9 ± 2.5 - 90.01 ± 3.2	--	--	Hossain et al., 2016
Indigenous	79.7 ± 3.6 - 93.8 ± 1.5	--	--	Jha et al., 2018
Indigenous	82.15 (78-86)	--	23.00 ± 1.9(skim milk) 18.2 ± 1.1 (tris)	Rahman et al., 2018
Indigenous	--	88.9±0.5(7% glycerol) 88.4±0.4(5% glycerol)	53.5±3.3(7% glycerol) 67.6±2.7(5% glycerol)	Jha et al., 2019
Indigenous	78.5	--	--	Khan et al., 2019
Indigenous	90.93 ± 0.74	80.73 ± 1.22	--	Asaduzzaman et al., 2021
Muzaffarnagari cross	91.27 ± 0.57	73.67 ± 0.86	48.1±0.76	Asaduzzaman et al., 2021
Indigenous	91.0 ± 3.6	--	--	Chaki et al., 2015
Muzaffarnagari cross	87.31 ± 0.39	--	--	Saha et al., 2021
		Sperm normal morphology (%)		
Native	95.17 ± 2.33	--	--	Pervage et al., 2009
Indigenous	84.2 ± 3.5 (80.7 ± 2.2 - 86.3 ± 3.7)	79.2 ± 2.9 (76.9 ± 2.3 - 81.0 ± 2.5)	70.9 ± 1.1 (68.5 ± 0.1-72.4 ± 0.6)	Mahmuda et al., 2015
Indigenous	84.2 ± 3.3	--	73.0±4.7(5% glycerol) 73.0±4.7(7% glycerol)	Chaki et al., 2015
Native	85.5 ± 0.7	--	--	Hossain et al., 2016
Indigenous	72.8 ± 3.9 - 86.4 ± .0	--	--	Jha et al., 2018
Indigenous	85.5	--	--	Khan et al., 2019
Indigenous	85.33 ± 0.60	82.66 ± 0.52	--	Asaduzzaman et al., 2021
Muzaffarnagari cross	85.27 ± 0.64	83.13 ± 0.74	80.2 ± 1.28	Asaduzzaman et al., 2021
Indigenous	77.5 ± 2.5 - 90.21 ± 2.2	--	--	Sharmin et al., 2021
Muzaffarnagari cross	88.00 ± 1.09	--	--	Saha et al., 2021
		Sperm membrane integrity (%)		
Indigenous	70-93	--	-- 42-57	Zohara et al., 2014
Native	84.7 ± 0.5	--	--	Hossain et al., 2016
Indigenous	87.9 ± 3.4 - 93.8 ±1.5	--	--	Jha et al., 2018
Indigenous	80 (76-83)	--	17.8 ± 2.1(skim milk) 12.3 ± 0.9(tris)	Rahman et al., 2018
Indigenous	--	84.3±0.8(7% glycerol) 84.1±0.8(5% glycerol)	47.3±3.2 (7% glycerol) 59.8±3.0 (5% glycerol)	Jha et al., 2019
Indigenous	81.93 ± 0.77	75.07 ± 0.87	--	Asaduzzaman et al., 2021
Muzaffarnagari cross cross	82.4 ± 0.77	69.73 ± .92	56.47 ± 1.15	Asaduzzaman et al., 2021
		Sperm acrosome integrity (%)		
Indigenous	84.7 ± 3.0 - 93.2 ± 2.0	--	--	Jha et al., 2018
Indigenous	--	87.8±0.7(7% glycerol) 87.6±0.7(5% glycerol)	57.5±3.2(7% glycerol) 69.5±2.0 (5% glycerol)	Jha et al., 2019
Indigenous	94.73 ± 0.75	92.6 ± 0.53	--	Asaduzzaman et al., 2021
Muzaffarnagari cross	95.8 ± 0.39	91.27 ± 0.37	77.4 ± 0.81	Asaduzzaman et al., 2021

Sperm morphology

The sperm morphology reveals the structural information of spermatozoa studied under light and electron microscopy techniques. These are highly potential cells as they carry improved genetic materials from male genitalia to female oocytes. Not less than 85% of spermatozoa should be considered for normal fertilization, according to Saeid et al. (2006). Hence, it is essential to recognize the proportion of normal and abnormal spermatozoa by evaluating sperm morphology.

Each component of sperm has morphologic properties, including a head, a mid-piece, and a tail.

The acrosome should make up between 40 and 70 % of the typical sperm head, with an oval shape and a consistent outline. A mitochondrial sheath and often extracytoplasmic material from the developing spermatid can be found in the mid-piece. An axial core comprises two central singlet microtubules surrounded by nine pairs of doublet microtubules, an outside ring made up of nine dense fibers encircled by the fibrous sheath, and a fibrous sheath makes up the tail. Ax et al. (2000) categorized sperm abnormalities into three categories: primary abnormalities (those affecting the sperm head and acrosome), secondary abnormalities (those involving the mid-piece cytoplasmic droplet), and tertiary abnormalities (those affecting the mid-piece cytoplasmic droplet) (tail damage). According to Evans and Maxwell (1987), head damage occurs during testis creation, mid-piece abnormalities occur during their maturation in the epididymis, and tertiary abnormalities arise due to improper semen management. Sperm morphological abnormality assessment is considered a prerequisite standard in liquid and frozen semen production for bulls in advanced countries such as the United States, Sweden, and the Netherlands (Arifiantini et al., 2006).

When 20% or more cells in a ram's sperm sample are aberrant, the ram's fertility is frequently questioned. Gil et al. (2003) judged sperm with less than 10% abnormalities typical for sheep. The number of aberrant sperm cells rises in the spring and then falls as the natural breeding season develops. Even when spermatozoa are kept at a constant temperature, Pickett and Berndtson (1978) found that morphological changes can occur as the spermatozoa age. Semen having more than 70% viable sperm is appreciated for a successful conception rate (Nilani et al., 2012). According to Fernandez-Abella et al. (2003) and Malama et al. (2013), a ram with less than 15% abnormal sperm is recommended for breeding purposes. The presence of immature sperm forms in a ram's sperm could suggest that it's been overused (Hafez and Hafez, 2013). According to de Paz et al. (2010), the association between ram sperm head morphometry and fertility is dependent on evaluation methods, with the best findings achieved using light microscopy with a digital camera and traditional image processing. The length of the mid-piece of ram sperm, on the other hand, had no bearing on fertility (Dhurvey et al., 2012). The average sperm head dimensions do not affect male fertility; nevertheless, changes in fertility rates across rams are strongly linked to the proportion of spermatozoa with short and elongated heads in an ejaculate (Baril et al., 1993; Benia et al., 2018). The sperm morphology for Bangladeshi ram's semen, as reviewed in the literature, is presented in Table 2.

Plasma membrane integrity

A healthy and active spermatozoon with an intact plasma membrane is necessary for fertilization at the period of capacitation, acrosome response, and fusion of spermatozoa and ovum. Therefore, spermatozoa with inactive membranes cannot fertilize an ovum (Panin and Mahabile, 1997). Spermatozoa with an intact plasma membrane can fertilize an oocyte after a series of intricate alterations in the female genital system (Hafez, 1993). As a result, plasma membrane integrity (PM) is critical for sperm survival in the female reproductive tract and sperm's ability to fertilize the oocyte by overcoming intracellular and extracellular barrier components (Graffer et al., 1988). Jeyendran et al. (1995) used the HOST test to assess sperm membrane integrity and biochemical activity and found that it was a better predictor of spermatozoa fertilizing capacity than motility in ram. The test is one of the most straightforward procedures for assessing the stability of sperm cell plasmalemma (Mitchell et al., 2004). Endosmosis (fluid influx) is accompanied by an increase in cell volume (swelling) and characteristic curling of the tail, which is likely due to the caudal regions being more flexible than the head if the plasma membrane is functioning and intact (Panin and Mahabile, 1997). On the other hand, a defective or chemically inactive membrane permits fluid to

freely flow across it without accumulating inside, resulting in no cytoplasmic swelling or tail curling. The reactivity of spermatozoa with the HOS solution varies with each animal and is dependent on the animal's composition, osmolarity, and incubation time (Cameron et al., 1987). Nalley and Arifiantini (2013) assessed Indonesian Garut ram semen (fresh) and found that 69.47% of swollen sperm showed swelling when subjected to the HOST test. The seminal plasma biochemical constituents might cause variation in reaction values (Verma et al., 1999). The sperm membrane integrity for Bangladeshi ram's semen, as reviewed in the literature, is presented in Table 2.

Acrosome integrity

The acrosome is an acidic secretory cap-like structure or organelle of spermatozoa made of membranes, available in various Golgi complex shapes, and loaded with hydrolytic enzymes (Hafez and Hafez, 2000). It covers over 60% of the sperm head's anterior half. During the passage within the female reproductive system after ejaculation, sperm undergo structural and metabolic changes known as capacitation (Pereira et al., 2000). Capacitation causes sperm to become hyperactive, make contact with the oocyte zona pellucida, experience an acrosome reaction, and commence oocyte plasma membrane fusion (Hafez, 1993; Naz et al., 2004). The secreted acrosomal enzyme hyaluronidase softens and desolates the cumulus cell matrix of the zona pellucida, allowing easy penetration of the spermatozoa (Neild et al., 2005), resulting in the fusion of the male and female nuclei (Witte and Schäfer-Somi, 2007). As a result, sperm must have an intact acrosome for the acrosome response to occur at a reasonable period to permit fertilization (Partyka et al., 2012). Because spermatozoa must keep their acrosomes intact until they reach the isthmus, where zona binding occurs, the acrosome reaction renders spermatozoa infertile (Patel and Siddiquee, 2013). Furthermore, good-quality sperm should have optimal acrosomal morphology for capacitation and acrosome response in the female reproductive tract to achieve fertilizing capabilities. Nonetheless, morphological abnormalities in sperm may have developed during their survival span, reducing and impairing their capacity to fertilize (Correa et al., 2007). A range of physical and chemical variables produce and are associated with morphological abnormalities. Freezing and thawing have the potential to harm the acrosome. It is widely known that sub-lethal sperm cell damage occurs during cryopreservation (Ricker et al., 2006), with intracellular ice production (Mazur, 1984) causing membrane deterioration and acrosome integrity loss. Despite its popularity as a cryoprotectant, glycerol has osmotic and toxic effects on cryopreserved cells. Because acrosomes are lower, membranes are disrupted, and cryo-capacitated sperm cannot fertilize oocytes, acrosomal integrity is essential for regular fertilization (Malama et al., 2013). It is recommended to use only ram semen having ≥ 90% acrosome integrity (Gundogan et al., 2010). Interference or phase-contrast microscopy and particular staining techniques can be used to examine it (Williams et al., 1991). Other methods include triple stain, Naphthol yellow/Erythrosine B staining (Chacarov and Mollova, 1976), Eosin-B, and fast green (Selvaraju et al., 2008). Selvaraju et al. (2008) combined the hypo-osmotic swelling test and Giemsa staining as HOS-G, in which both the acrosomal integrity and HOST test can be studied from a single slide. Acrosomal integrity is also assessed using fluorescent probes (tetracycline with antibodies and lectins with antibodies). The sperm acrosome integrity for Bangladeshi ram's semen, as reviewed in the literature, is presented in Table 2.

EVALUATION OF FROZEN SEMEN QUALITY

The ram semen is frozen in Bangladesh by using a hand-made box freezer or a commercial programmable bio-freezer. In a box freezer, the freezing is done in liquid nitrogen vapour in a special box and then the straws are transferred into the cryocan at-196°C. Generally, a two-step dilution method (semen is added to part

A diluent and then calculated diluent B is poured to a previous dilution after two hours) is used to freeze the semen (Chaki et al., 2015; Jha et al., 2019). Generally, homemade diluent: Tris-citrate-fructose-egg yolk and commercial diluent: Triladyl are used for ram semen freezing (Rekha et al., 2018; Jha et al., 2019). Several factors such as age, breed, testicular biometry, preservatives, and production process may affect semen production and preservation quality. Some authors studied the preservation effects of skim milk and Tris-citrate on the quality of chilled and frozen-thawed indigenous ram semen (Rahman et al., 2018). Sharmin et al., (2021) studied the quality of ram semen about age, body weight, and scrotal size. Different percentages of glycerol affect the quality of frozen semen following different thawing times (Chaki et al., 2015, Jha et al., 2019). Male fertility and semen quality are of significant importance, especially in the artificial breeding system. Therefore, it is mandatory to evaluate the semen in the production center before using it in the operational Centre. Various seminal attributes are considered in the quality testing of the produced semen. Rowe et al. (1993) stated that sperm viability and motility percentage need to be evaluated before its use. Moussa (1999) noted that the sperm functional tests- hypo-osmotic swelling test (HOS-test) and permeability test need to be performed for semen quality. The frozen semen quality of Bangladeshi ram as reviewed in the literature is shown in Table 2.

CONCLUSIONS

Examining the features and attributes of the ejaculated semen, which significantly impacts flock performance, improves a breeding ram's or sire's production potential. Semen evaluation tests stated in this review bear the importance of predicting the ram quality and discriminating its semen between acceptable or unacceptable margins by the livestock stakeholders.

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AUTHOR CONTRIBUTIONS

Mohammad Asaduzzaman conceived the topic and tabulated all data, while FY Bari supervised, reviewed, and edited the final paper.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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