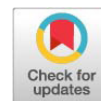


Research Article



Media Effects on Sperm Motility Percentage of Indigenous, Mozaffarnagri and Dorper Ram During Frozen Semen Production

MUHAMMAD RAKIBUL HASAN¹, RADWAN ISLAM¹, FARHANA NAZNIN², TANVIR AHMED SUJON¹, JAYONTA BHATTACHARJEE¹, FARIDA YEASMIN BARI^{1*}

¹Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh, Bangladesh; ²School of Agriculture and Rural Development, Bangladesh Open University, Gazipur-1705.

Abstract | The use of high-quality ram semen in artificial insemination programs has the potential of improving the reproductive performance and genetic gains in Bangladesh. This research aimed to use the semen of three different ram breeds in two semen extenders, Tris and Triladyl, and to evaluate semen quality in prefreezing and post-thawed stages. A total of 15 Indigenous, Mozaffarnagri, and Dorper rams of three breeds were reared, and semen was collected using the AV method. Semen was evaluated immediately after collection, and motility, progressive motility, and poor motility parameters were assessed separately for each medium. Tris egg yolk citrate media were prepared in the lab, and Triladyl® was used according to its directions. The results showed that the Dorper rams had the greatest semen volume, sperm concentration, motility, and viability, even though the variation between the different breeds was not significant. Dorper (79.10%) and Indigenous (78.90%) had the highest progressive motility, followed by Mozaffarnagri (66%). Membrane integrity and viability were a little more in Mozaffarnagri and Dorper than in Indigenous, but the difference was not significant. Triladyl enhanced the semen quality of all breeds with the highest consistency in prefreezing (72.68%) and post-thaw (46.99%) motility. Triladyl also increased progressive motility, and in the case of Dorper, it was highest in the two stages. Triladyl was better than Tris in terms of pregnancy rates in Indigenous (50%), Mozaffarnagri (50%), and Dorper (66%). Such results indicate that Triladyl is superior to Tris with respect to increasing sperm motility and fertility rates, and Dorper has the best performance in most of the semen parameters that are needed for breeding regimens to maximize the fertility in sheep artificial insemination programs.

Keywords | Motility, Extender, Semen evaluation, Cryopreservation, Bangladeshi sheep

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***Correspondence** | Farida Yeasmin Bari, Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh, Bangladesh; **Email:** faridabari06@gmail.com

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INTRODUCTION

Fertilization of the egg *in vivo* requires sperm motility as sperm should travel through the female reproductive tract to fertilize the egg. Motility plays an important role in assessing the fertilization mechanisms, especially in artificial insemination (AI) (Suarez and Pacey, 2006; Puglisi *et al.*, 2012). It is important that sperm motility can be regarded

as one of the main parameters of semen quality and male fertility (Kathiravan *et al.*, 2011; Batellier *et al.*, 2001), and semen assessment is an important routine measure of male fertility. It has been reported to have positive correlations between sperms motility and ram fertility (Kozłowska *et al.*, 2022). Fernandes *et al.* (2021) recommended that, in general terms, the egg yolk-base semen extender was more beneficial to the cryopreservation of rams' semen.

Underlying reasons for the interaction effect between semen extenders on various spermatozoa parameters should be investigated, possibly in new studies with molecular research to further optimize egg yolk-based as well as in egg yolk-free-based semen extenders.

Various breeds of rams have differing semen freezability rates with some breed such as Merino having high post-thaw survivability rates and some having lower rates such as Slovak Dairy, Native Wallachian, and Improved Wallachian rams (Vozaf *et al.*, 2022). Every breed will need certain extenders to be optimally frozen. Extenders such as Tris which are made out of yolk, are mainly used because they help to prevent the cold damages of sperm (Sevilla *et al.*, 2025). Some more recent studies are looking to other options such as skim milk extender or soy lecithin, and antioxidants such as glutathione and sugars such as trehalose are proving to be effective at lengthening the lifespan of sperm (Iqbal *et al.*, 2016; Bashar *et al.*, 2025).

Cryopreservation has detrimental consequences on semen quality such as decreased motility, membrane integrity, and viability and the extender type is important to the efficacy of semen preservation (Purdy *et al.*, 2020). Breed, age, and semen type (fresh, chilled, and frozen) are some of the factors that influence the success of cryopreservation (Gaunand *et al.*, 2014). Individuation The use of synchronized estrus is aimed at optimum timing of AI, which is normally 12–18 hours after estrus onset (Madrigali *et al.*, 2021). It still undergoes research on the impacts of various extenders on semen traits in other breeds of rams, including Kail sheep (Hameed *et al.*, 2024).

Semen storing is influenced by the extender, temperature, and/or time, and cryoprotectants have a crucial impact on protecting sperm in the process of storage (Layek *et al.*, 2016). Cryoprotectants increase the permeability of sperm membranes and the temperature shock resistance (Batellier *et al.*, 2001; Layek *et al.*, 2016). Aggregated spermatozoa decrease reduces the cryoprotectant penetration, which minimizes the damage (Spanner *et al.*, 2025). Through this experiment, the aim was to examine the effects of two extenders on the reproductive performance and the quality of the sperm in the various breeds of sheep in Bangladesh through AI.

MATERIALS AND METHODS

ANIMAL MANAGEMENT

A total of fifteen rams of three different breeds, Indigenous (n= 5), Muzaffarnagri (n= 5), and Dorper (n= 5) were selected, purposively, on a number of criteria, i.e., health, age (12–18 months) and lack of previous reproductive complications yet all groups had a rating of an average

body condition score (BCS) of between 3.0 and 3.5. Housing conditions, feeding conditions and management conditions were kept the same to reduce environmental variation.

All procedures that handled animals was conducted following the institutional and international regulations approved by: Bangladesh Agricultural University Animal Welfare and Ethics Committee (Approval No. AWEEC/BAU/2024(01)).

SEMEN ANALYSIS AND EVALUATION

Semen collection was achieved through the artificial vagina (AV) technique as mentioned by Jha *et al.* (2019) with the regular set of recommended procedures. Following morphological analyses were done such as the semen volume, motility, sperm concentration, membrane integrity, and viability in fresh semen. All tests were performed according to the procedure outlined in the previous experimental work of Jha *et al.* (2019) and Valente *et al.* (2010).

DILUENT PREPARATION

Semen samples were diluted in a two-step method using homemade Tris-based egg yolk diluents (20%, v/v) and 5% glycerol by following Rekha *et al.* (2016) process and a one-step method for a commercial diluent Triladyl®, which contains double distilled water and egg yolk.

FROZEN SEMEN PRODUCTION TECHNIQUE

In this experiment, one step freezing technique were followed. A styrofoam box was used with a round hole at the corner of the lid to add liquid nitrogen. A hand-made metal stand were designed to place the semen straw. The wire net holding semen straw was kept in the styrofoam box (pre-cooled at +5°C). Loaded straws were placed on the net. Lid was closed immediately and six litre liquid nitrogen (6 cm deep) was poured and left for 12 min to decrease the temperature of suspended semen straw from +5°C to -140°C. They were then frozen in LN vapour according to the methods of freezing technique of Jha *et al.*, 2019 and stored in liquid nitrogen.

ARTIFICIAL INSEMINATION AND PREGNANCY DIAGNOSIS

Thirty-six (n=36) ewes were given two doses of 0.8 mL (150 µg) intramuscular (im) prostaglandin F2α (PGF2α) analogue (Ovuprost, Bayer New Zealand Ltd., Auckland, New Zealand) 9 days apart for estrous synchronization according to Zohara *et al.* (2014). TCAI was performed after 48–52 hours of 2nd dose injection with previously made frozen semen. Pregnancy was diagnosed by ultrasonography using a digital trans-abdominal ultrasonic transducer 5.0 MHz (Model Magic 5000, Art NO. 303700, Germany) after 50 days of TCAI.

STATISTICAL ANALYSIS

All the statistical analyses were carried out by R Studio (2024 version). The normality of data was checked with the help of the Shapiro-Wilk test. The three breeds of ram were compared in one-way Analysis of Variance (ANOVA) and later post hoc test of Tukey Honest Significant Difference (HSD) post hoc test.

RESULTS

FRESH SEMEN EVALUATION

The three ram genotypes had similar characteristics of semen with Dorper rams having a little higher semen volume, sperm concentration, motility and viability, though the differences are minimal and they are not statistically significant (Table 1).

The volume of semen of the indigenous (0.696 ± 0.10 ml), Mozaffarnagri (0.70 ± 0.11 ml), and Dorper (0.786 ± 0.02 ml) was similar with Dorper having the highest volume of semen. Dorper recorded the highest sperm concentration ($3098.98 \pm 28.35 \times 10^6/\text{ml}$), to Mozaffarnagri, and Indigenous. Progressive motility of Dorper (79.10 ± 0.83) and Indigenous (78.90 ± 1.06) did not differ with Mozaffarnagri was the lowest ($66 \pm 1.50\%$). Membrane integrity of Mozaffarnagri and Dorper ($81.08 \pm 0.33\%$ and $80.22 \pm 1.73\%$, respectively) had a little higher membrane integrity as compared to Indigenous ($76.72 \pm 1.42\%$). Sperm viability was high in the cases of Indigenous ($79.32 \pm 0.52\%$), Mozaffarnagri ($80.30 \pm 0.55\%$), and Dorper ($80.94 \pm 0.38\%$) with a slight numerical difference between them.

Table 1: Average value of evaluated fresh semen.

Ram	Volume (ml)	Concentration ($\times 10^6$)	Motility (%)	Membrane permeability (%)	Viability (%)
Indigenous	0.696 ± 0.10	2723.6 ± 62.39	78.904 ± 1.06	76.72 ± 1.42	79.32 ± 0.52
Mozaffarnagri	0.7 ± 0.11	2872.16 ± 28.71	78.66 ± 1.50	81.08 ± 0.33	80.3 ± 0.55
Dorper	0.786 ± 0.02	3098.98 ± 28.35	79.1 ± 0.83	80.22 ± 1.73	80.94 ± 0.38

MOTILITY EFFECTS OF PREFREEZING STAGE USING TRIS MEDIA

The Dorper breed was the fastest in average motility (65.80) with the least variation (SD = 3.12) so it is very active as well as stable. The mean motility was similar in the indigenous and Muzaffarnagri breeds (62.10 and 62.0, respectively) with increased variability (Table 2).

Dorper was steadier again with the largest percentage of progressive mean motility (44.90) and the smallest variability (SD = 1.73). The least mean (39.40) with a high

variability was recorded in Muzaffarnagri, whereas the intermediate mean percentage was 43.10 percent and the variability was also moderate in Indigenous. Dorper had the greatest mean poor motility characteristics (19.70%) but had more variability (SD = 2.71). Mean scores were lower in Indigenous and Muzaffarnagri (17.40 and 17.10) although Muzaffarnagri had a greater range of variance than Indigenous did (Table 2).

Table 2: Descriptive statistics for motility, progressive, and poor traits across different breeds using tris media.

Trait	Breed	Mean	Std. deviation	Std. Error	95% confidence interval
Motility	Indigenous	62.10	7.34	2.33	56.84-67.36
	Dorper	65.80	3.12	0.97	63.57-68.03
	Muzaffarnagri	62.00	7.23	2.3	56.80-67.20
Progressive	Indigenous	43.10	6.15	1.95	38.70-47.50
	Dorper	44.90	1.73	0.55	43.66-46.14
	Muzaffarnagri	39.40	5.15	1.63	34.38-43.02
Poor	Indigenous	17.40	2.17	0.69	16.06-18.74
	Dorper	19.70	2.71	0.86	17.61-21.79
	Muzaffarnagri	17.10	3.31	1.05	14.73-19.47

PREFREEZING STAGE USING TRILADYL® MEDIA

Prefreezing motility of the Indigenous breed had a mean of 69.46 and a standard deviation of 10.11 which was high. The 95 percent confidence interval was between 62.22 and 76.69. Comparatively, the mean of 72.69 and lower variability (SD= 8.38) were observed in the Dorper breed with a confidence interval of 66.69 and 78.68. The mean of Muzaffarnagri breed was similar (70.95) with a standard deviation of 10.09, with confidence interval of 63.73 to 78.16 (Table 3).

Table 3: Descriptive statistics for Prefreezing (Triladyl®) across different breeds.

Traits	Breed	Mean	Std. deviation	Std. error	95% confidence interval
Pre-freezing	Indigenous	69.46	10.11	3.19	62.22 – 76.69
	Dorper	72.69	8.38	2.65	66.69 – 78.68
	Muzaffarnagri	70.95	10.09	3.19	63.73 – 78.16

POST-THAW MOTILITY COMPARISON

Prefreezing motility increased in all breeds when Triladyl was used as compared to Tris. The most prefreezing motility was observed with Triladyl (72.68 ± 8.37), Mozaffarnagri (70.95 ± 10.08) and Indigenous (69.46 ± 10.11). The standard deviation of motility (variability) of Dorper was lower than the other breeds, which implies that the motility of Dorper was more consistent under Triladyl. There was an improvement of post-thaw motility associated with Triladyl relative to Tris in all breeds.

Table 4: Comparison of sperm motility between two media.

		Indigenous		Mozaffarnagri		Dorper	
		Tris	Triladyl®	Tris	Triladyl®	Tris	Triladyl®
Prefreezing	Motility	62.10±7.36	69.46±10.11	62.0±7.27	70.95±10.08	65.80±3.11	72.68±8.37
	Progressive motility	43.10±6.15	49.10±7.33	39.40±5.15	52.10±6.41	44.90±1.73	51.10±5.91
	Poor motility	17.40±2.17	16.40±4.32	17.10±3.31	18.40±7.32	19.70±2.71	19.63±9.40
Post-thaw motility	Motility	43.25±15.37	45.95±10.47	41.92±10.0	44.97±7.77	45.46±10.36	46.99±7.51
	Progressive motility	29.90±10.85	32.80±7.32	27.20±7.73	29.00±6.29	31.00±7.24	32.5±5.46
	Poor motility	12.1±4.25	13.1±3.35	11.40±2.84	12.40±2.67	13.3±2.54	14.00±1.94

Post-thaw motility was highest with Triladyl (46.99±7.51) then with Indigenous (45.95± 10.47) and Mozaffarnagri (44.97±7.77). Triladyl enhanced motility in all the breeds with Dorper exhibiting the most consistency (smallest standard deviation) in comparison to other breeds (Table 4).

The progressive motility was found to increase in all breeds when Triladyl was used as opposed to Tris. Dorper was most progressive in terms of prefreezing (44.90%) and post-thaw (31.00%), with the Indigenous and Mozaffarnagri coming close in both stages. Progressive motility of Mozaffarnagri exhibited the most significant change between prefreezing (39.40%) and post-thaw (29.00%) which showed that the motility was better preserved on thawing with Triladyl. All breeds had an enhanced progressive motility with Dorper recording the highest in prefreezing conditions and post-thawing conditions, implying that it is more adaptable to freezing-thaw conditions (Table 4).

The Table 5 shows the percentages of the pregnancy rate of three breeds (Indigenous, Mozaffarnagri and Dorper) in two conditions (Tris and Triladyl). The pregnancy rate of the Indigenous breed under Tris and Triladyl was 42 and 50 percent, respectively. Mozaffarnagri breed had a pregnancy rate of 56% (7/12) with Tris and 50% (6/12) with Triladyl. Tris and Triladyl breeding give a 56% (7/12) and 66% (8/12) Dorper pregnancy rate respectively. This data entails the comparison of the pregnancy rates among the three breeds based on the various breeding modes and gives an insight into their reproductive performance when in various conditions.

Table 5: Comparison of sperm motility and pregnancy rate (%) between two media.

Breed	Tris		Triladyl®	
	Motility	Pregnancy rate	Motility	Pregnancy rate
Indigenous	43.25±15.37	42 (5/12)	45.95±10.47	50 (6/12)
Mozaffarnagri	41.92±10.0	56 (7/12)	44.97±7.77	50 (6/12)
Dorper	45.46±10.36	56 (7/12)	46.99±7.51	66 (8/12)

DISCUSSION

This research was conducted to compare sperm motility, progressive motility, and poor motility in the Indigenous, Mozaffarnagri, and Dorper breeds under two extenders (Tris and Triladyl) during prefreezing and post-thaw stages. These results provide insights into the variations in sperm quality before and after freezing, which is crucial for artificial insemination (AI) success in livestock breeding.

Indigenous breed showed increased motility with Triladyl (63.1±2.5) compared to Tris (48.10±3.0), which may be due to improved resistance to cold shock (Jha *et al.*, 2019). Dorper sperm demonstrated a similar trend, with improved motility (72.68±8.37) when using Triladyl, supporting findings that it enhances sperm integrity during cryopreservation (Shah *et al.*, 2023).

Post-thaw motility generally decreased, as expected, but the Indigenous breed retained more motility with Triladyl (45.95±10.47), indicating partial protection against freezing damage. These findings demonstrate the superior cryoprotective efficacy of Triladyl® over the Tris extender in maintaining post-thaw semen quality (Solangi *et al.*, 2026; Shah *et al.*, 2023). Mongolian showed minimal reduction in motility (67.60%), suggesting better preservation during cryopreservation (Sun *et al.*, 2020). Dorper sperm exhibited the highest post-thaw progressive motility (46.99±5.91), consistent with studies showing that Dorper sperm has better post-thaw viability (Najjar *et al.*, 2016). Sperm post thaw motility is variable and varied breed to breed and media effect which support this study stated by some researcher like (Saha *et al.*, 2022; Vozaf *et al.*, 2022).

The pregnancy rate also improved with Triladyl, particularly in the Indigenous breed (42% to 50%) and Dorper (56% to 66%), indicating that Triladyl not only preserves motility but enhances fertility. Some other research results suggest that if semen preserving methods can be improved and AI timing programs developed, breeding efficiency in Assaf breed of sheep will be beneficial (Halawa *et al.*, 2025). In contrast, Mozaffarnagri maintained a constant pregnancy rate of 50%, suggesting resilience to extender

CONCLUSION

This research showed that the three genotypes of rams had good semen quality with Dorper rams having better ejaculate volume, sperm concentration, and viability. The outcomes of post thaw motility using two separate media indicated that Triladyl offered better sperm motility than Tris particularly in Dorper with high pregnancy rate. This study had several limitations, like field fertility outcomes were not measured, and only two specific extenders were compared despite of other commercially available extenders. However, these results highlight the significance of using the correct semen extender and breeding stock to achieve the best results with the artificial insemination (AI) programs with Triladyl as an ideal option to preserve sperms and increase fertility in the Indigenous and Dorper breeds.

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NOVELTY STATEMENT

The novel contribution of this research is a breed specific comparative assessment of laboratory made Tris extender with commercial Triladyl for ram semen cryopreservation and post-thaw motility, were examined in combination with pregnancy status after artificial insemination. The Triladyl seems a comparatively better producing frozen ram semen especially in the Bangladeshi context, which is also useful for Dorper semen.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Muhammad Rakibul Hasan and Farida Yeasmin Bari. The first draft of the manuscript was written by Muhammad Rakibul Hasan, Farhana Naznin and Jayonta Bhattacharjee and Farida Yeasmin Bari revised and corrected the manuscript. Md. Redwanul Islam and Tanvir Ahmed Sujon were involved directly in laboratory work and help in data analysis. All authors read and approved the final manuscript.

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DATA AVAILABILITY

The datasets generated during analysis and during the current study are not publicly available due to further next research is ongoing but are available from the corresponding author on reasonable request.

GENERATIVE AI AND AI ASSISTED TECHNOLOGY STATEMENT

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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