



Effect of diluters on frozen semen production of Black Bengal Goat

MA Rimi, T Akter, MM Akhtar and MAMY Khandoker[✉]

Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

ARTICLE INFO

Article history:

Received: 05 September 2023

Revised: 13 December 2023

Accepted: 17 December 2023

Published: 31 December 2023

Keywords:

Black Bengal goat, Semen, Diluter, Cryopreservation, Conception rate

Correspondence:

M. A. M. Yahia Khandoker ✉:
yahiakhabg@bau.edu.bd

ISSN: 0003-3588



ABSTRACT

The present study was undertaken to investigate the efficacy of three diluters (Triladyl, Andromed and Tris) for the production of frozen semen in Black Bengal goat and their effect on conception rate. Over the course of 6 months, data from six bucks were collected. Each collected sample was diluted with Triladyl, Andromed and Tris diluter, filled into 0.50 ml straws, sealed and equilibrated at 5°C for freezing. The motility, viability and morphology were evaluated after dilution and in every step of cryopreservation (after equilibration, after freezing and after thawing). It became clear that motility and sperm viability of the frozen thawed semen differed significantly ($p < 0.05$) but differed insignificantly ($p > 0.05$) immediate after dilution among the diluters. The motility of frozen thawed semen was $42.50\% \pm 1.71$, $34.67\% \pm 1.67$ and $28.71\% \pm 1.55$ in Triladyl, Andromed and Tris diluter, respectively. The viability of frozen thawed semen was $40.51\% \pm 1.78$, $32.24\% \pm 1.52$ and $26.84\% \pm 1.56$ in Triladyl, Andromed and Tris diluter, respectively. In case of morphology, it differed insignificantly ($p > 0.05$) among the diluters in every step of cryopreservation. Higher percentage of normal spermatozoa was found in Triladyl based diluter (87.11%) in frozen thawed semen. After 15 days of sample preparation, the motility of prepared sample was again checked to ensure the quality of frozen semen in liquid nitrogen (LN). It differed insignificantly ($p > 0.05$) with frozen thawed semen after 24 hours of production. The obtained conception rate was 38% for Triladyl, 35% for Andromed and 27% for Tris diluter. The results of the study revealed that cryopreservation of buck semen revealed good results with Triladyl diluter followed by Andromed and Tris diluter.

Copyright © 2023 by authors and Bangladesh Journal of Animal Science. This work is licensed under the Creative Commons Attribution International License (CC By 4.0).

Introduction

Bangladesh has only one goat breed known as the Black Bengal goat. Bangladesh is home to one of the richest treasures precious Black Bengal goats. Bangladesh's heritage, as well as one of its potential genetic resources is the Black Bengal goat. Black Bengal goat is world famous due to its adaptability, fertility, prolificacy, delicacy of meat and superior skin quality (Husain *et al.*, 1996). It adapts well to hot and humid climates and

typically produces twins and triplets. But unfortunately, the availability of breeding bucks are gradually reducing day by day because most of the goat raiser castrates almost all the male kids at early age for economic and social reasons (Khandoker, 2007). As a result, same buck has been used generation after generation which has created greater chance of increasing inbreeding and hence lowering reproductive performances along with disseminating various venereal and infectious diseases (Husain, 2007). As a result,

How to Cite

MA Rimi, T Akter, MM Akhtar, MAMY Khandoker (2023). Effect of diluters on frozen semen production of Black Bengal Goat. *Bangladesh Journal of Animal Science* 52 (4): 105-113. <https://doi.org/10.3329/bjas.v52i4.70700>

providing artificial insemination (AI) using quality buck semen is the simplest way to exploit desirable germ-plasm.

Frozen sperm can be stored and even used long after the donor is dead. Thus, genetically superior male could be made accessible to all sectors of the animal production, thereby rapidly improving the quality of output from the sector (Afroz, 2005). Extensive use of frozen semen materially paved the way for better use of superior bucks to produce more offspring and thus ensuring genetic diversity and reducing the generation interval (Kalyani et al., 2015). AI needs fresh or quality preserved semen, and 95% of all AI is accomplished using preserved semen (Raheja et al., 2018). Thus, semen must be preserved in a perfect medium to maintain its quality (Raheja et al., 2018 and Hernandez-Avilés et al., 2020). Accordingly, it is necessary to develop and evaluate semen diluters used to preserve semen during chilling or cryopreservation (Santos et al., 2018).

Diluters have been used for both protection and maintenance of spermatozoa during cryopreservation of semen and to increase ejaculate volume. Good diluter must have equal osmotic pressure to the seminal plasma, buffering capacity, protect sperm from cold shock, nutrient for sperm metabolism, controlling microbial contamination, protect sperm against freezing, thawing damages and preserve sperm viability without more decline in fertility (Soltanpour and Moghaddam, 2014). Suitable extender also determines the prolificacy of Black Bengal does (Karim et al., 2018). To evaluate the efficacy of frozen semen production, it is necessary to choose an appropriate diluter. It appears logical to compare cryo-survival of spermatozoa in the preservation of buck sperm using different diluters. As a result, the selection of a suitable extender for preserving buck semen is essential for the adoption of AI in goats.

Although Black Bengal goats have been reared for many years, preservation of the buck semen has not been well established in Bangladesh. The cryopreserved semen technique for preserving the semen of buck is underutilized due to a lack of information and shortage in the field of AI. Though AI has become widely accepted in most industrialized countries' dairy cattle sectors, and is now also popular in Bangladesh, it has not yet achieved the same level of universal acceptance in Bangladesh's goat industries. However, interest in AI in goats has increased day by day after achieving its outstanding success in cattle. Fertilizing capacity of semen has always been thought to be one of the most important components of an AI program. So, to bring any AI

program into economic success, use of poor-quality semen of buck must be avoided in AI. For this, selection of a suitable diluter is very much important to maintain the good quality semen.

Therefore, the present investigations were designed to compare the efficacy of Triladyl based, Andromed based and Tris based (locally manufactured) diluter for production of frozen semen of Black Bengal goat and to compare the effects of diluents on conception rate of Black Bengal goat.

Materials and methods

Experimental site

The present research work was conducted at the Artificial Insemination (AI) center under the Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh during the period from August, 2021 to February, 2022.

Selection of Breeding bucks and managements

Six adult Black Bengal bucks were selected based on their ability to produce semen having greater than 80% morphologically normal spermatozoa and greater than 80% motility. The bucks used were aged between 12-18 months. The body weight of bucks ranged between 16.68 to 20.91 kg. The bucks were reared isolated from the does. They were allowed to graze for 7-8 hours daily and received green grass *ad-libitum*. Each buck was fed with germinated gram (50 gm/buck/day) and concentrate (300 gm/buck/day). There was always access to clean and safe water.

Semen collection and evaluation

The bucks were trained to ejaculate semen in artificial vagina (AV) (Minitube Germany). Semen was collected twice a week. After collection, semen was kept at 37 °C in water-bath until the media and reagents were added with it. After collecting the semen individual ejaculate was evaluated for volume (cc), mass motility (%), sperm concentration (million/cc), live and dead sperm count (%) and normal and abnormal sperm count (%).

Preparation of diluters

Triladyl diluter

Triladyl diluter is a commercial complete diluter for cryopreservation of semen. It is comprised with several components, which further supports the freezing and post thawing survival of semen. A stock solution for Triladyl diluter was prepared by dissolving 20% of Triladyl solution (contains tris, citric acid, sugar, buffers, glycerol, water and

Diluters effect on frozen goat semen

antibiotics), (Minitube, Germany), 20% egg yolk and 60% distilled water.

Andromed diluter

Andromed diluter is egg yolk free commercial concentrated diluter for cryopreservation of semen. It is comprised with phospholipids, tris, citric acid, sugars, antibiotics, buffers, glycerol and purest water which further supports the freezing and post thawing survival of semen. A stock solution for Andromed diluter was prepared by dissolving 20% of Andromed solution (Minitube, Germany) and 80% distilled water.

Egg yolk tris citrate diluter

A stock solution for tris-glucose-citrate diluents was prepared by dissolving tris, glucose and citrate in 70 ml distilled water. The stock solution was sterilized by filtration and preserved at 4-7°C for maximum 2 weeks. At the day of semen

collection, fresh well-churned egg yolk (2.0 ml) and glycerol (1.0 ml) were added with stock solution to make 10 ml of complete medium. Penicillin and streptomycin sulfate was added at the rate of 1000IU/ml and 1mg/ml respectively.

Dilution and preparation of semen for cooling

After collection of semen, motility and concentration of sperm were assessed and semen was diluted with the cryodiluent (diluter + cryoprotectant) to reach a final concentration of 100 million sperm per dose. After dilution motility, viability and morphology of sperm were checked. In this experiment three diluters (Triladyl, Andromed and Tris based diluter) were used.

Filling and sealing

Semen was then packed manually into 0.5 ml straws and the laboratory ends of the straws were sealed with polyvinyl chloride (PVC) powder.

Table 1: Cost of ingredients required for preparing of Triladyl diluter

Ingredients	Company/Manufacturer	Cost	Cost for preparation of 10 ml diluter
Triladyl solution	Minitube GmbH	50000tk/750ml	133.33tk/2ml
Egg yolk		10tk/10ml yolk	2tk/2ml
Distilled water		10tk/liter	0.06tk/6ml
Total			135.39tk
Cost per dose			6.78tk

Table 2: Cost of ingredients required for preparing of Andromed diluter

Ingredients	Company/Manufacturer	Cost	Cost for preparation of 10 ml diluter
Andromed solution	Minitube GmbH	6000tk/200ml	60tk/2ml
Distilled water		10tk/liter	0.08tk/8ml
Total			60.08tk
Cost per dose			3.004tk

Table 3: Cost of ingredients required for preparing of Tris diluter

Ingredients	Company/Manufacturer	Cost	Cost for preparation of 10 ml diluter
Tris	Qualikems Fine Chemicals Pvt. Ltd	896tk/100gm	3.25tk/0.363gm
Citric acid	Aldrich-chemical Company	5000tk/100gm	9.95tk/0.199gm
Glucose	Pran	10tk/25gm	0.02tk/0.05gm
Glycerol	Bio Basic Canada Inc.	4000tk/500ml	8tk/ml
Egg yolk		10tk/10ml	2tk/2ml
Pronapen	Renata Limited	44.59tk/2.5gm	0.11tk/10000IU
Streptopen	Renata Limited	75tk/2.5gm	0.3tk/10mg
Distilled water		10tk/liter	0.07tk/7ml
Total			23.70tk
Cost per dose			1.19tk

Equilibration

After filling, the straw was placed in refrigerator at 4-5°C for 4 hours for equilibration. At the end of equilibration the motility, viability and morphology

of sperm were checked and only samples with more than 60-70% motility were used for freezing.

Freezing

After equilibration, the straws were placed horizontally on a rack and transferred to the ice box to be frozen in vapor 5 cm above the liquid nitrogen (LN) for 20 minutes (Jha et al., 2019). After 20 minutes, the frozen straws were moved into the canister within the LN container at -196°C until use for AI. After freezing, motility, viability and morphology of sperm were checked to determine that the sample was eligible to transfer into LN or not.

Thawing

After 24 hours, straws were retrieved from the LN2 container using forceps and thawed in water bath at 37°C for 12 seconds. The straws were wiped and cut near the cotton plugged end. One drop of semen was placed on to a glass slide and the post-thaw motility was assessed under microscope. Viability and morphology were also checked. Other straws were kept at optimum level of LN and after 15 days motility was checked again.

Insemination of does

Artificial insemination (AI) with frozen semen was performed at BAU AI center by trained AI technician. The does to be inseminated were detected in estrus by the farmers based upon behavioral signs. During AI, the vulva region of estrus doe was cleaned with tissue paper. The doe was restrained by holding her hind legs upward. After that with the aid of a tubular speculum lubricated with non-spermicidal gel and equipped with a frontal light source, the external opening of cervix was visualized.

Straw of frozen-thawed semen was loaded in AI gun which covered with plastic sheath and placed carefully into os of the cervical canal. Each frozen straw (0.5 ml) contained 100 million spermatozoa (Afroz, 2005). After complete deposition of semen, AI gun was removed from the vagina. In total, 37 does were inseminated with frozen semen.

Conception rate measurement

Conception rate was recorded as the percentage of does that had not returned to estrus within 42 days (two cycles) after AI. This conception rate was also ensured by taking the history from the owner, then conception rate was calculated by using the following formula:

$$\text{Conception rate} = \frac{\text{Number of does conceived}}{\text{Number of does inseminated}} \times 100$$

Statistical analysis

The data generated from this experiment were entered in Microsoft worksheet and expressed as percentage, organized and processed for further analysis. Analysis was performed with the help of SPSS 23 (Statistical Package for Social Sciences).

Results

Motility of cryopreserved semen with different diluters

After collection, dilution, equilibration, freezing and cryopreservation in liquid nitrogen (LN) at -196°C sperm motility was observed. Table 4 showed that mean of the motility immediate after dilution (with Triladyl, Andomed, Tris based diluter) ranged from 81.71%-83.67%. Higher sperm motility was observed in Andromed diluter (83.67%) followed by Triladyl diluter (82.50%) and Tris diluter (81.71%) and did not differ significantly ($p>0.05$).

Motility after equilibration at 4-5°C for 4 hours ranged from 73.29%-75.67% among three diluters which differed non-significantly ($p>0.05$). Higher sperm motility was observed in Triladyl diluter (75.67%), followed by Andromed diluter (75.33%) and Tris diluter (73.29%) (Table 4).

After 20 minutes of freezing in LN, on an average sperm motility was found 37.86%-51.67%. There was a significant ($p<0.05$) difference in the mean value of motility after freezing among three diluters. Higher sperm motility was observed in Triladyl diluter (51.67%) and lower in Tris diluter (37.86%) (Table 4).

Mean motility of sperm in frozen thawed semen diluted with different diluter differed significantly ($p<0.05$). Higher sperm motility was observed in Triladyl diluter and lower in Tris diluter. Sperm motility observed in Triladyl, Andromed and Tris diluter were 42.50%, 34.67% and 28.71% respectively (Table 4).

Viability of cryopreserved semen with different diluters

According to the Table 5, the mean viability of sperm immediate after dilution (with Triladyl, Andromed and Tris based diluter) higher viable sperm was recorded in Triladyl diluter (84.46%) followed by Andromed diluter (84.10%) and Tris diluter (82.21%) and the difference was insignificant ($p>0.05$) among the diluters.

The average viability of the sperm after equilibration at 4-5°C for 4 hours and after 20 minutes of freezing ranged from 72.76%-78.57% and 36.14%-52.15% respectively. In both step higher viable sperm was found in Triladyl diluted semen which had significant ($p<0.05$) difference with rest of the diluters. Lower viable sperm was found in Tris based diluted semen which had significant ($p<0.05$) difference with Triladyl diluter but non-significant ($p>0.05$) with Andromed diluter (Table 5).

The mean viability in frozen thawed semen diluted with different diluter differed significantly ($p<0.05$). Higher sperm viability was observed in

Diluters effect on frozen goat semen

Triladyl diluter and lower in Tris diluter. Sperm viability observed in Triladyl, Andromed and Tris diluter were 40.51%, 32.24% and 26.84% respectively (Table 5).

Morphology of cryopreserved semen with different diluters

After collection, dilution, equilibration, freezing and cryopreservation in LN at -196°C sperm morphology was observed. The morphology among three diluted semen in every step of cryopreservation of sperm did not differ significantly ($p>0.05$). Higher normal sperm was found in Triladyl diluted semen followed by

Andromed and Tris diluted semen in every step of cryopreservation of sperm (Table 6).

Observation of frozen thawed semen motility after 15 days

After 15 days of frozen semen production with three different diluters, the motility of remaining straw was checked again to compare with the motility of frozen thawed semen. The motility of sperm differed non-significantly ($p>0.05$) between 24 hours and 15 days. Higher sperm motility was recorded in Triladyl based diluter (41.67%) and lower sperm motility was recorded in Tris based diluter (26.67%) (Table 7).

Table 4: Effect of different diluters on motility (%)

Diluters	Mass motility (%)			
	Immediate after dilution	After equilibration (4 hrs at 4-5°C)	After freezing (20mins in 6L N)	Frozen thawed semen
Triladyl (6)	82.50±1.71	75.67±1.28	51.67±2.11 ^a	42.50±1.71 ^a
Andromed (6)	83.67±0.88	75.33±0.92	43.33±1.05 ^b	34.67±1.67 ^b
Tris (7)	81.71±0.92	73.29±0.89	37.86±1.49 ^c	28.71±1.55 ^c

Each value represents the mean percentage (±SEM); Values with different superscripts (a,b,c) in the same column differ significantly ($p<0.05$); Parenthesis indicates the number of observations.

Table 5: Effect of different diluters on sperm viability (live sperm) (%)

Diluters	After dilution	After equilibration (4 hrs at 4-5°C)	After freezing (20mins in 6L N)	Frozen thawed semen
Triladyl (6)	84.46±1.32	78.57±1.65 ^a	52.15±2.90 ^a	40.51±1.78 ^a
Andromed (6)	84.10±1.30	74.07±1.11 ^b	41.31±1.23 ^b	32.24±1.52 ^b
Tris (7)	82.21±1.26	72.76±1.32 ^b	36.14±1.54 ^b	26.84±1.56 ^c

Each value represents the mean percentage (±SEM); Values with different superscripts (a,b,c) in the same column differ significantly ($p<0.05$); Parenthesis indicates the number of observations

Table 6: Effect of different diluters on morphology (Normal sperm) (%)

Diluters	After dilution	After equilibration (4 hrs at 4-5°C)	After freezing (20mins in 6L N)	Frozen thawed semen
Triladyl (6)	90.27±0.48	89.22±0.67	88.16±0.62	87.11±0.70
Andromed (6)	89.69±0.30	88.94±0.31	88.05±0.20	87.01±0.15
Tris (7)	89.99±0.41	88.70±0.29	87.24±0.22	86.22±0.23
Level of Significance	NS	NS	NS	NS

Parenthesis indicates the number of observations; NS, non-significant

Table 7: Observation of frozen thawed semen motility

Parameters	Mass motility (%)		
	Triladyl (6)	Andromed (6)	Tris (7)
Frozen thawed semen after 24hours	42.50±1.71	34.67±1.67	28.71±1.55
Frozen thawed semen after 15days	41.67±1.67	32.33±0.67	26.67±1.67
Level of Significance	NS	NS	NS

Parenthesis indicates the number of observations; NS = Non-significant

Conception rate

To assess the conception rate, frozen semen was used for AI and three different diluters (Triladyl, Andromed and Tris) were used where each dose contained 100 million spermatozoa per 0.5 ml of straw and conception rate was calculated on non-return basis. Total 37 does were inseminated of them 18 with Triladyl, 13 with Andromed and 6 with Tris. After insemination, the number of non-returned does were 9, 6 and 2 in case of Triladyl, Andromed and Tris, respectively. The conception rate is presented by pie diagram as shown in Figure 1. Higher conception rate was obtained using Triladyl diluter (38%) followed by Andromed (35%) and Tris (27%).

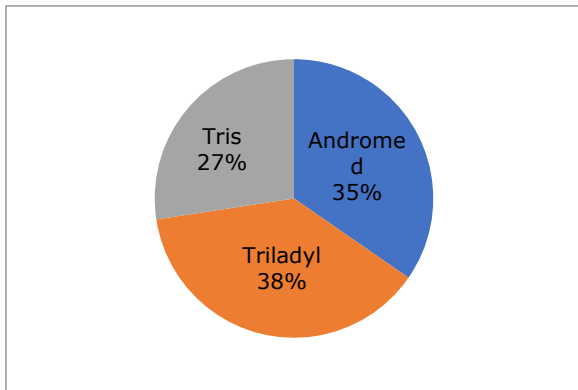


Figure 1: Conception rate using different diluters

Cost for preparation of different diluters

The pie chart showed that for preparation Triladyl diluter per doses of semen it required 6.78 Taka. On the other hand for Andromed and Tris diluter it required 3.004 Taka and 1.19 Taka respectively per dose. It can be said that Tris diluter is much cost effective.

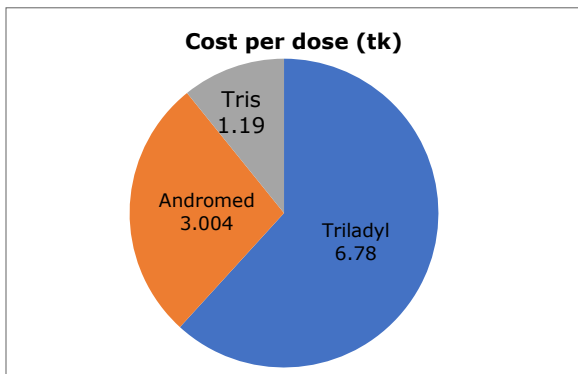


Figure 2: Cost for preparation of different diluters

Discussion

Motility of cryopreserved semen

The addition of diluents, filling and sealing of straw was done in room temperature. After 4 hours of equilibration the motility (75.67% in

Triladyl diluents, 75.33% in Andromed diluents and 73.29% in Tris based diluents) of spermatozoa at pre-freezing stage declined from raw semen which is very usual. This result also supports the results of Bane et al., (2004). Afroz et al., (2008) recorded 65.00-66.675% motility in Triladyl diluter and 63.33%-70.00% in Tris diluter. On the other hand Karim et al., (2018) recorded 67.70%-70.50% motility in Tris diluter. Both results are lower than that of result of present findings. Equilibration period of 4 hours in this study showed better rate of sperm motility which is in agreement with statement of Leite et al., (2010) found 4 hours equilibration time yielded the most desirable for total motility, progressive motility and percentage of sperm with intact plasma and acrosome membranes after post thaw and after 4 hours of equilibration. Therefore, an equilibration period of 4 hours might be suitable for freezing of goat semen.

In this study the freezing time was 20 minutes in an ice box filled with 6L LN. After freezing motility were found 51.67%, 43.33% and 37.86% in Triladyl, Andromed and Tris diluter, respectively which is lower than that of with Faruque et al., (2007). This difference could be due to the manual freezing of semen.

After freezing the straws were transferred to LN container. The sperm motility after thawing was 42.50% in Triladyl diluter, 34.67% in Andromed diluter and 28.71% in Tris based diluter. Post thaw motility values are similar to those of Bane et al., (2004), Jha et al., (2019), Apu et al., (2012), Afroz et al., (2008) and Faruque et al., (2007), indicating the freezing method was successful. In case of Tris diluter Afroz et al., (2008) obtained 6.00%-6.67% and Rahman et al., (2018) obtained 14.83% post thaw motility which is lower than that of present findings.

Viability of cryopreserved semen

Viability of diluted semen after equilibration obtained in the present study was 78.57%, 74.07% and 72.76% in Triladyl, Andromed and Tris based diluter, respectively. The result is similar to that of Kalyani et al., (2015).

The sperm viability after thawing varied from 26.84%-40.51% among three diluters (Triladyl, Andromed and Tris), which supports the result of Bane et al., (2004) and Motamedi-Mojdehi et al., (2014). Karim et al., (2018) reported 42.73% viable sperm after thawing in Tris diluter and Jha et al., (2019) reported 71.8% viable sperm after thawing in Triladyl diluter and 53.5% viable sperm in Tris diluter which is much higher than that of results of present study.

Morphology of cryopreserved sperm

Diluters effect on frozen goat semen

The result of these findings in case of abnormal spermatozoa after equilibration was consistent with the findings of Kalyani *et al.*, (2015) who found 10.68% abnormal spermatozoa in Black Bengal buck semen.

After thawing abnormal spermatozoa obtained in present study was 12.89%, 12.99% and 13.78% in Triladyl, Andromed and Tris diluter respectively which supports the findings of Apu *et al.*, (2012) and Faruque *et al.*, (2007), but slightly higher than the findings of Bane *et al.*, (2004). The normality of sperm after thawing was 87.11% in Triladyl based diluter, 87.01% in Andromed based diluter and 86.22% in Tris based diluter which is much higher than that of results of Karim *et al.*, (2018) and Dorado *et al.*, (2007).

Observation of frozen thawed semen motility after 15 days

Motility of frozen thawed semen after 15 days obtained in the present study was 41.67%, 32.33% and 26.67% in Triladyl, Andromed and Tris diluter respectively which is nearly close to the motility of frozen thawed semen after 24 hours and which is fit for use. The finding of motility of frozen thawed semen after 15 days is similar to the findings of Mansur *et al.*, (2018).

Conception rate

For assessment of conception rate, frozen thawed semen was used in artificial insemination (AI) for farmers does. Three different diluters (Triladyl, Andromed and Tris) were used where each dose contained 100 million spermatozoa per 0.5 ml of straw. The higher conception rate was obtained using Triladyl diluter followed by Andromed and Tris in the order of 38%, 35% and 27%, respectively. From the quality tests of semen after dilution, it was found that motility, viability and normality were higher in semen diluted with Triladyl than the other two. That's can be the reason of having more conception while Triladyl based semen was used.

This observation supports the result of Gacitua and Arav (2005) who reported 38.9% pregnancy rates using frozen-thawed semen. On the other hand, Dorado *et al.* (2007) obtained 42.9% conception rate with frozen semen which is in close agreement with this study.

Karatzas *et al.* (1997) and Mara *et al.* (2007) also reported higher conception rate than the result of the present study. The higher conception rate recorded by these researchers could be attributed to the evaluation of conception rate on a large population, whereas in this study fertility rate was collected on a non-return basis and the sample size employed was too small.

Cost for preparation of different diluters

Cost is a crucial issue in goat frozen semen production. Because most of the farmers do not use AI method due to the higher cost of frozen semen. The cost is high because of using double doses of frozen semen. From the figure 2 we can see that Tris diluter is cost effective than the commercial diluters. It required 1.19 tk for per dose preparation. Tris diluter is cost effective because only locally manufactured ingredients are used in the preparation of this diluter.

Conclusion

The study was carried out to compare the efficacy of three diluters for frozen semen production of Black Bengal goat. The results of the study clearly demonstrated that frozen thawed semen quality significantly varied among different diluters but in case of morphology it did not differ significantly. Highest fertility was observed in Triladyl diluter and cryopreservation of buck semen offers good results with Triladyl diluter followed by Andromed and Tris diluter. Semen must be preserved in a perfect medium to maintain its quality. So, emphasis should be given on the selection of suitable diluters for the cryopreservation of semen. This study will help to select a suitable diluter.

Acknowledgements

We are gratefully acknowledging Ministry of Science and Technology, Government of the People's Republic of Bangladesh for funding.

Author's contribution

Mst. Afsana Rimi: Conceptualization, design of the study, methodology, investigation, analysis and interpretation of data, writing-original draft, writing-review and editing. **Tasmina Akter:** Conceptualization, design of the study, investigation, analysis and interpretation of data, revision and drafting of the manuscript, final approval of the version to be submitted. **Mst. Mahomudha Akhtar:** Conceptualization, design of the study, investigation, analysis and interpretation of data, revision and drafting of the manuscript, final approval of the version to be submitted. **M. A. M. Yahia Khandoker:** Conceptualization, design of the study, investigation, revision and drafting of the manuscript, final approval of the version to be submitted.

Conflicts of interest

The authors declare that there is no conflict of interest of any person, company or any aspect of the impact of the manuscript.

Data Availability

All the necessary data used in this research will be made available as per the authorization of the authors.

Compliance of ethical standards

This research complies with the ethical standard required for the research in Bangladesh in relation to the handling of biological material.

Consent for publication

All authors are fully agreed to publish this research in Bangladesh Journal of Animal Science.

References

- Afroz S (2005). Cryopreservation of buck semen. MS thesis, Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh.
- Afroz S, Islam M, Khandoker MAMY, Akter QS (2008). Cryopreservation of Black Bengal buck semen: Effects of diluents and freezing on sperm motility and morphology. *Animal Science Journal*, 79: 550-553.
<https://doi.org/10.1111/j.1740-0929.2008.00563.x>
- Apu A, Husain S, Khandoker M, Rahman A, Notter D (2012). Investigation on seminal attributes and fertility of Black Bengal goat. *Bangladesh Journal of Animal Science*, 37: 17-24.
<https://doi.org/10.3329/bjas.v37i2.9877>
- Bane KB, Takarkhede RC, Sahatpurej SK, Kuralkar SV, Bondp SW (2004). Effect of different glycerol levels in tris egg yolk citrate diluent on freezability of buck semen. *Indian Journal of Animal Sciences*, 74: 741-742.
- Dorado J, Rodri'guez I, Hidalgo M (2007). Cryopreservation of goat spermatozoa: Comparison of two freezing extenders based on post-thaw sperm quality and fertility rates after artificial insemination. *Theriogenology*, 68: 168-177.
<https://doi.org/10.1016/j.theriogenology.2007.04.048>
- Faruque MH, Bari FY, Siddiqui MAR, Shamsuddin M (2007). Fertilizing capacity of buck (*Capra hircus*) semen frozen with different concentrations of egg yolk. *Journal of the Bangladesh Agricultural University*, 5: 95-104.
- Gacitua H, Arav A (2005). Successful pregnancies with directional freezing of large volume buck semen. *Theriogenology*, 3: 931-938.
<https://doi.org/10.1016/j.theriogenology.2004.05.012>
- Hernández-Avilés C, Love CC, Serafini R, Ramírez-Agámez L, Friedrich M, Ghosh S, Varner DD (2020). Effects of glucose concentration in semen extender and storage temperature on stallion sperm quality following long-term cooled storage. *Theriogenology*, 147: 1-9.
<https://doi.org/10.1016/j.theriogenology.2020.02.007>
- Husain SS (2007). Preservation of buck semen and their use in artificial insemination for rapid genetic improvement of rural goat population. Final Report, *Bangladesh Agricultural University Research Systems*, Mymensingh.
- Husain SS, Horst P, Islam ABMM (1996). Phenotypic selection on the improvement of growth performance of Black Bengal kids. *Asian-Australasian Journal of Animal Sciences*, 9: 149-153.
<https://doi.org/10.5713/ajas.1996.149>
- Jha PK, Alam MGS, Al-Mansur MA, Naher N, Islam T, Bhuiyan MU, Bari FY (2019). Cryopreservation of Bangladeshi ram semen using different diluents and manual freezing techniques. *Cryobiology*, 89: 35-41.
<https://doi.org/10.1016/j.cryobiol.2019.06.001>
- Kalyani R, Singh L Gojen, Sarkar Bidhan (2015). Cryopreservation of Black Bengal buck semen by Tris-based extenders containing different levels of egg-yolk. *Annals of Veterinary and Animal Science*, 2: 47-54.
- Karatzas G, Karagiannidis A, Varsakeli S, Brikas P (1997). Fertility of fresh and frozenthawed goat semen during the non-breeding season. *Theriogenology*, 48: 1049-59.
[https://doi.org/10.1016/S0093-691X\(97\)00331-2](https://doi.org/10.1016/S0093-691X(97)00331-2)
- Karim MF, Khandoker MAMY, Husain SS (2018). Fact findings about the frozen buck semen characters freezing with EYC and Tris extender and productivity of Black Bengal does as the potential genetic resource in Bangladesh. *Research in Agriculture Livestock and Fisheries*, 5: 341-350.
<https://doi.org/10.3329/ralf.v5i3.39583>
- Khandoker MAMY (2007). Conservation of Black Bengal goat as the potential genetic resource in Bangladesh. Annual Research Report (2006-2007). *USDA-Bangladesh collaborative research project*, 44: 2.
- Leite TG, do Vale Filho VR, de Arruda RP, de Andrade AF, Emerick LL, Zaffalon FG, Martins JA, de Andrade VJ (2010). Effects of extender and equilibration time on post-thaw motility and membrane integrity of cryopreserved Gyr bull semen evaluated by CASA and flow cytometry. *Animal Reproduction Science*, 120: 31-38.
<https://doi.org/10.1016/j.anireprosci.2010.04.005>
- Mansur MA, Alam MGS, Jha PK, Rimon MA, Naher N, Bari FY (2018). Conception rate following intra-cervical artificial insemination using frozen semen at field level in indigenous sheep of Bangladesh. *Asian Journal of Medical and Biological Research*, 4: 55-62.
<https://doi.org/10.3329/ajmbr.v4i1.36822>
- Mara L, Dattena M, Pilichi S, Sanna D, Branca A, Cappai P (2007). Effect of different diluents on

Diluters effect on frozen goat semen

- goat semen fertility. *Animal Reproduction Science*, 102: 152-157.
<https://doi.org/10.1016/j.anireprosci.2007.02.007>
- Motamedi-Mojdehi R, Roostaei-Ali Mehr M, Rajabi-Toustani R (2014). Effect of different levels of glycerol and cholesterol-loaded cyclodextrin on cryosurvival of ram spermatozoa. *Reproduction in Domestic Animals*, 49: 65–70.
<https://doi.org/10.1111/rda.12225>
- Raheja N, Choudhary S, Grewal S, Sharma N, Kumar N (2018). A review on semen extenders and additives used in cattle and buffalo bull semen preservation. *Journal of Entomology Zoology Study*, 6: 239-245.
- Rahman MS, Gofur M, Rahman MM, Bari FY, Juyena NS (2018). Effect of skim milk and tris-citrate extenders to preserve the semen of indigenous ram of Bangladesh. *Asian Journal of Biology*, 5: 1-11.
<https://doi.org/10.9734/AJOB/2018/39448>
- Santos M, Soares F, Moreira M, Beirão J (2018). Evaluation of different extenders for the cold storage of eager (*Argyrosomus regius*) semen. *Aquaculture Research*, 49: 2723-2731.
<https://doi.org/10.1111/are.13733>
- Soltanpour F, Moghaddam G (2014). Effect of diluents on storage of ram semen. *Journal of Agri-food and Applied Sciences*, 2: 179-183.