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A Comparative Study of Fresh and Frozen-Thawed Semen Quality in Relation to Fertility of Black Bengal Goats

Research Article

A.S. Apu[1*], M.A.M. Yahia Khandoker[2], S.S. Husain[1,2], M. Fakruzzaman[2] and D.R. Notter[3]

[1] Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh
[2] Patuakhali Science and Technology University, Dumki, Patuakhali, Bangladesh
[3] Department of Animal and Poultry Science, Virginia Polytechnic Institute and State University, USA

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*Correspondence E-mail: auvijit_bau@yahoo.com

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ABSTRACT

Semen from six adult male Black Bengal goats (*Capra hircus*) was collected to compare the fresh and frozen-thawed semen quality and investigate its relationship with the fertility. Each collected samples was divided into two parts: One part of semen was used as fresh and another part was filled into 0.50 ml straws, sealed, cooled (5 ˚C) and equilibrated for freezing. In both cases, semen was diluted with commercial Triladyl diluent (a Tris based diluter). The motility and morphology of fresh and frozen-thawed semen was subjectively evaluated by one operator. It was revealed that motility and sperm abnormality of the frozen semen differed significantly ($P<0.05$), but differed insignificantly in fresh semen between males. Besides these, fresh and frozen-thawed sperm motilities also differed significantly ($P<0.01$) between males which varied from 70.83±1.54 to 74.23±1.59% and 44.17±2.39 to 52.31±1.08%, respectively. A significantly ($P<0.01$) higher sperm abnormalities was observed in frozen-thawed semen (11.18±0.42 to 16.55±0.09%) than that of fresh semen (8.82±0.24 to 9.71±0.52%). Cervical inseminations were performed with fresh and frozen-thawed semen in 997 and 1004 female goats, respectively, both at Nucleus Breeding Flock (NBF) and four project areas. Fresh semen showed significantly ($P<0.01$) higher kidding rates (59.8%) than that with frozen-thawed semen (43.9%). The motilities of fresh and frozen-thawed semen were positively correlated (0.526 and 0.987; $P<0.01$), whereas the proportion of morphologically abnormal spermatozoa was negatively correlated (-0.530 and -0.776) with fertility of Black Bengal goats. Males with a higher motility and lower proportion of abnormal spermatozoa provided better fertility.

KEY WORDS Black Bengal, fertility, frozen-thawed, sperm abnormality, Tris diluter.

INTRODUCTION

Bangladesh possesses a tropical goat breed popularly known as Black Bengal goat. They are dwarf in size and noted to be famous for their adaptability, fertility, prolificacy, delicacy of meat and superior skin quality (Husain *et al*. 1996). It is well adapted to hot and humid climate and usually produces twins and triplets. However, it has been observed that there exists sizeable genetic variation with the traits between individuals within and between locations (Amin *et al*. 2000). These variations can be exploited for genetic improvement using selective breeding within the breed (Husain *et al*. 1996; Akhter *et al*. 2000). But it is a matter of great concern that unlike cattle, goat raisers castrate almost all of their male kids at their early age. Consequently, availability of breeding bucks become squeezed day by day (Amin, 1999). Therefore, provision of Artificial Insemination (AI) using selected buck semen is the easiest

way of exploiting desirable germplasm as well as overcome the existing problem within short span of time. Moreover, it is possible to preserve the present genetic material for long time in a biologically safe deposit vault (Johnston and Lacy, 1995). The development and widespread use of frozen semen materially paved the way for greater use of superior bucks. Moreover, Genome Resource Banking (GRB) through semen cryopreservation is a fundamental conservation strategy for any potential genetic resource. In combination, reproductive biotechnologies like AI allow to produce more offspring from the selected pairs and thus ensuring genetic diversity and reducing the interval between generations.

Though AI has gained widespread acceptance in dairy cattle industries of most developed countries and now-a-days also popular in Bangladesh. But it has not yet received such universal acceptance in goat industries of Bangladesh. Moreover, AI in goats with cryopreserved semen is still not as developed as it is with dairy cows.

Goats present a wider range of fertility when frozen-thawed semen is used, varying from 3% up to 70% (Corteel, 1973). Irrespective of the process used for freezing, differences have been observed between males regarding the freezability and fertility of semen, so they could be classified as 'good freezers' or 'bad freezers'. This variability is relatively independent of prior semen quality, and the semen of certain individuals consistently freezes with less cryoinjury than that of others (Corteel *et al*. 1987). Besides these, successful fertilization depends on a lot of factors like semen quality, their processing, number of spermatozoa per dose, time of insemination relative to ovulation (Dauzier, 1966), site of semen deposition, duration of sperm transport in the female genital tract and time of survival of male and female gametes. In addition, the physiological condition of the doe, parity, their nutritional status also affect the fertility.

On the other hand, fertility after cervical insemination with fresh and frozen-thawed semen also shows variable results in different goat breeds between the reports (Karatzas *et al*. 1997; Gacitua and Arav, 2005) in the world. These inconsistent reports prompted us to compare the fertility of fresh and frozen-thawed buck semen in Black Bengal goat breed as well as find out the relationship of fertility with semen quality to improve this goat breed.

MATERIALS AND METHODS

Animals

Six adult Black Bengal male goats were selected based on body weight, scrotal circumference (SC), libido and also their ability to produce semen having greater than 80% morphologically normal spermatozoa with satisfactory motility and concentration. The bucks aged between 18 to 22 months. The body weight and SC of bucks were 19.0 to 25.0 kg and 17.0 to 22.0 cm, respectively. These bucks were managed and raised under confinement as an intensive system. They were housed in individual pens of one square meter in a galvanized iron sheet shed with a wooden slatted floor raised above the ground level. The house was provided with necessary arrangements for feeding and watering and for sufficient access to fresh air. The bucks were kept under zero grazing management and stall fed twice daily on a diet consisting of Napier, German and/or Maize fodder *ad libitum*. The feed was supplemented with commercial concentrate in pellet form in the morning and again in the afternoon at the rate of 400 gm/buck/d (crude protein content: 120 g/kg DM and energy content: 10.4 MJ ME/kg DM). The breeding bucks were also supplied with germinated gram (20 gm/buck/d). They were allowed for exercise for 1 to 2 hours daily. Clean and safe water was made available at all times. Throughout this study, the nutrition of bucks remained uniform. A general management program including dipping, disease prevention, and hoof trimming was applied. All bucks were vaccinated against Peste des Petits Ruminants (PPR) and dewormed routinely with *Ivermectin* twice yearly.

Preparation of extender

Triladyl, a Tris based diluter is a complete mixture of diluter including cryoprotectants for the extension of semen. A stock solution of commercially available Triladyl based diluents (Minitiub, Germany) was prepared by mixing 20% of Triladyl diluents, a medium made up of 3.8% Tris (w/v), 2.2% citric acid (w/v), 0.6% glucose (w/v), Aqua bidistillata, Acidum, Citricum, Gentamycin, Tylosin, Spectinomycin, Lincomycin (8.4%), 5% glycerol (w/v) and 15% egg yolk and 65% distilled water.

Semen collection, evaluation and processing

Male goats were trained to ejaculate in artificial vagina (AV) at heterosexual mount. Semen was collected using AV (41 ˚C) twice a week within 8.00 to 8.30 A.M into a pre-warmed (30 ˚C) graduated collection vial. Semen volume was measured directly from the graduation of the collection vial. Then, the freshly collected semen was immediately transferred to the laboratory and emerged in a water bath at 37 ˚C until the media and reagents were added with it. Concentration of spermatozoa was determined by hemacytometer method (Herman and Madden, 1963). Then, the collected semen sample was extended with commercial Triladyl diluter maintaining a concentration of 200×10^6 spermatozoa per ml diluted semen and splitted into two equal parts. Sperm motility of the extended semen was assessed microscopically by examining a uniform drop of

semen placed on a clean, pre-warmed slide. Semen samples were stained with Rose Bengal stain and examined microscopically for sperm morphology (Herman and Madden, 1963).

For the preparation of frozen semen, another part of extended semen was filled into medium French Polyvinyl straws (0.50 mL) containing 200×10^6/mL spermatozoa. The straws of semen were subjected to computer assisted freezing protocol using software Cryogenesis V5. The straws were placed in freeze controlled cryochamber to be frozen in liquid nitrogen vapor for 30 minutes and then stored in the liquid nitrogen container at -196 ˚C until used for AI. After 24 h of storage in liquid nitrogen, sample of the frozen straws were retrieved from the LN container using tweezers and thawed in a thermostatic water bath at 37 ˚C for 12 seconds. The post-thaw motility and abnormality were examined as described before (Herman and Madden, 1963).

Artificial insemination (AI)

To observe the effect of male goat as well as measure the difference of fresh and frozen-thawed sperm motility, morphology and fertility, semen was collected from six bucks. One hundred ejaculates from each male goat was taken for the study and after necessary processing, a total of 997 does were inseminated with fresh semen and 1004 does were inseminated with the frozen-thawed semen at Nucleus breeding flock in Bangladesh Agricultural University Artificial Center and four project areas. All of the does were inseminated by single insemination at their natural estrus. Estrus behavior of the does were monitored twice daily (6:00 am and 5:00 pm) using teaser buck (Teaser buck is the castrated male that has no capability to mate). Immobilization of the female when mounted by the male was considered to be sign of occurrence of estrus (Mauleon and Dauzier, 1965). After showing the behavioural sign of estrus, AI was performed after 24 hours to maintain proper timing which was close to their ovulation (Rao and Bhattacharyya, 1980). Fertility was calculated as the percentage of inseminated females actually kidding.

Statistical analyses

The data generated from this experiment were entered in microsoft Excel worksheet, organized and processed for further analysis. Descriptive statistics were performed to calculate mean, standard errors and percentages. One way analysis of variance (ANOVA) was performed to observe the effects of individual adult male goat on sperm motility and morphology according to the method of Steel and Torrie (1980). Moreover, paired sample t-test was used to find the difference between fresh and frozen parameters of semen and kidding rate. Besides these, Pearson's correlation coefficients were estimated to find out the relationship between semen quality and fertility. All statistical analyses were performed using SAS (1998) software.

RESULTS AND DISCUSSION

Fresh and frozen-thawed semen quality of Black Bengal goat is presented in Table 1. Significant statistical difference ($P<0.01$) was observed between fresh and frozen-thawed semen quality. On the other hand, individual buck showed significant effect ($P<0.05$) only in frozen-thawed sperm motility and abnormality but insignificant in fresh stage. Fresh semen motility of six adult male Black Bengal goats ranged from 70.83±1.54 to 74.23±1.59%, whereas frozen-thawed sperm motility varied from 44.17±2.39 to 52.31±1.08%. Besides these, sperm abnormalities were from 8.82±0.24 to 9.71±0.52% in fresh semen and 11.18±0.42 to 16.55±0.09% in frozen-thawed semen.

The fertility of male Black Bengal goats was assessed through kidding rate of goats using fresh and frozen-thawed semen which is also shown in Table 1. Statistical analysis showed that kidding rate using fresh semen (59.8%) was significantly ($P<0.01$) higher than that with frozen-thawed semen (43.9%).

Table 2 showed that fresh and frozen-thawed sperm motility was positively correlated (0.526 and 0.987; $P<0.01$), whereas the proportion of morphologically abnormal spermatozoa was negatively correlated (-0.530 and -0.776) with fertility of Black Bengal goats.

Semen quality of fresh and frozen-thawed semen

The present study compared the fresh and frozen-thawed semen quality in respect of fertility levels of Black Bengal goats. Motility and structural integrity represent a good test for evaluating semen quality from the view point of cell functionality (D'alessandro *et al*. 2001). Therefore, we assessed sperm motility and abnormality at before and after freezing. Sperm motility and abnormality in fresh semen of different adult male goats showed similar result as reported by other investigators (Karatzas *et al*. 1997; Afroz *et al*. 2008). On the other hand, the average values of frozen thawed sperm motility and abnormality obtained in this study fall within the ranges that other authors have taken to indicate that the freezing method has been successful (Karatzas *et al*. 1997; Gacitua and Arav, 2005**;** Dorado *et al*. 2007; Afroz *et al*. 2008) and may be considered acceptable semen quality for commercial use of frozen-thawed goat spermatozoa. However, the results indicated that sperm motility decreased and abnormal spermatozoa percentage increased following cryopreservation. During freezing and thawing, many factors can affect the maintenance of spermatozoa function such as freezing method, eq-

Table 1 Fresh and frozen-thawed semen quality and fertility of Black Bengal goats

Buck ID	Motility (%)		Abnormal spermatozoa (%)		Kidding rate (%)	
	Fresh	Frozen-thawed	Fresh	Frozen-thawed	Fresh	Frozen-thawed$^{\Psi}$
BBG1	73.5±1.30	47.50±2.00^{ab}	8.82±0.24	13.94±0.31^{bc}	56.3 (72/128)	44.2 (65/147)
BBG2	70.83±1.54	44.17±2.39^{b}	9.44±0.13	16.55±0.09^{a}	53.0 (96/181)	35.6 (47/132)
BBG3	74.23±1.59	52.31±1.08^{a}	8.89±0.33	12.84±0.29^{c}	68.6 (109/159)	53.4 (109/204)
BBG4	72.92±1.29	48.33±1.98^{ab}	8.75±0.13	11.18±0.42^{d}	62.8 (93/148)	46.5 (74/159)
BBG7	73.33±1.67	46.67±2.11^{ab}	9.71±0.52	13.91±0.30^{bc}	57.2 (107/187)	41.8 (74/177)
BBG11	71.11±1.39	46.11±1.62^{b}	9.22±0.31	14.79±0.74^{b}	61.3 (119/194)	38.9 (72/185)
Pooled	72.65±0.56	47.52±1.12	9.14±0.13	13.87±0.38	59.8 (596/997)	43.9 (441/1004)
T-test	**		**		**	

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
** P<0.01.
Parenthesis indicates total number of kidding divided by total number of does inseminated.
$^{\Psi}$ Kidding rate using frozen-thawed semen showed significant difference between the male goats (P<0.05).

uilibration periods and cooling rate (Maxwell and Salamon, 1993). In agreement with previous studies (Salamon and Maxwell, 1995), the freezing thawing process reduces motility to a lesser degree than structural integrity. This means that the plasma and acrosome membranes are more vulnerable than the parts of the spermatozoa involved in locomotion (Salamon and Visser, 1972).

Table 2 Relationship between fresh and frozen-thawed semen quality with fertility of Black Bengal goats

Form of semen	Relation	Correlation coefficient
Fresh	Motility-Abnormality	-0.399
	Motility- Fertility	0.526
	Abnormality-Fertility	-0.530
Frozen-thawed	Motility-Abnormality	-0.704
	Motility- Fertility	0.987**
	Abnormality-Fertility	-0.776

** P<0.01.

In this study, Triladyl extender (a Tris-based extender) provided beneficial cryoprotection of motility and morphology of the spermatozoa during the freezing procedure. It is possible that cryopreservation process may be affected not only by diluent's components, such as buffer system and osmotic pressure, but also by interactions between the various components of the extenders (Garde *et al*. 2003). In this context, frozen-thawed samples were diluted with the same extender as used fresh in order to obtain a correct interpretation of sperm parameters in post-thaw data (Dorado *et al*. 2007).

Fertility

Fertility was calculated on the basis of kidding rate by using fresh and frozen-thawed semen. The overall kidding rate achieved with fresh semen (59.8%) is regarded satisfactory and lies within the range (55 to 65%) as observed by some authors in different breeds of does (Karatzas *et al*. 1997; Gacitua and Arav, 2005). On the other hand, the overall kidding rate with frozen thawed semen found in our present study was 43.9%. This fertility rate is also similar to those results obtained by several investigators in different breeds: 43.3% in Angora goat (Ritar and Salomon, 1983), 42.9% in Florida goat (Dorado *et al*. 2007), 39.1% in Cashmere goat (Ritar *et al*. 1990) and 38.9% in Saanen goat (Gacitua and Arav, 2005).

On the other hand, difference in fertility using frozen-thawed semen was found significant between different male goats but in fresh semen no significant difference was observed. This variability in fertility of frozen semen can be attributed to differences in freezability, results of chilling injury (Drobnis *et al*. 1993) and fertilizing capacity of the semen. Some buck's semen are more affected by cryoinjury than others (Corteel *et al*. 1987) which is probably attributable to their membranes biochemical and biophysical properties (Arav *et al*. 2000). Besides these, the form of semen (fresh and frozen-thawed semen) significantly influenced the fertility. The kidding rate with fresh semen was significantly higher (59.8%) than that with frozen thawed semen (43.9%).

The previous studies also showed significant difference between these two forms of semen (Ritar and Salomon, 1983; Karatzas *et al*. 1997). Fertility after cervical insemination with frozen-thawed semen is relatively low compared with fresh semen (Maxwell and Salamon, 1993). Most mammalian species show a reduction in fertility after cervical insemination with frozen semen, which is associated with the damage that occurs during cryopreservation that reduces motility to 40-50%, thereby requiring an increase in the number of sperm necessary to achieve reasonable fertility (Watson, 2000).

CONCLUSION

The results of the study clearly present the significant difference of fresh and frozen thawed semen quality and fertility of Black Bengal goats. Furthermore, frozen thawed semen quality significantly varied between different male go-

ats. Therefore, emphasis should be given on the selection of high quality ejaculates of male adult Black Bengal goats with higher motility and lower abnormal spermatozoa to improve the fertility as well as conserve the present diversity of this breed for future use.

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