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*Research Article*

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# Pregnancy Rate of Black Bengal Does Inseminated With frozen-thawed Boer and Jamnapari Buck Semen

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**Abstract**

The quality of frozen-thawed semen collected from two different breeds of the buck was assessed based on plasma membrane functional integrity percentages, progressive motility percentages, sperm kinetic velocity and fertility after artificial insemination (AI) in Black Bengal Does. The fitness of a commercial semen extender (Andromed) was also evaluated in comparison to a traditional semen extender (Tris-citrate-fructose buffer). Collected and examined semen were used for AI in 36 Black Bengal does with natural heat. Results showed that the sperm plasma membrane functional integrity, progressive motility, sperm kinetics parameters such as beat cross frequency (BCF), linearity (LIN), straightness (STR), average path velocity (VAP) in fresh Boer semen was significantly higher than that of Jamnapari. There were no significant ($P > 0.05$) differences between frozen-thawed semen of Boer and Jamnapari buck in terms of CASA parameters except for BCF, LIN and VAP. However, Andromed diluted semen was found better in quality than that of semen with traditional extender irrespective of breeds of bucks. The study reports that there is no significant difference among pregnancy rates of Black Bengal does inseminated with Boer and Jamnapari buck semen and satisfactory pregnancy rate could be obtained after AI with Andromed diluted frozen-thawed buck semen than that of traditional semen extender. So, the Andromed could be an alternate extender in freezing buck semen.

**Keywords:** Fertility, Frozen-thawed semen, Crossbred bulls, extender.

## INTRODUCTION

Selective breeding of animals to improve the species requires an artificial insemination (AI) program using semen from males with high genetic merit (Roberts and Foote, 1989). AI is a reproductive technology that has made possible use of best breeding males (Januskauskas and Zilinskas, 2002). The success of AI depends on the maintenance of viability, motility and fertilizing capacity of spermatozoa during storage. For this purpose, sperm cryopreservation extenders are used. Fertilizing capacity of spermatozoa enhances when extenders are used (Shamsuddin *et al.*, 2000). Extender with cryoprotectant agent has been used to provide some protection to spermatozoa and to minimize the adverse effects of cryopreservation (Katila, 1997). Routine semen analysis allows evaluation of the fertility potential of a male and seminal material quality. Membrane integrity is an indicator of sperm vitality and it is necessary to maintain sperm function. Semen cryopreservation is an effective technology for improving breeding programs (Yodmingkwan *et al.*, 2016). The success of cryopreservation depends upon many factors, these are types of extender, cooling rate, thawing rate, packaging,

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interactions between cryoprotectant and even the individual animal variation (Andrabi and Maxwell, 2007; Clulow *et al.*, 2008; Cooter *et al.*, 2005).

In this circumstance, superior goat selection seems to be very important and alternative approach to boast up the production potential. Consequently, during selection of breeding buck special attention should be given on sperm quality to increase pregnancy rate. Although the boer and Jamanapari breeds are available in Bangladesh, there is no study on their semen quality parameters and pregnancy rate in Black Bengal goats. The study was thus designed to determine and compare the fertility of Black Bengal does inseminated with fresh and frozen semen of Boer and Jamnapari bucks. The effects of different semen extenders in bucks was also evaluated.

# MATERIALS AND METHODS

## Selection of breeding bucks

Two adult pure breeds of Boer and Jamnapari buck of 24 to 36 months of age and 45-58 kg body weight with good physical and reproductive health were selected for the study.

The bucks were kept in individual pens and provided plenty of green fodder (1 kg/buck/day), fresh water and formulated concentrate at the rate of 1.0 kg/buck/day. They were allowed for grazing and exercise for 1 to 2 hours daily.

## Preparation of semen extender

The manually prepared extender used in this study was composed of TRIS (2.42% w/v), fructose (1% w/v), citric acid (1.36% w/v), egg yolk (2.5% v/v), glycerol (7% v/v), streptomycin (100 mg/ml) and penicillin G (100 μg/ml) respectively (Qureshi *et al.*, 2013). The commercial extender used in the study was AndroMed® and has been prepared according to manufacturer instructions (Minitub, Germany).

## Semen Collection

Semen was collected from bucks by Artificial Vagina (AV) method after stimulating with an estrus doe. Semen was collected once a week during the study period. At least one ejaculate from each buck was collected and assessed for semen volume and color. The collected semen was then shifted immediately to a water bath at 37° C until the media were added.

## Macroscopic evaluation

The volume of semen was recorded by reading the graduated mark of the collection vial in milliliter. Color was observed by the naked eye in the collecting tube immediately after collection.

## Microscopic evaluation

To evaluate the mass activity, a drop (0.5μl) of semen was placed on a slide (37°C) without any cover slip and examined under microscope equipped with phasecontrast optics 10x. The mass activity was scored into 5 scales Scale 1: no motion; Scale 2: free spermatozoa moving without forming any waves; Scale 3: small, slow moving waves; Scale 4: vigorous movement with moderately rapid waves and eddies; Scale 5: dense, very rapidly moving waves and distinct eddies. Motility of spermatozoa was examined by a drop of 0.5μl semen diluted with TRIS at 1:4 ratios. The solution was placed on a clean pre- warmed slide (+37°C) and covered with a cover slip. The motility was determined by eye-estimation of the proportion of spermatozoa moving progressively straight forward at higher magnification (400x) and expressed as percentage. The concentration of spermatozoa (million/ml) was determined by using spectrophotometer. Semen samples were diluted with distilled water (1:400) to kill the spermatozoa. Photometer was set at zero by using normal saline water and then 20 μL semen samples was mixed in 2 ml of normal saline water (Atiq *et al.*, 2011).

## Analysis of kinetic velocity of semen

The motility characteristics of fresh spermatozoa were analyzed by Computer Assisted Semen Analyzer (CASA) (IVOS II, IMV Technologies, France) after dilution with Phosphate buffer saline (PBS). Motility analysis was carried out in 5μl of semen samples placed onto a pre-warmed (37°C) microscopic slide covered with 18 mm × 18 mm coverslip. A minimum of 200 spermatozoa from one drop was analyzed for each sample. Various motion parameters of spermatozoa like motility (MOT %), progressive motility %, straight-line speed, curvilinear velocity, linearity, lateral head displacement and average path velocity, straightness % and beat cross frequency were evaluated and recorded. CASA settings used for this study: Progressive STR %-60, Progressive VAP (μm/s) -50, Head size max ($\mu m^2$)-70, Head size min ($\mu m^2$)-6, Illumination Intensively Visible-2562, Camera gain -300, Stage temperature:

37°C, Frame capture speed (Hz)-60, Frame count-30 & Calibration (objective magnification; X-1.21 & Y-1.2).

### Plasma membrane integrity test

A hypo-osmotic swelling test (HOST) was used to evaluate the functional integrity of the sperm plasma membrane. This was performed by incubating 20 µl semen with 200 µl of a 75 mOsm/L hypoosmotic solution (made of 13.5g fructose and 7.35g sodium citrate in 1000 ml distilled water) at 37°C for 60 min. After incubation, 5µl of the mixture was spread with a coverslip on a warm slide. A total of 200 sperms were evaluated in different microscopic fields at 40X objective. The percentage of spermatozoa with swollen and curled tails was recorded as HOST (+ve) cells.

### Preservation of semen

After collection and evaluation of semen, the ejaculates of each goat were diluted with TRIS-based egg yolk extender and AndroMed extender. One step dilution method was used to freeze the semen in this experiment. Within the wide range of semen density in buck semen, most of the ejaculates being processed are extended at a dilution ratio between 1:4 and 1:8. Diluted semen samples were drawn into 0.25 ml French straws (Minitub, Germany) and sealed with polyvinyl alcohol powder. The sealed straws were placed in the refrigerator at +4°C for further equilibration for 2 hours. After equilibration, the straws were frozen in liquid nitrogen vapors in a special box for 5-6 minutes. After that straws were transferred into cryocan at -196°C.

### Artificial Insemination

Semen was inseminated transcervically in does after observing natural heat. Semen was thawed by plunging the frozen straws in water bath at 38° – 40°C for 10-12 seconds (Salamon and Maxwell, 2000). Before loading the semen in the AI gun, the gun was warmed by rubbing it with a paper towel to avoid cold-shocking of semen.

### Pregnancy diagnosis by ultrasonography

All inseminated does were monitored for non-return to estrus 17-21 days following insemination. The does which were in non-return to estrous were allowed for Transrectal ultrasonography (MUIV ultrasonography, Bionet®, Korea) using transducer frequency 5 mHz within 45-50 days of post insemination.

### Statistical analysis

All values relating to semen evaluation parameter were expressed as mean ± standard error mean (SEM). Two way analysis of variance (ANOVA) was done to find out significant differences in frozen-thawed semen parameters of the buck. Chi Square test was used to determine statistical differences in pregnancy rates of does in different groups. All the statistical analyses were done using SPSS 20.0. P-value of 5% was considered as level of significant.

## RESULTS

The present study focused on the quality of Boer and Jamnapari bucks semen determined by the fertility rate of Black Bengal does inseminated with fresh and cryopreserved semen processed with Tris based and Andromed extenders. The volume, mass activity, concentration of spermatozoa, total motility and sperm plasma membrane functional integrity of fresh semen of Boer and Jamnapari was evaluated. The sperm plasma membrane functional integrity in Boer semen ($67.82 \pm 1.14\%$) was significantly higher than that of Jamnapari ($60.14 \pm 1.48\%$) (Table 1).

Table 1: Comparison on general characteristics (Mean ± SE) of fresh buck semen between Boer and Jamnapari

| Parameters | Boer | Jamanapari |
|---|---|---|
| Color | Creamy white | Milky white |
| Volume (ml) | $1.09 \pm 0.15$ | $0.94 \pm 0.10$ |
| Mass activity (0-5 scale) | $3.89 \pm 0.20$ | $3.56 \pm 0.18$ |
| Concentration (X $10^6$ per ml) | $3070.56 \pm 88.19$ | $2778.44 \pm 110.62$ |
| Total motility (%) | $78.83 \pm 1.77$ | $75.82 \pm 1.56$ |
| Plasma membrane functional integrity (%) | $67.82 \pm 1.14^{**}$ | $60.14 \pm 1.48$ |

** Indicates highly significant ($P < 0.01$) variation between rows.

The progressive motility in Boer semen ($69.92 \pm 1.25$) was higher compared to Jamnapari ($65.48 \pm 1.29$) (Table 2). Sperm kinetics parameters such as amplitude of lateral head displacement (ALH), beat cross frequency (BCF), linearity (LIN), straightness (STR), average path velocity (VAP),Curvilinear velocity (VCL) and straight-line velocity (VSL) was also

studied. The BCF, LIN, VAP and STR were significantly higher in Boer semen than Jamanapari (Figure 1).

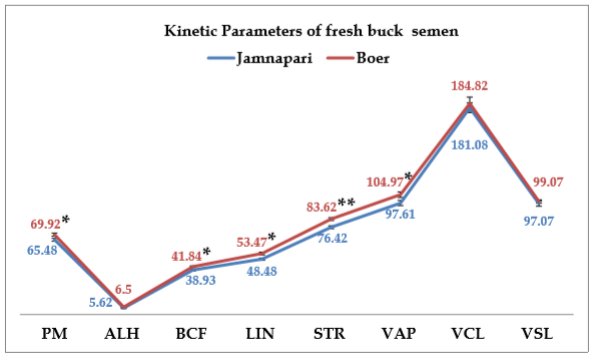


Figure 1: Comparison between Boer and Jamnapari fresh buck semen on the basis of kinetic velocity parameters. ** Indicates highly significant ($P < 0.01$) variation between rows; * Indicates significant ($P < 0.05$) variation between rows.

Kinetic parameters of frozen-thawed semen of two bucks regarding the effect of extenders used for freezing of boar semen was evaluated. The significant difference was observed in plasma membrane functional integrity, progressive motility, BCF, LIN, STR, VAP, VCL and VSL. When Andromed was used in the cryopreservation of semen and values were considered between breeds, we observed no significant differences in all values of motility parameters, except BCF, LIN and VAP. Moreover, no significant difference was observed in values of sperm ALH irrespective of extenders and breeds (Figure 2 and 3).

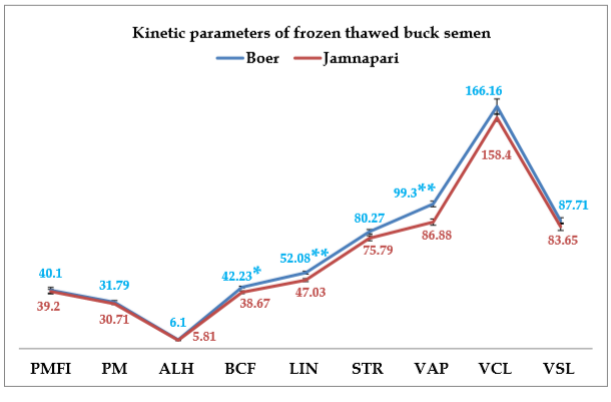


Figure 2: Comparison on kinetic parameters (Mean ± SE) of frozen-thawed buck semen diluted with Andromed between Boer and Jamnapari. ** Indicates highly significant ($P < 0.01$) variation between rows; * Indicates significant ($P < 0.05$) variation between rows.

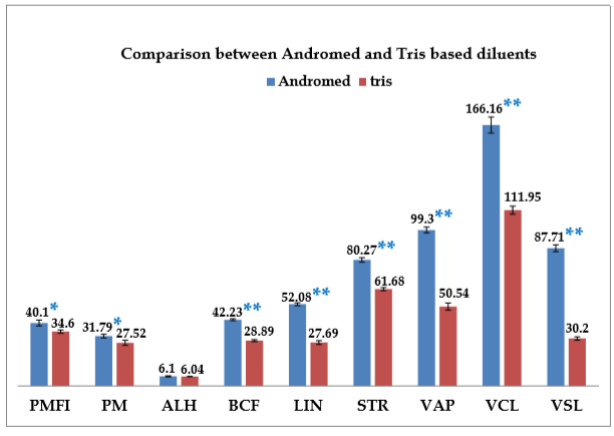


Figure 3: Comparison between Andromed and Tris diluent on the basis of kinetic parameters (Mean ± SE) of frozen-thawed Boer buck semen. ** Indicates highly significant ($P < 0.01$) variation between rows; * Indicates significant ($P < 0.05$) variation between rows.

### Pregnancy rates in Black Bengal does

The pregnancy rate of fresh semen was higher than frozen semen in both breeds of buck, while the boer semen had better result. In the case of frozen semen, pregnancy rate was better in Jamanapari (Table 2).

Table 2: Comparison on general characteristics (Mean ± SE) of fresh buck semen between Boer and Jamnapari

| **Breeds of semen inseminated to Black Bengal Goat** | **Pregnancy rate in Black Bengal Does (N=36)** | | **P-value** |
|---|---|---|---|
| | **Fresh (n=17)** | **Frozen-thawed (n=19)** | |
| Boer (n=19) | 100.00% (9/9) | 50.00% (5/10) | 0.01* |
| Jamanapari (n=17) | 87.50% (7/8) | 55.50% (5/9) | 0.04* |
| P-value | 0.27 | 0.80 | |

** Indicates highly significant ($P < 0.01$) variation between rows.

## DISCUSSION

In this study, the volume, mass activity, concentration of spermatozoa, total motility and plasma membrane functional integrity of fresh semen of Boer were $1.09 \pm 0.15$, $3.89 \pm 0.20$, $3070.56 \pm 88.19$, $78.83 \pm 1.77$, and $67.82 \pm 1.14$ while in the semen of Jamnapari were

$0.94 \pm 0.10$, $3.56 \pm 0.18$, $2778.44 \pm 110.62$, $75.82 \pm 1.56$ and $60.14 \pm 1.48$. However, the values recorded in the present study were almost similar to those reported by Salmani *et al.* (2014). Fresh goat sperm cell motility generally ranges from 80 to 90% and the post freezing sperm cell motility ranges from 23 to 65% among different goat breeds (Gacitua and Arav, 2005). In this study, sperm concentration of Boer was higher than Jamnapari. The plasma membrane functional integrity was significantly higher in Boer spermatozoa compared to Jamnapari goats. This variation could be due to the breed differences and the HOS test is not only indicative of whether the plasma membrane is intact but also an indicator of plasma membrane potentiality to be osmotically active (Colenbrander *et al.*, 2003). We observed significant in kinematics parameter of fresh semen of two breed, except ALH, VCL and VSL. Evaluation of motility and kinetic values is very important to determine sperm ability to transport of spermatozoa from site of deposition to site of fertilization. There were significant differences between Boer and Jamnapari on CASA parameters except for ALH, VCL and VSL. AI depends on maintenance of viability, motility and fertility of spermatozoa during storage. For this purpose, sperm cryopreservation extenders are used. The composition of extender and suitable cryoprotectants are important factors for successful semen cryopreservation (Curry *et al.*, 1994). In this experiment, two extenders, namely, AndroMed and Tris based citrate (2.5% egg yolk) were compared for the cryopreservation of semen from Boer and Jamnapari buck. Post-thaw motility of spermatozoa is the most widely used parameter for judging the quality of frozen semen and the potential fertility of the semen. The plasma membrane integrity of post thawed spermatozoa of Boer breed was better in Andromed than Tris extender. Results of this investigation showed that progressive motility percentage of frozen-thawed semen processed in Andromed ($31.79 \pm 1.25$) was significantly higher than TRIS-egg yolk extender ($27.52 \pm 1.65$). The progressive motility obtained in this study is higher than the values reported by Janett *et al.* (2005). In case of frozen thawed semen, higher value of hypo-osmotic swelling test integrity was recorded in Boer than Jamnapari by diluted with AndroMed in this study. Lower values of different parameters of semen evaluated for frozen-thawed semen compared to the fresh may be resulted from biophysical reaction occurred during cryopreservation. Semen cryopreservation causes ultra-structural, biochemical and functional damage to spermatozoa leading in lower motility and viability. In addition, adding diluents to semen reduces the concentration of certain components, ions and compounds in seminal plasma changing the viability of sperm. Buck seminal plasma contains an enzyme secreted by the bulbo-urethral glands, which in the presence of egg yolk, by hydrolysis, leads to the formation of lysophosphatidylcholines – which are toxic to sperm. Bulbourethral glycoprotein-60 secretion (BUSgp60) has a triacylglycerol hydrolysis activity that decreases sperm motility and quality of motion by disrupting cell membranes (Pellicer-Rubio and Combarnous, 1998). This lethal interaction between the seminal plasma and egg yolk in semen extender supplemented with osmotic disturbance,resulted in deceased semen quality. These losses must be compensated by incorporating the required components in the diluent formulations. Therefore, goat spermatozoa involves special attention in order to maximize post-thaw viability and this would be improved by a comprehensive knowledge on the freezability of goat spermatozoa in term of motility, viability and finally fertility of frozen-thawed spermatozoa. The processing of goat semen for artificial insemination or conservation requires the development or modification of appropriate protocol for a diluent. Citrate is the salt of choice as it improves the solubility of protein fractions in egg yolk by its chelating characteristics. According to Ritar *et al.* (1990) goat semen has been cryopreserved using the Tris-based extender, which includes a secure egg yolk margin to prevent coagulation and provide nutrients such as protein to sperm cells. Other studies indicated that Triscitric acid could provide the most adequate buffering scheme and act as a better diluent for goat spermatozoa (Mishra *et al.*, 2010). Chehadeh *et al.* (2001) found that Tris was the best diluent after semen dilution to maintain buck sperm motility (77.08%). Salamon and Ritar (1982) considered Tris hydroxymethyl amino methane, which is an important component of Tris diluent, is principally responsible for prolonging the preservation time by creating a buffer zone in and outside the spermatozoa. Additionally, the fructose content of the yolk Tris diluent may also assist maintain osmotic pressure and provide sperm metabolism with nutrient. Regarding buck breeds in this research and their impact on motility and viability of post-thaw semen, regardless of diluents and freezing protocol, the mean values of post-thaw semen motility, ALH, STR, VCL and VSL after thawing showed no significant distinctions for distinct buck breeds. Accordingly, the genetic influencing traits of young buck semen manufacturing are evaluated by Furstoss *et al.* (2009). They stated that there was no important impact on post-thaw semen motility

between buck breeds. Washing of buck semen to remove the seminal plasma before dilution and freezing is considered a prerequisite when using diluents containing egg yolk. However, semen washing is time consuming, may impact sperm viability and may result in loss of sperm (Gacitua and Arav, 2005). Pregnancy rate of Black Bengal does which were inseminated by fresh semen was higher (100% and 87.5%) than frozen buck semen (50% and 55.5%) collected from Boer and jamunapari bucks, respectively. This observation supports with the result of Khalifa and El-Saidy (2006). Dorado *et al.* (2007) also observed 55.90% conception rate in the Black Bengal goat using frozen semen made with Tris diluter. However, reduced fertility after vaginal or cervical AI with frozen-thawed semen seem to impaire sperm transport and decrease viability of spermatozoa in the female genital tract (Houdeau *et al.*, 2008). Therefore, to attain decent fertility rates, it is essential to boost the recovery of excellent quality sperm cells following semen cryopreservation (Gacitua and Arav, 2005). In addition, pregnancy rate of does were comparatively lower by Boer buck semen to compare with Jamnapari semen when inseminated with frozen thawed semen. In a study by Nordstoga *et al.* (2010) tested the effect of semen containing egg yolk or in AndroMed and obtained higher non-return rate (NRR) and kidding rates of 37.3% and 24.5%, respectively, after AI with semen processed with milk-based extender than that inseminated by semen diluted in AndroMed diluent. No statistically significant difference have been found in fertility rate between the two buck semen, although the fertility results, were somewhat better in Jamunapari when AndroMed was used. The lack of a significant difference in fertility rate between the two breeds may indicate that AndroMed could be an alternate extender for buck semen, thereby simplifying the handling procedure at the buck station. The fertility results after trans-cervical deposition of frozen-thawed buck semen could be considered as satisfactory. However, the sample size used for AI was very small. So, further study demands to optimize this results using larger number of does for insemination.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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