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Research article

Effects of different extenders on preservation of Jamunapari buck semen

Md. Moktadir Billah Reza, Usha Yadav, Chandra Jit Yadav, Md. Maksud Ul Alam, Bhajan Chandra Das, Tanjila Hasan and Azizunnesa*

Department of Medicine and Surgery, Chattogram Veterinary and Animal Sciences University, Khulshi, Chattogram- 4225, Bangladesh

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Corresponding author:
Cell: +8801716955099
E-mail: azizunnesa@cvasu.ac.bd

A B S T R A C T

The aim of this study was to investigate the effects of different extenders on the quality of Jamunapari buck semen. Semen was collected from three adult Jamunapari bucks by Artificial Vagina (AV) method once a week. Immediate after collection, fresh semen was evaluated for recording its quality including volume, color, density, concentration, mass motility, pH and membrane potentiality. Each semen sample was divided into two groups; one for chill and another for cryo-preservation. Three different types of semen extenders; Tris (T), Skim milk (S) and Skim milk D-glucose (SD) were added in each group of semen dividing into three parts and then preserved accordingly. Chilled semen was evaluated on day 2 and day 4 of chilling at refrigerator (4ºC) and frozen semen on day 5 and day 12 of cryopreservation at liquid nitrogen (LN_2). Preserved semen was evaluated for motility, viability, membrane potentiality (HOS test) and morphology. Average volume, mass motility, and sperm concentration were 1.22±0.26 ml, 4.01±0.67and 3.13±0.27×10^9/ml, respectively. The motility, viability, normal morphology and membrane potentiality and pH were 86.67.5±3.00, 82.13±2.18, and 96.52±1.69, 83.2±3.20, and 6.74±0.28, respectively. The quality of chill semen was the best in Tris-based extender ($P<0.05$) followed by Skim milk D-glucose based extender and skim milk-based extender. Frozen semen quality was also maintained at a higher level ($P\leq0.05$) in Tris-based extenders compared with the other two extenders studied. It was concluded that a Tris-based semen extender was the best choice for the preservation of Jamunapari buck semen in comparison with Skim milk D-glucose and skim milk-based extender.

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1. INTRODUCTION

The common extender used for cryopreservation of goat semen is either egg yolk or non-fat dried skim milk. But buck semen has some differences in the cryopreservation method compared with other domestic species because of negative interactions between the phospholipids of the egg yolk or the milk-based extender and the bulbourethral gland buck secretions in seminal plasma (Purdy, 2006). Buck semen preservation adding commonly used semen extender is difficult to maintain the quality of semen because of egg yolk coagulating enzyme which has an adverse effect on buck sperm (Leboeuf et al., 2000; Purdy, 2006). As a result,

buck semen gives rise to its coagulation and sperm die due to toxicity of sperm cells (Purdy, 2006; Sen et al., 2015). Also, there is a protein secretion from the bulbourethral gland named BUSgp60 that reduces motility and vitality of cooled and frozen sperm extended in milk-based extenders (Sias et al., 2005). Recently researchers are trying to replace the egg yolk by adding a soya-based extender containing trehalose to overcome the adverse effects of egg yolk (Narwade et al., 2017). Separation of seminal plasma by low-speed centrifugation and cryopreservation might be another method to dilute the adverse effect of egg yolk coagulating enzyme (Sen et al., 2015).

Black Bengal, Jamunapari and crossbreds (Black Bengal × Jamunapari) are available in our country. The total goat population of our country is 24. 2 million (DLS, 2012) and they contribute 8% of the total milk production (BBS, 2012). Despite a high density of livestock population, the country suffers from an acute shortage of livestock products like milk, meat and eggs. Jamunapari goat is suitable for meat and milk production. However, a huge number of goats sire suffering from infertility due to lack of proper reproductive management and traditional breeding methods (Ball and Peters, 2004). Though AI in cattle is ancient in Bangladesh, still now it has not gained popularity in our country in the goat sector due to the lack of ready or preserved semen. So, preservation of buck semen and performing AI in goat has crucial demand in the goat sector which may accelerate the conception rate with rapid genetic merit. If any convenient and superior quality buck semen extender can be produced for cryopreservation and AI methods will be established and implemented successfully in goats, it would allow increasing and improving the goat production which will meet up the protein requirement and create a sustainable goat industry which has an economic impact in the country. From the literature, it seems very little work has been done in Jamunapari buck semen preservation by adding different types of extenders to observe their effects. Therefore, we were interested to carry this study to evaluate semen extenders which will help to preserve Jamunapari buck semen by maintaining optimum quality for AI in goats.

2. MATERIALS AND METHODS

Animals and semen collection

The study was conducted at the Andrology Laboratory of the Department of Medicine and Surgery, Faculty of Veterinary Medicine, CVASU, Chattogram, Bangladesh. Three Jamunapari bucks were purchased locally from the goat owner by personal communication. Semen was collected from adult bucks by the Artificial Vagina (AV) method once a week. A total of 27 ejaculates were collected from buck during the monsoon season (October 2019 – January 2020). The collection was always performed in the morning (7:00 – 8:00 AM). After collection, semen was kept at 33-35°C in a water bath until the fresh semen evaluation and media were added with it.

Experimental design

For freezing, each semen sample was divided into three groups named Tries based (T), Skim milk (S) and Skim milk D-glucose (SD) depending on the extender used. Extenders used for chilling and cryopreservation of semen were prepared following the standard protocol (Narwade et al., 2017; Gojen et al., 2016). Chilled semen samples were evaluated on the 2nd and 4th day of chilling at the refrigerator and frozen on the 5th and 12th day of cryopreservation.

Extender preparation

Tris (T) extender was prepared using Tris (GPR®, BDH Laboratory Ltd., England), citric acid (Emprove®, Merck Ltd., Germany), fructose (D- Fructose®, Merck Ltd., India), deionized water and stored at 4°C temperature as stock solution. For making the final extender on the day of semen collection; egg yolk, penicillin and streptomycin were added to the stock solution. After then, the extender was divided into Part-T1 (without glycerol) and Part-T2 (with 7% glycerol). Skim milk (S) extender was prepared using non-fatty milk powder (Shape Up®, New Zealand Dairy Product Ltd. Bangladesh) (10%, w/v) and deionized water, heated to 95°C for 10 min, after cooling to room temperature, the solution was stored at 4°C temperature as a stock solution. Skim milk D-glucose (SD) extender was prepared by adding 10.0 g skim milk, 0.2 g D-glucose (D-glucose®,

Merck Ltd., India) in 100 ml deionized water and heated at 91ºC for 10 min. This solution was cooled to room temperature and stored at 4°C temperature and used as a stock solution. On the day of semen collection, for making final extenders of S and SD, a similar protocol was applied which was used for preparing T extender. However, for the SD extender egg yolk was not added. All the glycerol added parts of extenders such T2, S2 and SD2 were kept at the refrigerator (4°C) until use and parts of extenders without glycerol (T1, S1 and SD1) were kept at 33-35°C in a water bath. For the chilling of semen, all these three types of extenders were prepared without providing glycerol accordingly and namely Tc, Sc and SDc.

Semen chilling, freezing and evaluation

Each ejaculate was examined macroscopically and microscopically. Mass motility (0–5) and concentration (10^9/ml) of spermatozoa were evaluated. Chilling of semen was done by adding the required volume of extenders were prepared without adding glycerol. For preservation and evaluation, each collected semen sample was divided into six parts, three for chilling and another three for freezing and kept in six separate eppendorf tubes. At first, three eppendorf containing semen was extended with extender parts Tc, Sc, and SDc and chilled at 4°C until evaluation. Chilled semen was evaluated on the 2nd and 4th day of preservation. Before evaluation, the chilled semen was kept at room temperature for 4-5 min to adjust the environmental temperature. For freezing, another three eppendorf containing semen sample was mixed with the extender part T1, S1 and SD1 separately at room temperature and cooled at 4ºC for 2 hrs, then extender part T2, S2 and SD2 were added, respectively. Thereafter, the extended semen (T_F, SD_F, S_F) was loaded, named, sealed and held for the next 2 hrs at 4°C. After 4 hrs of equilibration, all the straws were given nitrogen vapor and stored in liquid nitrogen. On the 5th and 12th day of preservation, frozen semen straws were thawed and evaluated for motility, viability, membrane potentiality by hypo-osmotic swelling (HOS test) and morphology (Rose Bengal Dye) (Ax et al., 2000). Eosin-nigrosin staining was used to determine the viability of spermatozoa (Mortimer, 1994). The HOS solution was prepared by mixing 9.0 g fructose and 4.9 g sodium citrate into 1000 ml deionized water. At least 200 spermatozoa were observed in different microscopic fields to calculate the proportion of reacted sperm (Revell and Mrode, 1994).

Statistical analysis

The data generated from this study were entered in a Microsoft Excel® spreadsheet, organized and processed for further analysis. Descriptive statistics were performed to calculate mean, standard deviations and percentages. Two-way ANOVA was done to evaluate the effects of three different extenders and two different preservation times on quality of spermatozoa. All statistical analyses were performed using STATA 13.0 (StataCorp 2013, College Station, TX, USA) statistical software. The level of significance was observed at the 5% level.

3. RESULTS AND DISCUSSION

Very limited report is available in Jamunapari buck semen. Our present study observed that the quality of fresh, chilled and cryopreserved semen in Jamunapari buck was at satisfactory level. For quality evaluation, volume, sperm concentration, mass motility, viability, individual motility, morphology and membrane potentiality of the fresh semen from experimental buck were evaluated (Table 1). The most advantageous of fresh semen quality is very crucial for preservation to maintain greater quality. Primarily, semen volume, concentration, motility, morphology and membrane potentiality are the important parameters that lead to ensure good quality of semen preservation for AI. The studied Jamunapari buck produced 1.22±0.26 ml semen. This result is consistent with others who showed 1.49±0.19 ml semen from Jamunapari bucks (Islam et al., 2019) but is inconsistent with Kharche et al. (2013) who observed 0.54±0.04 ml semen. The sperm concentration and motility were $3.13\pm0.27\times10^9$/ml and 86.67±3.00, respectively. One of the studies showed that the mass motility and concentration of Jamunapari buck semen were 3.58±0.14, and $3.57\pm1.05\times10^9$, respectively (Kharche et al., 2013). The viability, normal morphology and membrane potentiality were 82.13±2.18,

96.52±1.69, and 83.2±3.20, respectively which may point out the quality level of semen was good. So, the present results would carry a good message to the goat farmers if they would be interested to breed their does artificially using preserved semen.

Table 1. Quality of fresh semen in Jamunapari bucks (n=27)

Semen parameters	Measurement
Volume (ml)	1.22±0.26
Color	Creamy
Density (0-5 score)	4.00±0.15
Mass motility (0-5 score)	4.01±0.67
Individual motility (%)	86.67±3.00
Conc. of spermatozoa (10^9/ml)	3.13±0.27
Live spermatozoa (%)	82.13±2.18
Dead spermatozoa (%)	17.87±1.18
Normal spermatozoa (%)	96.52±1.69
Abnormal spermatozoa (%)	3.48±0.78
Membrane potentiality (HOS Test) (%)	83.2±3.20

The study observed the effects of three different extenders on the quality of chilled semen at day 2 and day 4 during chilling. Quality of spermatozoa including motility, viability, and membrane potentiality of chilled semen was the best ($P<0.05$) in Tris-based extender followed by Skim milk D-glucose based extender and skim milk-based extender both on day 2 and day 4 of chilling. The results were similar in trend in normal morphology of spermatozoa at day 4 of chilling but at day 2 of chilling Tris-based extended semen provided the greatest percentages of normal morphology ($p<0.01$) followed by skim milk-based and Skim milk D-glucose based extender. The effect of type of extender on chilled semen quality in goats was highly significant ($p<0.01$) throughout the preservation at 4ºC (Udeh and Oghenesode, 2011). Effects of different types of extenders on chilled goat semen varied. Some reporters found Tris-based extenders maintained better sperm quality in goat semen compared with skim milk extenders (Dorado et al., 2007). Others observed that skim milk extenders provided better semen quality in comparison with Tris-based extenders (Mohammed et al., 2012; Salvador et al., 2007). In our study, it was observed that the best quality of semen was maintained in Tris-based extender compared with skim milk. It could be due to a protein called SBUIII secreted from the bulbourethral gland of the goat which negatively reacted with a milk-based extender (Nunes et al., 1982). It was expected and also observed that the increased time of chilling from day 2 to day 4 significantly decreased ($P\leq0.01$) the quality of sperm in any extenders studied in this research (Table 2).

Cryopreservation of buck semen is challenging because of buck seminal plasma which contain an enzyme that may be detrimental to the sperm cell (Yusoff et al., 2011; Ferreira et al., 2014). It is well known that many of the researchers trying to establish a suitable extender for the preservation of buck semen. For this reason, several authors added different types of extenders to observe the effects of extenders (Nor-Ashikin and Abdullah, 2011; Mara et al., 2007). For the consistency of the current global research, we studied to find out the suitable extender for Jamunapari buck semen preservation. Tris-based or milk-based extenders are commonly used for semen extension and preservation. As buck seminal plasma negatively interacts with egg yolk or milk-based extenders (Purdy, 2006), so that we were studied three different extenders including Tris-based with egg yolk, skim milk with egg yolk and skim milk with D glucose. In the present study, the effects of three different extenders on the quality of frozen semen were evaluated. The results revealed that the type of extenders affected the quality of frozen semen significantly. Tris-based extender maintained the best sperm quality in liquid nitrogen at -196°C followed by Skim milk D-glucose based and skim milk-based extender ($P\leq0.05$). The present results are consistent with the observations of Dorado et al. (2007) who observed that tris-based extender produced better frozen-thawed sperm quality compared to the skim milk extender. Between two skim milk sources, it was observed that skim milk D glucose produced significantly greater semen

quality (P≤0.05) compared with skim milk based with egg yolk extenders in terms of motility, viability, membrane potentiality and normal morphology (Table 3). It might be due to the disadvantageous effects of egg yolk with buck seminal plasma which ultimately produced lower frozen semen quality. From the literature, it was known that egg yolk is detrimental for goat spermatozoa (Leboeuf et al., 2000; Purdy, 2006). The two different freezing times from day 5 to day 12 was not affected the quality of post thawed frozen semen (P≥0.05) in any extenders studied here (Table 3). It recommended that the quality of frozen semen was not decreased with increased preservation time.

Table 2. Quality of chilled semen in Jamunapari buck in three different extenders in two different preservation times (n=27)

Parameters (%)	**Semen extenders**	**Chilling time**	
		Day 2	**Day 4**
Individual motility	SDc	77.46^{bA}±1.2	60.0^{bB}±1.77
	Sc	75.91^{bA}±1.01	57.07^{cB}±1.1
	Tc	81.26^{aA}±1.69	62.3^{aB}±1.53
Viability	SDc	78.42^{bA}±0.82	61.53^{bB}±2.05
	Sc	73.68^{cA}±1.04	59.56^{bB}±1.02
	Tc	81.09^{aA}±1.48	62.31^{aB}±2.13
Normal spermatozoa	SDc	75.1^{cA}±1.59	62.8^{bB}±1.35
	Sc	78.19^{bA}±1.18	60.56^{cB}±2.45
	Tc	84.12^{aA}±1.96	71.74^{aB}±2.29
HOST positive	SDc	75.05^{bA}±1.16	59.55^{bB}±0.95
	Sc	73.01^{cA}±1.81	57.6^{bB}±1.54
	Tc	80.55^{aA}±1.52	59.8^{aB}±1.38

*Values indicate mean ± S.D, HOST: Hypo-osmotic swelling test, SDc: Skim milk D-glucose, Sc: Skim-milk based Tc: Tris-based. *Superscript (^{a,b,c}) indicate a significant difference (P < 0.05) within individual parameters in a column in respect of extenders (SDc,Sc,Tc) with chilling time. *Superscript ($^{A,\ B}$) indicates a significant difference (P < 0.05) within individual parameters in a row in respect of chilling time (Day 2 and Day 4) with extenders.

Table 3. Post thawed quality of frozen semen in Jamunapari buck in different extenders in two different preservation times (n=27)

Parameters (%)	**Semen extenders**	**Cryopreservation time**	
		Day 5	**Day 12**
Individual motility (%)	SD_F	61.64^{bA}±1.25	60.13^{bA}±1.43
	S_F	56.12^{cA}±1.87	55.05^{cA}±1.63
	T_F	74.67^{aA}±0.76	72.77^{aA}±0.65
Live spermatozoa (%)	SD_F	65.28^{bA}±1.56	64.17^{bA}±1.32
	S_F	54.39^{cA}±0.89	52.22^{cA}±0.81
	T_F	76.99^{aA}±1.96	75.16^{aA}±2.21
Normal spermatozoa (%)	SD_F	71.08^{bA}±1.68	70.26^{bA}±0.39
	S_F	72.04^{bA}±3.78	70.53^{bA}±3.94
	T_F	79.81^{aA}±3.00	77.73^{aA}±2.45
HOST positive	SD_F	64.08^{bA}±0.92	63.15^{bA}±1.22
	S_F	52.69^{cA}±1.12	50.03^{cA}±1.65
	T_F	75.73^{aA}±1.26	$74.42\ ^{aA}$±0.98

*Values indicate mean ± S.D, HOST: hypo-osmotic swelling test, SD_F: Skim milk D-glucose, S_F: Skim milk based, T_F: Tris-based. *Superscript (^{a,b,c}) indicate a significant difference (P≤0.05) within individual parameters in a column in respect of extenders (SD_F,S_F,T_F) with cryopreservation time.*Superscript ($^{A,\ A}$) indicate non-significant difference (P > 0.05) within individual parameters in a row in respect of freezing time (Day 5 and Day 12) with extenders.

4. CONCLUSION

It is summarized that the quality of Jamunapari buck fresh as well as preserved semen is satisfactory. The tris-based extender is the best for the preservation of Jamunapari buck semen followed by skim milk D-glucose and skim milk-based extenders. Further field assays are necessary for the evaluation of fertility by using preserved semen.

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