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COMPARATIVE EFFICACY OF TWO EXTENDERS ON POST-THAW SPERM CHARACTERISTICS OF CRYOPRESERVED BLACK BENGAL BUCK SEMEN

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A B S T R A C T

The research work was conducted at the Artificial Insemination (AI) Center under the Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh to compare the efficacy between Egg Yolk Citrate (EYC) and Tris extenders for cryopreserved semen production in Black Bengal buck. The parameters of semen characteristics included percentage of individual motility (progressive), normal and live sperm and sperm concentration (billion/ml). Statistical analysis of fresh semen of individual buck effect was found to be significant ($p<0.01$) on normal sperm percentage but insignificant on individual motility, sperm concentration and live sperm percentage. After dilution with EYC extender, individual bucks had significant effect ($p<0.05$) on sperm motility and but insignificant on motility after cooling with and without glycerol. In the same way with Tris extender, it was insignificant ($p>0.05$) on diluted semen motility and motility after cooling with and without glycerol. Motility and morphology of the sperm after equilibration and thawing showed insignificant difference among the bucks using EYC extender. On the other hand, variation in the motility after equilibration and thawing was found significant ($p<0.05$) using Tris extender and insignificant on normal and live sperm percentages.

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INTRODUCTION

Artificial Insemination (AI) is the choice of tool to disseminate superior genes from individuals free from specific diseases and thereby improving the production of offspring. An additional important benefit of AI is the opportunity of breeding them outside their natural breeding seasons (Petersons 2007). AI in goats has increased interest because only few bucks of good genetic merits are available for breeding (Herman *et al*., 1995). Globally more than 100 million AIs in cattle, 40 million in pigs, 3.3 million in sheep and 0.5 million in goats are performed annually (Donovan and Hanrahan, 1999). The widespread use of AI has allowed accurate genetic evaluation and rapid dissemination of genetic merit on a national and international basis to bring benefit to both breeders and consumers. Artificial insemination can be used to improve goat production through adoption of selective breeding. Various factors like breeding soundness of male, semen quality, sexual health of female, accuracy, and efficiency of estrous detection, method of semen preservation and storage, skill of AI technician, suitable extender used etc. play an important role in an AI program. For the better propagation of goat, there should have good breeding buck. But unfortunately, there is severe shortfall of stud bucks all over the country, especially in the rural areas, where more than 80% goats are being reared by the farmers. It is evident that about 30% heated does remain without service due to lack of mature breeding bucks available in the locality. In most situations, bucks are being kept by only the few traditional people and they are often genetically poor with unknown pedigree. Same buck has been used generation after generation which has created greater chance of increasing inbreeding depression and hence lowering reproductive performances along with disseminating of various venereal and infectious diseases (Husain, 2007). Selective breeding is necessary for genetic improvement of goat and this requires controlled artificial insemination with known genetic merit.

Farmers castrate almost all the male kids at an earlier age for economic and social reasons in Bangladesh. Consequently, availability of breeding bucks became squeezed. Therefore, there remains no chance for judging breeding soundness and fertility of the buck (Husain, 2007). Now it is well established that the selection of good quality bucks and their widespread use could improve the overall production potential of goats (Husain, 2007). The best quality bucks could only be exploited rapidly through AI. Provision of AI with selected buck semen could be an easiest source of spreading desirable germplasm with short span of time as well as save the traditional breeds from extinction or severe erosion of genetic biodiversity. Artificial Insemination (AI) is the most powerful tool for livestock improvement ever available to the breeder (Robert and Foote, 1989). Increased milk, meat and skin production could be achieved by the development of AI (Robert and Foote, 1989). For future breeding purpose, the chilling semen could not be used. This has reduced or arrested metabolism of spermatozoa and thereby prolonged their fertile life**.** At present, buck scarcity is a common problem for breeding Black Bengal does in rural areas. Besides, due to socio-economic reason bucks are not normally reared in our country. Only some traditional people used to keep bucks for natural mating and some bucks are being used for generation after generation which increases inbreeding problem. As a result, our Black Bengal goat is a losing concern from breeding and reproductive point of view. AI is a breeding vehicle which is possible through frozen semen production.

To conduct it, selection of suitable extender is essential. Furthermore, little research has been conducted on this topic in Bangladesh. Considering this real scenario, this research has been taken to select suitable and economic extender for frozen semen production. Therefore, the development of suitable extender for preserving buck semen is essential for adoption of AI in goat. An extender should not only increase the volume of semen but also be capable of prolonging the life span of spermatozoa without compromising with the fertilizing capacity. It is also stable, economic and easy to prepare. A number of diluents are used for buck semen preservation. Amiri (1997) found that Tris-Fructose-Egg-Yolk (TFEY) and Glucose-Citrate-Egg-Yolk (GCEY) are better diluents than skim milk. Type of extender used determines the success of cryopreservation. Therefore, it seems rationale to test the efficacy of different extender in the preservation of buck semen.

MATERIALS AND METHODS

Selection of breeding bucks and management

The study was conducted at the Artificial Insemination (AI) Center under the Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh. A total of six adult Black Bengal bucks were selected from Nucleus breeding flock (NBF) based on body weight, libido, reaction time, scrotal circumference (SC), volume and also their ability to produce semen having greater than 80% morphologically normal spermatozoa with satisfactory motility, live spermatozoa and concentration. The age, body weight and scrotal circumference (SC) of bucks were 15 to 28 months, 19.0 to 25.0 kg and 17.0 to 22.0 cm respectively. The bucks were reared in individual pen (4×2.5 sq.ft) and they were fed with Napier, Ipil-Ipil and/or German grass twice daily as per requirement. The feed was supplemented with commercial concentrate in pellet form (crude protein content: 120g kg^{-1} (DM) and energy content: 10.4 MJ ME kg^{-1} (DM) in the morning and again in the afternoon at the rate of 100 g $buck^{-1}$. They were allowed for grazing and exercise for 1 to 2 hours daily. Clean and safe water was also made available at all the time. The breeding bucks were also supplied with germinated gram (20g $buck^{-1}$ day^{-1}).

Preparation of extender

Egg yolk-citrate extender was used for the extension of semen. Before use, the egg yolk-citrate extender was prepared according to Herman and Madden (1963). A stock solution for tris-glucose egg yolk diluents was prepared by dissolving tris-glucose and citrate in 85 ml distilled water.

Semen collection and evaluation

Collection of semen was done with artificial vagina maintaining optimum temperature about 41-43^{o}C. After complete mounting over the teaser male (with seeking movement of the penis), the artificial vagina was touched with the glans penis. Semen was collected twice a week within 8.30 AM from each buck after cleaning the prepuce with antiseptic solution (Savlon). The individual ejaculate was evaluated immediately after collection using the method described by Herman and Madden (1963). One part of the splitted semen sample was diluted with egg yolk-citrate and another part with Tris diluter in a way so that 100 million motile spermatozoa per insemination dose were prevailed. Then the diluted semen was evaluated for motility (%), normal and abnormal sperm count (%), live and dead spermatozoa.

Statistical analysis

The data generated from this experiment were entered in Microsoft Excel worksheet, organized and processed for further analysis. Analysis was performed with the help of Statistical Analysis System Computer Package (SAS, 1998, version 6.03. SAS Institute Inc. Cary NC, 25-109 USA).

RESULTS AND DISCUSSION

The semen characteristics for diluted semen of the experimental bucks are presented in Table 1

Table 1. Evaluation of semen diluted with EYC diluter

Buck no.	Diluted semen motility (%) (Mean ± SD) (n = 30)	Motility after cooling (%)	
		Without glycerol	With glycerol
B3	70.00±0.00 ^{b}	67.00±2.00	63.75±7.75
B4	72.50±2.45^{ab}	70.20±2.10	70.00±3.10
B11	72.00±2.00 ^{b}	67.10±0.56	68.75±2.00
B30	75.00±0.00 ^{a}	71.10±0.35	70.75±2.00
B32	73.75±2.45 ^{a}	70.20±2.13	70.70±3.16
B39	72.50±2.45^{ab}	70.00±2.00	70.00±3.16

Means with different superscripts within the same column differ significantly ($p<0.05$);
SD = Standard deviation; n = Number of observations

Evaluation of semen diluted with EYC extender before freezing (magnification 100x)

After evaluation of fresh semen, one part of the splitted semen sample was diluted with the EYC diluter. Sodium citrate can disperse the fat globules in the egg yolk and make observation of individual sperm possible on microscopic examinations (Herman et al*.,* 1994). With regard to the diluted semen, individual bucks showed significant difference ($p<0.01$) on volume and normal spermatozoa percentage and significant ($p<0.05$) difference was found on diluted semen motility with EYC extender. Table-2 shows that the highest percentage of diluted semen motility was observed in buck B30 (75.00±0.00) and the lowest in buck B3 (70.00±0.00) with EYC diluter. On the other hand, the highest motility percentage without glycerol was reported in buck B30 (71.10±0.35) and the lowest in buck B3 (67.00±2.00). Similarly, the highest motility percentage with glycerol was obtained in buck B30 (70.75±2.00) and the lowest in buck B3 (63.75±7.75). This result is in agreement with the studies of pervious works (Das et al*.,* 2006, Afroz, 2005 and Banu et al*.,* 1988).

Evaluation of semen diluted with Tris extender before freezing (magnification 100x)

Present study showed that the highest diluted semen motility percentages was in buck B30 (75.75±2.20) whereas the lowest was in buck B3 (73.50±.1.25).

Table 2. Evaluation of semen diluted with Tris diluter

Buck no.	Diluted semen motility (%)(n=30)	Motility after cooling	
		Without glycerol (%)	With glycerol (%)
B3	73.50±.1.25	69.80±2.00	67.70±2.20
B4	74.00±1.50	71.00±1.90	70.00±3.15
B11	74.25±2.00	72.00±1.80	70.50±2.20
B30	75.75±2.20	74.00±2.20	72.25±2.20
B32	73.80±2.20	72.50±2.00	70.50±2.00
B39	74.50±2.20	72.00±2.20	70.00±3.15

SD = Standard Deviation; n = Number of observations

And the motility percentage without glycerol was the highest in buck B30 (74.00±2.20) and the lowest was in buck B3 (69.80±2.00).Whereas the motility percentages with glycerolated portion was the highest in buck B30 (72.25±2.20) and the lowest was in buck B3 (67.70±2.20) (Table 2). This study indicates that it is almost similar to Singh et al., (1996) and Afroz (2005).

Evaluation of frozen semen (magnification 400x)

Means along with standard deviation (SD) of semen motility diluted with Tris and EYC extender, motility after freezing, motility after thawing and live and normal sperm percentages are furnished in Table-3.

Semen motility diluted with EYC and Tris extender

Different experiments in this regard were performed and obtained better sperm viability with this extender (Dutta et al., 1996, Singh et al*.,* 1996 Afroz, 2005 and Janett et al., 2005).

Motility after equilibration

The analysis of variance showed that the sperm motility after equilibration with EYC did not differ significantly among the bucks ($p>0.05$) (Table 3). This is in agreement with the findings of other investigators (Singh et al.1996; Dutta et al*.,* 1996, Biswas, 2001; Arriola and Foote, 1987 and Ahmad and Foote, 1985). The analysis of variance showed that the sperm motility after equilibration with Tris differ significantly ($p<0.05$) among the bucks (Table-4). The difference could be due to the difference in the type of extender, percentage of egg yolk, glycerol, equilibration time and thawing.

Table 3. Comparative Evaluation of Frozen semen (using Tris and EYC extender)

Diluter	Buck no.	Motility after equilibration (%) (mean ± SD) (n=30)	Motility after thawing ((%)	Morphology	
				Live sperm (%)	Normal sperm (%)
EYC	B3	57.50±8.00	37.50±4.90	29.65±2.49	75.89±2.59
	B4	66.25±2.45	43.75±3.74	35.41±8.66	77.07±2.27
	B11	63.75±2.00	42.50±5.83	33.33±10.98	78.53±1.45
	B30	67.50±2.45	50.00±5.49	41.75±4.33	82.96±2.20
	B32	67.40±2.45	47.50±5.10	41.75±3.40	79.48±0.92
	B39	66.25±3.16	46.25±2.45	31.50±2.44	76.70±3.87
TRIS	B3	66.50±2.00 [a]	48.75±5.48 [b]	40.19±2.46	74.65±2.68
	B4	68.75±2.00 [a]	57.50±2.45[a]	42.52±3.10	75.22±2.33
	B11	69.00±0.00 [a]	56.25±2.45 [a]	41.11±2.13	77.53±2.51
	B30	70.50±3.74 [b]	56.25±2.00 [a]	48.00±6.27	84.32±1.43
	B32	70.00±0.00 [a]	57.50±0.46[a]	42.44±3.84	82.06±0.85
	B39	69.00 ±0.00 [a]	57.50±2.45 [a]	42.12±6.59	77.56±.18

Means with different superscripts within the same column differ significantly (p<0.05)
SD= Standard Deviation; n = Number of observations

Motility after thawing

The analysis of variance revealed that the differences of sperm motility after thawing with EYC among the bucks were found insignificant (p>0.05) (Table-4) whereas it was found to be significant (p<0.05) with Tris diluter. This observation agrees with the result of Singh et al., (1996); Dutta et al*.,* (1996) who found 58.33±1.67 to 60.00±2.89% sperm motility after thawing.

Live sperm percentage (%)

In this study, in case of Tris extender, the highest and lowest live sperm percentages was in buck 30 (48.00±6.27) and B3 (40.19±2.46%) respectively and in EYC it was as 41.75±3.40 and 29.65±2.49% in buck B30 and B3 respectively. This result agrees with Singh et al., (1996); Dutta et al*.,* (1996).

Normal sperm percentages

This study showed that the highest and lowest normal sperm percentage using Tris extender was 84.32±1.43 and 74.65±2.68% in buck B30 and B3 orderly whereas in case of egg yolk citrate extender it was 82.96±2.20 and 75.40±0.92 % in buck B30 and B3 respectively. This supports the finding of Singh et al., 1996; Dutta et al*.,* 1996.

Table 4. The comparison of semen attributes between EYC and Tris for frozen semen production

Parameters	Extender		Level of significance
	EYC	Tris	
Motility after equilibration	64.78 ± 3.81	68.96 ± 1.38	*
Motility after thawing	44.58 ± 4.38	55.63 ± 3.42	**
Live sperm (%)	35.57 ± 5.16	42.73 ± 2.73	*
Normal sperm (%)	78.44 ± 2.57	78.56 ± 3.85	NS

P** <0.01, P* <0.05, NS = Non-significant

The results revealed from statistical analysis that the motility after equilibration and live sperm percentages differed significantly ($p<0.05$) when EYC and Tris extender were considered for frozen semen production (Table 4). There was also significant variation ($p<0.01$) between EYC and Tris on motility after thawing. No significant variation was found on normal sperm percentages.

CONCLUSION

In case of diluted semen with EYC extender, individual bucks had significant effect ($p<0.05$) on diluted semen motility and but insignificant on motility after cooling with and without glycerol. On the other hand, it was insignificant ($p>0.05$) on diluted semen motility and motility after cooling with and without glycerol with Tris extender. It revealed from statistical analysis that the motility after equilibration and live sperm percentages differed significantly ($p<0.05$) when EYC and Tris diluter were considered for frozen semen production. There was also significant variation ($p<0.01$) between EYC and Tris on motility after thawing. No significant variation was found on normal sperm percentages. Significant variations of semen parameters were observed between extender to extender and buck to buck. No significant variation was found on normal sperm percentages. The efficacy of tris extender is better than that of EYC extender taken into considerations all semen attributes though further study is to be needed for more economic confirmations.

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