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MK Goswami
Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India

S Sinha
Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India

BC Deka
Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India

M Bhuyan
Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India

RK Biswas
Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India

Corresponding Author:
MK Goswami
Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India

Effect of addition of vitamin E and caffeine on quality of frozen goat semen

MK Goswami, S Sinha, BC Deka, M Bhuyan and RK Biswas

Abstract
Semen from Sirohi and Beetal bucks (three each) maintained at Goat Research Station, Assam Agricultural University, Burnihat were collected. Study on the effect of adding 2mM vitamin E and 2mM Caffeine in tris extender before and after freezing on the quality of frozen semen were conducted. Sperm motility and live sperm were studied using conventional method. Live intact acrosome was found out using Eosin-Nigrosin-Giemsa stain. HOST- reacted spermatozoa were recorded using hypo-osmotic solution having 150 mOsm/L. The overall mean percent sperm motility, live sperm, live intact acrosome, HOST-reacted sperm were significantly (P<0.05) higher after equilibration containing Vitamin E than in Caffeine as well as between treatments after freezing containing Vitamin E than in that containing Caffeine. The activities of ALT (Alanine transaminase) and AST (Asperate transaminase) (u/L) in extracellular medium were significantly (P<0.05) lower after freezing in tris extender containing vitamin E than in that containing Caffeine.

Keywords: frozen semen, vitamin E, caffeine

Introduction
India occupies second position in terms of goat population and goat meat production in the world (FAOSTAT 2019) [5], with 34 registered goat breeds in India. Chevon (goat meat) which is free from religious and social taboo constitutes a major part of non-vegeterian diet. Thousands of goats are being slaughtered every year for meat purpose and people in general prefer the meat of male goats. This has resulted in acute scarcity of male goats for breeding. The local goats of Assam are mostly of non-descript type that are reared for the purpose of both meat and milk production. Assam Hill Goat is much popular because of its unique qualities like high degree of fecundity combined with ability to breed throughout the year and resistance to various common diseases. Goat husbandry provides glimpses of future hope for employment generation, nutritional security and prosperity to the millions of small and marginal farmers in the country.

Being smaller in body size, the native goats of Assam are of poor production potentialities in terms of meat and milk in comparison with that of other recognized goat breeds of India *viz.* Beetal, Jamunapari, Sirohi, Barbari *etc*. This can be improved by crossbreeding with bucks of superior breeds of goat through Artificial Insemination

Several studies have revealed tris extender to be the extender of choice for freezing of goat semen and the addition of antioxidants can help in minimizing the cryo-injury. Spermatozoa are protected by antioxidants present in the seminal plasma or in spermatozoa themselves to prevent oxidative damage during freezing and thawing processes (Kim and Parthasarathy, 1998) [9]. Use of antioxidant in semen extender was realized long back (Graham and Pace, 1967) [6] for controlling oxidation in order to maintain sperm motility and different antioxidants used as additives in semen extender have been reported to improve the quality of frozen semen (Sinha *et al*., 1994; Priyadharsini *et al*., 2011; Lalrindintluanga, 2012; Mazumdar, 2012; Dewry, 2014; Hazarika, 2014) [21, 13, 10, 11, 4, 7]. However, there is diversity of opinion about the best additive to be incorporated in extender for freezing of semen.

Materials and Methods
Three Sirohi and three Beetal bucks aged three to eight years maintained at Goat Research Station, Assam Agricultural University, Burniihat were used in the study. The bucks were thoroughly examined for sexual and general health prior to the study. The bucks were stall fed and maintained under uniform feeding and management practices throughout the period of study.

Semen was collected from each buck once or twice a week with the help of a standard artificial vagina.

Semen extending, freezing and Thawing

The ejaculates having volume of more than 0.8ml and initial sperm motility more than 70 per cent were only processed for further preservation. A total of 30 ejaculates each from Beetal and Sirohi breeds of goat were used to study the effect of additives on quality of frozen semen adopting split sample technique. A Tris-based extender (Tris 254mM, citric acid 78mM, fructose 70mM, egg yolk 15% (v/v), glycerol 6% (v/v), pH 6.8) was used as the base extender. Ejaculate was split into three parts and diluted at 37^0 C with the base extender containing @ 2 mM, vitamin E and caffeine @ 2mM. The remaining part was extended in tris extender without additive which served as control. The extended semen was cooled gradually to 5°C @ 1°C per 3 minutes and equilibrated in cold handling cabinet for 4 hours at 5°C.

The diluted semen samples were aspirated into 0.25mL French mini straws and sealed with polyvinyl alcohol powder and equilibrated at 5ºC for 3.5 hours. A few straws were taken out from the water and dried using pre-cooled (5ºC) towel for evaluation. The remaining straws after equilibration were frozen in liquid nitrogen vapor for 10 min and then plunged into liquid nitrogen for storage. After being stored for 16 hours the frozen straws were thawed individually (37^0C for 30 seconds) in a water bath, for microscopic semen evaluation.

Post thawing semen evaluations

Each semen sample was evaluated for sperm motility, live sperm per cent, live intact acrosome, Hypo Osmotic Swelling Test (Host)-reacted sperm, ALT and AST after equilibration and freezing by standard methods.

Statistical analysis

The results expressed as the mean ± SE. Means were analyzed using a two-way analysis of variance, followed by a Duncan's post hoc test to determine significant differences in all the parameters recorded between groups using SAS Enterprise Guide 4.2 version. Differences with values of $P < 0.05$ were considered to be statistically significant.

Results and Discussion

Sperm motility

The mean post thaw sperm motility was the highest after equilibration in Beetal and Sirohi goat semen extended with tris extender (Table 1) and after freezing (Table 2) containing vitamin E followed by caffeine and control. The present highest value of sperm motility after freezing of Beetal goat semen was close to that (63.75±0.56) reported by Dewry (2014) [4] but lower than that (65.17 ± 0.30) obtained by Hazarika (2014) [7] in Beetal goat semen extended with tris extender containing 2 mM vitamin E. The present value of sperm motility in Beetal goat semen obtained after freezing with tris extender containing 2 mM vitamin E was higher than that obtained by Lalramdintluanga (2012) [10] with 2mM vitamin E (57.75 ± 0.82%) and Dewry (2014) [4] with 5mM (55.00 ± 0.57) and 7 mM (51.00 ± 0.45) vitamin E in Beetal goat semen. The post thaw sperm motility of Sirohi goat semen observed in tris extender containing 2 mM vitamin E in the present study was higher than that reported by earlier workers in Sirohi goat semen frozen in tris extender supplemented with vitamin E at a concentration of 4.5mM (37.40 % by Priyadarshini *et al.,* 2011; 29.50 and 37.00 % by Saraswat *et al.*, 2012a, b) [13, 14, 15] and 3.5mM (32.00% by Saraswat *et al*., 2012b) [15].

In the study the mean sperm motility after freezing of Beetal and Sirohi buck semen in tris extender containing 2mM caffeine (Table 2) was higher than 58.20±0.84 per cent obtained by Sinha *et al.* (1995) [22] in Beetal goat semen extended with 2 mM concentration and 46.50±0.91 per cent reported by Agarwal *et al*. (2010) [1] in Sirohi goat semen extended with 7 mM concentration. This variation might be due to the differences in extender components, processing, freezing and thawing procedures used.

Live sperm

The mean post thaw live sperm per cent was the highest after equilibration in Beetal and Sirohi goat semen extended with tris extender (Table 1) as well as after freezing (Table 2) containing vitamin E followed by caffeine and control (no additive). The present highest value of live sperm obtained after freezing of Beetal goat semen with extender containing 2 mM vitamin E was comparable to that reported by Lalramdintluanga (2012) [10] and Dewry (2014) [4] with 2 mM (63.42 ± 0.82 and 64.29 ± 0.38) and Hazarika (2014) [7] with 6 mM (63.92 ± 0.28) but lower than that recorded by Hazarika (2014) [7] with 2 mM (70.15 ± 0.35) and higher than that obtained by Dewry (2014) [4] with 5mM (59.82±0.30) and Hazarika (2014) [7] with 8 mM (58.48 ± 0.37) concentrations of vitamin E in Beetal buck semen.

The highest live sperm obtained in the present study after freezing of Sirohi buck semen extended in tris extender containing 2 mM vitamin E was higher than that reported by Saraswat *et al*. (2012a) [14] in Sirohi goat semen frozen in tris extender containing 4.5 mM (42.80 ± 0.65) vitamin E. In the present study the mean value of live sperm obtained after freezing of Beetal and Sirohi goat semen in tris extender containing 2 mM caffeine was lower than that reported by Sinha *et al*. (1995) [22] in Beetal with 2 mM (68.70 ± 0.63) and higher than that recorded by Agarwal *et al*. (2010) [1] in Sirohi goat semen with 7 mM (50.16 ± 1.14) caffeine.

Live Intact acrosome

In the present study, the mean post thaw live intact acrosome percent was highest after equilibration in Beetal and Sirohi goat semen extended with tris extender containing vitamin E followed by caffeine and control (Table 1) as well as after freezing (Table 2). The present highest value of live intact acrosome obtained after freezing of Beetal buck semen extended with tris extender containing 2 mM vitamin E was comparable to that reported by Dewry (2014) [4] at 2 mM (45.24 ± 0.42) but lower than that obtained by Hazarika (2014) [7] at 2 mM (48.73 ± 0.36) and higher than that recorded by Dewry (2014) [4] at 5 mM (39.54 ± 0.35) or 7 mM (37.46 ± 0.36%) and Hazarika (2014) [7] at 6 mM (43.18 ± 0.28) or 8 mM (36.67 ± 0.41) concentrations.

HOST-reacted sperm

The mean percentage of HOST-reacted sperm was the highest after equilibration in Beetal and Sirohi goat semen extended with tris extender containing vitamin E followed by caffeine and control (Table 1) as well as recorded after freezing of Beetal and Sirohi buck semen in tris extender containing vitamin E followed by caffeine and control (Table 2). The present highest value of Host-reacted sperm recorded after freezing of Beetal buck semen with 2 mM vitamin E was

comparable to that reported by Hazarika (2014) [7] at 6 mM (51.28 ± 0.28) and Dewry (2014) [4] at 2 mM (52.89 ± 0.23) but lower than that reported by Hazarika (2014) [7] at 2 mM (55.47 ± 0.32) concentrations. Host-reacted sperm in Beetal goat semen obtained after freezing with 2 mM vitamin E in the present study was higher than that reported by Lalramdintluanga (2012) [10] with 2 mM (47.62 ± 0.97), Dewry (2014) [4] with 5 and 7 mM (48.99 ± 0.32 and 44.98 ± 0.40) and Hazarika (2014) [7] with 8 mM (46.52 ± 0.47) concentrations. The highest post thaw Host-reacted sperm recorded in Sirohi goat semen extended with tris extender containing 2 mM vitamin E in the present study was higher than that recorded by Saraswat *et al*. (2012a) [14] in Sirohi at 4.5 mM concentrations (20.90 ± 0.62). Due to lack of available literature the present value of Host reacted sperm in tris extender containing caffeine could not be compared.

Table 1: Semen Characteristics (Mean* ± Se) after Equilibration of Beetal and Sirohi Buck Semen in Tris Extender Containing Different Antioxidants

Groups	Control			Vitamin E			Caffeine			P value
	Beetal	Sirohi	Overall	Beetal	Sirohi	Overall	Beetal	Sirohi	Overall	
Sperm Motility (%)	71.97 ±0.40	72.03 ±0.38	72.00 ^{b} ±0.27	75.63 ±0.45	76.10 ±0.38	75.87 ^{a} ±0.29	73.30 ±0.48	69.97 ±2.38	71.63 ^{b} ±1.22	**
Live Sperm (%)	75.44 ± 0.44	73.45 ±0.32	74.45 ^{b} ±0.30	77.06 ±0.68	75.99 ±0.43	76.52 ^{a} ±0.41	74.87 ±0.51	73.50 ±0.28	74.18 ^{b} ±0.30	**
Live intact acrosome (%)	73.82 ±0.40	74.13 ±0.34	73.97^{c} ±0.26	77.46 ±0.35	77.46 ±0.31	77.46^{a} ±0.23	75.73 ±0.41	75.10 ±0.32	75.41^{b} ±0.26	**
HOST- reacted sperm (%)	70.96 ±0.23	70.38 ±0.10	70.67^{c} ±0.13	72.37 ±0.26	71.69 ±0.23	72.03^{a} ±0.18	71.38 ±0.26	71.05 ±0.15	71.21^{b} ±0.15	**

a, b, c, Means bearing different superscript differ significantly within the same row (p<0.01**, p<0.05*)

Extracellular release of ALT and AST activities

In the present study the extracellular ALT and AST activities in frozen thawed Beetal buck semen in tris extender containing 2 mM vitamin E, 2 mM caffeine and no additive (control) in Sirohi buck semen are shown in Table 2. The lowest value of extracellular release of ALT (23.79 ± 1.71 U/L) and AST (49.28 ± 0.80 U/L) obtained in frozen thawed Beetal semen with 2 mM vitamin E in the study was lower than that reported by Dewry (2014) [4] (35.67 ± 0.31 and 251.08 ± 0.41 U/L) and Hazarika (2014) [7] (173.25 ± 0.30 and 216.86 ± 0.32 U/L) in frozen Beetal semen with the same concentration of vitamin E. Lower values of GPT (ALT) and higher values of GOT (AST) than that observed in the present study were reported in frozen thawed semen of Sirohi bucks (Sharma *et al.*, 2013) [16]. The mean values of ALT in frozen thawed semen of Beetal and Sirohi bucks observed in the present study were within the range (21.96 to 220 units per ml) reported for different breeds of bucks (Sinha *et al.*, 1996; Shakeel, 1999; Sivaselvam *et al.* 2000) [23, 17, 25]. The variations in leakage of ALT and AST could be attributed to differences in breeds (Singh *et al*., 1985; Sinha *et al*., 1988) [20, 24], individual variations between the bucks of same breed (Tuli *et al*., 1991) [27], age of bucks (Tiwari, 2000) [26], rate of dilution as well as composition of diluents (Singh *et al*., 1993; Singh *et al*., 1996) [19], glycerol levels (Bonadonna *et al*., 1974) [3], equilibration periods (Joshi *et al*., 1990), cooling rates (Bhosrekar, 1975) [2] and freezing rates (Graham and Pace, 1967; Mohan, 1982) [6, 12].

Table 2: Semen Characteristics (Mean* ± Se) after Freezing of Beetal and Sirohi Buck Semen in Tris Extender Containing Different Antioxidants

Groups	Control			Vitamin E			Caffeine			P Value
	Beetal	Sirohi	Over all	Beetal	Sirohi	Over all	Beetal	Sirohi	Over all	
Sperm Motility (%)	60.00 ±0.28	60.10 ± 0.55	60.05 ^{b} ±0.30	62.57 ±0.44	63.00 ±0.46	62.78^{a} ±0.32	61.80 ±0.29	62.17 ±0.56	61.98 ^{a} ±0.31	**
Live Sperm (%)	60.86 ±0.14	61.07 ±0.25	60.97 ^{c} ±0.14	64.27 ±0.31	64.43 ±0.35	64.35 ^{a} ±0.23	61.97 ±0.26	62.06 ±0.25	62.02 ^{b} ±0.18	**
Live intact acrosome (%)	44.55 ±0.52	44.35 ±0.63	44.45^{b} ±0.40	46.51 ±0.43	47.01 ±0.61	46.76^{a} ±0.37	44.77 ±0.57	45.57 ±0.55	45.17^{b} ±0.39	**
HOST- reacted sperm (%)	50.17 ±0.15	50.30 ±0.15	50.24^{c} ±0.11	51.58 ±0.18	51.46 ±0.25	51.52^{a} ±0.15	50.69 ±0.14	50.78 ±0.17	50.73 ^{b} ±0.11	**
ALT (U/L)	30.80 ± 1.50	35.50 ±2.15	33.15 ±1.34^{a}	23.79 ± 1.71	28.60 ± 1.72	26.19 ±1.08^{b}	31.41 ± 0.77	32.21 ± 1.50	31.81± 0.65^{a}	**
AST (U/L)	58.17 ±1.92	45.47 ±1.35	51.82 ±1.43^{a}	49.28 ±0.80	41.33 ±1.15	45.30 ±0.86^{b}	52.19 ±0.88	49.23 ±1.26	50.71± 0.79^{a}	**

a, b, c, Means bearing different superscript differ significantly within the same row (p<0.01**, p<0.05*)

Conclusion

Addition of Vitamin E in Tris extender significantly improved post thaw semen quality as compared to caffeine and control.

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Disclosure statement

No potential conflict of interest was reported by authors.

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