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Research Article

EFFECT OF VITAMIN-E SUPPLEMENTATION ON QUALITY OF BEETAL BUCK SEMEN DURING STORAGE AT 4°C

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ABSTRACT

The objective of this study was to evaluate the role of vitamin E in improving the seminal parameters during storage for 72 h. The semen ejaculates were collected by artificial vagina from nine bucks (Beetal) during the normal reproduction season. The samples were centrifuged and seminal plasma was discarded. The sperm pellet was diluted with Tris based extender and divided into 4 groups. Group 1 was taken as control and groups 2, 3 and 4 were supplemented with tocopherol @ 0.1mM, 1mM and 3mM, respectively. The samples were evaluated for progressive motility, percent livability and acrosome integrity before and after liquid preservation for 72 h. After 72 h of storage, the decline in the progressive sperm motility was significant in groups 1, 2 and 3 and was non-significant in group 4. The progressive sperm motility, percent livability and percent intact acrosomes were significantly higher in group 4 as compared to control and other treated groups at 72 h of storage at 4°C. Thus, it can be concluded that vitamin E when supplemented to the semen samples @ 3mM concentration helps in maintaining the quality of seminal parameters during liquid preservation of semen at 4°C for 72 h.

Key words: Beetal buck, semen, oxidative stress, seminal parameters, vitamin E

Goat husbandry is the backbone of rural economy in India. It is important as it helps to sustain the livelihood of rural poor in difficult terrains characterized by spare vegetation, marginal land and a high incidence of poverty. Among various goat species, Beetal is an outstanding milch breed and is known for its ability to acclimatize to tropical harsh environment, resistance to diseases and capacity to convert food into meat and milk production. For improvement of genetic program in goat, artificial insemination (AI) with liquid or frozen semen is important. The quality of semen plays a major role in successful AI program as well as full exploitation of the productive potential of buck.

Spermatozoal membrane, being rich in polyunsaturated fatty acids (PUFA), is easily damaged by reactive oxygen species (ROS) or membrane lipid peroxides (Silva, 2006). The equilibrium between the amount of ROS produced and scavenged is related with the gamete cell stability and damage. Reactive oxygen species reduces the sperm freezing potential, dysfunctions the sperm by lipid peroxidation and affects the cell membrane integrity. These changes finally alter the cytological parameters of semen making them incompetent to fertilize with the ova (Budai *et al.*, 2014). The ROS can be neutralized by antioxidants which serve as defense mechanism against the lipid peroxidation of semen and help in maintaining the sperm motility and viability. Supplementation of antioxidants to the semen extenders have been reported to improve semen quality in goat (Sinha *et al.,* 1996).

Among non-enzymatic antioxidants like vitamin C, vitamin E, selenium, glutathione etc., vitamin E (α-tocopherol) is the major chain breaking and lipid soluble antioxidant that acts to support the cell defense mechanism. It readily donates the hydrogen from the hydroxyl (-OH) group on the ring structure to free radicals, making them unreactive. It is located primarily within the phospholipid bilayer of cell membranes and is effective in preventing lipid peroxidation i.e., oxidative deterioration of PUFAs in sperm membrane. The addition of vitamin E and glutathione, as primary antioxidants to the semen extender inhibit lipid peroxidation caused by ROS and thus prevent loss of sperm motility as reported in chilled boar spermatozoa (Cerolini *et al*., 2000) and frozen thawed bull semen (Jeong *et al*., 2009). Since very little work seems to have been done on addition of antioxidants to Beetal buck semen, the present study was carried out on *in vitro* addition of vitamin E to the semen extender in order to maintain the keeping quality of Beetal buck semen.

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MATERIALS AND METHODS

The semen ejaculates were collected from nine Beetal bucks at weekly intervals with the help of an artificial vagina during the normal reproductive season at Goat farm of this university. On day 0, ejaculates were collected and transferred to a water bath maintained at 37ºC, seminal plasma was separated by centrifuging the semen at 1500 rpm for 10 min at 16ºC and Tris - egg yolk extender was added to the semen pellet. Each sample was divided into four parts; first part was taken as control (Group 1) and other three parts were supplemented with vitamin E (α-tocopherol) @ 0.1mM, 1mM and 3mM (Groups 2, 3 and 4, respectively). The samples were evaluated for progressive motility, percent livability (Campbell *et al*., 1960) and acrosome integrity (Watson and Martin, 1972). Data thus obtained were analyzed by suitable statistical analysis following standard methods described by Snedecor and Cochran (1994) using SPSS-20.0, a statistical package (SPSS Inc., Chicago, IL, USA). All data were presented as mean ± standard error of mean (SE).

RESULTS AND DISCUSSION

Percent progressive sperm motility, live sperm percentage and percent intact acrosome in liquid preserved semen of Beetal bucks in control and vitamin E supplemented groups at 0, 24, 48 and 72 h of incubation are presented in Table 1. There was non-significant variation in progressive sperm motility among all four groups at 0 h. The progressive sperm motility declined at 24, 48 and 72 h of incubation at 4°C in groups 1, 2 and 3. However, in group 4, the decline in progressive sperm motility was very less till 72h incubation as compared to 0 h. This finding in the present study is supported by the report of Zeitoun and Al-Damegh (2015) who observed better motility with 5IU vitamin E in ram semen after 96 h of incubation at 5^0C in a refrigerator. This is also in consonance with the studies of Saraswat *et al*. (2012) and Andreea and Stela (2010) who also reported that supplementation of α-tocopherol/ vitamin E had a significant role in maintenance and sustaining the quality of frozen–thawed spermatozoa including motility in goat semen.

Percent live spermatozoa in semen in the present study at 0 h incubation were higher in group 4 (73.07±2.86), as compared to group 1 (62.22±3.22). In groups 1, 2 and 3, the percent live spermatozoa were comparable at 0 h, but this showed a significant decline at 72 h. However, in group 4, the decline in percent live spermatozoa was non-significant from 0 h till 72 h incubation. The observation in the present study is in accordance with the reports of other workers. Iqbal *et al.* (2015) observed that the inclusion of 5mM butylated hydroxytoluene (BHT) in tris- egg yolk extended Beetal buck semen showed mild improvement in viability of sperm. Significantly increased percent live spermatozoa were reported after supplementation of 5 IU/ml and 1mM/ml of vitamin E and glutathione respectively, in chilled-stored ram semen (Zeitoun and Al-Damegh, 2015). Supplementation of antioxidants (α-tocopherol) at 4.5mM in semen extender has been reported to increase the duration of activity of spermatozoa during freeze- thawing in Sirohi goat semen (Saraswat *et al.,* 2012).

Percent spermatozoa with intact acrosome in Beetal buck semen at 0, 24, 48 and 72 h of incubation at 4ºC was significantly higher in group 4 as compared to groups 1, 2 and 3. The results in the present study are in agreement with Saraswat *et al.* (2012) who reported the least damaged acrosome during freezing after addition of á-tocopherol at 4.5mM in Sirohi goat semen as compared to control, since vitamin E, being the major

Table 1
Seminal parameters (Mean±SE) of buck spermatozoa supplemented with vitamin E up to 72 h of incubation at 4°C

Seminal parameters	Treatment (hours)	Group 1	Group 2	Group 3	Group 4
Progressive sperm motility	0	64.22^{ax}±2.64	58.88^{ax}±2.73	53.55^{ax}±2.88	64.77^{bx}±1.74
	24	58.11^{ax}±2.66	46.22^{axy}±2.13	45.33^{axy}±2.60	62.00^{bx}±3.06
	48	47.55^{axy}±2.12	37.77^{ay}±2.9	35.00^{ay}±2.63	58.77^{bx}±2.54
	72	32.11^{ay}±1.33	25.00^{ay}±3.22	23.11^{ay}±3.40	55.88^{bx}±2.28
Live sperm percentage	0	62.22^{ax}±3.22	61.25^{ax}±4.54	56.63^{ax}±3.26	73.07^{bx}±2.86
	24	58.52^{ax}±3.18	49.22^{axy}±3.13	46.84^{axy}±2.94	66.36^{bx}±3.65
	48	38.22^{ay}±3.35	39.28^{ay}±3.75	35.85^{ay}±2.95	62.34^{bx}±2.84
	72	36.25^{ay}±1.84	22.83^{ay}±2.93	22.80^{ay}±3.58	60.95^{bx}±2.53
Intact acrosome percentage	0	60.12^{ax}±3.15	52.74^{ax}±2.07	45.10^{ax}±3.10	68.05^{bx}±3.22
	24	54.83^{ax}±3.16	44.57^{axy}±3.14	41.29^{ax}±2.99	59.95^{bx}±3.88
	48	48.42^{axy}±2.74	34.14^{ay}±4.43	30.46^{ay}±3.02	58.06^{bx}±3.06
	72	33.27^{ay}±2.01	24.48^{ay}±3.11	19.66^{ay}±3.33	56.51^{bx}±2.89

Different superscripts (a, b) within the row and (x, y) within a column for a parameter differ significantly ($p<0.05$). Group 1=Control; Group 2=Supplemented with vitamin E @ 0.1 mM; Group 3=Supplemented with vitamin E @ 1 mM; Group 4=Supplemented with vitamin E @ 3 mM

chain-breaking antioxidant, has the ability to scavenge all the reactive oxygen radicals, namely superoxide, peroxyl and hydroxyl radicals there by reducing lipid peroxidation and maintaining sperm membrane integrity.

Thus it can be concluded that supplementation of antioxidants in form of vitamin E @ 3mM in Tris extender helped in liquid preservation of buck semen up to 72 h at 4°C in refrigerator with higher progressive sperm motility, higher liveability percentage and higher percentage of sperms with intact acrosome thus offering protection to the spermatozoa from the free radicals generated during storage of semen.

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