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Research Article

EFFECT OF VITAMIN-E SUPPLEMENTATION ON SPERM MOTILITY IN SURTI BUCK SEMEN PRESERVED AT REFRIGERATION TEMPERATURE

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ABSTRACT

The objective of the study was to evaluate the effect of vitamin-E supplementation in Tris egg yolk citrate extender on sperm motility in Surti buck semen preserved at refrigeration temperature. The pooled semen from all four bucks was diluted with the Tris egg yolk citrate extender to make a final concentration of 200 $\times 10^6$ sperm/ml. The diluted semen was divided into five equal aliquots and each aliquot was treated with 0 mM (Control), 1 mM (T1), 2 mM (T2), 3 mM (T3) and 4 mM (T4) of vitamin-E and preserved at refrigeration temperature (4-5 ºC) up to 48 hours. Sperm motility was evaluated at 0, 24, 36 and 48 hours while, Motility degeneration rate (MDR) was calculated at 24, 36 and 48 hours post-chilling. The significantly ($p<0.01$) highest percentage of sperm motility whereas lowest percentage of MDR, irrespective of preservation time, was found in T3 (65.47±1.49 and 18.29±1.62, respectively) followed by control (56.48±1.67 and 28.37±1.98, respectively), T1 (55.23±1.82 and 29.71±2.07, respectively), T2 (53.2±1.68 and 31.85±1.84, respectively) and T4 (52.5±1.83 and 33.68±2.08, respectively) groups. Moreover, the sperm motility above 50 % was only maintained in T3 group (54.06 ± 2.75 %) at 48 hours post-chilling. Use of 3 mM vitamin-E as a semen additive in tris egg yolk citrate extender maintained the sperm motility above 50% till 48 hours at refrigerated temperature (4-5 ºC) implies its beneficial effect on buck semen preservation.

Keywords: MDR, Motility, Sperm, Surtibuck, Vitamin-E

Artificial insemination is extensively used for goat and sheep breeding with high genetic merit available to them (Foote, 2002). Sperm motility is core standard for any semen sample and based on it generally the samples are accepted or rejected before additional processing and storage for forthcoming usage in AI programme. During cryopreservation, there is extreme production of reactive oxygen species (ROS) which has been associated with reduced post-thaw motility, sperm viability, plasma membrane integrity and antioxidant status; resulting in male infertility and various sperm anomalies (Zhao and Buhr, 1995; Aitken *et al.,* 1998; Bilodeau *et al.,* 2001). Immotile sperm with abnormal morphology certainly fail to infiltrate the oocyte and in spite of good quality of oocyte, there is failure of zygote establishment, which in due course leads to financial losses to goat breeders and poor farmers as well. Thus, to combat the impairment taking place during cryopreservation, antioxidants are suggested to be incorporated in dilutors as additives.

Vitamin-E plays an important role as antioxidant to thwart the lipid peroxidation of biomembranes and aids in cryopreservation of semen, by which the preserved semen can be helpful for artificial insemination. One of the elementary functions of vitamin-E as an important endogenous and exogenous antioxidant is to preserve the cellular and sub cellular membranes integrity (Kale *et al*., 1999).Vitamin-E has been shown to inhibit the free-radical-induced injury to delicate sperm cell membranes as it is a foremost chain-breaking antioxidant (Sinclair, 2000) and forms a relatively stable complex such as tocopheroxyl radical. It readily bequeaths the hydrogen ion from the hydroxyl (-OH^-) group on the ring structure to free radicals, making them unreactive. Supplementation of freezing extender with the water-soluble vitamin-E analogue, Trolox, improved the motility of frozen–thawed boar spermatozoa (Pena *et al*., 2003).

Therefore, in pursuit of an antioxidant effect of vitamin-E, present study was performed to evaluate the effect of different concentrations of vitamin-E in Tris egg yolk citrate extenderon sperm motility in Surti buck semen preserved at refrigeration temperature.

MATERIALS AND METHODS

Collection of semen and its processing: Total four apparently healthy Surti bucks above 1 years of age maintained under All India Coordinated Research Project (AICRP) on Goat at Livestock Research Station, Navsari Agricultural University, Navsari were selected. The selected bucks were regularly maintained under proper healthy and hygienic conditions and well-fed. The selected bucks were housed in a common covered pen and separated from females. The bucks were trained to donate the semen in artificial vagina using female as a dummy. After completion of one-month training period, semen was collected regularly by artificial vagina twice a week from each buck for up to 8 weeks. Total 64 semen ejaculates (16 from each buck) were collected. Before semen collection, one false mount was given to the buck on female dummy secured in travis. Buck apron was applied to the buck to prevent touching of penile part to the hind part of dummy. The ejaculates were obtained in sterilized graduated glass tubes and transferred to the laboratory within 10 minutes.

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To eliminate individual buck variability and increase semen quantity, all the ejaculates were pooled during each semen collection. The pooled semen was diluted with the Tris egg yolk citrate extender to make a final concentration of 200×10^6 sperm/ml. The diluted semen was evaluated for initial sperm motility and semen samples with ≥ 70% motility were considered for further processing.

Experimental groups: To evaluate the effect of vitamin-E supplementation in Tris egg yolk citrate extender on sperm motility the extended semen was divided into five equal aliquots and each aliquot was supplemented with 0 mM (control), 1 mM (T1), 2 mM (T2), 3 mM (T3) and 4 mM (T4) vitamin-E. The semen sample treated with different concentrations of vitamin-E was preserved at refrigeration temperature up to 48 hours. In present experiment α-Tocopherol phosphate disodium salt (Sigma Aldrich), a water-soluble analogue of vitamin-E was used as semen additive having molecular weight of 554.65 g/mol.

Sperm motility: Sperm motility from the extended samples was examined at 0, 24, 36 and 48 hours by placing a small drop of the diluted sample on a warmed glass slide covered with cover slip by means of phase contrast microscope with warm stage (37 ºC). The proportion of progressively motile spermatozoa was expressed in percentage from 0 to 100 with intervals of 5%. To decide motility more precisely, minimum four to five different microscopic fields were assessed.

Motility degeneration rate (MDR): The motility degeneration rate (MDR) was calculated on the basis of sperm motility by using the following formula :

$$\text{MDR at various hours (\%)} = \frac{\text{Motility (0 hour)} - \text{Motility at various hours}}{\text{Motility (0 hour)}} \times 100$$

(Campos *et al*., 2004)

Corresponding values of motility at 24, 36 and 48 hours were substituted in the above formula to calculate MDR at various hours.

Statistical analysis: The data pertaining to motility and its degeneration were suitably tabulated and analyzed using R-3.3.2 software. The differences among the parameter means were performed using appropriate statistical methods *viz.,* ANOVA and DNMRT (Duncan's New Multiple Range Test). The mean differences were considered significant at $p<0.01$ and $p<0.05$.

RESULTS AND DISCUSSION

Sperm Motility (%): The initial and post-chilled sperm motility of Surti buck semen supplemented with different concentrations of vitamin-E in Tris egg yolk citrate extender was monitored in all the groups at different time intervals and presented in table 1.

The initial sperm motility (%) found at 0 hour differed non-significantly between various groups. Further, at 24 hours post-chilling the sperm motility (%) was found significantly ($p<0.01$) higher in T3 (69.69± 1.96) as compared to all other groups, however, it differed non-significantly between Control, T1, T2 and T4 groups. Similarly, at 36 hours post-chilling, the sperm motility (%) was found significantly ($p<0.01$) higher in T3 (62.5±2.42) as compared to other groups with non-significant differences between the Control, T1, T2 and T4 groups. Again at 48 hours post-chilling also, the sperm motility (%) was found significantly ($p<0.01$) higher in T3 (54.06±2.75) as compared to remaining groups, with non-significant differences between the Control, T1, T2 and T4 groups. The corresponding overall mean values of sperm motility (%) irrespective of various groups gradually declined with increasing preservation time as 71.94±0.8, 61.13±1.07, 51.5±1.2 and 42.81±1.21 at 0, 24, 36 and 48 hours, respectively.

Moreover, in control, T1, T2, T3 and T4 groups, the sperm motility (%) was significantly ($p<0.01$) higher at 0 (71.88±1.88, 71.25±1.80, 70.31±1.85, 75.63±1.64 and 70.63±1.64, respectively) as compared to 24 (61.56±1.63, 60.00±2.37, 57.5±2.09, 69.69±1.96 and 56.88±2.58, respectively), 36 (51.25±1.80, 49.69±2.72, 47.19±2.09, 62.5±2.42 and 46.88±2.45, respectively) and 48 (41.25±1.68, 41.56±2.84, 39.69±2.11, 54.06±2.75 and 37.5±2.09, respectively) hours post-chilling. Exceptionally, in T3 group, the sperm motility found was differ non-significantly between 0 and 24 hours of preservation time. Further, the sperm motility (%) recorded at 24, 36 and 48 hours post-chilling was significantly ($p<0.01$) highest in T3 (69.69±1.96, 62.5±2.42 and 54.06±2.75, respectively) as compared to any other group. Moreover, the above 50% sperm motility was only maintained in T3 group (54.06±2.75 %) and not in the any other group at 48 hours post-chilling preservation time. The corresponding overall mean values of sperm motility (%) irrespective of preservation time were found significantly ($p<0.01$) highest in T3 (65.47±1.49) as compared to control (56.48±1.67), T1 (55.23±1.82), T2 (53.2±1.68) and T4 (52.5±1.83) groups propose the beneficial effect of vitamin-E as an additive when supplemented at 3mM concentration in tris egg yolk citrate extender for preservation of buck semen at refrigeration temperature.

The findings of the present study were in very close agreement with the findings of Sarangi *et al.* (2017) who

Table 1

Effect of different concentrations of Vitamin-E and storage duration on motility (%) of Surti buck semen preserved at refrigeration temperature (Mean±SE)

Groups	Individual sperm Motility (%) (n=16)				Overall	F value	P value
	0 hr	24 hr	36 hr	48 hr			
C	$71.88\pm1.88_{w}^{a}$	$61.56\pm1.63_{x}^{b}$	$51.25\pm1.80_{x}^{c}$	$41.25\pm1.68_{x}^{d}$	$56.48\pm1.67_{x}$	57.032**	0.00
T1	$71.25\pm1.80_{w}^{a}$	$60.00\pm2.37_{x}^{b}$	$49.69\pm2.72_{x}^{c}$	$41.56\pm2.84_{x}^{d}$	$55.23\pm1.82_{x}$	27.22**	0.00
T2	$70.31\pm1.85_{w}^{a}$	$57.50\pm2.09_{x}^{b}$	$47.19\pm2.09_{x}^{c}$	$39.69\pm2.11_{x}^{d}$	$53.20\pm1.68_{x}$	42.384**	0.00
T3	$75.63\pm1.64_{w}^{a}$	$69.69\pm1.96_{w}^{a}$	$62.50\pm2.42_{w}^{b}$	$54.06\pm2.75_{w}^{c}$	$65.47\pm1.49_{w}$	17.404**	0.00
T4	$70.63\pm1.64_{w}^{a}$	$56.88\pm2.58_{x}^{b}$	$46.88\pm2.45_{x}^{c}$	$37.50\pm2.09_{x}^{d}$	$52.50\pm1.83_{x}$	40.812**	0.00
Overall	71.94 ± 0.8^{a}	61.13 ± 1.07^{b}	51.50 ± 1.20^{c}	42.81 ± 1.21^{d}	—	—	—
F value	1.483	5.7252**	7.6547**	7.7256**	—	—	—
P value	0.2158	0.00	0.00	0.00	—	—	—

$_{WZ}$ Means with different subscript within a column (between the groups) differs significantly at p<0.01.**

$^{a-d}$ Means with different superscript between a column (between time intervals) differs significantly at p<0.01.**

C - Control; T1 - Vitamin-E-1mM; T2 - Vitamin-E-2mM; T3 - Vitamin-E-3mM; T4 - Vitamin-E-4mM

Table 2

Effect of different concentrations of Vitamin-E and storage duration on percent Motility Degeneration Rate (MDR) of Surti buck semen preserved at refrigeration temperature (Mean±SE)

Groups	MDR (%) (n=16)			Overall	F value	P value
	24 hr	36 hr	48 hr			
C	$14.20\pm1.30_{x}^{c}$	$28.6\pm1.86_{w}^{b}$	$42.33\pm2.27_{w}^{a}$	$28.37\pm1.98_{w}$	57.749**	0.00
T1	$16.04\pm1.86_{wx}^{c}$	$30.81\pm2.36_{w}^{b}$	$42.27\pm2.84_{w}^{a}$	$29.71\pm2.07_{w}$	30.329**	0.00
T2	$18.34\pm1.82_{wx}^{c}$	$33.19\pm1.78_{w}^{b}$	$44.03\pm1.75_{w}^{a}$	$31.85\pm1.84_{w}$	52.462**	0.00
T3	$8.04\pm0.84_{y}^{c}$	$17.77\pm1.73_{x}^{b}$	$29.07\pm2.47_{x}^{a}$	$18.29\pm1.62_{x}$	33.962**	0.00
T4	$19.89\pm2.21_{w}^{c}$	$33.96\pm2.44_{w}^{b}$	$47.19\pm2.25_{w}^{a}$	$33.68\pm2.08_{w}$	35.266**	0.00
Overall	15.30 ± 0.86^{c}	28.87 ± 1.11^{b}	40.98 ± 1.24^{a}	—	—	—
F value	7.5499**	10.18**	8.7998**	—	—	—
P value	0.00	0.00	0.00	—	—	—

$_{WZ}$ Means with different subscript within a column (between the groups) differs significantly at p<0.01.**

$^{a-d}$ Means with different superscript between a column (between time intervals) differs significantly at p<0.01.**

C - Control; T1 - Vitamin-E-1mM; T2 - Vitamin-E-2mM; T3 - Vitamin-E-3mM; T4 - Vitamin-E-4mM

evaluated the role of vitamin-E (3mM) in improving the seminal parameters of Beetal buck semen stored at 4 °C for 72 hours. They also found significantly (p<0.05) higher value of sperm motility in a group supplemented with 3mM of vitamin-E (63.33±1.02 %) as compared control group (63.33±1.02 vs. 55.83±1.04%, 57.36±1.10 vs. 45.14±1.04 and 50.83±0.88 vs. 33.61±1.51, respectively) at 0, 24 and 48 hours post-chilling which strongly support the findings of present study. Moreover, their findings regarding maintenance of above 50 % sperm motility only in 3 mM vitamin-E supplemented (50.83 ± 0.88 %) group at 48 hours post-chilling preservation time was also in the close agreement with the present findings where, we had also found the above 50% sperm motility only in T3 (54.06±2.75 %) group at 48 hours post-chilling.

However, Anghel *et al.* (2010) studied the effect of various antioxidant additives in cryopreserved Alpine buck semen and reported significantly (p<0.05) higher value for post thaw sperm motility (57.5 ± 1.96 vs 51.00 ± 2.33 %) in 1mM vitamin-E supplemented as compare to control group. Likewise, Zamfirescu and Anghel (2010) also recorded significantly (p<0.05) higher values of post thaw sperm motility in 1mM vitamin-E supplemented (51.62 ± 0.38 vs 40.02 ± 0.55%) as compared to control group in cryopreserved Saanen buck semen. The above findings were not in agreement with the present findings as we found non-significantly lower value of overall sperm motility in 1mM (55.23±1.82%) vitamin-E supplemented as compared to control (56.48 ± 1.67 %) group.

Further, contrary to the findings of present study,

Saraswat *et al.* (2012) reported non-significantly higher values of post thaw motility in 4.5mM vitamin-E supplemented (29.50 ± 0.60 vs 28.7 ± 0.42 %) as compared to control group in cryopreserved Sirohi goat semen. The findings were in opposition to the findings of present study where, we had found lower motility with 4mM of vitamin-E supplementation.

Motility degeneration rate % (MDR %): The percentage of motility degeneration rate of Surti buck semen supplemented with different concentrations of vitamin-E in Tris egg yolk citrate extender was monitored in all the groups at different time intervals and presented in table 2.

The MDR (%) found at 24 hours post-chilling was significantly ($p<0.01$) lower in T3 (8.04±0.84) as compared to all other groups. Similarly, at 36 hours post-chilling also, the MDR (%) found was significantly ($p<0.01$) lower in T3 (17.77±1.73) as compared to remaining groups. Similar trend was also seen at 48 hours post-chilling also, where, the MDR (%) recorded was significantly ($p<0.01$) lower in T3 (29.07±2.47) as compared to other groups. Further, the MDR (%) recorded at 24, 36 and 48 hours post-chilling was significantly ($p<0.01$) lowest in T3 (8.04±0.84, 17.77±1.73 and 29.07±2.47, respectively) as compared to all other groups. The corresponding overall mean values of MDR (%) irrespective of various groups were gradually inclined with increasing preservation time as15.3±0.86, 28.87±1.11 and 40.98±1.24 at 24, 36, and 48 hours, respectively.

Moreover, in control, T1, T2, T3 and T4 groups, the MDR (%) was significantly ($p<0.01$) lower at 24 (14.2±1.3, 16.04±1.86, 18.34±1.82, 8.04±0.84 and 19.89±2.21, respectively) as compared to 36 (28.6±1.86, 30.81±2.36, 33.19±1.78, 17.77±1.73 and 33.96±2.44, respectively) and 48 (42.33±2.27, 42.27±2.84, 44.03±1.75, 29.07±2.47 and 47.19±2.25, respectively) hours post-chilling. Further, the MDR (%) found in various groups also differed significantly ($p<0.01$) between 36 and 48 hours post-chilling period. The corresponding overall mean values of MDR (%) irrespective of preservation time were found significantly ($p<0.01$) lower in T3 (18.29 ± 1.62) as compared to control (28.37±1.98), T1 (29.71±2.07), T2 (31.85±1.84) and T4 (33.68±2.08) groups suggesting a positive effect of vitamin-E as an additive when supplemented at 3 mM concentration in tris egg yolk citrate extender for preservation of buck semen at refrigeration temperature.

Since there is very scanty literature available regarding supplementation effect of vitamin-E on MDR, the discussion was carried out only on the basis of available literature.

Like present findings, Atara *et al.* (2018) also reported increasing trend in MDR with increase in preservation time as 6.65±0.24, 13.67±0.42 and 28.77±0.79 at 30, 60 and 120 min., respectively. However, contrary to the present findings, Aguiar *et al*. (2013) observed significantly ($p<0.05$) higher MDR at 2 hours after cooling (57.6±9.1%) than 48 hours after cooling (43.5±9.0 %) during dry season in non-defined breed of bucks. Further, they found non-significant difference in MDR at 24 hours after cooling (50.5±9.1 %) during dry season. Similarly, during rainy season also, they found non-significant difference in MDR after 2 hours (28.2±6.2 %), 24 hours (28.2±7.3 %) and 48 hours (27.1±7.4 %) of time intervals.

In conclusion, the highest sperm motility and lowest motility degeneration rate was found in treatment group supplemented with 3 mM of vitamin-E in tris egg yolk citrate extenders. Further, the above 50% sperm motility was also maintained in the 3 mM vitamin-E supplemented group up to 48 hours of preservation at refrigeration temperature proves the favourable effect of vitamin-E for buck semen preservation at refrigeration temperature.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest regarding the present research work.

REFERENCES

Aguiar, G.V., Van Tilburg, M.F., Catunda, A.G.V., Celes, C.K.S., Lima, I.C.S., Campos, A.C.N., Moura, A.A.A. and Araujo, A.A. (2013). Sperm parameters and biochemical components of goat seminal plasma in the rainy and dry seasons in the Brazilian Northeast: the season's influence on the cooling of semen. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia.* **65(1)**: 6-12.

Aitken, R.J., Gordon, E., Harkiss, D., Twigg, J.P., Milne, P., Jennings, Z. and Irvine, D.S. (1998). Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. *Biol. Reprod.* **59**: 1037-1046.

Anghel, A., Zamfirescu, S., Dragomir, C., Nadolu, D., Elena, S. and Florica, B. (2010). The effects of antioxidants on the cytological parameters of cryopreserved buck semen. *Romanian Biotechnol. Lett*. **15(3)**: 26-32.

Atara, V., Chaudhari, C., Ramani, U., Chaudhary, M. and Patel, D. (2018). Semen characteristics in young and adult Surti buck. *Indian J. Anim. Hlth*. **57(2)**: 219-224.

Bilodeau, J.F., Blanchette, S., Gagnon, C. and Sirard, M.A. (2001). Thiols prevent H_2O_2-mediated loss of sperm motility in cryopreserved bull semen. *Theriogenology.* **56**: 275-286.

Campos, A.C.N., Nunes, J.F., Monteiro, A.W.U., de Figueiredo, E.L., Pinheiro, J.H.T., Ferreira, M.A.L. and de Araujo, A.A. (2004). Viability of washed and unwashed goat sperm diluted in coconut

water, cooled and storage at 4 °C. *Rev. Bras. Cien. Vet.* **11(3)**: 178-182.

Foote, R.H. (2002). The history of artificial insemination: selection notes and notables. *J. Anim. Sci.* **80**: 1-10.

Kale, M., Rathore, N., John, S. and Bhatnagar, D. (1999). Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species. *Toxicol. Lett.* **105(3)**: 197-205.

Pena, F.J., Johannisson, A., Wallgren, M. and Martinez, H.R. (2003). Antioxidant supplementation in vitro improves boar sperm motility and mitochondrial membrane potential after cryopreservation of different fractions of the ejaculate. *Anim. Reprod. Sci*. **78(1-2)**: 85-98.

Sarangi, A., Singh, P., Virmani, M., Sahu, S. and Magotra A. (2017). Effect of vitamin-E supplementation on quality of Beetal buck semen during storage at 4 °C. *Haryana Vet*. **56(1)**: 95-97.

Saraswat, S., Jindal, S.K., Priyadharsini, R., Ramachandran, N., Yadav, S., Rout, P.K., Kharche, S.D. and Goel, A.K. (2012). The effect of antioxidants supplementation to cryopreservation protocol on seminal attributes and sperm membrane characteristics in Sirohi goat. *J. Physiol. Pharmacol. Adv.* **2(1)**: 49-58.

Sinclair, S. (2000). Male infertility: nutritional and environmental considerations. *Alt. Medicine rev.: J. Clinic. Therapeut.* **5(1)**: 28-38.

Zamfirescu, S. and Anghel, A. (2010). Researches regarding the ultrastructural modifications of sperm cells before and after freezing in different media. *Lucrari Stiinţifice Seria Zootehnie.* **53(15)**: 67-74.

Zhao, Y. and Buhr, M.M. (1995). Cryopreservation extenders affect calcium flux in bovine spermatozoa during a temperature challenge. *J. Androl.* **16**: 278-285.