EFFECT OF VITAMIN E SUPPLEMENTATION ON PLASMA MEMBRANE FUNCTIONAL INTEGRITY IN SURTI BUCK SEMEN PRESERVED AT REFRIGERATION TEMPERATURE

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Abstract

The present research work was carried out on four Surti bucks maintained under All India Coordinated Research Project (AICRP) on Goat at Livestock Research Station, Navsari Agricultural University, Navsari during December 2019 to March 2020 with the objective to evaluate the effect of vitamin E supplementation in tris egg yolk citrate extender on plasma membrane functional integrity 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 200×10^6 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°C) up to 48 hours to access the plasma membrane functional integrity at 0, 24, 36, and 48 hours. The significantly ($p < 0.01$) highest percentage of hypo osmotic swelling (HOS) reacted and lowest percentage of HOS non-reacted sperm count (%) irrespective of preservation time was found in T3 (73.63 ± 0.6 and 26.38 ± 0.6) followed by control (68.47 ± 0.9 and 31.53 ± 0.9), T1 (67.89 ± 0.85 and 32.11 ± 0.85), T4 (66.53 ± 0.67 and 33.47 ± 0.67) and T2 (63.97 ± 0.93 and 36.03 ± 0.93) groups. It can be concluded from the present study that plasma plasma membrane integrity of Surti buck semen was well maintained at refrigerated temperature up to 48 hours when extended with 3 mM of vitamin E.

Key words: HOS, Plasma membrane functional integrity, Sperm, Surti Buck, Vitamin E

INTRODUCTION

The goat is considered to be meticulously related to the sheep as a member of family *Caprinae*. India has an outstanding count of 148.88 million goats contributing 27.7% of the livestock population during the year 2019 in total livestock population (20th Livestock census, 2019). In Gujarat, the total goat population was 48.6 lakh and out of them 41.4 lakh were females. Surti goats are gifted with capacity to abide adverse climate and can be reared for milk and meat purpose. They offer main source of revenue to the poor farmers colonized around the south Gujarat region.

Artificial insemination turns out to be widely used technique for breeding goat and sheep (Foote, 2002), which requires rapid cooling, maintenance of motility, viability, plasma membrane integrity and several other parameters. The major drawback is the reduction in the quality of semen during freezing and thawing. Egg yolk has proven beneficial as a cryoprotectant of the sperm plasma membrane and acrosome against cold-shock, in association with the other components (Amirat *et al.*, 2004). Cryopreservation can induce an increased rate of lipid peroxidation in the sperm plasma

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membrane, causing an overall increase in the concentration of oxygen radicals (Baumber *et al.*, 2003). The reactive oxygen species (ROS) produced during cryopreservation leads to reduce post-thaw motility, viability, membrane integrity, antioxidant status, fertility and sperm functions (White, 1993; Zhao and Buhr, 1995; Aitken *et al.*, 1998; Bilodeau *et al.*, 2001). Thus, to minimize the deterioration during cryopreservation antioxidants are added as cryoprotectants.

Vitamin E is a major scavenger of lipid peroxy and alkoxyl radicals and demonstrated to be having a protective effect during semen storage (Tariq *et al.*, 2015). Vitamin E plays an important role as an antioxidant to prevent the lipid peroxidation of biomembranes and helps in cryopreservation of semen, thus the stored semen can easily be used in artificial insemination. Vitamin E has a preservative effect in Tris-citrate egg yolk (TEYC) extender for cryopreserved canine spermatozoa (Michael *et al.*, 2007). The α -tocopherol acetate, an analogue of Vitamin E, when used in tris-citric acid extender on cryopreserved Sahiwal bull semen reported higher values for HOS reacted sperm count (Batool *et al.*, 2012).

MATERIALS AND METHODS

Collection of semen and its processing: Total four healthy Surti bucks above 1 years (1.25 – 1.3 years) of age maintained under All India Coordinated Research Project (AICRP) on Goat at Livestock Research Station, Navsari Agricultural University, Navsari, Gujrat were selected for the study. 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 the training period, semen was collected regularly by artificial vagina twice a week from each buck 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. To eliminate individual buck variability and increase semen quantity, all the ejaculates were pooled during each semen collection. The pooled semen was diluted with 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 $\geq 70\%$ 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 concentration 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 554.65 g/mol.

Plasma membrane functional integrity: To evaluate the plasma membrane functional integrity of spermatozoa, hypo osmotic swelling test (HOST) was performed by mixing 0.1 mL of diluted semen with 1.0 mL of hypo-osmotic solution. The tube containing the mixture was incubated at 37°C for 30 minutes. A drop of semen from the mixture placed on a clean dry glass slide and covered with cover slip. The sperms characterized by varying degrees of coiling or swelling of tail were considered to have an intact plasma membrane (HOS reacted sperm) and the sperms without tail curling were considered to have damaged

membrane (HOST non-reacted sperm). Total 200 spermatozoa were counted in different fields and percentage of spermatozoa exhibiting tail curling (reacted) was calculated.

$$\text{HOST reacted sperm (\%)} = \frac{\text{No. of HOST reacted sperms}}{\text{Total no. of sperms counted}} \times 100$$

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

RESULTS

The initial and post-chilled HOS reacted and non-reacted sperm count 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 HOS reacted sperm counts (%) at 0 hours were 76.25 ± 1.44 , 73.88 ± 1.15 , 68.81 ± 1.67 , 78.31 ± 0.72 and 71.81 ± 0.87 in Control, T1, T2, T3 and T4 groups, respectively. It was observed that the value in the T3 group was significantly ($p < 0.01$) higher as compared to all treatment groups whereas, differed non-significantly with the control group. However, the HOS reacted sperm counts (%) found at 24, 36 and 48 hours post-chilling were significantly ($p < 0.01$) higher in T3 (75.5 ± 0.8 , 72.19 ± 0.71 and 68.5 ± 0.93 , respectively) as compared to Control (69.94 ± 1.29 , 66.19 ± 1.10 and 61.5 ± 0.99 , respectively), T1 (70.31 ± 1.19 , 66.00 ± 1.29 and 61.38 ± 1.4 , respectively), T2 (65.63 ± 1.69 , 62.38 ± 1.64 and 59.06 ± 1.6 , respectively) and T4 (68.44 ± 0.91 , 64.69 ± 0.9 and 61.19 ± 0.93 , respectively) groups. Further, the values of HOS reacted sperm count recorded in T3 group were significantly ($p < 0.01$) higher as compared to all

other groups when observed at any interval post-chilling.

The corresponding overall mean values of HOS reacted sperm count (%) irrespective of various groups were reduced with increasing preservation time as 73.81 ± 0.65 , 69.96 ± 0.64 , 66.29 ± 0.63 and 62.33 ± 0.64 at 0, 24, 36 and 48 hours, respectively.

Moreover, in control, T1, T2, T3 and T4 groups the HOS reacted sperm count (%) were significantly ($p < 0.01$) higher at 0 (76.25 ± 1.44 , 73.88 ± 1.15 , 68.81 ± 1.67 , 78.31 ± 0.72 and 71.81 ± 0.87 , respectively) as compared to 24 (69.94 ± 1.29 , 70.31 ± 1.19 , 65.63 ± 1.69 , 75.5 ± 0.8 and 68.44 ± 0.91 , respectively), 36 (66.19 ± 1.10 , 66.00 ± 1.29 , 62.38 ± 1.64 , 72.19 ± 0.71 and 64.69 ± 0.9 , respectively) and 48 (61.5 ± 0.99 , 61.38 ± 1.4 , 59.06 ± 1.6 , 68.5 ± 0.93 and 61.19 ± 0.93 , respectively) hours post-chilling. Exceptionally, in T1 and T2 groups the HOS reacted sperm count was found non-significantly different between 0 and 24 hours of preservation time. Further, the HOS reacted sperm count recorded in various groups that differed significantly ($p < 0.01$) at 24, 36 and 48 hours post-chilling.

The corresponding overall mean value of HOST reacted sperm count (%) irrespective of preservation time was significantly ($p < 0.01$) highest in T3 (73.63 ± 0.6) as compared to control (68.47 ± 0.9), T1 (67.89 ± 0.85), T2 (63.97 ± 0.93) and T4 (66.53 ± 0.67) groups.

The values of HOS non-reacted sperm count (%) found at 0 hour were 23.75 ± 1.44 , 26.13 ± 1.15 , 31.19 ± 1.67 , 21.69 ± 0.72 and 28.19 ± 0.87 in Control, T1, T2, T3 and T4 groups, respectively. It was observed that the value found in the T3 group was significantly ($p < 0.01$) lower as compared to all treatment groups, whereas, differed non-significantly with the control group. However, the HOS non-reacted sperm count (%) found at 24, 36 and 48 hours post-chilling were

Table 1. Effect of different concentrations of vitamin E and storage duration on plasma membrane functional integrity (%) of Surti buck semen preserved at refrigerated temperature (Mean±SE)

Groups	HOST reacted and Non-reacted spermatozoa (%)				Overall (n=64)	F value	P value
	0 hr	24 hr	36 hr	48 hr			
C	Reacted	76.25±1.44 ^a _{wx}	69.94±1.29 ^b _x	66.19±1.10 ^c _x	61.5±0.99 ^d _x	68.47±0.9 _x	0.00
	Non-reacted	23.75±1.44 ^d _{yz}	30.06±1.29 ^c _x	33.81±1.1 ^b _x	38.5±0.99 ^a _w	31.53±0.9 _x	
T1	Reacted	73.88±1.15 ^a _{xy}	70.31±1.19 ^a _x	66.00±1.29 ^b _x	61.38±1.4 ^c _x	67.89±0.85 _x	0.00
	Non-reacted	26.13±1.15 ^c _{xy}	29.69±1.19 ^c _x	34±1.29 ^b _x	38.63±1.4 ^a _w	32.11±0.85 _x	
T2	Reacted	68.81±1.67 ^a _z	65.63±1.69 ^{ab} _y	62.38±1.64 ^{bc} _y	59.06±1.6 ^c _x	63.97±0.93 _y	0.00
	Non-reacted	31.19±1.67 ^c _w	34.38±1.69 ^{bc} _w	37.63±1.64 ^{ab} _w	40.94±1.6 ^a _w	36.03±0.93 _w	
T3	Reacted	78.31±0.72 ^a _w	75.5±0.8 ^b _w	72.19±0.71 ^c _w	68.5±0.93 ^d _w	73.63±0.6 _w	0.00
	Non-reacted	21.69±0.72 ^d _z	24.50±0.80 ^c _y	27.81±0.71 ^b _y	31.5±0.93 ^a _x	26.38±0.6 _y	
T4	Reacted	71.81±0.87 ^a _{yz}	68.44±0.91 ^b _{xy}	64.69±0.9 ^c _{xy}	61.19±0.93 ^d _x	66.53±0.67 _x	0.00
	Non-reacted	28.19±0.87 ^d _{wx}	31.56±0.91 ^c _{wx}	35.31±0.9 ^b _{wx}	38.81±0.93 ^a _w	33.47±0.67 _x	
Overall (n=80)	Reacted	73.81±0.65 ^a	69.96±0.64 ^b	66.29±0.63 ^c	62.33±0.64 ^d	—	—
	Non-reacted	26.19±0.65 ^d	30.04±0.64 ^c	33.71±0.63 ^b	37.68±0.64 ^a	—	
F value	9.2451**	8.7711**	9.5942**	8.9202**	—	—	—
P value	0.00	0.00	0.00	0.00	—	—	—

** p<0.01; * p<0.05

^{w-z} Means with different subscript within a column (between the groups) differs significantly at p<0.05; p<0.01

^{a-d} Means with different superscript between a column (between time intervals) differs significantly at p<0.05; p<0.01.

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

significantly ($p < 0.01$) lower in T3 (24.5 ± 0.8 , 27.81 ± 0.71 and 31.5 ± 0.93 , respectively) as compared to control (30.06 ± 1.29 , 33.81 ± 1.1 and 38.5 ± 0.99 , respectively), T1 (29.69 ± 1.19 , 34.00 ± 1.29 and 38.63 ± 1.4 , respectively), T2 (34.38 ± 1.69 , 37.63 ± 1.64 and 40.94 ± 1.6 , respectively) and T4 (31.56 ± 0.91 , 35.31 ± 0.9 and 38.81 ± 0.93 , respectively). Further in T3 group, the values of HOS non-reacted sperm count recorded were significantly ($p < 0.01$) lowest as compared to all other groups when observed at any interval post-chilling.

The corresponding overall mean values of HOS non-reacted sperm count (%) irrespective of various groups were inclined with increasing preservation time as 26.19 ± 0.65 , 30.04 ± 0.64 , 33.71 ± 0.63 and 37.68 ± 0.64 at 0, 24, 36 and 48 hours, respectively.

Moreover, in Control, T1, T2, T3 and T4 groups the HOS non-reacted sperm count (%) were significantly ($p < 0.01$) lower at 0 (23.75 ± 1.44 , 26.13 ± 1.15 , 31.19 ± 1.67 , 21.69 ± 0.72 and 28.19 ± 0.87 , respectively) as compared to 24 (30.06 ± 1.29 , 29.69 ± 1.19 , 34.38 ± 1.69 , 24.5 ± 0.8 and 31.56 ± 0.91 , respectively), 36 (33.81 ± 1.1 , 34.00 ± 1.29 , 37.63 ± 1.64 , 27.81 ± 0.71 and 35.31 ± 0.9 , respectively) and 48 (38.5 ± 0.99 , 38.63 ± 1.4 , 40.94 ± 1.6 , 31.5 ± 0.93 and 38.81 ± 0.93 , respectively) hours post-chilling. Exceptionally, in T1 and T2 groups the HOS non-reacted sperm count were differ non-significantly between 0 and 24 hours of preservation time. Further, the HOS non-reacted sperm count recorded in various groups were also differed significantly ($p < 0.01$) at 24, 36 and 48 hours post-chilling.

The corresponding overall mean value of HOST non-reacted sperm count (%) irrespective of preservation time was found significantly ($p < 0.01$) lowest in T3 (26.38 ± 0.6) as compared to Control (31.53 ± 0.9), T1 (32.11 ± 0.85), T2 (36.03 ± 0.93) and T4 (33.47 ± 0.67) groups. However, significantly ($p < 0.01$) highest overall mean value of HOST non-reacted sperm count

was noted in T2 among all the groups.

DISCUSSION

The findings of present study were in close agreement with the findings of Hamedani *et al.* (2016) who reported significantly ($p < 0.05$) higher values of HOS reacted sperm count in 3mM vitamin E supplemented (80.60 ± 0.40 and 23.80 ± 0.73 %, respectively) as compared to control (71.40 ± 0.97 and 16.80 ± 0.91 %, respectively) group in chilled as well as frozen-thawed semen. However, their findings regarding significantly ($p < 0.05$) higher values of HOS reacted sperm count in 1mM (80.20 ± 0.20 and 26.8 ± 0.92 %, respectively) and 2mM (87.0 ± 1.22 and 34.6 ± 2.01 %, respectively) vitamin E supplemented as compared to control (71.40 ± 0.97 and 16.80 ± 0.91 %, respectively) group in chilled as well as frozen-thawed semen was contrary to the present findings where we found, significantly ($p < 0.05$) lower values of overall HOS reacted sperm count in 2mM (63.97 ± 0.93 %) and non-significantly lower in 1mM (67.89 ± 0.85 %) vitamin E supplemented as compared to control (68.47 ± 0.9 %) group.

However, Anghel *et al.* (2010) reported the significantly ($p < 0.05$) higher value for HOST reacted sperm count (55.00 ± 1.65 vs 49.75 ± 2.6 %) in 1.0 mM of vitamin E supplemented as compare to control group in cryopreserved Alpine buck semen. Likewise, Anghel and Zamfirescu (2010) also recorded a significantly ($p < 0.05$) higher values for post thaw HOS reacted sperm count in 1mM vitamin E supplemented (58.97 ± 0.95 vs 53.02 ± 0.98 %) as compared to control group in cryopreserved Merino Palas ram's semen during normal reproduction season (October - December 2009). Similarly, Kurmi *et al.* (2018) also reported a significantly ($p < 0.05$) higher HOS reacted sperm count in 1mM (52.26 ± 0.15 %) as well as 2mM (54.36 ± 0.17 %) vitamin E supplemented as compared to control (50.42 ± 0.18 %) group in frozen-thawed Chhotanagpuri ram semen. Same way, Saraswat *et al.* (2012) studied the effect of vitamin E supplementation at 4.5 mM concentration in tris

based extender on cryopreserved Sirohi goat semen and reported significantly ($p < 0.05$) higher values for HOST reacted sperm count in vitamin E supplemented (13.00 ± 0.67 vs $11.10 \pm 0.89\%$) as compared to control group. The above findings were incompatible with the present findings as we found lower values of overall HOS reacted sperm count in 1mM, 2mM and 4mM vitamin E supplemented as compared to control group.

In conclusion, the highest HOS reacted and lowest HOS non-reacted sperm count were found in treatment group supplemented with 3 mM of vitamin E in tris egg yolk citrate extender entails 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.

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