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Effect of Vitamin E on the Quality of Frozen Ram Semen

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ABSTRACT

The objective of the study was to investigate the effect of different concentrations of vitamin E on the quality of frozen-thawed Chhotanagpuri ram semen. A total of 36 ejaculates were collected from six healthy Chhotanagpuri rams with the help of a standard artificial vagina using a restrained ewe as a mount. Two false mounts were allowed before semen was collected. Each semen sample was diluted in Tris extender and divided into three fractions for supplementation of vitamin E at the concentration of 1mM, 2mM and no antioxidant. All the samples immediately after equilibration and 24 hours after freezing and thawing were evaluated for various microscopic parameters viz. sperm motility, live sperm percentage, plasma membrane integrity percentage, acrosomal integrity percentage, DNA integrity and lipid peroxidation assay was evaluated only in freeze thawed semen. Both the doses of vitamin E significantly ($P<0.05$) inhibited lipid peroxidation as indicated by malondialdehyde (MDA) production and improved significantly ($P<0.05$) the values of different seminal characters such as sperm motility, live sperm, plasma membrane integrity, acrosomal integrity and DNA integrity as compared to control Tris dilutor. However, 2 mM vitamin E was most effective as compared to 1mM vitamin E or control Tris dilutor. The percentage of sperm motility, live sperm, plasma membrane, acrosomal and DNA integrity in Tris dilutor containing vitamin E 2mM were found to be 77.83±0.23, 85.86±0.19,¬ 73.03±0.11, 73.81±0.16 and 85.78±0.19, and 56.81±0.41, 65.19±0.18, 54.36±0.17, 55.53±0.27 and 77.97±0.18 after equilibration and freezing, respectively. From the results of the present study, it could be concluded that the addition of vitamin E to Tris freezing medium could reverse the free radical- mediated oxidative damage and improve the quality of frozen thawed Chhotanagpuri ram semen.

Keywords: Chhotanagpuri Ram, Vitamin E, Lipid peroxidation, DNA integrity

Studies on sheep reproduction have gained importance because of its economic value. Rearing of Chhotanagpuri sheep in Jharkhand by the small and marginal farmers mainly for mutton purpose. For the better propagation of the breed, there should have good breeding ram. In the rural areas, same rams have been used generation after generation which has created greater chance of increasing inbreeding hence lower reproductive performances. In order to improve the genetic make-up of the breed it is important to study the reproductive efficiency as it will enhance proper selection of proven rams. Among the reproductive traits, semen quality plays a major role in determining fertility and reproductive efficiency livestock. A particular basic problem with the cryopreservation of ram semen is the high unsaturated fatty acid content of plasma membrane of spermatozoa and they lack a significant cytoplasmic component containing antioxidants. Therefore, ram sperm cells are highly susceptible to lipid peroxidation

by free radicals. Since lipid peroxidation induced by ROS directly damages the phospholipid components of cell membranes which is related to the impairment of sperm motility and decreases in the function of spermatozoa. In the terminal phase of this process stable compounds such as malondialdehyde are formed, which also have genotoxic effect (Burcham, 1998). However, addition of antioxidants in the freezing extender has been reported to improve the quality of semen by inhibition of lipid peroxidation caused by ROS in frozen thawed semen. Vitamin E is the first line of defense against the peroxidation of PUFAs contained in the cellular and sub-cellular membrane phospholipids because of its lipid solubility (Horton *et al.,* 2002). Therefore, the present study was conducted to determine the effect of two dose levels of vitamin E in reversing the free radical- mediated oxidative damage on the sperm motility, viability, plasma membrane integrity, acrosome status, DNA integrity and lipid peroxidation during freezing and thawing of Chhotanagpuri ram spermatozoa.

MATERIALS AND METHODS

The study was conducted on six healthy Chhotanagpuri rams aged between three to five years maintained at Instructional Farm Small Ruminants, Ranchi Veterinary College, Birsa Agricultural University, Kanke, Ranchi-834006. The animals were maintained under uniform feeding and housing throughout the period of study. A total of 36 ejaculates were collected from the rams twice a week with the help of an artificial vagina. Immediately after evaluation, the semen samples having volume 0.5 ml or more, mass activity (0 to 5 grades) 3+ or more, sperm concentration 3000 × 10^6 or more and progressive motility 70% or more were only used for the study. The ejaculates of each ram were diluted with Egg Yolk-Tris-Citrate-Fructose extender. Subsequent to dilution of semen in Tris dilutor, each semen sample was divided into three fractions for addition of different levels of vitamin E. Out of three fractions one fraction served as control in which no antioxidant was added, whereas in other two fractions of ejaculate extended with Tris extender vitamin E was added at concentration of 1mM and 2mM respectively. All the samples immediately after equilibration and 24 hours after freezing and thawing were evaluated for live sperm percentage, plasma membrane integrity, acrosomal integrity and DNA integrity (Nur *et al.,* 2011). The Lipid peroxidation level of sperm was measured by determining the malondialdehyde (MDA) production (Suleiman *et al.,* 1996) in freeze thawed semen.

Statistical analysis

The data were subjected to statistical analysis as per standard method (Snedecor *et al.,* 2004).

RESULTS AND DISCUSSION

The mean values of different spermatozoan parameters recorded are presented in Table 1. The present observation of highest post thawed sperm motility after adding vitamin E @2mM in freezing medium was in agreement with that reported in ram (Anghel *et al.,* 2010) and buck semen (Hazarika *et al.,* 2015 and Dewry *et al.,* 2015). The result was also in agreement with the reports in ram (Anghel *et al.,* 2009 and Anghel and Zamfirescu, 2010), buck (Saraswat*et al.,* 2012[a]) and bull semen (Bansal *et al.,* 2009) where significant improvement of post thawed sperm motility in the presence of vitamin E was recorded.

The mean percentage of live spermatozoa were significantly ($P<0.05$) higher for 2mM vitamin E than other concentration of vitamin E(Table -01) which was in corroboration with the findings of Anghel *et al.* (2010) in Merino ram and Hazarika *et al.* (2015); Dewry *et al.,* 2015 in buck. This finding is supported with the similar observation in ram (Anghel *et al.,* 2009 and Anghel and Zamfirescu, 2010), buck (Saraswat *et al.,* 2012), crossbred bull (Gupta and Prasad, 2004) and buffalo bull semen (Beheshti *et al.,* 2011) who found significant differences in post

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Table 1: Percentage of live sperm, sperm motility, HOST reacted sperm, acrosome, DNA integrity and MDA production (mean ± se) in Chhotanagpuri ram semen extended in Tris dilutor containing different concentrations of Vitamin after equilibration and freezing

Attributes	Vitamin E 1mM		Vitamin E 2mM		Control	
	After equilibration	After freezing	After equilibration	After freezing	After equilibration	After freezing
Sperm motility	75.03±**0.13**^{B}	53.69±**0.18**^{b}	77.83±**0.23**^{C}	56.81±**0.41**^{c}	70.14±**0.47**^{A}	50.14±**0.68**^{a}
Live sperm	83.06±**0.18**^{B}	62.96±**0.21**^{b}	85.86±**0.19**^{C}	65.19±**0.18**^{c}	79.93±**0.16**^{A}	60.10±**0.13**^{a}
HOST reacted Sperm	71.19±**0.15**^{B}	52.26±**0.15**^{b}	73.03±**0.11**^{C}	54.36±**0.17**^{c}	70.06±**0.12**^{A}	50.42±**0.18**^{a}
Acrosomal integrity	71.74±**0.20**^{B}	53.36±**0.19**^{b}	73.81±**0.16**^{C}	55.53±**0.27**^{c}	70.06±**0.12**^{A}	50.42±**0.18**^{a}
DNA integrity	84.61±**0.19**^{B}	76.83±**0.19**^{b}	85.78±**0.19**^{C}	77.97±**0.18**^{c}	82.97±**0.21**^{A}	75.19±**0.11**^{a}
MDA production (nmol/10^8 sperm)		4.84±0.02^{b}		4.09±0.02^{a}		5.73±0.02^{c}

Values bearing different superscript with in a row differ significantly (P<0.05)

thaw sperm viability in Tris extender containing different concentrations of vitamin E.

The present findings of HOST reacted sperm was highest in Tris extender containing vitamin E at the concentration of 2mM after equilibration and freezing followed by 1mM vitamin E and control (Table 1). The significantly (P<0.05) higher percentage of HOST reacted sperm after equilibration and after freezing in extender added with 2 mM vitamin E than in control recorded in the study was in agreement with the findings in ram (Anghel *et al.,* 2010) and buck semen (Hazarika *et al.*, 2015). Earlier several workers also recorded higher percentage of HOST reacted sperm after freezing in extender supplemented with vitamin E in ram (Anghel *et al.,* 2009 and Anghel and Zamfirescu, 2010) and crossbred HF bull (Muzafer *et al.,* 2012). On the other hand, no significant effect of addition of vitamin E on the post-thaw percentage of HOST-reacted sperm in buck semen was reported by Saraswat *et al.* (2012).

The present post thawed acrosomal integrity found after freezing with Tris extender containing 2 mM vitamin E was higher than that recorded in buck semen (Dewry *et al.,* 2015 and Hazarika *et al.,* 2015). Significant improvement in post-thaw incidence of intact acrosome was reported by earlier workers with 4.5mM of vitamin E in buck semen (Saraswat *et al.,* 2012) and 0.3mg/ml of vitamin E in bull semen (Rao *et al.,* 2012). The variations in the effect of vitamin E on acrosomal integrity might be due to difference in concentration of vitamin E, processing, freezing and thawing procedures used in different studies.

In the present investigation the values of DNA integrity were significantly higher for 2mM vitamin E than either 1 mM vitamin E or Tris alone. The present finding are in accordance with Thuwanut *et al.* (2008) who reported that the addition of vitamin E @ 5mM in Tris extender improved DNA integrity percentage in cat semen. Kalthur *et al.* (2011) also reported that supplementation of vitamin E (5mM) in freezing medium significantly improved the post-thaw DNA integrity in normozoospermic and asthenozoospermic human semen. On the contrary, Donnelly *et al.* (1999) in human could not find any significant difference in DNA integrity after addition of alpha tocopherol (40 and 60 µM) in freezing medium, however, he also reported that alpha tocopherol can provide significant dose-dependent protection against H_2O_2-induced DNA damage. Due to lack of available literature on the effect of vitamin E on DNA integrity after freezing in ram semen, the present findings could not be compared suitably with that of other workers.

The present MDA concentration produced

revealed a significant difference (P<0.01) in semen samples diluted with Tris extender containing different concentrations of vitamin E. The present values of MDA concentration produced in semen after freezing in Tris extender containing Vitamin E was very comparable with the findings of Anghel and Zamfirescu (2010) in Merino rams semen extended with Tris extender containing vitamin E 1mM (3.89±0.41 nmol/10^8 sperm). However, the present values of MDA production in semen after freezing was higher than that reported by Anghel *et al.,* (2010) in Merino ram semen diluted in Tris extender containing vitamin E at the concentration of 1mM and 2mM. Muzafer *et al.* (2012) reported significantly lower MDA production in bull semen diluted in Tris extender containing vitamin E @ 0.3mg/ml as 0.12±0.01 µmol/ml which was lower than the present findings. On the other hand, Asadpour *et al.* (2011) did not find significant change with the addition of the vitamin E to TEY extender compared with the control group in bull semen.

In this study, vitamin E supplementation in Tris Egg Yolk extender improved viability, motility, HOST reacted sperm, acrosome integrity and DNA integrity of frozen-thawed ram spermatozoa. The production of malondialdehyde (MDA), the end product of lipid peroxidation, also reduced as compare to control. The result may be explained based on the fact that vitamin E protects the spermatozoa against the harmful effects of oxidative stress induced by freezing-thawing. Vitamin E is the major chain breaking antioxidants in membrane. It scavenges all the three types of radicals, namely superoxide, peroxyl and hydroxyl which lead to the peroxidation of phospholipids in the mitochondria of sperm cell and thus to their ultimate immotility (Sinclair, 2000). By scavenging these radicals it breaks free radical chain reaction and forms a relatively stable complex. Vitamin E captures and eliminates oxygen radicals within the membrane and also intercepts peroxyl and alkoxyl radicals which are generated during conversion of lipid hydroperoxides which are fuel for peroxidative chain reaction, thereby preventing through cell membrane (Aitken and Clarkson, 1988). The result of the present study shows that addition of vitamin E in Tris freezing medium improved the percentage of sperm motility, live sperm, plasma membrane integrity, acrosomal integrity and DNA integrity, reduced the MDA production in the frozen thawed semen compared to the Tris control as freezing medium. 2mM vitamin E added in Tris dilutor appeared to be the best. Thus on the basis of the present result 2mM vitamin E may be recommended for supplementation in Tris freezing medium for freezing of Chhotanagpuri ram semen. However, further studies on large population is required to understand the effect of vitamin E in reversing the free radical-mediated oxidative damage to the frozen thawed Chhotanagpuri ram semen.

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