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Research Article

VITAMIN E AS AN ANTIOXIDANT ON MEMBRANE INTEGRITY OF CRYOPRESERVED SPERM IN DIFFERENT BREEDS OF BULLS

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Abstract: Aim: To study the effects of Vitamin E in different dose on the semen parameters during cryopreservation processes in different breeds of bulls. Material and Methods: Three different breeds of healthy bulls; Red Sindhi (CB) (n=6), Haryana (CB) (n=6) and Jersey (CB) (n=6) with same feeding practice belonging to the frozen semen bank, Khapuria, Cuttack, Odisha were selected for the study. In this study, four types of extender with different concentration of vitamin E (2.5mM, 5mM and 10mM) were used. Four types of straws were prepared and all these straws underwent programmed freezing (-196°C). After cryopreservation, straws were thawed in water bath maintained at a temperature of 37°C for 15 minutes. Then different tests were conducted like individual motility, live and dead count, normal abnormal count and acrosomal integrity test for different straws. Results: The effects of vitamin E on different sperm parameters like individual motility, live and dead, acrosomal integrity, and normal and abnormal percentage in dose wise (normal, 2.5, 5, 10 milli mole) varied significantly. Vitamin E at 5 milli mole shows maximum result while 2.5 milli moles have least result. Conclusion: On the basis of the present experiment, we can conclude that the dose wise effect of vitamin E is significant. Among all parameters, sperm motility comparatively increased after adding vitamin E at the dose rate of 5 mM/ml.

Keywords: *Vitamin E, Antioxidant, Cryopreserved sperm, Membrane integrity, Sperm motility*

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Introduction

Artificial insemination has a great importance in the development of dairy sector. In the last two centuries, the major advancement in dairy animal practice was due to the development of artificial insemination [1]. In artificial insemination, straws are stored at -196°C by using liquid nitrogen. This temperature has a detrimental effect on the ultra-structure, biochemical, and functional features of sperm cells. This low temperature has an impact of oxidative stress. Oxidative stress alters the membrane sulfhydryl status, lipid and phospholipid contents of crossbred bull spermatozoa [2]. During cryopreservation, different sperm parameters like motility, membrane integrity and fertilizing ability decrease. There is also decrease in freezing ability of sperm cells due to formation of ice crystals. This alternatively affects female reproductive performance [3]. There is reducing sperm concentration in semen to a critical condition. In present day, oxidative stress challenge in liquid bull semen, as it affects motility and livability of sperm during cryopreservation [4]. It is seen that natural antioxidants like tocopherol and ascorbic acids are responsible for maintenance of sperm activity during freeze-thaw process [5]. Natural antioxidants like alpha tocopherol are responsible for maintenance of sperm activity during freeze-thaw process at very low temperature. This is because alpha-tocopherol is a lipid-peroxidation (LPO) inhibitor [6, 7]. Different antioxidants such as vitamins C and vitamin E can also be used in semen preservation media to improve longevity and quality of sperm [8].

The main reason of poor quality semen is due to oxidative stress. Uncontrolled production of ROS leads to oxidative stress (OS) which is harmful to spermatozoa and also causes DNA damage leading to cell death. Therefore, addition of extra antioxidants to the extender during semen processing is essential. Among all natural antioxidants, vitamin E has maximum effectiveness against oxidative stress [7]. It directly quenches the free radicals and is the main scavenger of free radicals. It is one of the major protectors of spermatozoa membrane against ROS attack. Because of lipid solubility vitamin E acts as first line of defense against peroxidation. The present study was undertaken with three objectives, firstly to measure the effect of vitamin E at different doses on semen cryopreservation. Secondly to study the effect of vitamin E on different semen quality collected from different breeds and thirdly to measure the effect of vitamin E on semen of different breeds.

Ethical approval

This study was conducted at Frozen Semen Bank, Department of Fisheries & Animal Resources Development, Odisha under complete supervision of Deputy Director, Frozen Semen Bank, Khapuria, Cuttack.

Materials and Methods

Semen collection and processing

The whole study was performed in the Department of Physiology, Odisha Veterinary College with the help of Frozen Semen Bank, Khapuria, Cuttack, ODISHA. Three breeds – Red Sindhi, Haryana and Jersey belonging to the frozen semen bank were selected for the study. These bulls were within age group of 4- 6 years and maintained in optimal nutrition. From each bull, there were total of 6 collections of semen, so from each breed 6 x 6 = 36 collections. So the total sample collected from three breeds = 36 x 6 = 108 nos. Immediately after collection, raw semen was studied under spectrophotometer which indicates the concentration of sperm.

Extender Preparation

Four types of extender were prepared, with different concentration of vitamin E

(The product used for vitamin E is a HIMEDIA product with Empirical formula= C -29 H-50 0-2. DL-alpha Tocopherol Liquid :(±)-alpha- Tocopherol. CAS No 10191-44-0, Minimum assay 98.0 percent). Molecular weight of total product= 430.71 g. This means for preparation of one mole solution - 430.71 gm of the product should mix with liter of extender. For one milli mole solution = 430.71/ 1000 = 0.43071 gm of the product should mix in liter of extender. Purity of the product= 98 percent.1 milli mole concentration per lit of solution =100/ 98 x 0.43071 = 0.4395 gm in lit of solution (0.5 gm)

That means 0.5 gm of product should mix with liter of solution to make one milli mole of extender. For 2.5 milli mole concentration per lit of solution = 2.5x .5=1.5 gm per lit of solution For 5 milli mole concentration = 5 x 0.5 = 2.5 gm / lit of solution. For 10 milli mole vitamin E with extender = 10x.05 =5 gm / liter of extender.

Control extender-TRIS-24.2 gm, Citric acid-12.8 gm, Fructose-10 gm,Glycerol-70 ml , Distilled water-730 ml , Egg yolk-200 ml Streptomycin-0.5-1 gm, .Benzyl penicillin- 5 lakh to 10 lakh unit.

Second extender- control extender +2.5 milli mole vitamin E

Third extender- Control extender + 5 milli mole vitamin E

Fourth extender-Control extender + 10 milli mole vitamin E.

Preparation of straw

Four types of straw, one control straw, second with vitamin E at 2.5 milli mole concentrations, third 5 milli mole concentrations and fourth 10 milli mole concentration of vitamin E, were prepared for each bull. Then there is programmed freezing of these straws were done to -196 °C. These straws are kept in liquid nitrogen cryopreservation at least for 24 hours. After cryopreservation straws were taken out of cryocane. Immediately the straws were thawed in water bath maintained at a temperature of 37 °C for 15 minutes. Then after different tests are conducted like individual motility, live and dead count, normal abnormal count, acrosomal integrity test of different straws.

Test and procedure

Individual motility

A small drop of semen from straw was taken on a clean, grease free, preheated (37 °C) glass slide and a cover slip was placed over the drop. Then semen was examined under high power magnification 45X with thermostatically controlled 37 °C phase contrast microscope

Live and Dead Sperm count

Live and dead spermatozoa count was done by Eosin Nigrosin stain. One drop of semen taken in to the Ependruff tube and to these 4 drops of stain was added and was kept in incubator at 37 °C for 5 minutes. After 5 minutes, a drop of semen stain mixture was withdrawn and a smear was drawn in the clean grease free preheated glass slide. The slide was air dried and examined under phase contrast microscope at a higher magnification. The slide was examined for the occurrences of live normal spermatozoa, live abnormal and dead spermatozoa by counting 200 sperm cells. The result expressed in percentage of live spermatozoa. The live spermatozoa would not take stain and will be clearly seen in blue back ground. The dead spermatozoa took up acidophillic stain.

Normal and Abnormal sperm count

The percentage of abnormal spermatozoa was estimated by using Rose Bengal staining method. A clear grease free pre warmed glass slide was taken. A small drop of semen from the straw was taken in the glass slide. A thin uniform smear was drawn. Smear was stained with rose Bengal stain. It is kept for 10- 15 minute. The slide was washed with buffer solution and then air dried. The slides were observed under high magnification 100 X, under cidarwood oil. A total 200 number of spermatozoa were counted per smear and the percentage of normal sperm was counted. The type of abnormality was observed during examination of smear.

Acrosomal integrity Test

Giemsa staining technique used for acrosomal integrity test. A clean grease free glass slide was taken. A small drop of semen from the straw was taken on the glass slide. A thin uniform smear was drawn on the glass slide and allowed it for drying. The dried smear was fixed by putting 4-8 drops of formalin for 15 minutes. Then the slide transferred in to Giemsa staining jar. The slide was for 3-4 hour at 37 °C in the jar. After 4 hour the slide was taken out of the jar and rinsed under tap water. The air dried slide was observed under oil immersion lens. Intact acrosome expressed in percentage. The sperm having intact acrosome was identified and counted. The sperm showing no membrane damage were considered to be intact acrosome. Total 200 sperms were counted.

Statistical analysis

Analysis of data was performed using the Statistical System software package (SAS, USA, 2010). Data of different parameters were analyzed using non-parametric analysis of variance (ANOVA).

Result

The effect of vitamin E upon different sperm parameters in dose wise like without Vitamin E, Vitamin E at 2.5 millimole, 5 milli mole, 10 milli mole are measured. Effect of vitamin E upon different sperm parameters in dose wise is presented in the given table 1. In this table mean percentage of individual motility, Live Dead, Acosomal integrity and normal and abnormal percentage sperm are given after post thaw. This table indicates the effect of Vitamin E in Dose wise upon different sperm parameters like individual motility, Live Dead, Acosomal integrity and normal and abnormal percentage.

Effect of Vitamin E upon Individual Motility

The percent sperm individual motility ($p<0.01$) varied significantly with respect to different dose. There is change in motility percentage when Vitamin E is added in different Dose rate. Among different dose 5 milli mole shows best result. At 2.5 milli mole this has lowest impact upon individual motility. There is approximately 10 percent increase in individual motility when Vitamin E is added at dose rate of 5 milli mole. In dose wise 2.5 and 10 milli mole are not so significant, they only increase in 2 to 3 percent individual motility as compare to normal.

Effect of Vitamin E upon live Dead percentage

Livability percentage of normal sperm of different breed of cattle was 70 percent. Due to cold shock maximum sperm cells were dead. By adding Vitamin E at dose rate of 2.5 milli mole the motility is 73.55 %. Similarly at 10 milli mole the result was 72.88 %. Compare to 2.5 and 5 milli mole vitamin E 10 mill moles have better effect and livability percentage significantly increases. Live percentage is around 77.22 percent at 5 milli mole vitamin E.

Effect of Vitamin E upon acrosomal integrity percentage

Acrosomal integrity percentage at normal condition in absence of Vitamin E is 70.72 % , at 2.5 milli mole the value is 74.94 % and at 10 milli mole it about 72.88 % .That means 10 milli mole has better impact upon acrosomal integrity. At 5 milli mole the acrosomal integrity percentage is maximum and the value is 77%.

Effect of Vitamin E upon normal abnormal percentage

However, the effect of vitamin E upon normal abnormal percentage is completely reverse. It is highly significant at 10 milli mole ($p<0.01$). Not significant in 5 and 2.5 milli mole. At 10 milli mole the abnormal percentage is lowest. The same sperm without Vitamin E having abnormal percentage around 21 percent, when vitamin E added at a dose rate of 10 milli mole the abnormal percentage decreases to 14 percentages. At 2.5 and 5 milli mole Vitamin E the abnormal percentage decreases, but that are not so significant.

Discussion

Reactive oxygen species (ROS) causes the destabilization of sperm membrane and also affect the capacitating ability sperm; it also completely destroyed the integrity of sperm membrane. Sperm membrane is made up of phospholipids, so it vigorously reacts with ROS [9]. In the frozen semen bank of Odisha it is a common report that there is decrease in motility in post thaw after cryopreservation. So, to prevent this above effect we have used vitamin E as an antioxidant.

It was previously found that Vitamin E is used as an anti oxidant in treatment of Human Health and Some Diseases [10]. Vitamin C can also be added with extender, in most of frozen semen bank till today. Some previous study indicate addition of vitamin E is comparatively more better than vitamin C [8].

Vitamin E increase post thaw semen motility by 10-20 percent depending upon concentration in human semen for that reason vitamin E use along with the extender as per some workers. Use of vitamin E increases the utility of extender. Vitamin E has effect on bovine semen preservation when added with extender in frozen thawed semen [11].

Vitamin E effect changes depending on dose. It is seen that addition of vitamin E to cryo-preserved semen at different dose with the extender results in improvement of post-thaw motility [12]. Cryo-protectants and thawing procedure are associated with significant reduction in sperm motility induced by ROS and that effect can be avoided by adding vitamin E at different doses to the extender at rates like 5 milli mole 2.5 milli mole and 10 milli mole concentration. It was previously proved dose wise vitamin E has effect upon semen in case of chicken. Vitamin E addition to Chicken Semen increases the sperm quality during in Vitro Storage of Semen [13].

Supplementation of vitamin E 5 mM significantly improves the post-thaw motility and DNA integrity in normozoospermic and as the nozoospermic semen samples, vitamin E has almost best effect at 5 milli mole concentration [7]. Vitamin E has also potential against cancer and other chronic diseases because it captures the free radicals. It has best antioxidant effect. Studies revealed that vitamin E is a major chain breaking antioxidant in the sperm membrane and it appears to have a dose dependant protective effect [14,15] . Vitamin E the only lipid-soluble, chain-breaking anti-oxidant in human blood plasma and erythrocyte membranes [16].

At different dose rate vitamin E can be used as an antioxidant for the therapeutic agent in the treatment of male infertility and oligospermia condition. At 5 millimole vitamin E individual motility is highest. Similarly live dead percentage is more in 5 milli mole then 10 milli moles, then 2.5 milli moles. At 5 milli mole the livability percentage is more 77.22± 2.01, which correlates with our observation [7].

Vitamin E at a concentration of 10 milli mole per ml has protective effect on membrane function and enhances post thaw sperm motility by 10- 20 percent. According to Agrawal et al. acrosomal integrity percentage is more in 10 milli mole concentrations. The supplementation of Vitamin E in different concentration (10 milli mole/ml, 5 MM/ml, and 2.5 mM/ml increases the post thaw motility by 7-14 percent, 5 milli mole has best effect on the individual motility, and livability percentage . whereas 10 milli mole has best effect on the acrosomal percentage and abnormal percentage. Result of abnormal percentage is less in 10 milli Mole vitamin E, which corroborates our observation.

Acrosomal integrity percentage is more in 10 milli mole concentrations and normal abnormal percentage also significantly varies depending on dose. 10 milli moles maximally decreases the abnormal percentage

Conclusion

Vitamin at 5 milli mole has best effect upon the different semen parameters. It increases individual motility, livability percent, acrosomal integrity in significant amount. At this dose rate it also decreases abnormal spermatozoa. It should include during the extender preparation in semen processing lab.

Application of research: Study the effect of vitamin E on different semen quality collected from different breeds and thirdly to measure the effect of vitamin E on semen of different breeds

Research Category: Veterinary Physiology

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Cultivar / Variety / Breed name: Bulls

Conflict of Interest: None declared

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