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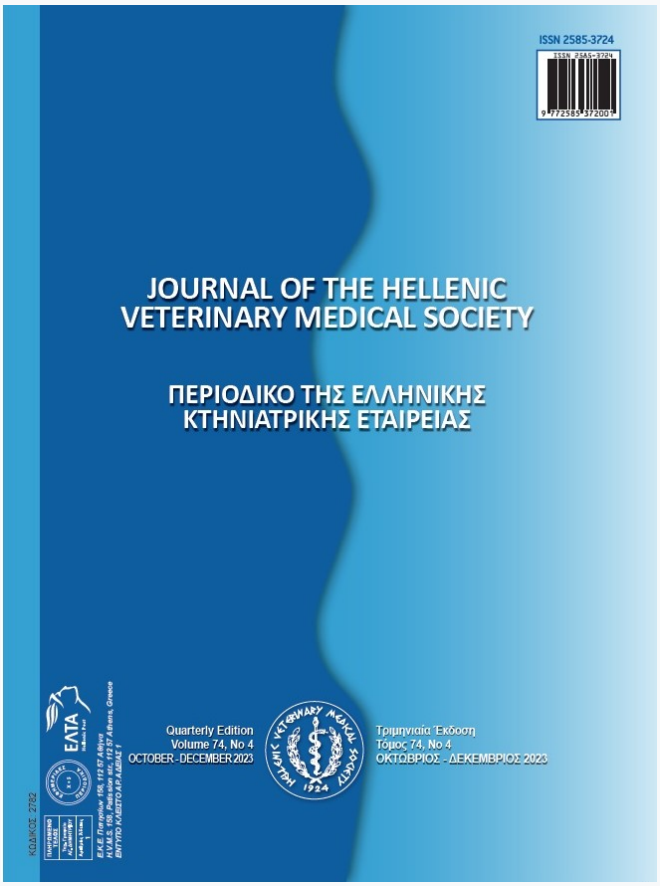

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Research article
Ερευνητικό άρθρο

Effects of α-Tocopherol on chilled quality parameters of Tharparkar bull semen extended in Lecithin and Tris based egg yolk extender

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ABSTRACT: This study was conducted to evaluate the effect of α-Tocopherol (Vit E) on chillled semen quality paramenters of Tharparkar bull. For the purpose of the present study, 52 ejaculates were collected from four bulls initially semen samples were evaluated for progressive motility, morphology and viability (Eosin-Nigrosin staining technique) and membrane integrity (Hypo-osmotic Swelling test). Samples having ≥70% of all above mentioned parameters were pooled and extended into a Lecithin based extender and Tris based egg yolk extender supplemented with α-tocopherol (Vit E) 0.02 mM and cooled at 4°C for 2 hours. The semen samples were subsequently assessed for progressive motility, morphology, viability and membrane integrity. Spermatozoa supplemented with α-tocopherol in Lecithin based extender showed improved parameters, compared to spermatozoa supplemented with α-tocopherol in Tris-based extender in terms of progressive motility (79.09±0.87 vs 74.69±0.94%), morphology (86.13±0.79 vs 82.81±1.00%), viability (82.13±1.20 vs 77.63±1.26%) and membrane integrity (81.40±0.77 vs 77.31±1.01%). In conclusion the addition of α-tocopherol improved the chilled quality parameters of Tharparkar bull semen extended in a Lecithin based extender as compared to Tris based egg yolk extender.

Keywords: Cryopreservation,Tharparkar Bull Semen, Chilled Semen, α-tocopherol, Lecithin based extender.

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INTRODUCTION

Tharparkar cattle was derived from Thar Desert (Chand, 2011) in Sindh, Pakistan. It is a *Bosindicus*, lyre-horned breed found in India-Pakistan border area of Thar Desert and considered a dual-purpose breed valued for its milk as well as draught utility (Godara *et a*l., 2015). It is considered highly resistant to tick-borne disease with good heat tolerance capacity, Tharparkar cattle breed is deep, alert and powerfully built & medium-sized with limbs straight & good feet (Sanjay *et a*l., 2018).

Cryopreservation is one of the techniques of assisted reproductive technologies in which semenis preserved for future use (Olaciregui *et a*l., 2014). This technique is widely used for domestic animals and humans (MotaFilho *et al.,* 2014). Cryopreservation accelerates genetic improvement, enhances animal production and contributes to eliminate contagious diseases (e.g. Brucellosis) and preserve endangered species (Bucak *et al.,* 2009). during cryopreservation the quality of semen is deteriorated, which leads to low fertilization rates (Ardon and Saurez, 2013). Cryopreservation induces thermal shock, toxic stress, osmotic disturbance, and ice crystals formation (Watson, 2000). During freeze-thawing Reactive Oxygen Species (ROS) are produced, while the accumulation of ROS and the lack of antioxidants result in DNA fragmentation. lipid peroxidation and premature capacitation (Miguel-Jimenez *et a*l., 2020; Upadhyay et al., 2021). Thus, the supplementaion of semen extender with additivess, such as antioxidants seems very promising to ensure the quality of semen.

An extensive effort has been made towards theimprovement of medias and preservation protocols during cryopreservation. Lecithin based extender (BIOXcell-™: IMV Technologies, L'Agile,France) is a mixture of several fatty acids i.e. Palmitic, Oleic and Stearic acids which maintains staibility of cell membrane and results in higher progressive motility compared to lab-based extenders. Moreover, the improved progressive motility parameters of spermatozoa extended in Lecithin based extender could be due to increased amount of glutathione (Stradaioli et al., 2007). The composition of Tris extenders is highly variable, while the microbial load and the presences of endotoxins invlove the risk of negative impact on sperm progressive motility and viability (Apu et al., 2012). In comparision to Lecithin based extender, studies suggest that egg yolk based extender is more effective for bull sperm viability and fertilizability (Crespilho et al., 2014; Kaka et al., 2015).

Various antioxidants have been employes, such as α-Tocopherol (Vit E), Vit C and Co-enzyme $Q_{10,}$ which act as scavengers and prevent cellular demage. α-Tocopherolis a lipid soluble antioxidant that helps to control lipid peroxidation, Adding of antioxidant vit E (α-Tocopherol) in the extender will inhibit the process of lipid per oxidation reaction that helps to manage the oxidation process of phosphorylation, which is responsible for the elevating level of ROS in the sperm.

Many studies have been addressed in exotic cattle breeds, but few of them refer to indigenous Tharparkar cattle bull. Therefore, this study was designed with a hypothesis that supplementation of α-Tocopherol into a Lecithin based extender and Tris-based egg yolk extender would improve chilled quality parameters of Tharparkar cattle bull semen.

MATERIALS AND METHODS

Bulls preparation &Collection of Semen

For the purpose of this study four Tharparkar bulls, 4-5 years old were housed under semi-intensive farming system at Deparent of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Sindh, Pakistan. Routine vaccination and drenching were followed according to farm schedules. Total 52 ejaculates (n=13) were collected with the help of Artificial Vagina (Internal Temprature 45-48 °C and air pressure of 35-44 mmhg) from september to early december 2021. After semen collection, samples were shifted to SPU (Semen Production Unit) for macroscopic and microscopic semen analysis. Moreover, samples with progressive motility, morphology and viability ≥70% were pooled and extended and further assessed.

Semen evaluation

Macroscopic Semen evaluation

Volume

The volume of semen was observed through visual examination with the help of graduated tube.

Colour

Visual examination was done for judgment of colour however semen was categorized as Milky, Creamy white and Translucent.

pH

pH was determined by a digital pH meter.

Microscopic Semen evaluation

Wave motion

The wave motion was assessed on a clean warm dry slide by putting a drop of undiluted semen under low power of magnification (10X) with phase contrast microscope (Nikon, Germany). Wave sample was recorded and classified as described by Rehman et al., (2012).

Table 2.1: Wave Motion Assessment Scale Mass Progressive Motility

Scale	Assessment
0	Nill mass activity
+	<20% of sperm motion
++	40-60% showing movement with the slow wave
+++	Wave showing more intense and movement 60-80%
+++++	Wave making eddies describing movement 80-100%

Progressive Motility

For the assessment of percentage of progressive motility, semen diluted with normal saline (1:100). One drop of diluted semen was taken and putted on pre-warmed slide by applying a cover slip on it. One hundred spermatozoa were randomly selected spermatozoa moving backward or in circle were not counted. The results were expresses as %, samples with ≥70% progressive motility was treated for further evaluation.

Concentration of sperm

Concentration of sperm was determined with a haemocytometer.

Sperm morphology and Viability

As per standard staining procedure of sperm, morphology was determined as described by (Björndahl et al., 2003). From each sample four smears were prepared and 100 spermatozoa were evaluated in terms of morphology and viability. Viability and abnormalities were assessed in 100X magnification.

Membrane integrity

Hypo Osmotic Swelling (HOST) test was used to determine membrane integrity of fresh samples, according to Revell and Mrode, (1994).

Experimental design of semen extension

Ejaculates qualifying the standard criteria of Macroscopic parameters: volume (3-6ml), colour (V/E), pH (6-7) and Microscopic parameters (concentration ($1x10^9$/ml), progressive motility (≥70%), morphology and viability (≥70%) and membrane integrity (≥70%) were pooled and diluted in the Tris based egg yolk and Lecithin based extender then divided into four groups consisting of Tris control,Tris+ 0.2mM α-tocopherol (Vit E), Lecithin based extender control and Lecithin based extender +0.2mM α-tocopherol (Vit E). Rate of dilution was based on initial sperm concentration and it was adjusted to have 20 million spermatozoa in 0.25 ml straw. α-tocopherol (Vit E)0.2mM concentration was selected from the study conducted by (Kaka, 2015b). Diluted semen samples were cooled at 4°C for 02 hours.

Extension of semen

Each semen sample was diluted with Tris based egg yolk extender and Lecithin based extender as described by Kaka, 2015a.

Equilibration

The equilibration was completed within 2 hours at 4°C.

Chilled semen evaluation

Chilled semen evaluation were followed after thawing at 37°C for 30 seconds in which progressive motility, morphology, membrane integrity and viability of the spermatozoa were evaluated.

Statistical analysis

Collected data were subsequently subjected to one-way analysis of variance (ANOVA) using Statistics (2006) and LSD was used to determine difference among means of different groups.

RESULTS

Chilled semen assessment of Tharparkar bull semen (Mean % ± SEM) in Lecithin based extender and Tris based egg yolk extender supplemented with and without vit E (α-Tocopherol) 0.02 mM.

The Mean (±SE) progressive motility were significant among all groups ($P<0.05$). However addition of α-Tocopherol showed improved progressive motility of spermatozoa in Lecithin based extender (79.09±0.87) followed by Tris based egg yolk extender (74.69±0.94), compared to Lecithin based extender (73.55±1.20) and Tris control (71.72±1.28) as are depicted in Figure 3.1. The Mean (±SE) Morphology was also significantly different in all groups ($P<0.05$), Tris-Control, Tris + α-Tocopherol, Lecithin

based extender + Control and Lecithin based extender + α-Tocopherol, However, numerically highest and improved values were observed in Lecithin based extender + α-Tocopherol (86.13±0.79) as compared to Tris-Control (78.90±1.01), Tris supplemented with α-Tocopherol (82.81±1.00) and Lecithin based extender Control (80.81±0.96) as shown in Figure 3.2.

Futthermore, Improved viability was also noted in Lecithin based extender (82.13±1.20) supplemented with α-Tocopherol as compared to Tris-Control (74.04±1.31), Tris supplemented with α-Tocopherol (77.63±1.26) and Lecithin based extender Control (76.02±1.28) and there were no significant difference among groups (P<0.05) Figure 3.3. The Mean (±SE) Membrane Integrity of each group is detailed Figure 3.4, The significant difference was observed among all groups (P<0.05), Tris-Control, Tris + α-Tocopherol, Lecithin based extender + Control and Lecithin based extender + α-Tocopherol, among the all groups numerically highest improved membrane integrity value were observed in Lecithin based extender + α-Tocopherol (81.40±0.77) as compared to Tris-Control (73.18±1.29), Tris supplemented with α-Tocopherol (77.31±1.01) and Lecithin based extender Control (75.31±1.20). The fluctuation in results might be due to variance in the techniques of preservation, dilution, temperature, intrinsic and extrinsic factors such as breed, age, climate, environment, management and so on so forth (Sansone et al., 2000).

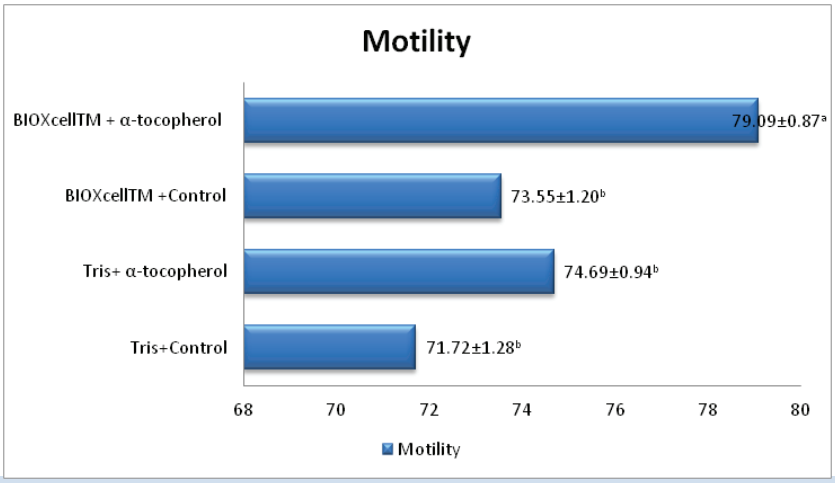

Figure 3.1 Progressive motility of Tharparkar bull chilled semen (Mean % ± SEM) extended in Lecithin based extender and Tris based egg yolk extender supplemented with or without 0.02mM α-tocopherol (Vit E)

ab: values with different superscripts shows significant difference (P<0.05).

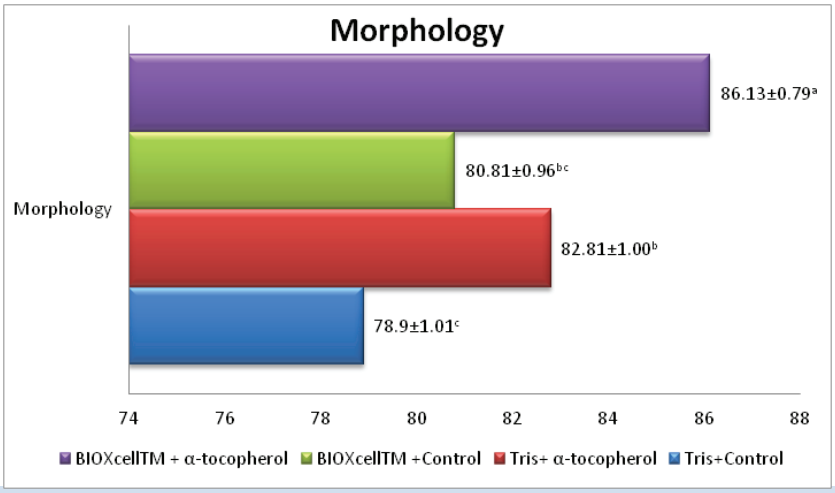

Figure 3.2 Chilled semen Morphology of Tharparkar bull semen (Mean % ± SEM) in Lecithin based extender and Tris based egg yolk extender supplemented with and without α-tocopherol (Vit E)0.02 Mm/ml.

abc: values with different superscripts shows significant difference (P<0.05).

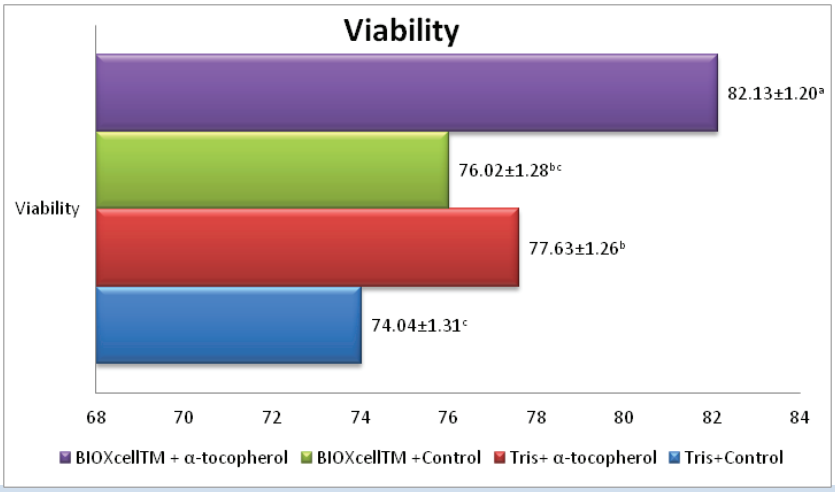

Figure 3.3 Chilled semen Viability of Tharparkar bull semen (Mean % ± SEM) in Lecithin based extender and Tris based egg yolk extender supplemented with and without α-tocopherol (Vit E)0.02 Mm/ml.

abc: values with different superscripts shows significant difference (P<0.05).

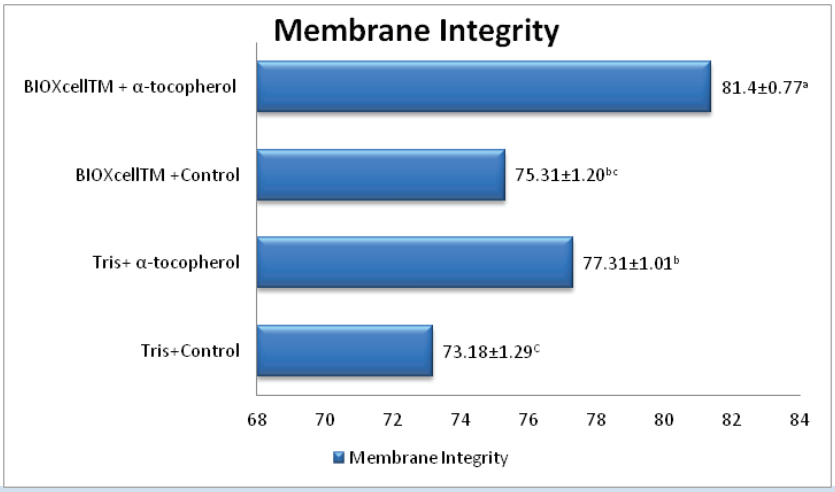

Figure 3.4 Chilled semen Membrane Integrity of Tharparkar bull semen (Mean % ± SEM) in Lecithin based extender and Tris based egg yolk extender supplemented with and without α-tocopherol (Vit E)0.02 Mm/ml.

abc: values with different superscripts shows significant difference (P<0.05).

DISCUSSION

Chilled Semen Assessment

Generally it is considered that the cryopreservation process itself reduces more than 50 percent of the Progressive Motility and viability of spermatozoa. During the different stages of cryyopreservation spermatozoa face chemical osmotic and thermal stresse (Leboeuf et al., 2000). Addition of α-tocopherol into semen resulted improvement in quality parameters of frozen-thawed bull spermatozoa i.e. Progressive motility, morphology, viability and membrane integrity in both Lecithin based extender and Tris based egg yolk extender. Sperm parameters were improved in all groups supplemented with α-tocopherol (Vit E)0.02 Mm/ml. The spermatozoa showed improved progressive motility parameter in Lecithin based extender supplemented with α-tocopherol (Vit E) 0.02 Mm/ml, The obtained results agreed with previous studies observed by Ansari et al., (2012), However, the value were higher than Kaka et al., (2015a) Thari Semen and Yadav et al., (2019)Hariana Bull.

Morphological study of spermatozoa showed improved quality parameters Lecithin based extender supplemented with α-tocopherol (Vit E)0.02 Mm/ml. The obtained results agreed with the findings of Kaka et al., (2015) and Yadav et al., (2019). However, results of current obtained morphology were higher than Ansari et al., (2012). The viability of spermatozoa is one of the fundamental characteristic which is linked with maturation of spermatozoa (Kathiravan et

al., 2011). The greater viability of spermatozoa were seen in Lecithin based extender supplemented with α-tocopherol (Vit E)0.02 Mm/ml which agreed with results carried out by Yadav et al., (2019) and current findings were lower than the values obtained by Ansari et al., (2012) and Kaka et al., (2015).

Membrane Integrity of spermatozoa were also assessed and it was highest in Lecithin based extender supplemented with α-tocopherol (Vit E)0.02 Mm/ml. The obtained value of spermatozoa membrane integrity is in agreement with results observed by Yadav et al., (2019) and the present value of current study is lower than the results observed by Ansari et al., (2012) and Kaka et al., (2015).

Addition of α-tocopherol (Vit E) at concentration of 0.02 Mm/ml improves chilled semen quality parameters of Tharparkar bull semen in both extenders, Meanwhile most of findings suggests that egg yolk extender are more effective over Lecithin based extender based extenders (Crespilho et al., 2014),These variations in results might be due tovariance in the techniques of preservation, dilution, temperature, intrinsic and extrinsic factors such as climate, environment, management and use of extenders with different compositions or it depends on the use of antioxidants. Meanwhile use of various concentrations of antioxidants presents variations in results (Sansone et al., 2000).

CONCLUSION

Supplementations of α-Tocopherol **(**Vit E) within Lecithin based extender and Tris-based egg yolk extender resulted improved Chilled quality parameters of Tharparkar Bull Semen.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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