ROLE OF SELENIUM AND VITAMIN E IN LACTOSE BASED EXTENDER ON SEMEN CRYOPRESERVATION OF BUFFALO BULL (*BUBALUS BUBALIS)*

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ABSTRACT: This study was designed to evaluate the supplementation of various concentrations of selenium and vitamin E and combinations in lactose egg-yolk-glycerol based extender on cryosurvival of buffalo bull semen. The semen of four healthy donor bulls and ejaculates (n= 80) were processed and pooled. The collected semen was extended with the experimental extenders and then frozen. The frozen straws of semen were thawed and analyzed for motility, viability, acrosomal integrity and functional integrity of sperm. The study indicated that combined use of selenium and vitamin E @ 2µg/ml + 0.75 mg/ml supplemented in extender had significantly ($P<0.05$) increased sperm motility, viability, acrosomal integrity and functional membrane integrity. Selenium (2µg/ml) in freezing extender also improved semen cryosurvival. The provision of selenium (3µg/ml) alone and in combination with Vitamin E (1 mg/ml) did not provide any beneficial impact on sperm cryosurvival. It was concluded that addition of selenium and vitamin E alone in fructose lactose egg yolk glycerol extender increased the cryosurvival of buffalo sperms.

Keywords; Selenium, Vitamin E, Buffalo bull semen, Extender.

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INTRODUCTION

Cryopreservation technique provides opportunity to preserve semen from the elite males and propagate the genome worldwide at economic conditions. Chatterjee *et al* (2001) reported that cryopreservation of semen induces the oxidative stress on function and structure of sperm. The production of excessive reactive oxygen species (ROS) damages the sperm by lipid per oxidation in the sperm cell during freezing (Hashem *et al*., 2013; Arabi *et al.,* 2001). Rising levels of ROS species like superoxide, hydroxyl and hydrogen peroxide significantly affect the semen quality parameters like motility, morphology as well as sperm functions and DNA (Hellstrom *et al*., 1994).

The vitamin E acts as an antioxidant in various metabolic reactions of cell (Partyka, *et al.,* 2012b; Scott, 1997) by capturing peroxyl free radicals (Anonymous, 2000) and by protecting sub cellular membrane (Koyuncu and Yerlikaya, 2007). In deep freezing technique, vitamin E improved the sperm motility, function of mitochondria and sperm membrane integrity due to reduction of ROS (Moghbeli *et al.,* 2016; Pena *et al.,* 2004). Selenium and vitamin E combination has positive interaction to maintain different body functions (Combs, 1992). Similarly, Se supplementation in freezing diluters helps to counteract the negative effects of freezing on sperm (Dorostkar *et al.,* 2012).

Previously, many researchers have used vitamin E or selenium alone to study their protective effects against ROS and damages in semen freezing (Aminipour *et al.,* 2013; Kamran *et al.,* 2012). The objectives of this study were to highlight protective effects of selenium and vitamin E on structural protection of buffalo bull semen against the unfavorable environment of cryopreservation.

MATERIALS AND METHODS

Fructose-lactose-egg yolk-glycerol extender was prepared as described by Chaudhari and Mshelia (2002). For 100 ml of extender, 56.25 ml of 11% lactose solution, 18.75 ml of 6% fructose solution, 20 ml egg yolk, 5 ml glycerol, benzyl penicillin 1000 I.U/ml of extender and streptomycin 1mg/ml of extender were taken. The extender was divided into seven equal parts (Table 1).

Table 1. Composition of freezing extender with different dose rates of sodium selenite and Vitamin E added in the extender

Group	Antioxidant (per ml of extender
A	Control = 0.0
B	Selenium = 2µg/ml
C	Selenium =3µg/ml
D	vitamin E =0.75 mg/ml
E	vitamin E =1 mg/ml
F	Selenium + vitamin E =2µg/ml +0.75 mg/ml
G	Selenium + vitamin E =3µg/ml +1 mg/ml

Four healthy Nili-Ravi buffalo bulls were selected at the semen production unit (SPU), Qadirabad, Sahiwal, Pakistan. The bulls were maintained under common managemental and feeding practices of farm. Semen was collected early in the morning twice a week using artificial vagina for ten weeks. The ejaculates of semen were stored in water bath on 37°C for initial assessment immediately after collection. Semen samples having over 60% motility were chosen for further study. After initial assessment, the ejaculates from four bulls were pooled to eliminate individual difference, divided in seven equal aliquots and diluted with the experimental extenders. Final sperm concentration was kept 20 x 10^6/0.5 ml after dilution (Chatterjee *et al.,* 2001).

The temperature of diluted semen was reduced from 37°C to 4°C in a cold cabinet in two hours and further equilibrated for four hours at 4°C (Andrabi *et al.,* 2006). The semen straws of 0.5 mL capacity were packed and placed horizontally on rack at 5 cm higher on the liquid nitrogen vapors for eight minutes. The frozen straws of semen were plunged into liquid nitrogen container and stored on this temperature until further evaluation. Four straws from each replicate were used to determine the semen quality. The sperm motility was measured by putting small drop of extended semen on warmed slide. This slide was enclosed with the help of cover slip to examine at 40X of phase contrast microscope as described earlier (Asr *et al.,* 2011). Sperm viability was measured by placing the quantity of 50 µL of the semen and 50 µL of Eosin and Nigrosin stain on slide (Zemjanis, 1970). The smears were observed under phase contrast microscope at 400X. The spermatozoa acrosomal integrity was monitored by mixing 500 µL of semen with 50 µL of 1% formaldehyde citrate in a test tube. A single drop of semen was observed below stage contrast microscope at 1000X as described by Asr *et al.* (2011) to count two hundred sperms with acrosome abnormalities. The functional integrity was measured by using hypo osmotic swelling solution consisted of 7.35 grams of tri-sodium citrate dihydrate along with 13.51 grams of fructose in 1000 mL of distilled water (Jeyendran *et al.,* 1984). Spermatozoa with swelled/coiled tailed were considered as viable with normal membrane.

Statistical analysis: The data was subjected to one way ANOVA. The comparisons in groups were checked by Duncan's Multiple Range Test. The level of $P<0.05$ was selected as significant.

RESULTS AND DISCUSSION

Mean results regarding the effects of vitamin E and selenium on cryopreservation of buffalo bull semen have been shown in Figure 1. Results showed that the extender B and F significantly ($P<0.05$) enhanced post-thaw buffalo bull semen in terms of sperm motility percentage, viability, acrosome integrity and sperm functional integrity. The semen extended in A, D, and E type extenders did not improve the sperm parameters. However, the post-thaw sperm quality decreased significantly ($P<0.05$) in C and G type extenders.

The freezing and thawing of semen results into the chemical and physical damages to the membrane of spermatozoa due to oxidative stress which consequently declines the sperm motility (Rahman *et al.,* 2014; Bilodeau *et al.,* 2001). There was significant rise in sperm motility in the extender supplemented with Se at the concentration of 2µg/ml. Similar observations have been reported in Jersey bulls, rams and buffalo bulls (Aminpour *et al.,* 2013; Dorostkar *et al.,* 2012; Seremak *et al.,* 1999; Siegel *et al.,* 1980). The improvement in motility of sperm may be associated with the increased enzymatic rate of ATP utilization by Se supplementation and regeneration pathway of sperms which are assessed by motility and oxygen consumption of sperm (Pratt *et al.,* 1980).

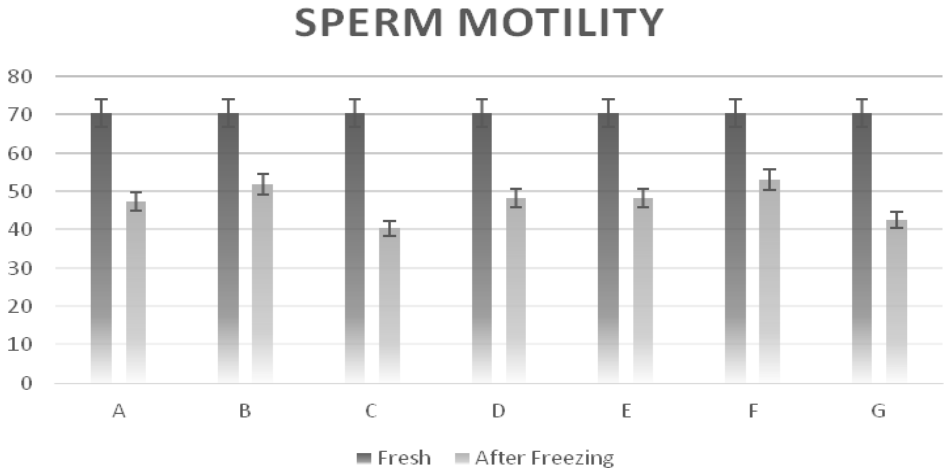

Figure 1. Effects of Se and vitamin E on sperm motility of buffalo bull

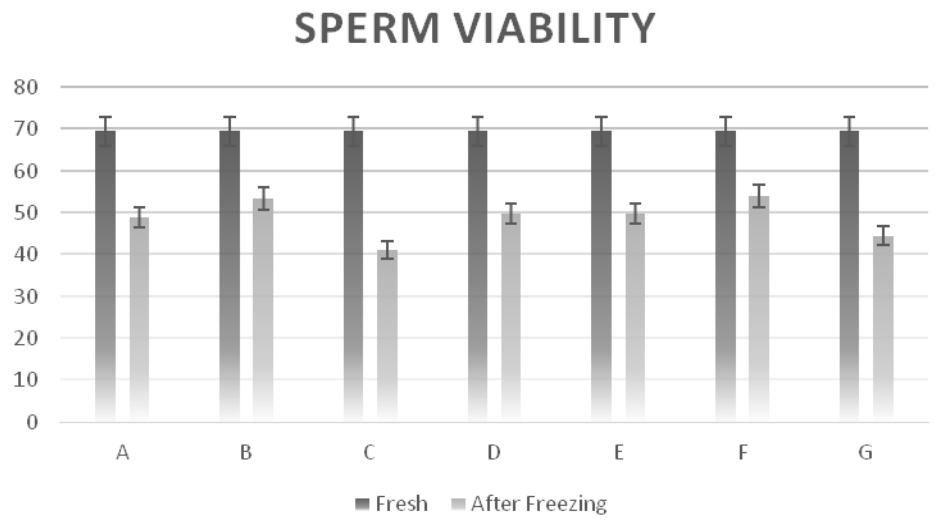

Figure 2. Effects of Se and vitamin E on viability of post-thawed sperm on buffalo bull

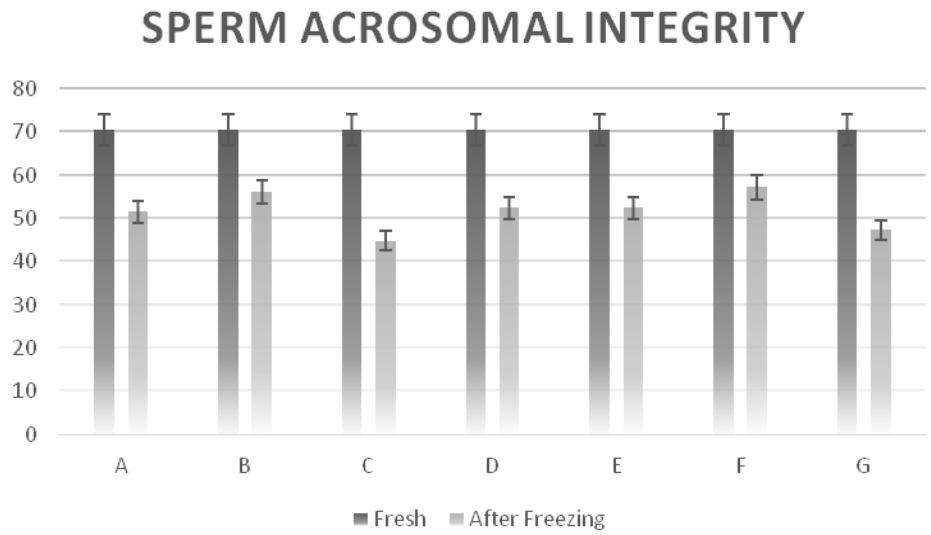

Figure 3. Effects of Se and vitamin E combination on acrosomal integrity of post-thawed sperm of buffalo bull

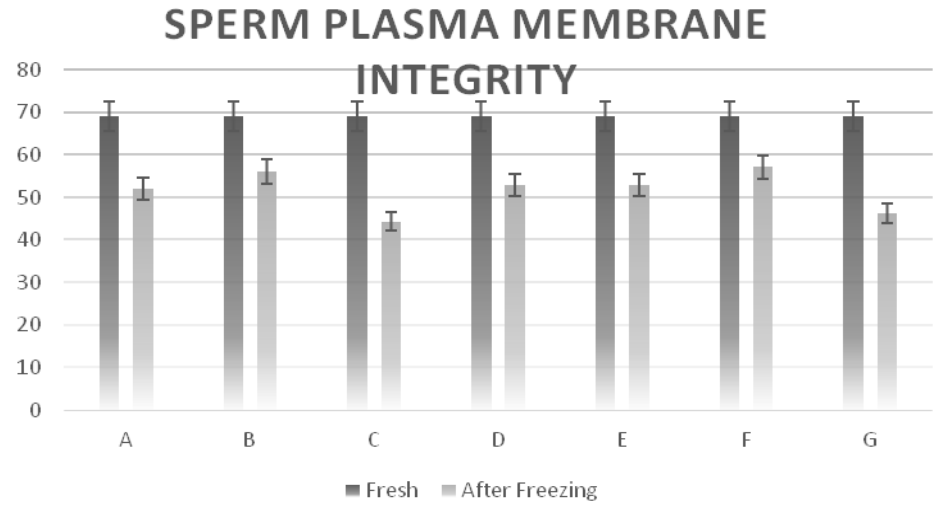

Figure 4. Effects of Se and vitamin E combination on functional integrity of post-thawed sperms of buffalo bull

Membrane of sperm is composed of huge amount of unsaturated fatty acids which are always at a risk to the lipid peroxidation. The higher rate of lipid peroxidation leads to membrane damages, disorders of cell functions and decrease sperm motility (Ball *et al.,* 2001). The supplementation of Vit E at the concentration rates of 0.75 mg and 1 mg per ml significantly increased percent sperm motility. These findings were similar to the previous reports in Murrah buffalo bulls (Raina *et al.,* 2002), Merino rams (Anghel *et al*., 2013) and bovine (Hu *et al.,* 2010). This increased sperm motility by vitamin E might be related to antioxidant action of vitamin E that scavenges the excessive ROS produced during lipid peroxidation (Anghel *et al.,* 2013).

A significant enhancement in sperm viability was observed by inclusion of Se@2µg/ml and vitamin E@0.75-1 mg/ml in freezing extenders. Similar findings have been observed earlier in water buffaloes, ram and boar (Anghel *et al.,* 2013; Dorostkar *et al.,* 2012; Sansores *et al*., 2011). The possible reason behind the enhanced viability by Se and vitamin E supplementation might be antioxidant capacity of these two antioxidants to lessen up the concentration of lipid peroxidation by interacting within chain during the procedure of oxidative stress (Khan *et al*., 2012; Kadirvel *et al.,* 2009; Beconi *et al.,* 1993).

Acrosomal integrity is one of the major parameter to analyze the functional status of membrane (Silva and Gadella, 2006). Premature cryocapacitation of sperm with standard cryopreservation before freezing is related to the change in the acrosomal integrity and makes sperm incapable to fertilize egg (Sansores *et al.,* 2011). Higher sperm acrosomal integrity was observed when Se was used with 2µg/ml in the freezing extender. These finding are corroborate with a pervious report in boar (Tareq *et al.,* 2010). Se plays important roles in antioxidant system of cells and protects the cells by forming catalytic site for antioxidant enzymes e.g. GPx (Alvarez and Storey, 1989). This protective action of Se may save the sperm against oxidative stress produced in the sperm freeze-thawed technique (Kempna *et al.,* 2004). Similarly, addition of Vit E with dose of 0.75 and 1 mg/ml in this study has significantly improved sperm acrosomal integrity. The present findings were alike to the previous findings in domestic pigs (Moghbeli *et al.,* 2016; Uc *et al.,* 2010), bulls (Andrabi *et al.,* 2008) and boars (Sansores *et al.,* 2011). The most likely reason of higher acrosome integrity rate might be due to the antioxidant property of vitamin E that maintains sperm acrosomal integrity by reducing the excessive ROS produced during the cryopreservation process (Hashem *et al.,* 2013; Ordonez, 2008).

The plasma membrane of sperm plays an important function in fertilization of spermatozoa (Jeyendran *et al.,* 1984). Higher sperm functional membrane integrity rate following the supplementation of Se in freezing diluents could be associated with antioxidant capacity of Se that scavenged the ROS produced during process of freezing and sustain its integrity and fluidity (Aiteken and Fisher, 1994). Generally, the cold shock to sperms results into initiation of the chain reaction of free radical formation that in turn damage structural component of plasma membrane (Alkhedaide *et al*., 2016; Fonseca *et al.,* 2005). Similar sperm membrane protective potential of Se has been observed earlier in different species (Angrimani *et al.,* 2017; Dorostkar *et al.,* 2012; Gutierrez *et al.,* 2008). Likewise, the Vit E supplementation with dose of 0.75 or 1 mg/ml in lactose based extender has increased post-thaw sperm plasma membrane integrity. These results were in close agreement to the previous findings in bucks (Wahjuningsih and Rachmawati 2012), bovine bulls (Kamran *et al*., 2012; Hu *et al.,* 2010) and boars (Sansores *et al.,* 2011). The vitamin E might increase the plasma membrane integrity against surplus ROS production as result of cryopreservation and thawing of sperms (Beconi *et al.,* 1993).

The combined protective effects of these two antioxidants on buffalo bull sperms have been first time studied. The results showed that Se and Vit E supplementation at the dose rate 2µg/ml and 0.75 mg/ml in semen extender provide better post-thawed sperm quality. Contrarily, Siegel *et al.* (1980) documented that combination of Se and Vit E had no impact on bovine sperms cryosurvival and this detrimental effect of Se and Vita E could be related with supplementation of inappropriate dose level in freezing extender.

In the present study, higher doses of Se or Vita E had detrimental effect of sperm survival during the freezing procedure. Similar findings are observed by Seremak *et al.* (1999) in rams and Dorostkar *et al.* (2012) in water buffalo bulls. This detrimental effect on sperm might be due to toxic dose level that caused the destruction of different process e.g. cellular respiration in mitochondria in sperm (Hawkes and Turek, 2001).

Conclusion: The findings of this study may propose that the supplementation of selenium and vitamin E in extender for cryopreservation has beneficial effect on post-thawed motile sperms, viability and structural integrity of buffalo bull semen. High dose of vitamin E and Se can be dangerous. This extender can be used to inseminate the buffaloes to evaluate the fertility of bulls.

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