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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(12): 06-08

www.thepharmajournal.com
Received: 04-10-2021
Accepted: 06-11-2021

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Effect of lycopene on acrosomal integrity of bull spermatozoa during freeze-thaw process

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Abstract
The present study was designed to determine the effect of lycopene as an additive in Hariana bull semen for cryopreservation. Eight ejaculates collected from four Hariana bull (n= 32) were evaluated and those which appears to be apparently normal, having ≥ 3.0 mass motility and ≥ 70% progressive motility were processed further for cryopreservation. Neat semen was extended in egg yolk tris glycerol (EYTG) was splits in to four aliquots and grouped as Group I: control (without lycopene), Group II, Group III and Group IV as treatment group with 0.25 mM, 0.5 mM and 1 mM lycopene respectively. Semen evaluated for acrosomal integrity by Giemsa stain at pre-freeze and post-thaw stage showed that supplementation of 0.25 mM lycopene (Group II) in semen significantly (P<0.01) decreased per cent acrosome reacted spermatozoa at both stages.

Keywords: bull semen, cryopreservation, Hariana, lycopene, antioxidant, acrosomal integrity

Introduction
Artificial Insemination with frozen semen has been proved to be the best tool worldwide for mass genetic improvement through dissemination of superior germplasm. The membranous structures of the spermatozoon (plasma membrane, outer acrosomal membrane and mitochondrial membrane) are highly sensitive to the freeze–thawing process (Bucak *et al*., 2015) [4]. These structures being composed of 65–70% thermodynamic phospholipids (fatty acids) result in an irreversible phase change from the liquid phase into the gel phase in the event of the cooling of the membranes (Watson, 2000) [19]. The instability caused by these changes leads to cold shock and oxidative stress-induced damage in the cell. Spermatozoa and the seminal plasma contain several antioxidants, which provide protection against the toxic effects of free radicals (Alvarez & Storey, 1983; Jeulin *et al*., 1989) [2, 10]. However, following the freeze–thawing process, this antioxidant system fails in protecting spermatozoa against oxidative damage and the toxic effects of free radicals (Chatterjee *et al*., 2001; Gadea *et al*., 2004) [6, 8]. Due to these reasons, with an aim to reduce oxidative damage and the toxic effects of free radicals during the freeze-thawing of sperm, semen extenders are supplemented with various antioxidant compounds (Alvarez & Storey, 1983; Uysal & Bucak, 2007; Bucak *et al*., 2010) [2, 18, 5]. Lycopene a carotenoid is considered as one of the most potent antioxidants (DiMascio *et al*., 1989; Miller *et al*., 1996; Mortensen *et al*., 1997; Woodall *et al*., 1997) [11, 12, 22] with a singlet-oxygen quenching ability twice as high as that of β-carotene and 10 times higher than that of α-tocopherol (DiMascio *et al*., 1989). The present paper deals with the effect of different concentrations of lycopene (Sigma-Aldrich: PHR1770) on acrosomal integrity of spermatozoa, during freeze-thaw process.

Materials and Methods
Present study was conducted at Semen Biology Lab, University Instructional Livestock Farm Complex (ILFC), College of Veterinary Sciences and Animal Husbandry, U.P. Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura (U.P.) which is situated in a semiarid zone of Northern India.
Thirty two ejaculates from four Hariana bulls were processed for cryopreservation, after dividing them in four groups. Group I was considered as control where as Group II, III & IV contain lycopene in concentration of 0.25 mM, 0.5 mM & 1 mM respectively. The samples were subjected for equilibration of 4 hours at 4 ºC. Equilibrated sample were cryopreserved in LN_2 for 7 minutes and subsequently plunged into LN_2 for storage.

After 24 hours of storage sample were thawed at 37 ºC for 45 sec. The thawed samples were subjected to preparation of semen smear which was stained with Giemsa as per the method described by Hancock (1952). The stained smear was than evaluated under 100x magnification and acrosome was studied adopting method of Watson (1975) [20].

Results

The percentage of intact acrosome in pre-freeze samples were significant ($P<0.01$) higher in group II compare to control (Group I) or other treatmented groups (Group II, III & IV). Similar effect was observed in post-thaw sample. The best post-thaw percentage of acrosome was 66.04±0.81 per cent in 0.25 Mm supplement lycopene groups. The respective value in control group was 63.16±0.78 per cent (Table 1 & Figure 1).

Table 1: Effect of different concentrations of lycopene on per cent Spermatozoa with intact acrosome at prefreeze and post-thaw stages of Hariana bull semen (Mean±SE=32)

Stage	Group I (Control)	Group II (0.25mM)	Group III (0.5mM)	Group IV (1mM)
Pre-freeze	70.56±0.68^{b} (62.7 - 74.5)	73.36±0.78^{a} (60.7 - 84.4)	58.81±0.99^{c} (46.8 - 70.4)	48.88±0.85^{d} (40.2 - 62.5)
Post-thaw	63.16±0.78^{b} (53.2 - 69.0)	66.04±0.81^{a} (51.6 - 76.2)	50.00±0.98^{c} (38.2 - 64.8)	40.42±0.84^{d} (29.5 - 48.8)

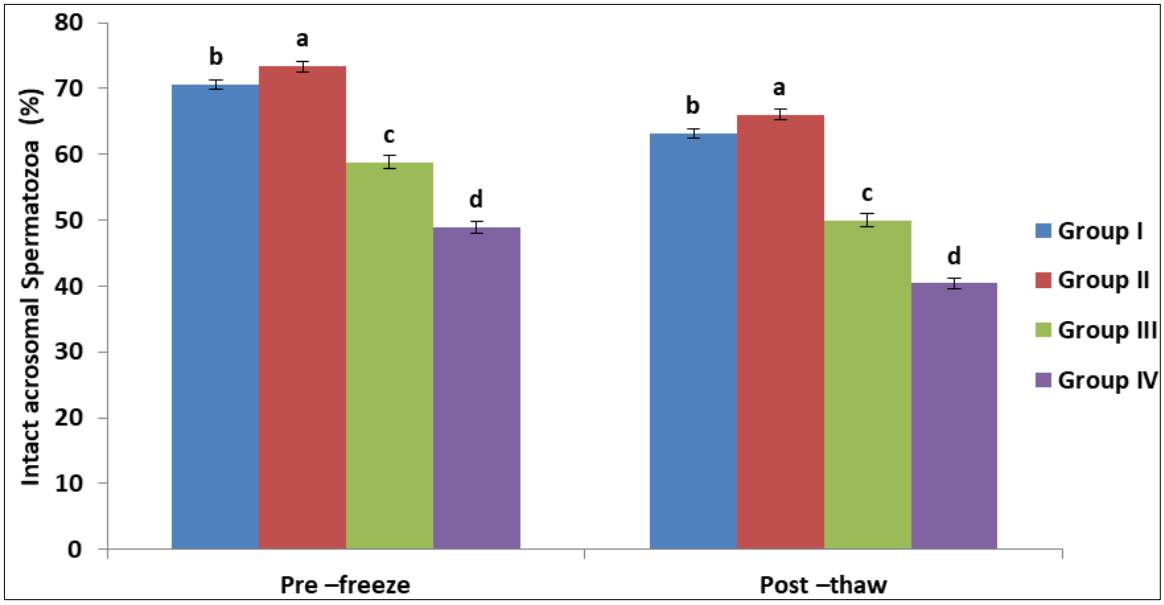

Fig 1: Graphical presentation of effect of different concentrations of lyocopene (Group I- control, Group II- 0.25mM, Group III- 0.5mM and Group IV- 1mM) on per cent spermatozoa with intact acrosome of Hariana bull semen during freeze-thaw process.

Discussion

The acrosome reaction is a process which takes place in living spermatozoa in response to natural inducers. Acrosome serves as the limiting structure of fertilisation which regulates the spermatozoa fusion with the oocyte and mediates its penetration into the oocyte. Any damage to acrosome and acrosome structures causes loss of functional competence of acrosome and ultimately a failure of fertilisation (Wells *et al*., 1970) [21].

In the present study at pre-freeze stage, lycopene in the concentration of 0.25 mM was found to be highly significant ($P<0.01$) as compare to the control and other lycopene treated groups. The same trend was observed at post-thaw stage. Thus 0.25 mM lycopene was found to be effective compared to the other two doses used. Our study further suggests that increase the concentration of lycopene had a negative effect on parameters.

Tvrda *et al*. (2017) [17] used lycopene in the concentration of 1.5 mM for bull semen and found a positive effect of lycopene. The per cent acrosomal integrity recorded was 85.20±0.42. Similarly, AI- Sarray *et al*. (2019) [1] have reported a positive effect of lycopene on acrosomal integrity. Whereas in other studies, (Uysal and Bucak, 2007; Rosato *et al*., 2012 and Bucak *et al*., 2015) [18, 4, 13] no significant (P>0.05) effect of lycopene was observed.

Freeze-thaw process induces the generation of free radicals which target the acrosome to bring out acrosome damage as well as mediate acrosomal exocytosis causing preterm acrosome reaction. Various acrosomal defects have been linked to cause reduced fertility (Anderson *et al*., 1990; Thundathil *et al*., 2000; 2001; 2002) [3, 14-16].

Summary and Conclusion

At both pre-freeze and post-thaw stages, per cent intact acrosome was highly significant ($P<0.01$) in group II (0.25 mM) as compared to I (control), III (0.5 mM) and IV (1 mM) groups. At higher concentration (0.5 mM & 1 mM) of lycopene, a negative influence of lycopene was observed compared to control. The post-thaw per cent intact acrosome in treatment group II was 66.04±0.81.

Addition of lycopene @ 0.25 mM to egg yolk based extender improved the freezability of Hariana bull spermatozoa in terms of acrosome integrity.

Acknowledgement

I am thankful to U.P. Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), Mathura (U. P) for providing the all required facilities which were necessary to conduct this piece of research.

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