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Effect of the Addition of Insulin Like Growth Factor-I (IGF-I) on Cryopreservation of Hariana Bull Semen

Research Article

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ABSTRACT

This experiment was designed to study the effect of insulin like growth factor-I (IGF-I) on cryopreservation of Hariana bull semen. For this purpose, semen ejaculates were collected from four Hariana bulls using artificial vagina at biweekly interval. The semen samples which possess more than 70% initial progressive motility and above 500 million/mL spermatozoa concentration was subsequently subjected to processing for experiment. Semen samples were extended in egg yolk tris glycerol (EYTG) extender and split into four groups. IGF-I was added at 50 ng/80 × 10^6 spermatozoa, 100 ng/80 × 10^6 spermatozoa and 150 ng/80 × 10^6 spermatozoa in the group II, III, IV respectively and group I as control (without IGF-I). Semen samples were evaluated at pre-freeze and post-thaw stage for percent individual progressive motility, percent live spermatozoa, percent hypo-osmotic swelling (HOS) positive spermatozoa, percent spermatozoa with intact acrosome. A significant ($P<0.05$) higher individual progressive motility, viability, HOS response and acrosomal integrity was observed at pre-freeze and post-thaw stage by using 150 ng/ml IGF-I. Concentration of 150 ng/mL IGF-I was found to be more beneficial in cryopreservation of Hariana bull spermatozoa as evidenced by post-thaw seminal parameter. It was concluded that IGF-I can be added to extender for improving cryosurvial of Hariana bull spermatozoa.

KEY WORDS cryopreservation, Hariana bull, IGF-1, post-thaw, pre-freeze.

INTRODUCTION

The major limiting factor in mammalian semen preservation which deteriorated the semen quality (El-Sisy *et al.* 2007) and fertility (Vishwanath and Shannon, 1997) was oxidative stress. This resulted in the production of reactive oxygen species molecules like nitric oxide, hydroxyl, hydrogen peroxide, superoxide, peroxy nitrile (Baumber *et al.* 2005). IGF-I is a single-chain, mitogenic protein found mostly in the highly proliferative cells. In the testis, IGF-I is secreted from the sertoli cells and leydig cells under the control of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), respectively (Lejeune *et al.* 1996).

IGF-I has been suggested to be an important factor for the germ cell development, maturation and motility of the spermatozoa (Henricks *et al.* 1998; Vickers *et al.* 1999). Further, IGF-I concentrations in the seminal plasma of healthy breeding bulls were positively correlated with fertilization/pregnancy rates (Sauerweina *et al.* 2000). Addition of IGF-I to the semen improves sperm quality, sperm kinematic parameters, mitochondrial membrane potential and reduces lipid peroxidation levels in frozen thawed spermatozoa (Selvaraju *et al.* 2009; Sang-Min *et al.* 2014). So keeping in view of the above facts about IGF-I, the present

study has been designed with the objective to evaluate the effect of IGF-I on physico-morphological properties of Hariana bull spermatozoa following cryopreservation.

MATERIALS AND METHODS

Semen ejaculates were collected from four healthy breeding Hariana bulls of the age group between 7.5-8.5 year and weighing between 450-500 kg body weights. A total of 8 ejaculates from each bull were collected (total ejaculates from all bulls 8×4=32). Semen collection was made bi-weekly from each bull with the help of artificial vagina (AV) on dummy animal between 7.0 to 8.0 a.m. in summer and 8.30 to 9.00 a.m. in other seasons. The temperature of AV was maintained at 40 to 42 ˚C by filling warm water of 45 ˚C. The fresh semen sample collected by means of artificial vagina was evaluated for ejaculate volume (mL), mass motility (0-5 scale), sperm concentration (million/mL), individual progressive motility (%) and live sperm (%).

The semen additive IGF-I and other chemicals used in this study were obtained from (Sigma, St Louis, USA). Semen was diluted in Tris diluent upto 80 × 10^6 spermatozoa/mL of semen sample. The aliquot was divided into following 4 groups (one control and three treatments groups). Group I: Control (without addition of IGF-I). Group II: Treatment with 50 ng/mL IGF-I/80 × 10^6 spermatozoa. Group III: Treatment with100 ng/mL IGF-I/80 × 10^6 spermatozoa. Group IV: Treatment with 150 ng/mL IGF-I/80 × 10^6 spermatozoa. French top bull mini straws (0.25 mL, 135 mm length and 2 mm diameter, IMV) of different colors were used. Automatic straw filling and sealing machine (IMV, France) was used for filling of semen into the straws and sealing it. Filling and sealing were done at room temperature. Vapour freezing of remaining semen straws was performed by using programmable biological cell freezer. After vapour freezing semen straws plunged into liquid N_2 at -196 ˚C. Frozen straws were thawed after 48-72 hr. of freezing at 37 ˚C for 30 sec. Immediately after thawing post-thaw evaluation of individual progressive motility, percentage viability, hypoosmotic swelling test and acrosome integrity were conducted.

The individual progressive motility of the spermatozoa was observed under high power phase objective lens (40X) on a thermostatically controlled stage maintained at 37 ˚C. Live and dead spermatozoa were counted as per method described by Bloom (1950) and Hancock (1951) was followed. Dead spermatozoa differentiated by their ability to get stained by Eosin dye. The hypo-osmotic sperm swelling test was performed according to the methods described by Jeyendran *et al.* (1984). Sperm tail curling is recorded as an effect of swelling due to influx of water. Acrosome integrity was judged by Giemsa staining technique as per the methodology described by Watson (1975).

Statistical analysis was carried out by using the SPSS 16 package (Chicago, USA) (SPSS, 2011).

RESULTS AND DISCUSSION

In the present study, the mean value of ejaculate volume (mL), mass motility (0-5 scale), sperm concentration (million/mL), individual progressive motility (percent) and live sperm (percent) of Hariana bull semen at fresh stage were 5.32 ± 0.17, 3.61 ± 0.06, 1356.69 ± 39.98, 80.31 ± 1.09 and 86.75 ± 0.93 respectively (Table 1).

In present study, mean percent of individual progressive motility of spermatozoa in group IV (supplemented with IGF-I 150 ng/mL) was 81.09 ± 0.70 and 54.38 ± 1.0 at pre-freeze and post-thaw stages of cryopreservation, respectively. It was significantly (P<0.05) higher as compared to other groups at pre-freeze and post-thaw stages of cryopreservation.

Selvaraju *et al.* (2016) reported that slow progressive motility of spermatozoa was significantly (P<0.05) higher in the concentration of 150 ng/mL IGF-I than control group at post-thaw stage of cryopreservation of Murrah buffalo semen.

The IGF-I receptors are present on the bovine spermatozoa and the seminal plasma. IGF-I binds to the IGF-I receptor to influence sperm motility (Henrick *et al.* 1998). One possible way that IGF-I maintains motility is through energy metabolism. IGFs have been shown to increase glucose uptake, lactate production, pyruvate dehydrogenase activity and conversion to glucose-6-phosphate (Stewart and Rotwein, 1996).

In the present study the mean percent of live spermatozoa of group IV (supplemented with IGF-I 150 ng/mL) were 85.97 ± 0.61 and 61.66 ± 0.95 at pre-freeze and post-thaw stages of cryopreservation, respectively (Table 2). There was significantly (P<0.05) higher percent viability of spermatozoa as compared to other groups at both stages of semen cryopreservation. Selvaraju *et al.* (2016) found significantly (P<0.05) positive effect of IGF-I on livability of spermatozoa at post thaw stage with concentration of 150 ng/mL IGF-I as compared to control group (0 ng/mL IGF-I) in Murrah buffalo bull semen. IGFs in neuronal cells prevent mitochondrial dysfunction when exposed to glutathione depleting agents, maintain calcium homeostasis and increase cell survival (Recio-Pinto *et al.* 1996; Sortino and Canonico, 1996). If IGFs act as antioxidants, this would then result in increased sperm viability (Wainer *et al.* 1996).

In the present study, it was observed the percentage of hypo-osmotic swollen spermatozoa was significantly (P<0.05) higher in group IV (supplemented with IGF-I 150 ng/mL) as compared to other groups in pre-freeze and post-thaw stages (Table 3).

Table 1 Effect of IGF-I on percent individual progressive motility (Mean±SE, n=32) of Hariana bull spermatozoa at pre-freeze and post-thaw stage

Stage of semen freezing	Group I	Group II	Group III	Group IV
Pre-freeze	75.16^{a}±0.91	73.44^{a}±0.61	75.47^{a}±0.65	81.09^{b}±0.70
Post-thaw	46.56^{a}±0.79	47.03^{a}±1.11	48.59^{a}±1.46	54.38^{b}±1.0

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 2 Effect of IGF-I on percent viability (Mean±SE, n=32) of Hariana bull spermatozoa at pre-freeze and post-thaw stage

Stage of semen freezing	Group I	Group II	Group III	Group IV
Pre-freeze	82.66^{a}±0.80	81.13^{a}±0.77	82.03^{a}±0.64	85.97^{b}±0.61
Post-thaw	54.66^{a}±0.68	54.59^{a}±1.09	56.84^{a}±1.28	61.66^{b}±0.95

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 3 Effect of IGF-I on percent HOS response of Hariana bull spermatozoa at pre-freeze and post-thaw stage (Mean±SE, n=32)

Stage of semen freezing	Group I	Group II	Group III	Group IV
Pre-freeze	64.47^{a}±0.63	65.19^{a}±1.09	65.03^{a}±0.68	70.25^{b}±0.77
Post-thaw	47.72^{a}±0.72	49.47^{a}±0.56	48.28^{a}±0.48	55.44^{b}±0.81

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 4 Effect of IGF-I on intact acrosome percent of Hariana bull spermatozoa at pre-freeze and post-thaw stage (Mean±SE, n=32)

Stage of semen freezing	Group I	Group II	Group III	Group IV
Pre-freeze	77.66^{a}±0.94	75.84^{a}±0.72	80.94^{b}±1.00	83.69^{c}±0.51
Post-thaw	51.26^{ab}±0.62	49.75^{a}±1.21	52.88^{b}±0.91	58.09^{c}±0.84

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

However, no significant difference observed in percentage of hypo-osmotic swollen spermatozoa between group I, II and III at pre-freeze and post-thaw stages of cryopreservation. Selvaraju *et al.* (2016) reported a significant (P<0.05) positive effect of IGF-I (supplemented with IGF-I 150 ng/mL) on sperm functional membrane integrity (%) during incubation at 4 ˚C for 4 hr as compared to control in Murrah bull spermatozoa. Jeyendranan *et al.* (1984) has revealed the fact that some physiological process in fertilization (acrosome reaction, capacitation, fusion of sperm with ovum) demands active membrane which do not take place with inactive membrane. This statement suggested that structural and functional integrity of sperm membrane are crucial for the viability of spermatozoa (Lechniak *et al.* 2002).

The percent intact spermatozoa were significantly (P<0.05) higher in group IV (supplemented with IGF-I 150 ng/80×10^{6}) as compared to other three groups including control at pre-freeze and post-thaw stages (Table 4). Selvaraju *et al.* (2009) reported a significant (P<0.05) positive effect of IGF-I on acrosome intactness in the group supplemented with IGF-I at 100 ng/mL) and the group supplemented with IGF-I 150 ng/mL as compared to control at post-thaw stages of cryopreservation. Kumar *et al.* (2019) reported supplementation with 250 ng/mL IGF-I resulted in improved membrane intactness as compared to control after cryopreservation of semen from normal buffalo bulls.

The insoluble pool of calmodulin present in the acrosome, might regulate influx of calcium in acrosomal region to minimize the cells from cryocapacitation and may maintain the intact acrosome (Schlingmann *et al.* 2007).

CONCLUSION

It was concluded that IGF-I can be added to extender for improving cryosurvial of Hariana bull spermatozoa. Concentration of IGF-I 150 ng/mL was found to be more beneficial in cryopreservation of Hariana bull spermatozoa as evidenced by post-thaw seminal characteristics. Further *in vivo* study need to prove the beneficial effect of IGF-I on semen cryopreservation.

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