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Insulin Like Growth Factor-1 Fortification Improves Post Thaw Sperm Quality and Fertility of Goat

Short Communication

K. Sharma[1,2], R. Ranjan[1*] and S. Gupta[2]

[1] Department of Animal Physiology and Reproduction, Institute of Indian Council of Agricultural Research (ICAR)-Central Institute for Research on Goats, Farah, Mathura, Uttar Pradesh, India
[2] GLA University, Mathura, UP, India

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*Correspondence E-mail: ravi.ranjan@icar.gov.in

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ABSTRACT

Insulin like growth factor-1 (IGF-I) has an antioxidant effect to maintain sperm motility. The present study designed to evaluate the effect of different levels IGF-1 on semen characteristic after cryopreservation of buck semen. Fifty ejaculates were collected and were extended with tris- citric acid- fructose diluent with 10% egg yolk and 6% glycerol with sperm concentrations of 4×10^8 mL^{-1}. The effect of IGF-1 concentration showed significant differences ($P<0.05$) on most of studied traits (sperm motility, live sperm count, acrosomes integrity, hypo osmotic swelling positive spermatozoa, malondialdehyde (MDA)) and were significantly ($P<0.05$) highest in 250 ng/mL of IGF-1in post thaw semen. The post thaw progressive motility, live, hypo osmotic swelled and acrosome intact sperm increased by 25.26%, 26.77%, 30.35% and 25.52%, respectively in 250 ng/mL IGF1supplemented group in compare with control group. The 250 ng/mL IGF-1 supplemented group showed the lowest MDA content ($P<0.05$) in compare with other studied groups. The fortification 250 ng/mL IGF-1 reduced the MDA content by 31.16% more than the control group. The results suggested that IGF-1 protects sperm membrane lipid peroxidation by lowering MDA production by reducing the harmful effect of reactive oxygen species. Eight out of ten goats were conceived and kidded by using supplemented of 250 ng/mL IGF-1 and three goats were kidded by non-supplemented (control) frozen semen straw through artificial insemination (AI). The success of kidding was 40.00% in supplemented group (250 ng/mL IGF-1) than 30.00% in non-supplemented group (control) on actual kidding basis.

KEY WORDS acrosome, antioxidant, goat, IGF-1, malondialdehyde.

INTRODUCTION

Insulin like growth factor 1 (IGF-1) is a differentiating polypeptide that plays an important role in the regulation of reproductive functions (Jones and Clemmons, 1995) and is responsible for the development of germ cell, sperm maturity and motility (Vickers *et al*. 1999). It acts as a regulator in spermatogenesis (Spiteri-Grech and Nieschlag, 1993) and is the main component for maintaining sperm motility through energy uptake or antioxidant effect. It has been proven that IGF-1 increase the cryosurvial of Haryana bull spermatozoa (Singh *et al*. 2020) and the motility of Yak semen (Pan *et al*. 2015).

Different types of additives had been added to goat semen diluent to improve sperm motility, membrane integrity and fertility of the sperm (Ranjan *et al*. 2017; Singh *et al*. 2020; Ranjan *et al*. 2022). Cryopreservation of buck semen reduces sperm motility, acrosome membrane integrity and fertility of sperm (Ranjan *et al*. 2020; Ranjan *et al*. 2022). The frozen semen artificial insemination (AI) is used for the preservation, conservation and propagation of superior goat germplasm. The adoption index score by AI was 42.50% in

India (Gunaseelan *et al*. 2018) and around 38% with frozen semen AI (Ranjan *et al*. 2020).

MATERIALS AND METHODS

The objective of the present study was to improve the fertility of post thaw sperm by the addition of various concentrations of IGF-1 in Jamunapari goat semen diluent. Goat semen diluent comprised of tris (7.208 g), citric acid (3.804 g), fructose (2.000 g), streptomycin (200 mg), penicillin (120 mg) and triple distilled water (200 mL). Hen fresh egg yolk (10% v/v) and glycerol (6% v/v) was added into the working semen diluent for goat semen freezing.

Proven bucks (n=12) were used for semen collection twice a week by the artificial vagina. The colour, volume, consistency and mass motility of ejaculates was assessed. Ejaculates with mass activity ≥ +4 were pooled of the same buck and made 7 equal groups and fortified with IGF-1: Control (without IGF-1), 50 ng/mL, 100 ng/mL, 150 ng/mL, 200 ng/mL, 250 ng/mL and 300 ng/mL. The diluted semen was filled and sealed in 0.25 mL French mini straws at 5 ˚C then was vapour frozen and finally plunged into liquid nitrogen (Ranjan *et al*. 2020). Sperm progressive motility was observed under 200X magnification of phase contrast microscope. The using Eosin – Nigrosine stain was used to calculate live and dead sperm (Hancock, 1951; Ranjan *et al*. 2009a). The sperm acrosome intactness was observed by Giemsa staining protocol (Watson, 1975; Ranjan *et al*. 2014).

The functional membrane integrity of sperm was examined (Revell and Mrode, 1994; Ranjan *et al*. 2009b) and at least 200 sperm were counted for the calculation of strong coiled sperm per cent (Ranjan *et al.* 2017). Lipid peroxidation was determined by estimating malondialdehyde (MDA) production using thiobarbituric acid (TBA) (Kumaresan *et al*. 2006).

MDA (µmol/mL)= optical density × 10^6 × total volume (3 mL) / 1.56 × 10^5 × test volume (1 mL)= optical density × 30 / 1.56

Trans cervical AI was done in twenty natural oestrous goats with 250 ng/mL of IGF-1 frozen semen. Ten goats were inseminated with non-supplemented control frozen semen. The conception rate was estimated on actual kidding basis. Statistical analysis was done by the General Linear Model of SPSS Package 20 (SPSS, 2011). The factorial model included the effect of IGF-1 concentration as an independent variable and percent post thawed sperm motility, live sperm count, acrosome intact sperm, hypo osmotic swelling positive sperm and MDA as dependent variables. Post Hoc Test Duncan LSD T3 and Homogeneity test was conducted to assign different superscript to variables based on significant difference at $P < 0.05$ between different variables.

RESULTS AND DISCUSSION

The progressive motile, live, hypo osmotic swelled and acrosome intact sperm were 83.33 ± 0.71, 83.60 ± 1.33, 79.21 ± 1.37 and 75.24 ± 0.93 % respectively in fresh goat semen. The malondialdehyde production was 9.68 ± 0.53% in fresh goat semen. The progressive motile, live, hypo osmotic swelled, acrosome intact sperm and MDA were significantly higher ($P<0.05$) in the 250 ng/mL IGF-1 supplemented group than other groups (Table 1). The post thaw progressive motility, live, hypo osmotic swelled and acrosome intact sperm increased by 25.26%, 26.77%, 30.35% and 25.52% respectively in 250 ng/mL IGF-1 supplemented group from the control group. The MDA content was significantly lower ($P<0.05$) in 250 ng/mL IGF-1 supplemented group than in other groups. The fortification of 250 ng/mL IGF-1 reduced the MDA content by 31.16% more than the control group.

Artificial insemination (AI) was carried out with 250 ng/mL IGF-1 supplemented semen in twenty natural oestrous goats. Ten goats were inseminated with control frozen semen straw. Eight goats were conceived and kidded by using supplemented of 250 ng/mL IGF-1 and three goats were kidded by non-supplemented (control) frozen semen straw. The success of kidding was 40.00% in supplemented group (250 ng/mL IGF-1) than 30.00% in non-supplemented group (control) on an actual kidding basis (Table 2).

Maintaining sperm motility and their survivability up to fertilization is a big challenge in assisted reproduction technology. The addition of a specific concentration of IGF-1 250 ng/mL has improved the post thaw sperm motility, viability, sperm membrane integrity, acrosome intactness than the control group. The supplemented group have less lipid peroxidation and protected the structures and functions of spermatozoa. Our earlier studies also proved fortification of different types of additives in goat semen diluent significantly improved semen quality (Ranjan *et al*. 2020).

There was a significantly higher live sperm count in the treatment group indicated IGF-1 reduced reactive oxygen species (ROS) production due to its antioxidant property. IGF-I (100 ng/mL) and IGFII (250 ng/mL) were significantly improved sperm motility (Henricks *et al*. 1998). IGF-I present in seminal plasma showed regulatory function by affecting sperm capacitation and motility (Henricks *et al*. 1998). Fortification of IGF-1 in buffalo semen showed increased progressive sperm motility (Selvaraju *et al*. 2016).

Table 1 Effect of IGF-1 fortification on post thaw semen qualities (Mean±SE)

Concentration (ng/mL)	Post-thaw sperm motility %	Live sperm %	HOST %	Acrosome intact sperm %	MDA (µM)
0	41.25±1.96^{bc}	43.63±1.92^{bc}	41.18±1.90^{c}	44.99±1.86^{bc}	13.22±0.89^{c}
50	37.92±1.56^{c}	41.60±1.88^{c}	42.32±1.84^{bc}	42.47±1.74^{bc}	19.51±0.97^{b}
100	44.58±1.89^{b}	49.55±2.43^{ab}	48.33±2.46^{bc}	49.14±2.36^{b}	22.39±1.25^{ab}
150	40.83±2.37^{bc}	43.66±2.79^{bc}	42.11±2.42^{bc}	43.18±2.75^{bc}	23.28±1.09^{a}
200	42.50±1.68^{bc}	44.63±2.20^{bc}	44.54±2.19^{bc}	44.42±2.25^{bc}	24.41±1.03^{a}
250	51.67±1.28^{a}	55.31±1.41^{a}	53.68±1.88^{a}	56.47±1.91^{a}	9.10±0.77^{d}
300	39.17±2.12^{bc}	40.13±3.12^{c}	41.53±2.33^{c}	40.42±2.26^{c}	19.59±0.79^{b}

HOST: hypo-osmotic swelling test and MDA: malondialdehyde.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 2 Effect of IGF-1 fortification on post thaw semen qualities (Mean±SE)

Concentration (ng/mL)	Kidding %
0	30
250	40

IGF-1 receptors in sperm (Naz and Padman, 1999) cause the chemokinetic effect of IGF-I and maintain sperm motility by activation of IGF-I receptors (Zangeronimo *et al.* 2013). In addition, IGF increases the intracellular calcium content by increasing the ion transport cause higher sperm motility (Miah *et al.* 2008). The specific concentration of IGF-1 250 ng/mL has improved the post thaw sperm acrosome integrity compared to our study's control group. There was a significant (P<0.05) positive effect of 100 ng/mL and 150 ng/mL IGF-I supplementation on acrosome intactness as compared to control fortification of 250 ng/mL IGF-I improved sperm membrane intactness than control in buffalo (Kumar *et al.* 2019).

Reactive oxygen species (ROS) generated during cryopreservation (Chatterjee and Gagnon, 2001) increased spermatozoa susceptibility to oxidative stress (White, 1993). ROS produced by dead sperm cannot be controlled by a specific concentration of IGF-1. The IGF-1 fortification-controlled lipid peroxidation proved by low MDA production in our present study. Thus, IGF-1 showed antioxidant property and protect the sperm from the detrimental effect of ROS in this study. The sperm membrane is polyunsaturated fatty acids rich and makes them very susceptible to oxidative damages cause secondary products formation. These secondary products cause to oxidation to sperm proteins which are responsible for capacitation via the carbonylation process (Lone *et al.* 2019).

CONCLUSION

In conclusion IGF-1 may be used to get best post- thaw semen quality. Fortification of specific concentration of IGF-1 to sperm medium enhanced viability and fertility possibly through reduced free radical mediated oxidative stress. Further, lowering MDA in spermatozoa proves its antioxidant property.

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