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Biotin fortification to sperm preparation medium enhances the motility and longevity by reducing lipid peroxidation in cryopreserved sperm

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Goat semen cryopreservation leads to poor structural and functional characteristics of spermatozoa. Several efforts have been made to enhance the post-thaw motility and sperm function by using pharmacological agents, vitamins and antioxidants. Biotin (vitamin B7 or vitamin H), is an essential micronutrient required for the normal growth and development of the body. It acts as a coenzyme in all carboxylation reactions involved in the biosynthesis of fatty acids, gluconeogenesis, metabolism of the branched-chain amino acids and *de novo* synthesis of purine nucleotides. Reactive oxygen species (ROS) cause increased rates of lipid peroxidation and consequent loss of sperm motility during long term storage (Aitken 2017). Various additives have been found to maintain sperm motility and fertilizing capacity (Ranjan *et al.* 2017, Singh *et al.* 2020). Freezing and thawing of buck semen causes changes in sperm motility, plasma membrane and acrosome intactness thereby, reducing the fertilizing ability of spermatozoa (Ranjan *et al.* 2020).

The objective of the present study was to increase the life and fertility potential of goat cryopreserved semen by reducing oxidative stress through addition of biotin in sperm preparation medium. The sperm preparation medium was composed of 3.604 g tris, 1.902 g citric acid, 1 g fructose, 100 mg streptomycin, 60 mg penicillin and 100 mL triple distilled water. The pH was adjusted to 6.8. Hen egg yolk 10% (v/v) and glycerol 6 % (v/v) was added freshly to the basic extender and mixed well for preparing working sperm medium. Semen samples were collected twice a week using artificial vagina after stimulating in front of an estrous doe. Immediately after collection, volume, colour, consistency and mass motility of ejaculates was assessed. Ejaculates of same buck with mass motility +4 and above were pooled and divided into 5 equal aliquots, viz. Control (non-supplement), 1 µM, 2 µM, 3 µM and 4 µM biotin. Sperm concentrations were adjusted to 1×10^8/ml and diluted semen was equilibrated at 5°C for 4 h before being frozen in liquid nitrogen (Ranjan *et al.* 2015, 2020). The progressive motility was calculated by considering the average values of two independent experts. The live and dead spermatozoa were calculated by using Eosin–Nigrosine stain (Hancock 1951, Ranjan *et al.* 2009a). The acrosome integrity of spermatozoa was assessed by using Giemsa stain (Watson 1975, Ranjan *et al.* 2014). Membrane integrity of spermatozoa was examined by hypo-osmotic swelling (HOS) test (Revell and Mrode 1994, Ranjan *et al.* 2009b). Lipid peroxidation level of spermatozoa was determined in frozen–thawed semen samples by measuring the malondialdehyde (MDA) production, using thiobarbituric acid (TBA) as described by Kumaresan *et al.* (2006) with slight modifications in sperm concentration and incubation time. The MDA concentration was determined by the specific absorbance coefficient (1.56×10^5/molcm^{-3}).

The percent of progressive motile spermatozoa, live

$$\text{MDA produced } (\mu\text{mol/ ml}) = \frac{\text{OD} \times 10^6 \times \text{total volume (3 ml)}}{1.56 \times 10^5 \times \text{test volume (1 ml)}} = \frac{\text{OD} \times 30}{1.56}$$

spermatozoa, hypo-osmotic swelled spermatozoa and acrosome integrity was 81.76±0.85, 81.61±1.60, 76.13±1.58 and 74.63±1.65, respectively, in fresh semen. The post-thaw semen quality decreased significantly ($P<0.05$) after cryopreservation. The results showed that the progressive motile spermatozoa percent was significantly higher ($P<0.05$) in 3 µM (47.67±1.88) than 1 µM (38.33±2.42) and 2 µM (38.67±2.26) biotin concentrations. The percent of live spermatozoa (51.90±2.28), hypo-osmotic swelled spermatozoa (51.86±1.96) and acrosome integrity (52.02±2.32) was significantly higher ($P<0.05$) in 3 µM than other biotin concentrations (Fig. 1).

The MDA concentration (lipid peroxidation index) was 7.53±0.44 µM in fresh semen. MDA concentration (µM) was significantly lower ($P<0.05$) in 3 µM biotin (14.13±2.19) than other biotin concentrations in post-thaw frozen semen (Fig. 1).

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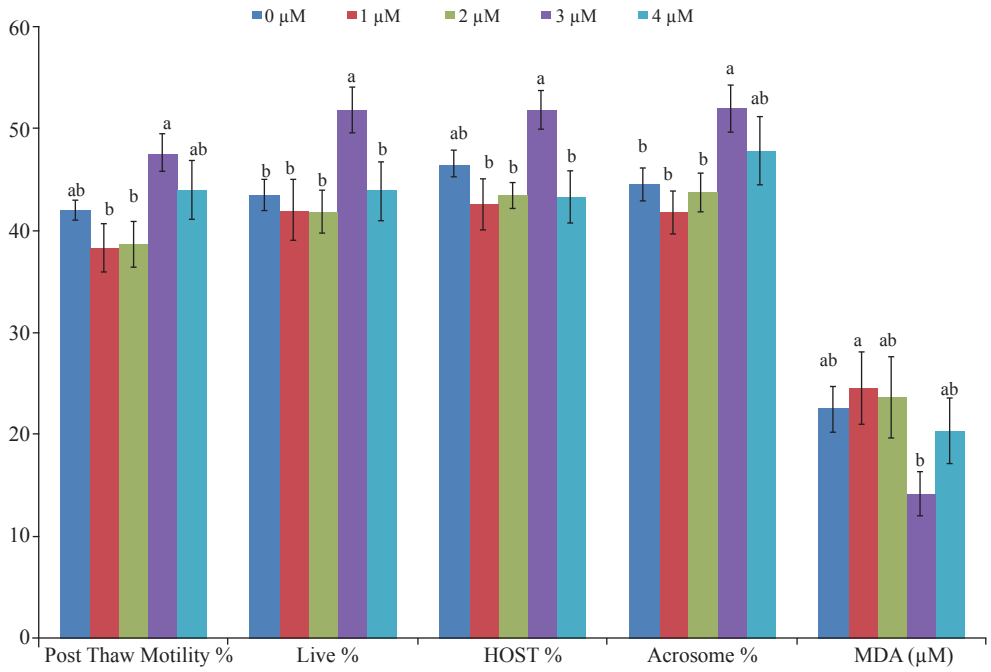

Fig. 1. Effect of biotin on post-thaw semen quality (Mean±SE). *Means value with different superscript differ significantly within a column (P<0.05).

There was a decline in post-thaw sperm qualities in frozen-thawed spermatozoa under *in vitro* conditions. The addition of specific concentration of biotin (3 µM) improved the post-thaw qualities, viz. motility, live cell count, membrane integrity, acrosome integrity as compared to control group and reduced lipid peroxidation and thus protected the structures and functions of spermatozoa efficiently. The incorporation of catalase, vitamin C, and chlorpromazine in semen extender improved the post-thaw semen quality in buck semen (Ranjan *et al.* 2015, 2020).

The pre-freezing and freezing-thawing significantly decreased the semen qualities due to ROS generation (Chatterjee and Gagnon 2001). The reduction in the seminal parameters might be due to the overproduction of ROS from continuously increasing number of dead sperm cells that cannot be checked by particular concentration of biotin. The addition of biotin reduced the lipid peroxidation as evidenced by low MDA production. Thus biotin acts as an antioxidant and protect the sperm from the harmful effect of ROS. The significantly higher motility obtained in the treatment group may be due to the effect of biotin in enhancing the metabolism of energy and protein. Supplementing biotin to sperm preparation medium can significantly enhance the post-thaw sperm motility and their longevity *in vitro*. Biotin helped in maintaining a higher percentage of progressive motility and *in vitro* survival in frozen-thawed semen samples (Kalthur *et al.* 2012).

SUMMARY

Biotin supplementation to the sperm cryopreservation medium appears to be beneficial in enhancing the *in vitro* sperm survival without affecting the fertilizing ability. Since, biotin is an essential micronutrient, it may be a safer sperm motility enhancing agent. We inferred that the fortification of 3 µM biotin significantly (P<0.05) reduced the detrimental effects of freezing stress on motility, viability, membrane integrity and acrosome integrity of buck sperm. This particular concentration also showed significant (P<0.05) improvement in protection of sperm membrane by lipid peroxidation. Therefore, biotin may be a promising and safe alternative agent to enhance the post-thaw sperm quality in an ART. However, further studies are required to elucidate the exact molecular mechanism behind biotin-induced sperm motility enhancement.

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