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Supplementation of Oxyrase in Extender Preserves Physico-Morphological Characteristics of Crossbred Hampshire Boar Semen

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ABSTRACT

The aim of present study was to investigate the effect of Oxyrase, an oxygen scavenger, on sperm functional attributes in extended liquid preserved boar semen. In the experiment, we studied the effect of Oxyrase supplementation on sperm progressive motility, viability, functional integrity of sperm plasma membrane and acrosome integrity in Hampshire crossbred boars. The sperm functional parameters were assessed at 0, 3 and 5 d of incubation following Oxyrase supplementation (0.125 IU/mL). The results of study revealed that Oxyrase supplementation significantly ($p<0.05$) improved the percentage of sperm functional parameters in comparison to non-treated group. It was concluded that Oxyrase at 0.125 IU per ml of extended liquid preserved semen has a beneficial effect on the sperm functional attributes in crossbred Hampshire boar.

Key words: Boar, semen, Oxyrase, sperm functional attributes, Hampshire.

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INTRODUCTION

Artificial insemination (AI) has been instrumental in dissemination of superior germplasm in farm animals over the last few decades (Vishwanath, 2003). Currently, AI with liquid semen from elite boars is the most commonly used reproductive technology for genetic up gradation of indigenous pigs in the smallholder pig production system (Kadirvel *et al.*, 2013). Since, cryopreservation of boar semen compromises the fertility and reduces the litter size; only 1% inseminations are being carried out with frozen-thawed semen (Yeste, 2015). The extended liquid

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semen stored at 15-18°C is commonly used worldwide for AI at the farm and field level. Liquid storage is preferred over cryostorage as boar spermatozoa are inherently susceptible to cold shock due to low cholesterol and phospholipid ratio (White, 1993). However, the metabolic activity of spermatozoa at 15-18 °C is not inhibited completely, leading to reduced shelf life. The marked metabolic activity reduces the availability of nutrients and antioxidants, and also results in generation of reactive oxygen species (ROS) at the level of mitochondria and plasma membrane (Szymanowicz *et al*., 2019). Beltsville Thawing Solution (BTS) extender, which is commonly used for liquid storage, preserves the quality of boar semen for up to 3 days (Johnson *et al*., 2000). Extending the quality of liquid semen beyond the storage limit may enhance the dissemination of superior germplasm, as most of the households in north eastern hill region are located in remote areas. Oxyrase is an *E coli* membrane fragment that acts as oxygen scavenger and thus, protects the spermatozoa against ROS mediated damage (Ngou *et al*., 2020). However, no study has reported its efficacy for the preservation of liquid boar semen, yet. Keeping this in view, it was hypothesized that Oxyrase supplementation could deoxygenate the semen extender by reducing the availability of oxygen during semen preservation, thereby reducing ROS levels and subsequent improvement in sperm quality attributes. Therefore, the present study was designed with an objective to evaluate the effect of Oxyrase supplementation on boar semen quality parameters during liquid storage at 15-18 °C.

MATERIALS AND METHODS

The research was carried out at the Division of Animal and Fisheries Sciences, ICAR Research Complex for NEH Region, Umiam, Meghalaya- 793103. A total of 12 semen ejaculates from four healthy Hampshire crossbred boars were collected by gloved hand method. The semen samples with mass activity ≥3+ and sperm progressive motility ≥70% were chosen for experiment. The semen samples were diluted in BTS @ 1:2 and split into two equal aliquots. One aliquot was supplemented with Oxyrase at the dose of 0.125 IU/mL (Ngou *et al*., 2020) was kept as treatment group, while another aliquot was maintained as control group (without Oxyrase). The semen samples were preserved at 15 °C for 5 days and sperm quality parameters were assessed on 0, 3 and 5 days. The semen was evaluated as per standard protocol for sperm motility, live sperm count by Eosin-Nigrosin staining, plasma membrane integrity by hypo-osmotic swelling (HOS) test, and acrosome integrity by Giemsa staining (Singh *et al*., 2022).

Statistical analysis

The experimental data was analyzed by repeated measures ANOVA using SPSS 20.0 and results were expressed as Mean ± standard error (Mean ± SE). Mean values between control and treatment groups were compared by Tukey›s multiple comparison test. The significance level was set at α=0.05.

RESULTS AND DISCUSSION

The progressive motility is one of the essential parameters for achievement of fertility. However, the sperm motility is known to decline during the storage of liquid semen at 15-18°C in boars. One of the limiting factors to maintain the sufficient percentage of progressively motile sperm is accumulation of ROS (Feng *et al*., 2020). It was observed in the present study that Oxyrase supplementation resulted in significantly ($p<0.05$) higher sperm progressive motility in treatment group in comparison to control group up to 5 days of storage (Table 1). The percentage of progressively motile sperm on fifth day in treatment group was comparable to third day progressive motility in control group. It has been observed that excessive ROS generation results in accumulation of lipid peroxidation products. These lipid aldehydes bind and adduct with the proteins resulting in the inhibition of the motor apparatus of the spermatozoa (Katiyar *et al*., 2022b). As Oxyrase supplementation results in reduction in the level of dissolved oxygen in the extender (Ngou *et al*., 2020), the ROS level might have been reduced, resulting in higher progressive motility in the treatment group. The sperm liveability percentage also followed the same pattern. The per cent liveability was significantly ($p<0.05$) higher in treatment group as compared to control group on all days of storage. These findings are consistent with Ngou *et al*. (2020) who also reported higher liveability following Oxyrase supplementation in case of Sahiwal bulls. Higher viability in the Oxyrase treated ejaculates might be due to lower ROS production as a result of decrease in the dissolved oxygen levels in extender. An intact sperm plasma membrane and acrosome integrity are other factors required for successful fertilization. Loss of membrane function may result in impaired fertility as an intact plasma membrane is required for successful fusion of male and female gametes (Chakrabarty *et al*., 2007; Kumar *et al.,* 2021; 2022).

Excessive ROS generation during storage of liquid boar semen at 15-18°C leads to deleterious changes in the sperm plasma membrane. It was evident in the present study that, Oxyrase supplementation resulted in significantly ($p<0.05$) higher plasma membrane and acrosome

Table 1. Effect of Oxyrase supplementation on seminal attributes of boar semen (Mean ± SE)

	1 hour (Zero day)		3 days		5 days	
Parameters	**Control**	**Treatment**	**Control**	**Treatment**	**Control**	**Treatment**
Progressive motility (%)	82.0± 0.82 [b]	87.50± 0.83 [a]	72.0 ± 0.82 [d]	76.50± 0.76 [c]	67.50 ± 0.83[e]	72.50± 0.83[d]
Viability (%)	86.20±0.48 [b]	89.10±0.71 [a]	74.60±0.36 [d]	78.65 ±0.53 [c]	70±0.58 [e]	74.0±0.29[d]
Plasma membrane integrity (%)	48.20± 0.85 [b]	57.40± 0.84 [a]	37.75 ± 0.81[d]	42.10± 0.81[c]	28.90[f]± 0.60	33.45± 0.55[e]
Acrosome integrity (%)	85.55 ± 0.38 [b]	88± 0.61 [a]	74.65 ± 0.51[d]	78.70 ±0.5 [c]	71.0± 0.76 [e]	75.10± 0.36[d]

Different ([a,b,c,d]) superscripts in a row differ significantly (p<0.05)

integrity on all days of storage as compared to control group (Table 1). Similar findings were reported by Ngou *et al*. (2020) in frozen-thawed Sahiwal bull semen. The ROS acts on the sperm plasma membrane resulting in lipid peroxidation. As a result of lipid peroxidation the plasma membrane becomes fragile and loss of function occurs (Katiyar *et al*., 2022a). The association of ROS production, induced oxidative stress due to lipid peroxidation, reduction in antioxidant defense system and thereby ultimately impaired seminal attributes and fertility have been established in different species (Mavi *et al*., 2019a; 2019b; 2020; Bisla *et al*., 2020; 2021; 2022; Ngou *et al*., 2020; Kumar *et al*., 2021; 2022; Katiyar *et al*., 2022a; 2022b; Rautela *et al*., 2022). As Oxyrase scavenges the oxygen which is the parent molecule of ROS, the reduced ROS levels following Oxyrase supplementation might have reduced the lipid peroxidation and thus higher sperm membrane and acrosome integrity in the treated ejaculates.

CONCLUSIONS

Based on the findings of present study it was concluded that enrichment of BTS extender with Oxyrase (0.125 IU/mL) improved the quality of Hampshire crossbred boar semen quality during short term preservation at 15°C.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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