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SEASONAL VARIATION IN GLUTATHIONE PEROXIDASE IN SEMINAL PLASMA OF KARAN FRIES (THARPARKAR X HOLSTEIN FRIESIAN) BULLS UNDER TROPICAL CLIMATIC CONDITIONS

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ABSTRACT

The present study was aimed to assess the seasonal variation in glutathione peroxidase (GPx) in seminal plasma of Karan Fries (Tharparkar x Holstein Friesian) bulls. Weekly ejaculates were collected by artificial vagina (42-45°C) during winter (December to mid February), spring (mid February-April), hot dry (May-June) and hot humid (July-August) seasons. Higher concentration of GPx was observed during hot humid season (65.74±0.93 pg mL^{-1}) than in winter (59.30± 0.60 pg mL^{-1}), spring (60.67±0.76 pg mL^{-1}) and hot dry (62.14±0.90 pg mL^{-1}) seasons. The percentage of live spermatozoa decreased during hot humid season as compared to other seasons. The acrosomal integrity also decreased during hot dry (78.7±0.6%) and hot humid (74.2 ±0.6%) seasons as compared to winter (83.1±0.6%) and spring (83.4±0.5%) seasons. The supplementation of antioxidant appears essential to Karan Fries bulls during summer season under tropical climatic conditions.

Keywords: Karan Fries bulls, glutathione peroxidase, seasons

INTRODUCTION

The purpose of cross breeding is to improve the productivity of local Indian cattle (Zebu) by artificial insemination with high productive performance of exotic breed (Holstein Friesian). It has been seen that the crossbred cattle carrying more than 50% exotic inheritance show good productive and reproductive performances (Chaudhry *et al.,* 1990). Therefore, bulls of F_1 generations having >50% exotic inheritance are maintained for sustainable growth of crossbred dairy cattle. However, heat stress is one of the major hindrances of crossbred cattle performances under tropical climatic conditions. Higher ambient temperature and humidity exhibit negative effects on spermatogenesis resulting in poor semen quality and fertility (Bhuiyan and Shamsuddin, 1998; Mathevon *et al.,* 1998). Good quality semen is very crucial for successful artificial insemination throughout the year. The embryonic death during summer may also contributed by spermatozoa (Setchell *et al.,* 1988). Valeanu *et al.* (2015) observed low percentage of live spermatozoa during summer than in spring season, and DNA fragmentation index percentage (DFI %) was higher in summer than spring. Several studies have reported significant effect of seasons on semen quality (Koonjanek *et al.,* 2007; Fiaz *et al.,* 2010). Routine analysis of semen is not able to give complete picture of bull

fertility as it is very difficult to detect bull's fertility. However, the multiple tests from different aspects give little indication.

Glutathione peroxidase (GPx) is one of the essential intracellular antioxidant enzymes (Combs *et al.,* 1975). Glutathione cycle has been shown by Kosower *et al.* (1971) as an effective intracellular defense mechanism against oxidant stresses in erythrocytes, lung (Chow and Tapel, 1972) and liver (Flohe and Zimmerman, 1970). Brown *et al.* (1977) found a positive correlation (r = 0.70) between enzyme activity of GPx and ejaculate volume. GPx improves metabolic activity and motility of spermatozoa and helps in maintaining spermatozoa in a de-capacitated state (Sinha *et al.,* 1996). GPx activities were higher in semen of poor quality during non-breeding season, and this higher activity is the indicator of protective mechanism to maintain sperm quality under adverse conditions (Cardozo *et al.,* 2006). Storey (1997) reported reduced sperm motility, viability in summer season. Seminal plasma can be one of the good indicators of semen quality for artificial insemination and *in vitro* fertilization. Antioxidant enzymes in seminal plasma play protective role against sperm membrane damage due to reactive oxygen species (ROS). Production of ROS is most common during spermatogenesis, as it is highly replicative process, capable of producing approximately 1000 sperm per second (Aitken and Roman, 2008). The present study was aimed to evaluate the impact of season on GPx concentration in seminal plasma of Karan Fires bulls.

MATERIALS AND METHODS

Five healthy Karan Fries bulls (4-6 year old) were selected from Animal Breeding Research Centre (ABRC), ICAR-NDRI, Karnal, Haryana (India). Maximum ambient temperature in summer in Karnal goes upto 48°C and minimum temperature in winter falls to 0°C with average annual rainfall 700 mm. The experiment was conducted from December, 2013 to August, 2014. Weekly ejaculates were collected by artificial vaginal during winter (December to mid February), spring (mid February to April), hot dry (May to June) and hot humid (July to August) seasons. Immediately after collection, 2 mL seminal plasma was taken in an eppendorf tube and centrifuged at 12000 rpm for 10 min. at 4°C. The supernatant was centrifuged again at 12000 rpm for 5 min at 4°C and supernatant was collected and kept -20°C until ELISA was carried out.

The percentage of live sperm was assessed by eosin-nigrosin (EN) stain (Blom, 1950). EN stain was prepared by proper mixing of eosin and nigrosin (1:5 ratio) in 10 mL sodium citrate buffer with the help of a magnetic stirrer at 70-80°C for 40-60 min. The content was filtered through filter paper and kept at 4°C. Then 2.5 µL neat semen and 20 µL EN stain were placed on clean pre-warm glass slide, mixed properly and 5-8 µL mixture drawn in a clean pre-warm glass slide. A very thin smear was made and air-dried. The dead sperm stained pink (eosin) and partially stained sperm were also considered as dead while live sperm did not stain. Spermatozoa (200) were counted under oil immersion from different fields. The percentage of acrosomal integrity was assessed as per Hancock (1951). Neat semen (3 µL) was placed on a clean glass slide and a thin smear was prepared, subjected to 5% Giemsa for overnight. The working solution of 5% Giemsa was prepared by mixing 5 mL Giemsa stock solution (Qualigens, India) with 7.5 mL stock solution A (0.06 M Na_2HPO_4 = 1.0673 g in 100 mL double distilled water) and similar amount of stock solution B (0.06 M KH_2PO_4 = 0.8165 g in 100 mL double distilled water). The total volume was raised to 100 mL by adding double distilled water. The pH of solution A and B was maintained at 6.8 and stored in clean glass bottles at room temperature. Approximately, 200 spermatozoa were counted under oil immersion from different fields. GPx estimation was carried out by bovine ELISA kits (SEA295BO 96 test, USCN Life science INC) as per the manufacturer's protocol. The sensitivity of assay kits was 7.2 pg mL^{-1}. The optical density was recorded using TECAN infinite PRO200 ELISA reader (Tecan Asia Pvt. Ltd., Singapore) at 450 nm. The intra- and inter-assay co-

efficient variation was <10%. The data was analyzed with help of SAS software version (9.1) [SAS Institute Inc., Cary, NC, USA]. One way ANOVA was used to estimate seasonal variation of GPx concentration and pair-wise comparison of mean was done by Tukey's multiple comparison test. Person co-efficient correlation was used between GPx concentration with acrosomal integrity and live spermatozoa.

RESULTS AND DISCUSSION

The seminal concentration of GPx was 59.30±0.60; 60.67±0.76; 62.14±0.90 and 65.74±0.93 pg mL^{-1} during winter, spring, hot dry and hot humid seasons, respectively. Complete and partially stained sperms were considered as dead and those without stain as live (Fig. 1). Spermatozoa with intact acrosome stained pink and reacted acrosome without colour (Fig. 1C). Higher concentration of GPx was observed during hot humid season than in other seasons (Fig. 2-A,B). The live spermatozoa were 66.23±0.88; 68.05±0.77; 65.86±0.77 and 62.42±0.75% during winter, spring, hot dry and hot humid seasons, respectively; and low percentage of live spermatozoa was observed during hot humid season [Fig. 2B] as compared to spring and winter seasons. The acrosomal integrity was 83.09±0.60, 83.40±0.48, 78.70±0.60 and 74.22±0.62% during winter, spring, hot dry and hot humid seasons, respectively. The acrosomal integrity also lowered during hot dry and hot humid seasons (Fig. 2C). A negative correlation (r = - 0.372) was observed between GPx and acrosomal integrity (Fig. 2D). The optimum spermatozoa production has been reported in bovine when testicular temperature is around 33-34°C (Wildeus and Entwistle, 1983; Barth and Bowman, 1994). This temperature might be well maintained during spring and winter seasons in crossbred bulls indicated by higher live spermatozoa, concentration, hypo-osmotic swelling test and lower sperm abnormalities in Karan Fires bulls as compared to summer season (Bhakat *et al.*, 2014). The testicular antioxidant defense system plays important role in maintaining sperm quality in challenged climatic conditions (Cardozo *et al.*, 2006). Adverse conditions such as heat stress enhance the production of reactive oxygen species (ROS) might be one of the reasons for lower lives spermatozoa and intact acrosome in Karan Fries bulls in the present study during hot humid season. Fluidity of sperm membrane is maintained due to high content of polyunsaturated fatty acids; therefore, sperm membrane is the common target point of ROS, causes lipid peroxidation and loses membrane fluidity. Bhakat *et al.* (2014) observed poor semen quality in Karan Fries bulls during summer season. Karan Fries bulls with half exotic inheritance may be affected by heat stress during summer under tropical environment. GPx is an effective intracellular antioxidant enzyme against oxidative stress (Kosower *et al.*, 1971). Glutathione peroxidase is involved in maintaining de-capacitated state of spermatozoa (Okamura *et al.*, 1997; Marti *et al.*, 2007). It prevents from cold-shock membrane injury during freezing (Barrios *et al.*, 2005) and is also involved in fructo-lysis of spermatozoa via glycolytic cycle (Slaweta and Laskowaska, 1987). The high concentration of GPx during hot humid season might be the response of heat stress. Higher GPx activity was reported during summer in Simmental bulls (*Bos taurus*) as compared to Nelore bulls (*Bos indicus*)

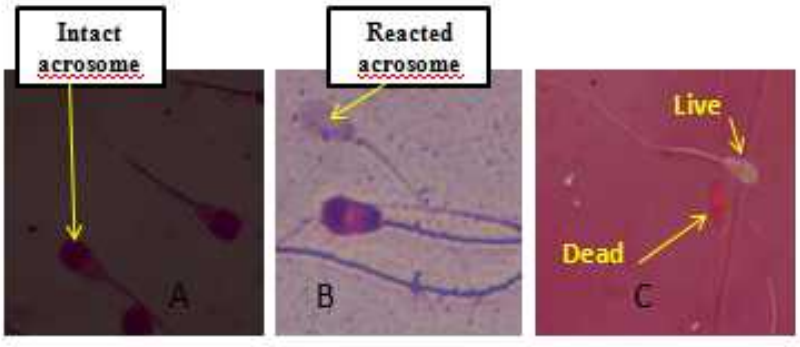

Fig. 1: Intact (A) and reacted (B) acrosome and live and dead (C)

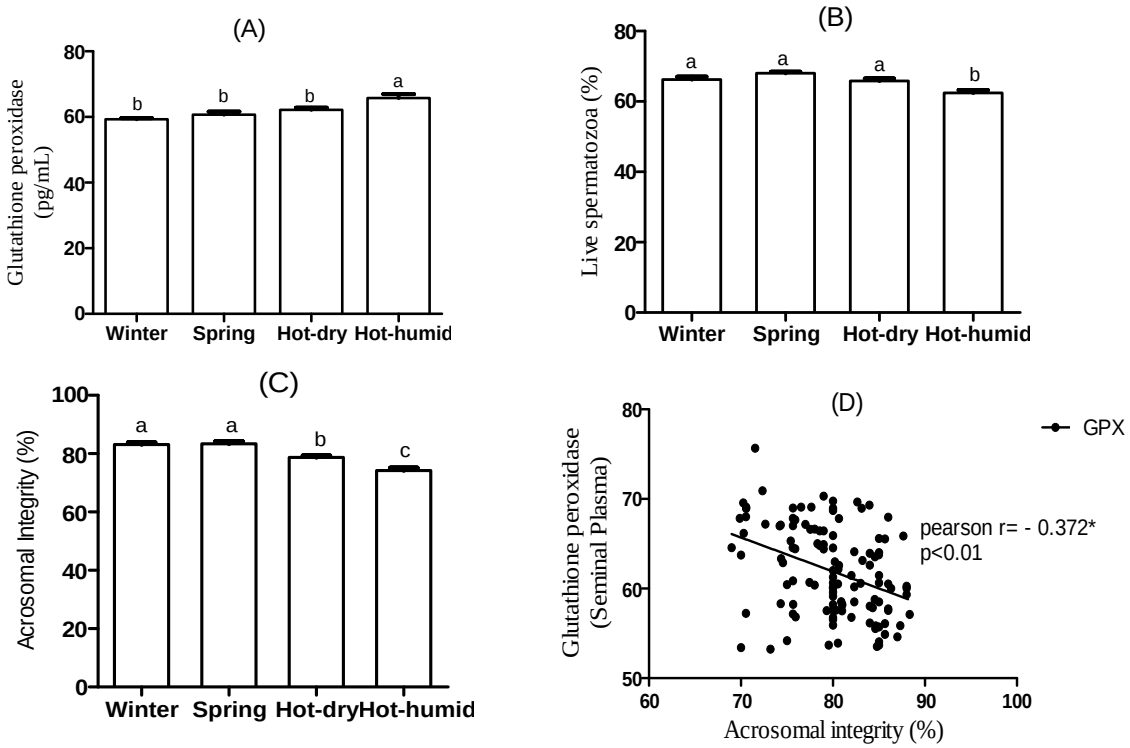

Fig. 2: Seasonal variation in glutathione peroxidase [GPx] (A), live spermatozoa (B), acrosomal integrity and correlation of GPx with acrosomal integrity (D).

raised under tropical climatic conditions and thiobarbituric acid reactive substance (malon-dialdehyde) also found positive correlation (r = 0.59, P = 0.021) with major abnormalities. They have concluded that GPx activity in Simmental bull's semen was insufficient to prevent deterioration of semen quality raise under tropical climatic conditions. In present study a negative correlation between GPx and acrosomal integrity was observed. The semen quality of Karan Fries bulls affected during summer season mostly hot humid season under tropical climatic conditions, therefore, special measures should be undertaken to ameliorate adverse effect heat stress on semen quality.

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