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Influence of season on seminal antioxidant enzymes in Karan Fries bulls under tropical climatic conditions

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Abstract: Availability of good quality semen is very crucial for sustainable development of the dairy sector throughout the year. Seminal antioxidant status plays an important role for sperm viability, acrosomal integrity, and motility and emerges as a prognostic tool for assisted reproductive technology. The present study was designed to evaluate the seminal antioxidant enzyme concentrations in Karan Fries bulls during different seasons. Five adult healthy Karan Fries (*Bos indicus* × *Bos taurus*) bulls were selected from the the Animal Breeding Research Centre of the Indian Council of Agricultural Research-National Dairy Research Institute, Karnal. Weekly ejaculates were collected using an artificial vagina (42–45 °C). The total antioxidant capacity, glutathione peroxidase, glutathione reductase, and superoxide dismutase were increased ($P < 0.05$) during the hot humid season compared to the winter and spring seasons. Malondialdehyde concentrations were also increased ($P < 0.05$) during the hot humid and hot dry seasons and positively correlated with major abnormalities ($r = 0.478$), while negatively correlated with sperm concentration ($r = -0.257$) and hypoosmotic swelling test ($r = -0.359$). The results indicate insufficiency of antioxidant enzyme concentrations to sustain the semen quality during the summer (hot humid and hot dry) season.

Key words: Season, antioxidant enzymes, Karan Fries bulls

1. Introduction

Global warming and climate change have become major threats for animal production and sustainable livestock production (1). Climate change is one of the most potentially serious environmental problems confronting the global community. Great economic losses may occur if the current management systems are not changed (2). High environmental temperature exerts a negative influence on the performance of livestock population (3). In tropical and subtropical regions high ambient temperature is the major constraint on animal production (2,4).

Seminal plasma is the secretary mixture of many components and factors that provide a suitable environment for maturation, viability, and fertilizing ability of spermatozoa. Seminal antioxidant enzymes are also essential for maintaining sperm viability (5,6), motility (7), and resistance to cold-shock damage (8,9). Production of reactive oxygen species (ROS) is most common during spermatogenesis, as it is a highly replicative process capable of producing approximately 1000 spermatozoa per second (10). ROS play a crucial role for eliminating abnormal spermatozoa during the process of spermatogenesis and also in spermatozoa functions such as motility, hyperactivation, capacitation, and zona binding (11–14); therefore, a moderate level of ROS is necessary. Overproduction of ROS damages the sperm membrane. The sperm membrane is very prone to free radical attacks as it contains a high amount of polyunsaturated fatty acids. Seminal plasma efficiently counteracts ROS and maintains a physiological range of ROS through antioxidants present in the seminal plasma. Instead of beneficial effects of ROS, sometimes they may be harmful to semen quality. ROS production is enhanced by adverse climatic conditions such as heat stress, which is one of the major threats of animal production. Semen quality of Karan Fries bulls is compromised during summer season as compared to other seasons of the year under tropical climatic conditions (15,16). Heat stress can reduce luteinizing hormone secretion, important for spermatogenesis (17). Testicular temperature in mammals should be 2–6 °C below body temperature, and 33 to 34 °C testicular temperature is optimum for suitable

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spermatogenesis in bovines (18,19). Screening of semen samples is done based on sperm motility for artificial insemination. Routine semen evaluation parameters cannot give complete information about semen quality; however, a combination of different tests can give some information about semen quality and the bull's fertility. Seminal antioxidants are good indicators of semen quality and emerge as a diagnostic tool for assisted reproductive technology; therefore, the present study was conducted to evaluate the concentration of antioxidant enzymes in the seminal plasma of Karan Fries bulls during different seasons under tropical climatic conditions.

2. Materials and methods

2.1. Experimental animals and sample collection

The study was conducted on five Karan Fries bulls (4–6 years of age, average body weight 655.2 ± 52.03 kg) maintained at the Animal Breeding Research Centre of the Indian Council of Agricultural Research-National Dairy Research Institute (ICAR-NDRI), Karnal, India. Experimental bulls were given a bath at least 40 min before semen collection. Bulls were subjected to physical exercise in a bull exerciser in the morning every other day. Semen samples were collected at weekly intervals from each bull during the winter (December to mid-February), spring (mid-February to April), hot dry (May to June), and hot humid (July to August) seasons using an artificial vagina (42–45 °C) in the early morning. Immediately after collection, semen samples were placed in a water bath (37 °C) to study semen evaluation parameters (mass motility, individual motility, noneosinophilic sperm count, acrosomal integrity, sperm concentration, and major abnormalities). All the experimental animals were maintained under uniform feeding and management regime practiced at the farm. The experimental bulls were offered roughages ad libitum and concentrate mixture at 2.5 kg per animal per day. The concentrate mixture consisted of maize 28%, ground nut cake 10%, mustard cake 13%, wheat bran 15%, rice polish 11%, deoiled soyabean 15%, millet (*Pennisetum glaucum*) 5%, mineral mixture 2%, and salt 1% with 16% crude protein (CP) and 70% total digestible nitrogen (TDN). Water was made available to the animals around the clock.

2.2. Temperature humidity index

The temperature humidity index (THI) was calculated using the formula THI = 0.72(W + D) + 40.6, where W is wet bulb temperature and D is dry bulb temperature in °C (20). The THI is considered as an indicator of thermal climatic conditions. Mild, medium, and severe stress was classified on the basis of THI range, i.e. 72–80 (mild), 80–90 (medium), and 90–98 (severe). Livestock species are comfortable at THI values between 65 and 72 (21).

2.3. Ethical permission

The experiment was approved by the institutional animal ethics committee constituted as per article number 13 of the rules of the Committee for the Purpose of Control and Supervision of Experiments on Animals laid down by the Government of India.

2.4. Semen analysis

Ejaculate volume was recorded just after semen collection in a graduated glass centrifuge tube. A drop of fresh semen sample was placed on preheated (37 °C) glass slide; a cover slip was placed on the drop and it was immediately observed under a phase contrast microscope (Nikon Eclipse E600, Tokyo, Japan) under low magnification (10×). The semen samples were graded on the basis of wave movement. Individual motility was assessed by diluting the neat semen with egg yolk medium (1:10). One drop of sample was put on a preheated glass slide (37 °C), a cover slip was added gently, and it was observed under a light microscope (40×, Labomed, Los Angeles, CA, USA). Noneosinophilic sperm were assessed by eosin-nigrosin (EN) stain as suggested by Blom (22). EN stain was also used for counting major sperm abnormalities (proximal cytoplasmic droplets, pyriform heads, folded/coiled tails, and middle piece defects). About 200 spermatozoa were counted under oil immersion. Spermatozoa having either completely or partially stained pink (eosin) heads were considered as dead sperm and the spermatozoa having completely unstained heads were considered as live spermatozoa. Acrosomal integrity was assessed by the method of Hancock (23). About 200 spermatozoa were counted under oil immersion by light microscope. The attached acrosome showed a purple color and detached acrosome without purple at the head of the spermatozoa. Sperm concentration was determined by hemocytometer (Neubauer improved, Marienfeld, Lauda-Königshofen, Germany).

2.5. Seminal plasma separation and antioxidant enzymes assay

Immediately after collection, 2 mL of semen sample was taken and centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatant was centrifuged again at 12,000 rpm for 5 min at 4 °C and the supernatant was collected and kept at –20 °C until ELISA was carried out.

Glutathione peroxidase (SEA295BO, USCN Life Science Inc.), glutathione reductase (BG-BVN11127, NovaTeinBio), superoxide dismutase (MBSO40427, MyBioSource), and total antioxidant capacity (MBS748686, MyBioSource) were determined by bovine ELISA kits as per the manufacturer's protocol. The sensitivity of the assay kits were 7.2 pg/mL, 2.0 U/L, 2.0 U/mL, and 1 ng/mL for glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), and total antioxidant capacity (TAC), respectively. Thiobarbituric acid reactive

substances [malondialdehyde (MDA): a product of lipid peroxidation] were determined by Quantichrom TBARS Assay Kit (DTBA-100, from Bioassay Systems, Hayward, CA, USA) as per the manufacturer's protocol. The optical density (OD) was recorded using a TECAN infinite PRO200 ELISA reader (TECAN Asia Pte. Ltd., Singapore) at 450 nm. The intra- and interassay coefficients of variation were <10%.

2.6. Statistical analysis

The data analysis was carried out with SAS software, version 9. (SAS Institute Inc., Cary, NC, USA). One-way ANOVA was used to estimate the seasonal variation of antioxidant enzyme concentrations in seminal plasma of Karan Fries bulls. The pairwise comparison of mean was done by Tukey's multiple comparison test. The graphs were prepared using Prism5 software.

3. Results

The average THI values were recorded as 61.39, 66.67, 80.68, and 81.64 during the winter, spring, hot dry, and hot humid seasons, respectively. The antioxidant enzyme concentrations in the seminal plasma increased ($P < 0.05$) during the hot humid season as compared to other seasons (Figure 1). The TAC concentrations were 91.70 ± 1.89, 86.59 ± 1.65, 96.17 ± 2.07, and 100.43 ± 1.78 ng/mL during the winter, spring, hot dry, and hot humid seasons, respectively. The GPx (65.74 ± 0.93 vs. 59.30 ± 0.60, 60.67 ± 0.76 pg/mL), GR (5.72 ± 0.13 vs. 4.90 ± 0.21, 5.12 ± 0.14 U/L), and SOD (69.10 ± 1.49 vs. 61.38 ± 0.81, 62.06 ± 0.81 U/mL) were increased ($P < 0.05$) during the hot humid season as compared to winter and spring. The individual motility, noneosinophilic sperm count, acrosomal integrity, and sperm concentration were significantly ($P <$

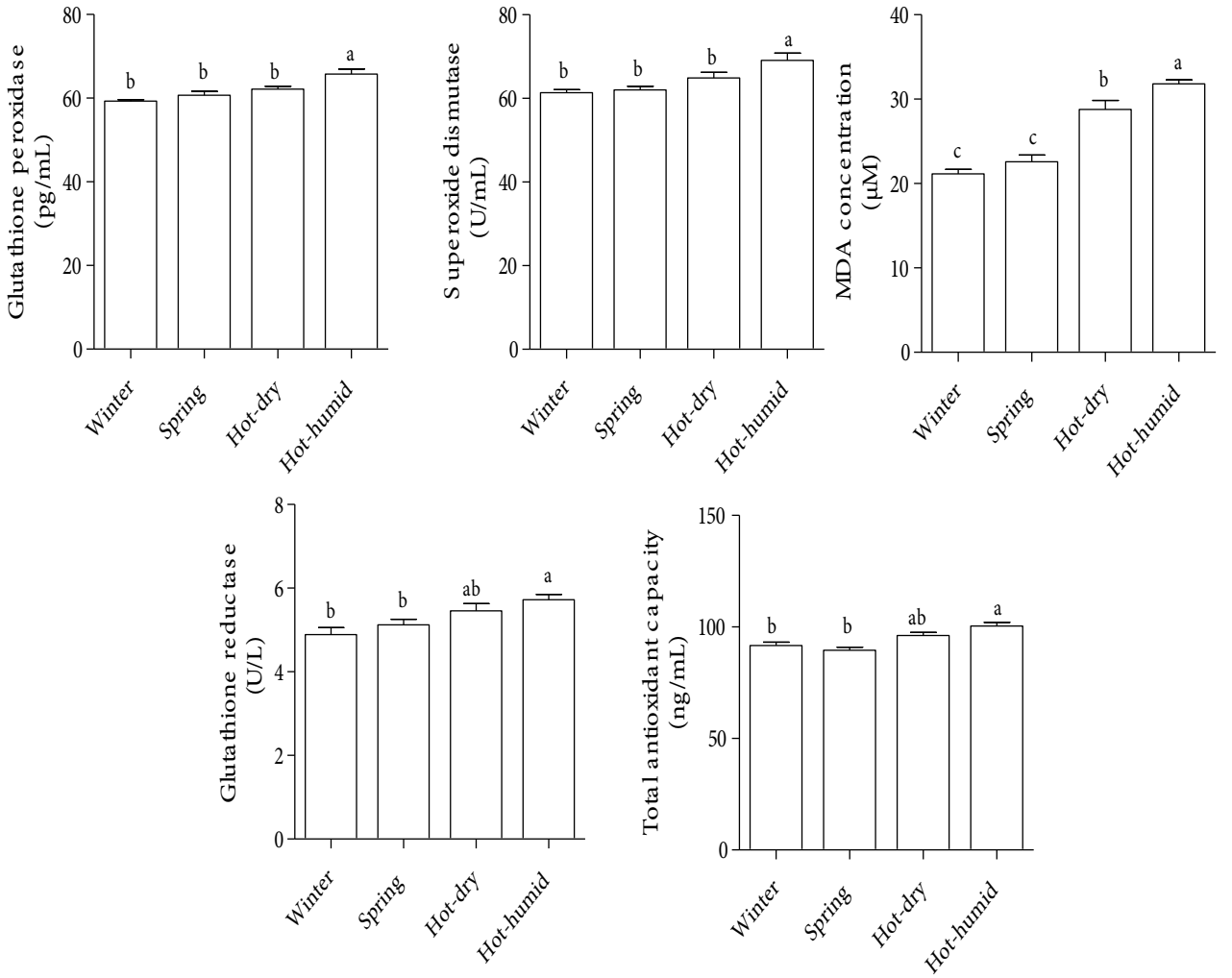

Figure 1. Seasonal variation of seminal antioxidant enzymes and MDA concentration in Karan Fries bulls under tropical climatic conditions.

0.05) lowered during the hot humid season as compared to spring; however, mass motility did not differ ($P < 0.05$) between the seasons. The major abnormalities were more common ($P < 0.05$) during the hot humid season as compared to other seasons. The MDA concentrations were also increased during the hot humid (31.81 ± 0.54 μM) and hot dry (28.72 ± 0.68 μM) seasons as compared to winter (21.16 ± 0.52 μM) and spring (22.60 ± 0.69 μM). MDA concentration showed positive correlation with major abnormalities ($r = 0.478$) and negative correlation with the hypoosmotic swelling test (HOST) ($r = –0.359$) and sperm concentration ($r = –0.257$) (Figures 2 A–2C).

4. Discussion

Sperm membranes are rich in unsaturated fatty acids (20:4 and 22:6), important for spermatozoa motility; however, they are vulnerable to ROS. Higher antioxidant enzyme concentration was observed in the hot humid season compared to other seasons ($P < 0.05$). This higher concentration of antioxidant enzymes might be to neutralize increased ROS, enhanced by heat stress during the hot humid (THI = 81.64) followed by the hot dry (THI = 80.68) season. Karan Fries (*Bos indicus* × *Bos taurus*) having 50% or more exotic inheritance showed poor adaption to the summer season under tropical climatic conditions. Better productive and reproductive performances were shown in crossbreeds having 50% exotic inheritance as compared to other genetic groups (24). Therefore, the F_1 generation or bulls with more than 50% exotic inheritance are maintained for sustainable growth of the dairy sector in India. Karan Fries bulls with half or more exotic inheritance are affected by heat stress during summer under a tropical environment, indicated by higher antioxidant enzyme concentrations in this study. It has been reported that European bulls (*Bos taurus*) have lowered fertility than Zebu bulls (*Bos indicus*) under tropical climatic conditions (25,26), and oxidative stress might be one of the reasons for reduced fertility (27). Higher

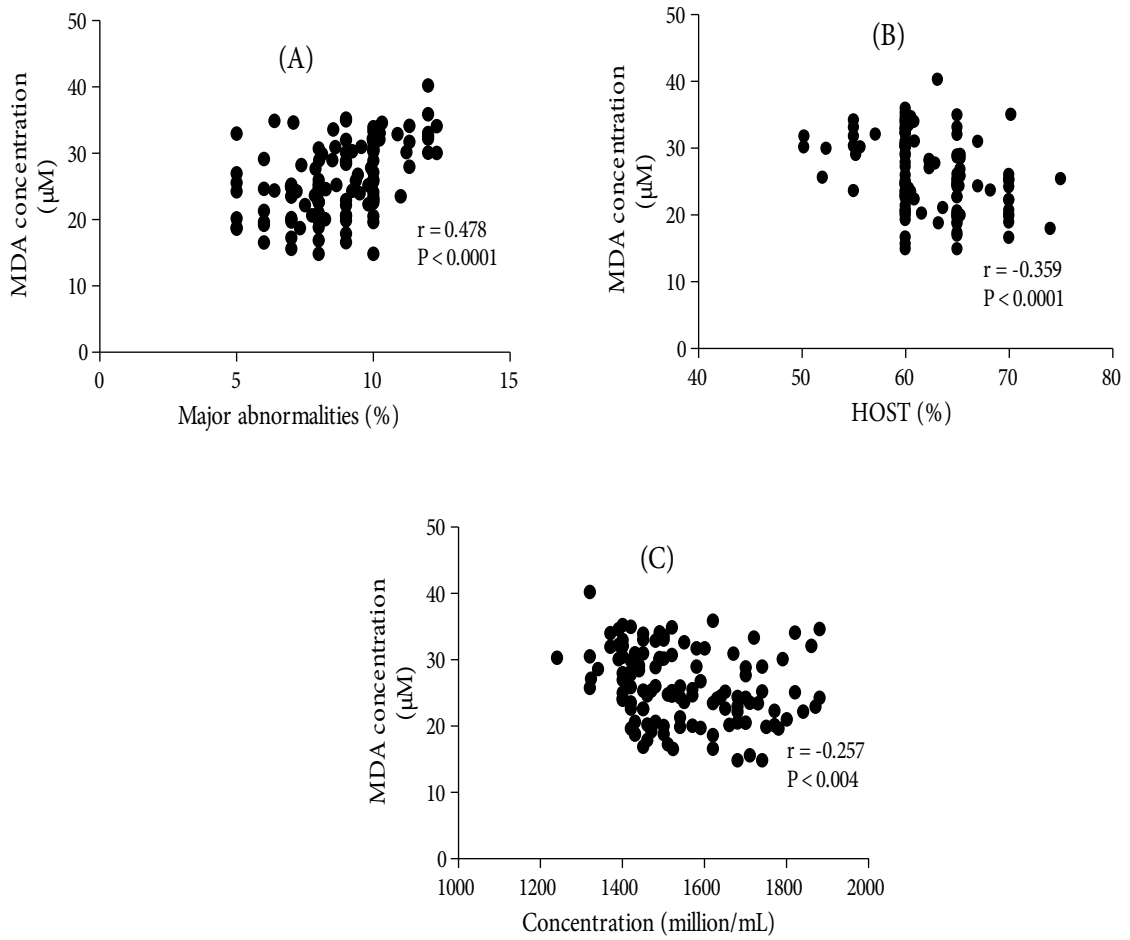

Figure 2. Correlation coefficient (Pearson) between seminal MDA concentration and major abnormalities (A), HOST (B), and sperm concentration (C).

concentrations of MDA were observed during hot humid and hot dry seasons compared to winter and spring in the present study; similar findings were reported by Yeni et al. (28) in ram seminal plasma during summer as compared to winter. Negative correlation was observed between MDA concentration and semen evaluation parameters (sperm concentration and HOST). MDA concentration was positively related with major abnormalities. The results of this study are in corroboration with those of Nichi et al. (27), who observed higher activity of antioxidant enzymes during the summer season in Simmental bulls (*Bos taurus*) as compared to Nelore bulls (*Bos indicus*). Higher rates of major abnormalities and lipid peroxidation (MDA) were also reported in Simmental bull semen under tropical climatic conditions during the summer season. Higher activities of GR, GPx, SOD, and catalase have been reported in semen of poor quality in the nonbreeding season; higher antioxidant enzymes in seminal plasma are indicators of a protective mechanism against ROS to maintain the fertilizing potential of spermatozoa under adverse conditions (29). Increase in testicular temperature (cryptorchidism) above normal causes high generation of ROS, resulting in DNA damage (30). The excess production of free radicals has an indirect consequence of impaired spermatogenesis and epididymal function, resulting in retention of excess residual cytoplasm (10).

The increased concentration of seminal antioxidant enzymes during hot humid and hot dry seasons in the present study indicates a negative effect on semen quality during the summer season in Karan Fries bulls under tropical climatic conditions. The higher concentration of MDA, positive correlation with major abnormalities, and negative correlation with sperm concentration and HOST indicate insufficiency of antioxidants in the seminal plasma. Therefore, special measures should be taken to ameliorate the adverse effect of heat on semen quality of Karan Fries bulls. Supplementation of antioxidants appears to be necessary in Karan Fries bulls for sustainable semen quality during the summer (hot humid and hot dry) season under tropical climatic conditions.

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