EFFECT OF Zn SUPPLEMENTATION (ZnSO₄) ON SPERM MORPHOMETRY OF MURRAH BUFFALO BULLS (BUBALUS BUBALIS)


ABSTRACT

The objective of the present study was to study the effect of zinc supplementation (ZnSO₄) on sperm morphometry of the Murrah buffalo bulls. Eight apparently healthy, sexually mature and clinically normal Murrah buffalo bulls of similar body weight and age group (nearly 3.0 to 6.5 years) were randomly divided into two groups: control and ING groups. The Diet for both the groups was the same except that the ING group was supplemented with 40 ppm zinc (zinc sulfate; analytical grade) for 150 days, including 30-day adaptation period. Zn was weighted as per the requirement of individual bulls and mixed with a weighed amount of concentrate mixture for feeding. The amount of Zn supplementation was adjusted at fortnight 14 intervals depending upon the total dry matter intake of individual bulls. Spermatozoa with intact acrosomes were selected and assessed using an immersion lens (1,000X) and standard illumination. The software made it possible to take linear measurements of each spermatozoon: head length, head width, head base, tail length, acrosomal cap length and acrosomal cap width. The results did not show any significant (P>0.10) effect of Zn supplementation on sperm morphometry. It can be concluded that Zn supplementation had no effect on sperm morphometry.

Keywords: buffalo, morphometry, sperm, zinc

INTRODUCTION

On a global scale, zinc (Zn) deficiency is the most widespread mineral deficiency and can occur through at least five mechanisms- inadequate intake, increased requirements, mal-absorption, increased losses and impaired utilisation. Sillanpaa (1982) concluded from the global study that deficiencies of Zn could be suspected in almost every country. Zn is an essential trace element in animal nutrition with a wide range of biological roles. It plays catalytic, structural or regulatory roles in the more than 200 Zn metalloenzymes that have been identified in biological systems. It plays important roles in polymeric organization of macromolecules like DNA and RNA, protein synthesis, cell division and stability of biomembranes (Chvapil, 1973). Zn plays a structural role in the formation of the so-called Zn fingers. Zn fingers are exploited by transcription factors for interacting with DNA and regulating the activity of genes. Another structural role of Zn is in the maintenance of the integrity of biological membranes resulting in their protection against oxidative injury. During spermatogenesis, a functional locomotor apparatus is formed in spermatozoa (Mohri and Ishijina, 1989) and a considerable amount of Zn is incorporated into the spermatozoa (Parizek et al., 1966). Flagellar Zn is located mainly within the outer dense fibers (Calvin et al., 1973), where it is bound to the sulphydryl groups of cysteine. In the course of epididymal
sperm maturation Zn content is reduced by approximately 60% (Kaminska et al., 1987) leading to increased stabilization of outer dense fiber proteins by oxidation of sulfhydryl groups to disulfide bridges (Calvin et al., 1973). Zn is also believed to regulate maturation of spermatozoa (Baccetti et al., 1976). Zn deficiency adversely affects sperm integrity (Merrells et al., 2009). With this in view, the following study was designed to study the effect of Zn supplementation on sperm morphometry of Murrah buffalo bulls.

**MATERIALS AND METHODS**

The investigation was carried out on eight apparently healthy, sexually mature and clinically normal Murrah buffalo bulls of similar body weight and age group (nearly 3.0 to 6.5 years). Bulls were randomly divided into two groups: control and ING groups. The Diet for both the groups was same except that the ING group was supplemented with 40 ppm zinc (zinc sulfate analytical grade) for 150 days, including a 30-day adaptation period. Except for Zn supplementation, the diets were the same for both the groups. Zn was weighted as per the requirement of individual bulls and mixed with weighed amounts of concentrate mixture for feeding. The amount of Zn supplementation was adjusted at fortnightly intervals depending upon the total dry matter intake of individual bulls.

Semen samples were diluted to 200 X 10⁶ sperm/mL. To avoid individual technician variation, one person measured all the parameters from the captured image. A Dual staining procedure initially developed by Sidhu et al. (1992) was used with some modification to identify the clear acrosome structure of buffalo spermatozoa. One hundred microliters of semen were mixed with 0.2 percent trypan blue (in TALP medium without BSA) and incubated for 10 minutes on a clean glass slide at 37°C. After the incubation period, smears of the semen were prepared gently on the glass slides and allowed to dry for 15 minutes at room temperature. A 0.72 percent (W/V) Giemsa stock solution was prepared by dissolving 1 g of Giemsa dye in glycerol-methanol mixture (54:84). One gram of Giemsa was diluted five times with distilled water (final concentration of Giemsa working solution is approximately 0.15%). The smears of spermatozoa previously stained with trypan blue were then stained with Giemsa for 1 hr at room temperature to evaluate the acrosomal status of the spermatozoa. Smears were dried between the folds of filter paper and stored. The dried smears were studied at 1000X under a light microscope using oil immersion without cover-glass. The slides were used for measurement within a week of preparation. A total of 700 spermatozoa were measured for the experiment.

**Image Analysis Measurements:**

Images were randomly selected from each slide by using an Nikon Eclipse E600 (Tokyo, Japan) microscope attached to an Nikon camera, interfaced to a PC computer and ACT1 software for measurement. The images were obtained by using

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A = 1.05 - 0.081 \times B^2 + 0.64 \times W \times L
\]

\[
e = \frac{L - W}{L - W}
\]

\[
SF = (1 - e) \times \frac{P^2}{4\pi A}
\]
100X objectives (oil immersion) in standard light transmission mode (transillumination). Only fresh images were used for the measurements. One speciality of this programme is that stored images cannot be used for measurements. The software was standardizing against a decimal scale. One hundred normal sperm were obtained for each bull from different days of semen sample to avoid any day-to-day variation.

Sperm morphology was quantified in terms of the following morphological features: head length (L), width (W), base (B), head area (A), perimeter (P), acrosomal cap length and width, tail length, ellipticity (e), shape factor (SF) (Ostermeier et al., 2001). The units for measurement variables were micrometers (μm); the ratios are without units. The head area, ellipticity and shape factor are defined in Equations. (1), (2) and (3), respectively.

The head shape was calculated as the ratio of head length and head width (Beatty and Napier, 1960). The width base is defined as the distance between the vertices of the base of the sperm head. Sperm head roundness was calculated as convex perimeter (Hunt et al., 1992).

Descriptive statistics (Systat 11.0) were performed on the data to determine normality. Statistical analysis was performed as per standard statistical methods (Snedecor and Cochran, 1989).

**RESULTS AND DISCUSSION**

Various types of sperm morphometric parameters are presented in Table 1. There was no significant (P>0.10) difference between control and ING group. Individual bull variation was not found for the head length, width, base, head area and shape (width:length), acrosomal cap length and width, tail length, perimeter, ellipticity and shape factor.

The results of the present study indicated that Zn supplementation had no effect on the sperm morphometry. This is the first study of this kind. Zn seems to play an important role in the physiology of spermatozoa; it has been reported to influence the process of spermatogenesis in ram (Underwood and Somers, 1969). Production of spermatozoa necessitates extensive cell division, which requires a large amount of Zn as it is involved extensively in nucleic acid and protein metabolism and is hence fundamental to cell replication and differentiation. Zn is also believed to regulate maturation of spermatozoa (Baccetti et al., 1976). Sperm morphometry, in combination with other objective traits, can be useful for developing a fertility index. Associations of abnormal spermatozoa with bull fertility have yielded varying results. Abnormal bull sperm morphology has been correlated with reduced
fertility (Sekoni and Gustafsson, 1987; Correa et al., 1997). In particular, the occurrence of abnormal sperm head morphology is associated with lower fertility in the bull (Saacke and White, 1972; Sekoni and Gustafsson, 1987). However, a number of other studies have shown no correlation between sperm morphology and fertility (Bratton et al., 1956; Linford et al., 1976) with clear associations between normal bull sperm morphology and fertility continuing to remain elusive (Johnson, 1997). The nucleus of the mammalian spermatozoa becomes highly condensed during the latter stages of spermatogenesis (Zambani, 1971). This condensation is accompanied by biochemical changes involving the replacement of histones by the more basic arginine and cystein rich protamines (Gledhill et al., 1966). The condensed sperm nucleus appears to be chemically more resistant than nuclei of other cells. This resistance or stability is probably due to the extensive disulphide (S-S) bridges existing between adjacent protamine molecules within sperm chromatin (Calvin et al., 1975). These disulphide bonds are formed during spermatozoan transit through epididymis. Spermatozoan nucleus remains in condensed form before fertilization. Zn has a high affinity for thiols (Valle, 1959) and that removal of Zn is facilitated by thiol reacting compounds in spermatozoa from rats (Calvin and Bleau, 1974) and men (Kvist and Eliasson, 1980).

Inadequate uptake of Zn may jeopardize the normal stability of the chromatin, and could result in, or at least signify, a reduced potential of the spermatozoan to contribute to a normal embryonic development (Kvist et al., 1998).

It can be concluded that Zn supplementation had no effect on sperm morphometry.

REFERENCES


