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Research Article

The Effects of Selenium Supplementation on the Production of Spermatozoa and Semen Quality of Riverine Buffaloes

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Abstract: This study determined the influence of different concentrations of organic selenium in the production of spermatozoa and semen quality of riverine buffaloes. Thirty (30) riverine buffalo bulls used for semen production at the National Bull Farm of the Philippine Carabao Center were allotted into six treatment groups. The first treatment (T1) with no supplementary selenium served as the negative control. The second (T2) and the third (T3) treatments were given 10% and 5% below the published selenium requirement of bulls, respectively. The fourth treatment (T4) serving as the positive control was given the normal level of selenium while the fifth (T5) and sixth (T6) groups were supplemented with 5% and 10% above the required selenium, respectively. The buffalo bulls were fed with rations composed of corn silage, rice straw, concentrates and molasses for three months under intensive system of management. Semen collection was done 2x a week using artificial vagina method and the semen volume, color, pH, sperm motility, sperm concentration and sperm morphology were evaluated. The results showed that T5 produced the highest values on semen volume (3.68 ml), sperm motility (71.82%), concentration (126.84 x 10^7) and viability (82.91%), respectively. This treatment has the lowest abnormal sperms of 10.55% when compared among the treatments with 22.89%. Overall results further revealed that the average sperm production and semen quality were improved by the different levels of dietary selenium supplementation. The color and pH of the semen collected from donor bulls were not significantly affected by selenium supplementation but the data gathered agreed with the published values.

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INTRODUCTION

Agricultural production system in developing countries is under pressure to fulfill the requirement of growing population, which has led to indiscriminate use of fertilizers resulting in severe deficiency of micro minerals in soil [1]. Moreover, some regions are naturally deficient in these micro minerals [2]. The deficiency of Selenium in soil and crop plants has been reported in many countries like India, China, Turkey and Pakistan [3]. The soil in the Indian subcontinent is also deficient in selenium. Selenium is an essential nutrient and indispensable element in growth and reproduction. Selenium helps in testicular growth and development of seminiferous tubules, spermatogenesis, steroidogenesis in testes, synthesis and secretion of follicular stimulating hormone (FSH) and luteinizing hormone (LH) [4]. The antioxidative property of Se prevents lipid peroxidation and stabilizes lysosomal membrane [5] and hence improves semen quality [6]. Selenium is present in the mid piece of spermatozoa and is associated with Cys-rich protein of the mitochondrial sheath [7]. A deficiency of Se causes changes in mid-piece architecture leading to breakage of head and tail of sperms and impaired sperm motility [8].

However, despite the current prominence of selenium in nutrition research, scientists are also interested on the influence of the selenium supplementation in the semen quality of riverine buffaloes [9]. Pathological factor and disorders of function can also play a part in reducing both semen quality and fertilization rates [10]. Selenium supplementation is one way to overcome the problem encountered in the sterility of the animals [11]. However, scientific studies regarding supplementation of Selenium to improve semen quality in buffaloes are lacking. Since Selenium is well known as an essential trace element, the problem is yet to be investigated as to its influence the production of spermatozoa and semen quality of buffaloes.

MATERIALS AND METHODS

Animals and Experimental Group

A total of 30 healthy riverine buffaloes that are already donating semen were used in the study. The experimental animals were divided into 6 groups (Table 1). The experimental buffalo bulls had an average age ranged from 3-7 years. Initial assessment of the riverine buffaloes was also included. All buffaloes were clinically free of the internal and external parasites. The testicular tone was glandular, both testes were in normal size and moved freely up and down within the scrotal pouches. Riverine buffaloes that were not supplemented with selenium served as the control (T1), the second group of buffaloes (T2) were fed with 10% below the Selenium requirement added to their feeds the third group (T3) were given 5% below the required selenium added to their feeds. The fourth group (T4) were supplemented with the required Selenium for reproducing animals 0.2mg/kg of Dry Matter Intake (DMI) [12]. On the other hand, the fifth and sixth group (T5 and T6) were given 5% and 10% above the required selenium intake, respectively.

Feeding and Management System

All buffaloes were fed daily with 2 kilograms of feeds and a standard silage/ haylage forage-based ration balanced in nutrients consisting of rice straw, corn silage, 0.02% of urea, 0.01% of salt, 0.01dicalcium phosphate and mineral blocks mixed with different levels of selenium based on EU maximum allowable level of Selenium for reproducing animals (poultry breeders, sows and dairy cow and buffaloes) [12]. The Selenium used was the commercially available organic Excential Selenium4000. The buffaloes were maintained on their diets for three months and semen collection followed.

Semen Collection

The schedule of semen collection at the bull farm is done twice a week that is every Tuesday and Friday using an artificial vagina method [15]. The semen that were collected every Friday from the donor bulls for three consecutive months were used in the study to determine if the supplementary selenium in the rations of the buffalo bulls have influenced the semen production and semen quality. The semen was collected in a graduated collecting tube containing a double walled glass fitted directly into the bottom of artificial vagina. Immediately after collection, the ejaculates were brought to the laboratory and were placed in a water bath at 37°C for semen evaluation.

Semen Evaluation

The fresh semen collected undergone rigid laboratory for examination. Semen samples were tested for various physical characteristics including volume, pH and color.

Semen volume

The volume of the semen was read based on the graduation in the conical tube. The volume, the donor and age were recorded.

Sperm Concentration

Counting sperm using haemocytometer is tedious. Using the IMV Accucell Photometer, IMV Technologies, L'aigle, Basse, Normandie, France sperm concentration can easily be determined. The photometer measures the amount of light absorbed by a sample, the more sperm are in the sample, the more light is absorbed. By generating a standard curve of absorbance versus sperm numbers, one can quickly and accurately measure sperm concentration without directly counting them.

Sperm Motility

The sperm motility of the ejaculate was estimated visually using the light microscopy developed at the Bull Farm of the Philippine Carabao Center [15]. The percentage of progressively motile cells were estimated using high power (400x) microscope with a pre-heated stage at 37°C to 40°C.The fresh semen was prepared as a thin film on a microscope slide, diluted with physiologic saline solutions so that the individual cells were visible. Table 2 presents the evaluation of the movement of sperm adapted from the protocol [13].

Sperm Morphology

Examination of sperm morphology was done by adapting a known protocol [14]. In the examination of sperm morphology, semen smears were prepared in each semen slide using the eosin-nigrosin stain solution, composed of 1% eosin and 5% nigrosin in 3% sodium citrate solution. Stained semen samples were evaluated using an inverted microscope. The shape of the head, neck or midpiece and the tail were examined. Sperm cells with abnormal morphology were counted. At least three hundred spermatozoa were observed on seven microscopic fields and the percentage of normal and abnormal sperms were calculated using the formula [15]. The abnormal morphology of bovine spermatozoa [16] (Figure 1) served as the reference in determining abnormal morphology in spermatozoa.

$$Percent\ abnormality = \frac{abnormal\ sperm}{total\ number\ of\ sperm\ observed} \text{x}100$$

Statistical Analysis

Data were statistically analyzed using Analysis of Variance and Comparison among means using Tukey's Test, using the SPSS/PC computer program (version 16.0; SPSS, Chicago, IL) and the research design used was Randomized Complete Block Design (RCBD), the animal's age, breed and initial semen volume acts as the blocking for this design to establish the influence of selenium supplementation in the semen quality and production of spermatozoa of riverine buffaloes.

RESULTS AND DISCUSSION

The study on the influence of selenium supplementation on the production of spermatozoa and semen quality of riverine buffaloes was conducted to evaluate and characterize spermatozoa ejaculated by riverine buffaloes supplemented with different concentrations of selenium added to their feeds. The riverine buffaloes were fed with different concentrations of selenium for three months and semen quality was evaluated. Semen quality such as volume, motility,

concentration, and abnormality were determined to assess which of the concentration of selenium could be utilized to collect good quality spermatozoa which are paramount in the fertility of buffaloes and development of Artificial Insemination (AI) technology.

Color and pH of Buffalo Semen

Table 1 presents the effects of selenium supplementation on color and pH of the buffalo semen. All the semen collected from buffalo bulls given different levels of Selenium supplement had average color ranging from milky white to creamy white which indicated that selenium supplementation did not affect the color of the buffalo bull's semen. The gathered data agreed with the report of Saito, (2005) that the color of good quality semen of bulls must be creamy, thick and milky. According to the report, any deviation from the published color is abnormal semen. Exception is semen from Holstein breeds where the color is yellowish.

The semen samples of donor bulls had average pH range from pH 6.3 to pH 6.6. The results implied closer pH values of normal semen or good quality semen ranging from 6.4 – 6.8, Saito (2005). Higher pH is observed in conditions when bulls are excessively used, with incomplete ejaculate or inflammatory conditions affecting the testes, epididymis, ampulla or seminal vesicle. The present study showed that different levels of selenium supplementation in buffalo bulls did not significantly affect the semen pH of the bulls.

Table1: Comparison of semen volume, color and pH in different levels of Selenium supplements

Treatment	Semen Volume (ml)			Average	Color	pH
	1st Month	2ndMonth	3rd Month			
T1	2.68 ± 0.51	3.26±0.75	3.22±0.61	3.06± 0.60	Creamy White	6.3
T2	2.58± 0.18	3.08±0.41	4.06 ±0.89	3.24±1.03	Creamy white	6.4
T3	2.92± 0.10	3.40±0.22	3.82 ±0.46	3.36±0.32	Milky White	6.4
T4	2.88±0.18	3.58±0.45	4.16±0.51	3.58±0.60	Milky White	6.5
T5	3.26±0.28	3.56±0.45	4.22±0.34	3.68±0.23	Creamy White	6.3
T6	2.82± 0.29	3.06±0.40	3.66±0.45	3.16±0.51	Milky White	6.6

Values are expressed as means ±standard error (n=30)

Semen Volume

Table 1 presents the volume of semen collected from buffalo bulls fed with different levels of selenium. The semen of buffaloes with 5% above the required selenium recorded the highest semen volume with a mean of 3.68ml on the other hand buffalo semen without supplementation had the lowest semen volume of 3.06ml. Analysis of variance found that there is no significant difference between the treatments. It can be noted however, that selenium supplementation 10% more than the required level resulted to lower semen volume. This conformed to the findings [17] that both the deficiency and excessive selenium supplementation have adverse effects to normal spermatogenesis thereby affecting sperm production. In buffaloes it is more of the adverse effect that is prominent.

The semen volume of buffaloes depends on age, breed, temperament, skill of technician and sexual stimulation. Normal volume of buffalo semen ranged from 2-8 ml [18]. The volume of sample obtained from buffaloes supplemented with different levels of Selenium was found to be within the reported ranges of semen volume. An increasing volume throughout the month was also observed in the semen samples of buffaloes supplemented with selenium. Selenium was correlated positively with an increase of semen volume, sperm count, motility, and morphology [19]. Results agreed with previous studies [20, 21]. Selenium has several functional roles in the testes. The first is its structural role in the development of the spermatogenesis and spermatozoal midpiece [22], second is in the development of the sertoli cell and their numbers [23], and third function is that it serves as a component of selenium-glutathione peroxidase. Dietary selenium supplementation produced a significant decrease in sperm lipid peroxidation and an improvement of sperm motility in infertile men [24]. Similarly, a recent study showed that treatment of infertile with idiopathic oligo-asthenoteratospermia, with 200 mg. selenium orally daily for 26 weeks, improves semen parameters [25]. On the other hand, the dose quantification of selenium supplementation is very important, because dietary selenium deficiency as well as excess supplementation induces multiple defects in mouse epididymal spermatozoa. A study showed that spermatozoa from Selenium-deficient mice presented an incomplete chromatin condensation showing an increase in occurrence of DNA strand breaks which also lead to reduction of semen volume produced [26].

Table 2: Comparison of sperm motility in different levels of Selenium supplements

Treatment	Sperm Motility (%)			Average
	1st Month	2nd Month	3rd Month	
T1	69.040± 2.32	69.520±2.19	62.040±2.72	66.82±1.5[b]
T2	62.540±5.24	66.020±3.78	74.520±1.79	67.68±3.45[a]
T3	63.520±3.20	70.020±0.88	72.280±2.99	68.58±2.04[a]
T4	63.520±3.76	65.500±3.30	72.280±1.55	67.01±2.30[a]
T5	67.020±2.64	72.020±1.02	76.520±1.33	71.82±1.09[a]
T6	63.540±3.5	68.780±3.81	72.780±2.28	68.36±2.81[a]

Values are expressed as means ± standard error (n=30)

In a column with different superscripts are significantly different at $P < 0.05$

Sperm Motility

Table 2 presents the motility of spermatozoa in different levels of selenium added to their feeds. As shown on the table, motility of the samples from buffaloes that received 5% above the required amount of selenium had the highest motility of 71.82% on the other hand buffalo semen without selenium supplements had the lowest sperm motility with a mean of 66.82%. Analysis of Variance in RCBD and Comparison among Means using Tukey's Test revealed that there is a significant difference between the motility of control and the treatment with different levels of selenium, indicating that the selenium supplementation significantly affected or improved the resulting percentage of motile and progressive spermatozoa in the semen samples collected.

Previous reports have demonstrated that males consuming diets low in selenium produce sperm with low motility [27]. The underlying cause of this reduced sperm quality is not known but may be the result of this development is during spermatogenesis [28]. One possible mechanism through which this could occur is via the activity of glutathione peroxidase. Selenium is an important component of glutathione peroxidase, which, in turn, is important for preserving the structural integrity of the sperm plasma membrane [29]. It is capable of neutralizing reactive oxygen species and thereby provides an important protective mechanism against lipid peroxidation for developing and mature spermatozoa [30, 31].

In a study conducted [32], it was suggested that a gradual increase in sperm motility was observed from the spermatozoa of cockerel supplemented with selenium. The increasing capacity for motility was attributed to the decrease in lipid peroxidation in the seminal plasma. The data proposed that organic Selenium protected sperm from free radical attacks which is translated in enhanced sperm motility. A study also showed that enhancing the antioxidant capacity of semen could present a major opportunity for improving male fertility [33].

Table 3: Comparison of sperm concentration in different levels of Selenium supplement

Treatment	Sperm Concentration (x10^7)			Average
	1st Month	2nd Month	3rd Month	
T1	73.76±9.45	89.38±7.47	104.86±6.11	89.34±6.37
T2	92.94±8.18	118.72±17.47	147.02±19.86	119.54±14.66
T3	104.98±11.45	98.52±11.13	107.78±16.97	103.74±10.92
T4	91.32±5.65	113.16±10.02	129.52±18.61	111.34±9.93
T5	100.72±5.23	132.12±13.74	147.78±9.57	126.84±8.44
T6	80.58±7.21	102.28±12.50	127.48±16.09	103.4±7.86

Values are expressed as means ± standard error (n=30)

Furthermore, the result of the study [34] suggested that there are positive effects of dietary supplementation with Selenium on semen characteristics and that organic Selenium supplementation may help ameliorate the negative effects of semen storage on characteristics of sperm motility. In addition, a study conducted [35] concluded that Selenium is vital to sperm antioxidant defenses and has demonstrated a positive effect on sperm motility. Moreover, Selenium was found to be an essential element to the formation of phospholipid-hyperoxide GSH-Px, an enzyme present in spermatids that becomes a structural protein comprising over 50% of mitochondrial capsule in the mid-piece of mature spermatozoa. Deficiencies of either substance can lead to instability of midpiece, resulting in defective motility.

Taken together, these studies indicate that the organic Selenium supplementation is essential for normal sperm development and improving semen quality characteristics, even under stressful conditions [18].

Sperm Concentration

The Table 3 shows that the concentration of sperm (x107) with different concentration of selenium was found to increase during the succeeding months of supplementation. The highest sperm concentration was found in treatment 5 (5% above the required selenium) with 126.84 (x107). Analysis of Variance in RCBD and Comparison among Means using Tukey's Test found that there is no significant difference between the sperm concentrations of samples in different levels of selenium.

The number of spermatozoa per ejaculate is very important in determining the optimum fertility [18]. Normal sperm concentration varies both within and between individuals even in the same species [36], but it is estimated [37] to be normally between 300-2500 million/ml of spermatozoa or higher for bull. In earlier studies conducted regarding the characterization of fresh ejaculated sperm of various breeds of bull, a high degree of variation in the sperm concentration was observed in the values [38].

Sperm concentrations of samples obtained from buffaloes supplemented with different levels of selenium were found to increase compared to the normal range in ejaculated samples. Results implied that sperm concentrations from buffaloes supplemented with different levels of selenium were enhanced in contrast to those not supplemented ones. These results were comparable with the result in a study conducted [23] in which they demonstrated that supplementation strategy improved sperm concentration, resulting in the improved fertilization in mature gilts. The reason behind this outcome is may be due to the mechanism of selenium to increase the number of sertoli cells and testicular reserves of animals [34].

Table 4: Comparison of sperm morphology of bulls in different levels of Selenium supplements

Treatment	Viability	Abnormality (%)		
		Initial	Final	Average
T1	71.864±2.94[a]	15.08±1.79 [a]	30.70±1.58[a]	22.89±1.69[a]
T2	75.327±5.69[a]	11.57±1.59 [ab]	10.93±1.74[ab]	11.57±1.67[ab]
T3	72.784±2.77[a]	10.55±1.33[b]	10.71±1.83[b]	10.93±1.58[b]
T4	74.066±2.58[a]	13.74±1.09 [ab]	9.86±1.14 [ab]	11.25±1.12[ab]
T5	82.905±4.33 [b]	12.10±0.67[b]	6.94±0.69[b]	10.55±0.68[b]
T6	76.322±4.62[a]	12.80±0.82[b]	8.65±1.50[b]	10.71±1.16[b]

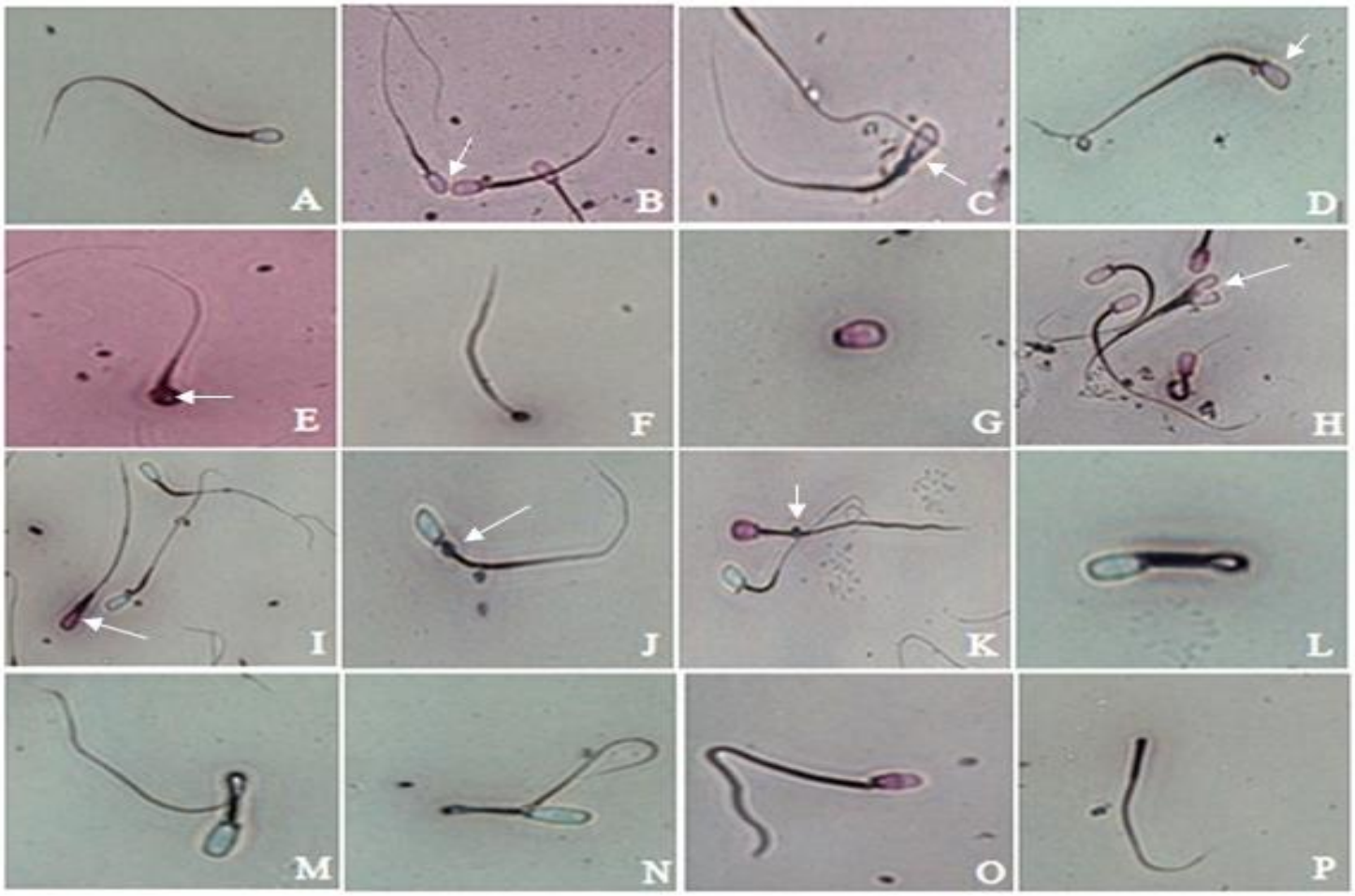

Fig. 1: Photomicrography of normal and abnormal morphology of Riverine buffaloes under HPO (a) Normal, (b) Knobbed acrosome (c) pyriform head (d) diadem defects (e) nuclear vacuole (f) microhead (g) detached head (h) double head (i) elongated head (j) proximal droplet (k) distal droplet (l) dag-like defect (m) distal reflex (n) dag-like defect (broken midpiece) (o) bent tail (p) detached tail

Sperm Morphology

An important part of any breeding soundness examination is an evaluation of sperm morphology. Table 4 presented the sperm morphology of bulls in different concentrations of Selenium added to feeds. Results showed that Treatment 5 (5% above the required selenium for mammals) had the lowest % abnormality (10.55%).

Percentage of live spermatozoa in the samples from treatment 1 was found to be 71.86% whereas for treatments 2, 3, 4, 5 and 6 were found to be 75.33%, 72.78%, 74.07%, 82.91%, and 76.32%, respectively. Statistical analysis showed significant difference between the viability of the spermatozoa. Results indicated that the sperm of the buffaloes with different levels of selenium has significant difference on the resulting percentage of viable sperm in the sample.

The percentage of viable spermatozoa from fresh ejaculated samples of various breeds or strains observed in earlier studies ranged from 70%-80% [38]. The viability of the samples obtained in this study was comparable to the results of the said earlier report.

Percent viable and normal (PVN) spermatozoa had been identified [40] as one of the most reliable indicators of sustainability of samples. Semen samples from bulls supplemented with 5% above the required selenium was found to be 10.55 % and had a relatively lower percentage of

abnormal spermatozoa as compared to other treatments. Literatures regarding characterization of semen ejaculates of riverine buffaloes imply that addition of selenium has positive influence in the morphology of the sperm [41]. Morphological evaluation of sperm ejaculates revealed that addition of selenium to feed reduced considerably the percentage of morphologically abnormal spermatozoa [22]. Furthermore, the results of other studies have confirmed those reported of other authors [42] that selenium prevents structural damage of spermatozoa cellular membranes.

Sperm Abnormality

Almost always, some spermatozoa from an ejaculate exhibit various form of deviation from normal morphology that, when present in great proportion, may adversely affect fertility [43]. Figure 1 showed some of the abnormalities observed in the samples from the bull supplemented with different concentration of selenium which include knobbed acrosome, pyriform head, diadem defects, nuclear vacuole, microhead, detached head, double head, elongated head, proximal droplet, distal droplet, dag-like defect, distal reflex, dag-like defect (broken midpiece) and detached tail.

As presented in Table 4, the percentage of morphologically abnormal spermatozoa in Treatment 1 (control) had the highest percentage abnormality of 22.89% while treatment 5 had the lowest percentage of abnormal sperm cells with 10.55% abnormality. Analysis of Variance revealed that there is a significant difference between the percentages of abnormalities obtained in semen samples. On the other hand, no region in particular showed a greater percentage of defects.

The percentages of abnormal spermatozoa observed from ejaculated sperm of various strains in the studies conducted [44] ranged from 15-20%. Results implied that the data gathered is within the considered normal range in ejaculated samples.

There is ample evidence that Selenium and various selenoproteins are essential in the reproductive functions of animals. As a component of mammalian enzymes such as glutathione peroxidases [45,46] and selenoproteins ([47], it plays a key role in a variety of biological processes including antioxidant defense [48] and fertility [49]. Deficiency or absence of selenoprotein can result in reduced concentration of Selenium in spermatogenic cells, leading to morphological defects in spermatozoa that are produced during spermatogenesis [50]. Another vital function of Selenoprotein is it acts as conjuction and moves along with spermatozoa throughout epididymis to protect against ROS (Reactive Oxygen Species) during the maturation process [51]. Thus, Selenium as a component of selenoproteins and selenoenzymes is involved in spermatogenesis by protecting spermatozoa from ROS. Gene knock-out studies of selenoproteins show that their absence is associated with abnormal sperm maturation leading to abnormalities in matured spermatozoa [52].

CONCLUSION

The study found out that supplementation of Selenium caused improvements in riverine buffaloes' semen production and semen quality. Thus, semen motility and semen quality were improved affected by dietary selenium supplementation of different concentration in terms of mean values at the time of collection. The study supplementing selenium to a diet provided additional benefit in semen production and sperm quality over the non-supplemented diet. Therefore, selenium may be tightly regulated by the bull's reproductive tract, thereby standardizing the effects of selenium on semen production and sperm quality.

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CONFLICTS OF INTEREST

The authors have reported no conflict of interest.

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