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EFFECT OF AGE AND SOME ENVIRONMENTAL FACTORS ON SPERM FERTILITY AND MOTILITY IN BULLS

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ABSTRACT

In the present investigation is mainly focused on the effect of age and seasonal variations on sperm fertility and motility in Buffalo bulls in Libya. The present study was carried out on 20 buffalo bulls divided into four age groups, including three healthy (less than 4, 5-10, and more than 12 year old animals), and one abnormal group (5-10 year old bulls having poor semen quality). The study was undertaken for a one year period divided into five seasons (dry summer, humid summer, autumn, winter and spring). Overall semen volume was varied and showed difference between age groups, although it was higher in autumn. Overall semen pH was showed variation in age and also depends on seasonal variation in different bulls. Color score was higher in less than 5 year old bulls. However, it was lower ($P<0.05$) in the abnormal group than in healthy groups but was lower in dry summer. Overall mass activity score was lower in aged bulls. However, it was very low in the abnormal than in healthy groups. Mass activity score was higher in dry summer and spring. Overall sperm motility showed no difference in healthy groups, but was lower in the abnormal group. The sperm activity is higher in winter than humid summer and autumn. Overall sperm concentration was higher in less than 5 year old bulls. However, it was lower in the abnormal group. Sperm concentration was higher in winter.

Key words: Semen, volume, pH, color, Mass activity, Motility, Sperm concentration, Buffalo bulls.

INTRODUCTION

Semen quality in bull sires reflects the degree of normality of the function of their testes, ducti epididymides and genital tract (including the accessory sex glands). The normality of the genital system also depends on the hormonal balance of the sire, which is sensitive to changes in health status, nutrition and management. Buffalo bulls breed the year round, conflicting results have been presented by researchers about semen quality and volume at different ages and during various seasons of the year.

Changes in these conditions influence sperm output, accessory sex gland secretion and epididymal function, all of which are reflected in the ejaculate as volume, sperm numbers or sperm characteristics (motility, morphology, viability etc.). The sperm quality in the collected ejaculate, regarded as the sum of these variables, will be normative for the quality of the processed (mostly cryopreserved) semen and, ultimately, of the semen's fertility when used in artificial insemination (AI). Furthermore, external cause such as seasonality also appears to influence sexual function, either through photoperiod [2]. Better semen quantity of buffalo bull has been reported during the hot and humid summer than in colder Months [6], but contrary observations have also been published [6]. Similarly, significantly ($P<0.05$) inferior [3] and superior semen quality has been reported during summer. Saeed [8] reported the best quality semen at 3-4 years of age. He concluded that the age of the bull and season of the year significantly affect semen characteristics but that variations in these parameters do exist even in the same season and at same age in different localities. Although information on semen quality with reference to age and season is available separately, no effort has been made study it comprehensively, including both healthy and abnormal bulls together. The present study was therefore planned with the following objectives: 1) to study the effect of age and season on semen volume and quality in healthy and abnormal (having poor semen quality) buffalo bulls;

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2) to study the differences in the above-mentioned parameters between healthy and abnormal buffalo bulls in Libya; 3) to investigate the relationship between semen characteristics in buffalo bulls in Libya.

MATERIALS AND METHODS

Location of the study

The present study was carried out at the Frozen Se-men and Artificial Insemination Centre of the Department of Livestock Development (DLD) in Azzayatuna University, Bani walid Libya.

Animals

This study was conducted on 20 buffalo bulls divided into four age groups, with four bulls in each group. The first three age groups, i.e., less than 4 years, 5-10 years and more than 12 years, comprised bulls with good quality semen. Bulls in the last group (6-10 years of age) were considered abnormal due to poor semen quality, as given below. The year was divided into five seasons. All bulls were kept under identical conditions of management, feeding and watering throughout the study period. The bulls were kept in sheltered paddocks with access to a small pond and had constant access to running water.

Clinical monitoring

The study started in July 2012 and was carried out until June 2013. A clinical history of each bull was taken at the start, including previous illnesses, mating behaviour and libido. Body condition score (BCS) was measured using a grading scale of 1–5, according to a current system for bulls[For the scoring, The appearance of the tail head, brisket and hump, the transverse processes of The lumbar vertebrae, The hips (trochanter major) and the ribs as well as the shape of the muscle mass between the hooks (tuber coxae) and pin (tuber ischii) were visually assessed.

Plasma membrane integrity

Sperm plasma membrane integrity (PMI) was evaluated using a hypo-osmotic swelling test (HOST). An aliquot of 100 µL of semen was suspended in 1000 µL of HOST solution (sodium citrate and fructose solution, 100 mOsmol/kg) and incubated at 35ºC For 45–60 min. After this incubation, 300–400 µL of the sperm suspension was fixed in a fixing medium (1 000 µL of HOST solution plus 5% formaldehyde) for later evaluation on wet smears. Two hundred spermatozoa per smear were counted under phase contrast light microscopy at × 400 magnification and The percentage of typical tail coiling/swelling was determined.

Spermatozoa morphology

An aliquot of each ejaculate was placed into labelled vials containing buffered formalin solution and mixed thoroughly for quicker fixation. A drop of raw semen was placed over a labelled slide and spread discontinuously to form dense ridges before drying (ridge smears). Thin smears were prepared from a physiological saline extended semen sample of the same ejaculate and spread out using a blunt-edged Slide (thin smears). Sperm morphology was evaluated on wet smears of the formalin-fixed spermatozoa and a phase contrast microscope (× 1 000) to detect percentages Of spermatozoa with heads (including acrosome and mid piece) and tail abnormalities as well as the presence of proximal and distal cytoplasmic droplets on 200 spermatozoa per sample. For the Evaluation of sperm head shape morphology, a total of 500 spermatozoa per thin slide were counted under light microscopy at × 1 000.

Meteorological Data

Ambient temperature (ºC), percentage of humidity, and rainfall (mm) for the present study period were obtained from Meteorological Department of the Ministry of Information and Communication Technology, Libya. The station was located near the bull station where the sires were stationed.

Owing to distinct mean maximum levels of ambient temperature, rainfall and humidity, for the purpose of the present study we arbitrarily divided the year into three seasons, namely (i) the rainy season: July– October; (ii) winter: November–February; and (iii) summer: March–June (Table 1).

Semen collection and analysis

Semen from all the experimental bulls was collected early in the morning before sunrise at fortnightly intervals, with the aid of an artificial vagina (AV). A total of two ejaculates were collected from each bull. The semen collected was brought to the laboratory immediately and was placed in a water bath at 37 °C. Two collected ejaculates were pooled and evaluated for total volume, color, mass activity, motility, pH and sperm concentration. Semen volume was recorded directly from graduated test tubes. The color of the semen was recorded as creamy, milky or watery, depending on the thickness of the semen and was assigned a numerical weight from zero to two for statistical analysis. A numerical weight of 2 was assigned to creamy, 1 to milky and 0 to watery samples.

The pH of semen was recorded with a pH meter (Checker 1 of HANNA, with 0.01- pH resolution and 0.2 accuracy). The mass activity of spermatozoa was recorded immediately after semen collection by examining a drop of semen on a warm slide at 100 × magnification under a microscope with attached stage warmer (temperature set at 37 °C). The score was calculated from 0 to 5. Motility, as a percentage of individually motile spermatozoa, was estimated by examining a drop of diluted fresh semen (with 2.9% sodium citrate solution) under a microscope at 400 ×. Motility was scored on the basis of the percentage of spermatozoa with normal forward progressive movement, while those showing circling movements or those oscillating at one place were regarded as immotile [1]. Sperm concentration was measured using an improved Neubaur haemocytometer [9].

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RESULTS AND DISCUSSION

Spermatozoa morphology

The data suggest that sperm quality in swamp buffalo AI sires, here in defined as sperm concentration, total spermatozoa per ejaculate, initial sperm motility and overall sperm morphology, did not vary statistically across the year under tropical conditions in Libya. Some individual sperm defects such as the proportions of sperm tail abnormalities, as well as the proportions of spermatozoa with intact membranes, showed significant variations over the year, however, with bull age and week of collection being the factors influencing these variations.

Semen volume

Overall ejaculatory volume observed during the present study was almost similar with that previously reported (4.10 ml.) However, many other workers previously reported the semen volume different in different bull types. Variation in semen volume reported by different workers might be due to differences in genetics, reproductive health status of bulls, age of bulls, frequency of collection, pooled volume, nutrition, season and management. Variations can also be due to skill of semen collector/attendant and temperature of AV. Volume of semen showed no significant differences between age groups. However, it was relatively high in adult bulls, followed by old bulls. Nordin [7] however, reported significantly higher ($P<0.05$) ejaculatory volume in adult and old than in young buffalo bulls. This indicates that buffalo bulls produce a maximum volume of semen around nine years of age and that thereafter it begins to decrease, probably due to the onset of senile changes. Higher ($P<0.05$) semen volume in autumn and low in humid summer recorded during the present study was in accordance with the finding in Iraqi buffalo and Gupta [5] in Surti buffalo bulls. However, higher volume in summer has been reported in Surti and Murrah [4]. Variation in different reports with regard to semen volume in different seasons might be due to a difference in genetics, number of observations made, and length of study period. An almost similar pattern of semen volume was observed in abnormal bulls during the present study, with slightly higher semen volume than healthy bulls of same age (6-10 years) and accorded with the findings of Settergen [10]. This suggests that semen volume does not depend on testicular status.

Semen color and pH

The color of semen studied was actually the thickness of the semen together with color of pigment. Similarly, in Nili-Ravi buffalo bull reported milky-white colored semen. The whitish or milky color of semen, reported to give an estimated concentration of 700,000 - 1,000,000 sperms/mm3 [13], was also indicated from present findings (1.00±0.50 106/_ l). The present study showed a better color during autumn (between milky to creamy) and in bulls of young age - an indication of good sperm concentration.

Semen pH observed during the present study was pH 6.51. The variation in overall semen pH may possibly be due to a difference in the duration of studies, number of observations made and quality of bulls.

Semen pH observed during the present study was lower in adult than in older bulls and was comparable with the findings of Terrezinha [12] have reported relatively lower pH in adult bulls. The pH during the present study was low in autumn while it was high ($P<0.05$) in winter, this was in accordance with the findings of Younis [13], who also reported low pH during autumn. The higher semen pH in winter but lower in spring and summer. It can be inferred from the present findings and other reports, that the fact that semen pH in buffalo bulls is higher in winter may be related to lowered sperm concentration

Semen pH during the present study was also lower in autumn than in other seasons in abnormal bulls, but was higher than healthy bulls. A pH of above 7.00 has been reported from bulls with less than 50% sperm motility. This indicates that semen pH has an inverse relation with sperm concentration.

Mass activity and motility

Overall mass activity observed during the present study was close to 3.93 and 3.53. The variation in the present may be due to a difference in judgement of mass activity, difference in total number of observations made, or quality of bulls. Mass activity observed during the present study was higher in adult than in older bulls and was comparable to the findings of Younis[13]. The higher mass activity in adult bulls was probably due to higher sperm concentration in these bulls, and low sperm abnormalities. Higher mass activity in autumn and spring than in humid summer and winter was in line with the findings of Younis [13] of higher ($P<0.05$) mass activity in autumn than in summer in Nili-Ravi buffalo bulls. As is obvious from the present findings and from some of the previous reports, it can be inferred that the water buffalo bull possesses better mass activity during autumn and lower in the winter season. The difference in motility in various reports could be due to variations in the judgement of motility, number of bulls studied, or difference of season in studies. The lower values both for mass activity and motility during the present study in winter could be because of the effect of cold on semen during collection, and bad handling due to poor conditions of management. Overall lower ($P<0.05$) mass activity and motility was observed in abnormal than in healthy bulls, with a non-significant difference between seasons in mass activity. However, motility was higher ($P<0.05$) in humid summer than in winter. Very low mass activity and less than 50% motility from various types of abnormal bulls (hypoplastic, *Pasteurella* infection of testes etc).

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Sperm concentration

Some authors [11] reported higher sperm concentration in buffalo bulls. Such variations can always be expected from workers working at different places with a variation in the number of animals selected, and which may be belonging to different age groups. However, it can be inferred from these reports that the buffalo bull produces semen with a sperm concentration of between 0.94 - 1.65×106/µl. Sperm concentration observed during the present study was lower (P<0.05) in old than in young bulls. However, some reported a non-significant difference in sperm concentration between bulls of young, adult and old age groups. The lower sperm concentration and mass activity in old bulls could be due to senility. Higher (P<0.05) sperm concentration in autumn and spring during the present study was similar to the higher sperm concentration during autumn and/or spring, reported by other researchers [14]. Higher sperm concentration during summer has, however, also been reported [5].

As indicated in most of the reports and results of the present study it can be concluded that sperm concentration in buffalo bulls is higher in milder seasons. Sperm concentration was lower (P<0.05) in abnormal than in healthy bulls. However, the pattern was similar, i.e., higher in spring and autumn, as observed in healthy bulls. These findings may be due to the also bulls showing inflammatory, degenerative and hypo plastic conditions of testes and severity of testicular lesions.

Table 1. Distribution of ejaculates collected from buffalo bulls (n = 5) in Libya between 1 July 2012 and 31 June 2013

Variable	Buffalo sires (No.)					Ejaculate totals
	I	II	III	IV	V	
Age (years)	5	6	6	14	18	
No. ejaculates	26	28	28	30	24	136
Ejaculates per season						
Rainy season	8	8	8	10	8	42
Winter	10	8	8	10	10	46
Summer	8	8	10	12	10	48

Table 2. Variables defining the seasons used in the present study, based on meterological data collected at the Meteorological Department of the Ministry of Information and Communication Technology,, Libya between 1 July 2012 and 31 June 2013 (mean ± SD)

Season	Temperature (mean maximum, ºC)	Rainfall (mean maximum, mm)	Humidity (mean maximum, %)
Rainy	28.1 ± 0.5	39.4 ± 30.1a	89.5 ± 0.5a
Winter	30.5 ± 1.0	0.7 ± 0.8	94.8 ± 2.5b
Summer	33.3 ± 1.0	16.3 ± 8.51c	91.4 ± 3.9c

Table 3. Relative score (0–3*) for presence of foreign cells (least square mean ± SEM) in 136 ejaculates collected from five AI-buffalo bulls in Thailand between 1 July 2012 and 31 June 2013

Presence of foreign cells*	Rainy season (*n* = 48)	Winter (*n* = 46)	Summer (*n* = 42) a, b
Epithelial cells	0.4 ± 0.1	0.6+	0.5
Spermatogenic epithelium	0.5 ± 0.0	0.4+	0.3+
Boat-shaped cells	0.8+0.0	0.7+	0.6 ± 0.1
Medusa cells	0.0	0.0	0.0
Pseudogiant cells	0.0	0.0	0.0
True giant cells	0.0	0.0	0.0
Chromatin plates	0.0	0.0	0.0
Leukocytes	0.0	0.0	0.0
Erythrocytes	0.0	0.0	0.0

CONCLUSION

It can be concluded from the present study, that age and testicular pathology has no influence on semen volume in buffalo bulls but that semen volume, sperm concentration and motility are significantly higher in milder seasons. The colour (thickness) and pH of the semen are good indicators of semen quality in both healthy and abnormal bulls. Finally it also concluded that age and some seasonal variations also showed influence on physical and chemical characteristics of the semen in bulls.

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