

## Effect of age and season on some semen parameters of Nili-Ravi buffalo (*Bubalus bubalis*) bulls

Muhammad Tariq Javed<sup>1\*</sup>, Ahrar Khan<sup>1</sup>, and Razia Kausar<sup>2</sup>

<sup>1</sup>Department of Veterinary Pathology Faculty of Veterinary Science

View metadata, citation and similar papers at [core.ac.uk](http://core.ac.uk)

University of Agriculture, Faisalabad, Pakistan

---

**JAVED, M. T., A. KHAN, R. KAUSAR: Effect of age and season on some semen parameters of Nili-Ravi buffalo (*Bubalus bubalis*) bulls. Vet. arhiv 70, 83-94, 2000.**

### ABSTRACT

This study was carried out on 16 buffalo bulls (*Bubalus bubalis*) divided into four age groups, including three healthy (less than 5, 6-10, and more than 11 year old animals), and one abnormal group (6-10 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  $4.67 \pm 1.62$  ml and showed a non-significant difference between age groups, although it was higher ( $P < 0.05$ ) in autumn. Overall semen pH was  $6.55 \pm 0.50$  and was lower ( $P < 0.05$ ) in the 6-10 than in the 11 year old bulls and the abnormal group. It was lower ( $P < 0.05$ ) in autumn while higher ( $P < 0.05$ ) in winter. Colour score was higher ( $P < 0.05$ ) 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 ( $P < 0.05$ ) in dry summer. Overall mass activity score was  $2.65 \pm 1.03$  and was lower ( $P < 0.05$ ) in more than 11 year old bulls. However, it was lower ( $P < 0.05$ ) in the abnormal than in healthy groups. Mass activity score was higher ( $P < 0.05$ ) in dry summer and spring. Overall sperm motility was  $56.89 \pm 0.65\%$  and showed no difference in healthy groups, but was lower ( $P < 0.05$ ) in the abnormal group. It was lower ( $P < 0.05$ ) in winter than humid summer and autumn. Overall sperm concentration was  $1.00 \pm 0.50$  millions/ml and was higher ( $P < 0.05$ ) in less than 5 year old bulls. However, it was lower ( $P < 0.05$ ) in the abnormal group. Sperm concentration was lower ( $P < 0.05$ ) in winter. The pH of semen showed negative ( $P < 0.001$ ) correlation with colour ( $r = -0.38$ ), sperm concentration ( $r = -0.37$ ), mass activity ( $r = -0.37$ ) and motility ( $r = -0.17$ ). Sperm concentration correlated positively ( $P < 0.001$ ) with mass activity ( $r = 0.86$ ) and progressive motility ( $r = 0.16$ ).

**Key words:** semen, volume, pH, colour, mass activity, motility, sperm concentration, buffalo bull, *Bubalus bubalis*, Pakistan

---

\* Contact address:

Dr. M. Tariq Javed, Department of Veterinary Pathology, University of Agriculture, 38040 Faisalabad, Pakistan, Phone 91 41 625 834; Fax: 91 41 762 497; E-mail: mtjaved@fsd.paknet.com.pk

## Introduction

Although buffalo bulls breed the year round, conflicting reports have been published about semen quality and volume at different ages and during various seasons of the year. Better semen quantity of buffalo bull has been reported during the hot and humid summer than in colder months (WIERZBOWSKI and TAHIR, 1980; SINGH et al., 1992), but contrary observations have also been published (LI et al., 1988). Similarly, significantly ( $P < 0.05$ ) inferior (ERB et al., 1942; BHATTACHARYA 1968; BHOSREKAR, 1980; RAO, 1984) and superior semen quality has been reported during summer (SINGH et al., 1992). SAEED (1988), 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, Table 1) Nili-Ravi buffalo bulls; 2) to study the differences in the above-mentioned parameters between healthy and abnormal Nili-Ravi buffalo bulls; 3) to investigate the relationship between semen characteristics in Nili-Ravi buffalo bulls.

Table 1. Characteristics of buffalo bulls having poor semen traits and grouped as abnormal

Bull No.	SC (Cm)	Sperm concentration (millions/ml)	Progressive motility (%)	Sperm abnormalities (%)	Dead sperm (%)
365	33.0	1.27	25	61.81	19.44
277	33.0	0.30	50	23.48	15.00
321	30.0	1.60	50	43.11	11.98
323	27.0	0.57	40	23.38	41.56

## Materials and methods

This study was conducted on 16 buffalo bulls (*Bubalus bubalis*) divided into four age groups, with four bulls in each group. The first three age groups, i.e., less than 5 years, 6-10 years and more than 11 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 (Table 1).

The year was divided into five seasons (JAVED, 1998). All bulls were kept under identical conditions of management, feeding and watering throughout the study period.

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, colour, mass activity, motility, pH and sperm concentration. Semen volume was recorded directly from graduated test tubes. The colour 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 (NAZIR, 1988). 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 (AHMAD, 1994). Sperm concentration was measured using an improved Neubaur haemocytometer (SALISBURY et al., 1978).

Obtained data were subjected to analysis of variance test by using SPSS computer software package (ANONYMOUS, 1996). The means were compared by Duncan's Multiple Range test, and correlation coefficients among different parameters were also worked out.

## **Results and discussion**

### *Semen volume*

Overall mean ejaculatory volume observed during the present study ( $4.67 \pm 1.62$  ml, Table 2) was almost similar to that reported (4.10 ml) for

Iraqi buffalo bulls (EL-WISHY, 1978). However, YOUNIS (1996) observed semen volume of  $2.80 \pm 0.07$  ml in Nili-Ravi buffalo bulls during a 6-month period, while MALIK et al. (1974) and AHMAD et al. (1984) recorded 3.20 and 3.40 ml semen volume, respectively, from the same breed. 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 (NAZIR, 1988; SODERQUIST et al., 1992). Variations can also be due to skill of semen collector/attendant and temperature of AV.

Table 2. Comparison of semen volume, colour and pH between buffalo bulls of different ages in various seasons of the year, and in abnormal bulls

Age groups	Summer		Autumn	Winter	Spring	Overall mean
	Dry	Humid				
Semen volume (ml)						
<5 years	$4.05 \pm 0.32^{Aa}$	$3.82 \pm 0.31^{Aa}$	$5.80 \pm 0.31^{Ab}$	$4.07 \pm 0.29^{Aa}$	$4.00 \pm 0.32^{Aa}$	$4.35 \pm 0.14^A$
6-10 years	$4.82 \pm 0.31^{Aabc}$	$3.95 \pm 0.31^{Aa}$	$5.85 \pm 0.31^{Ac}$	$4.46 \pm 0.28^{Aab}$	$5.71 \pm 0.33^{Bbc}$	$4.96 \pm 0.14^A$
>11 years	$4.67 \pm 0.31^{Abc}$	$3.40 \pm 0.31^{Aa}$	$6.40 \pm 0.31^{Ad}$	$4.04 \pm 0.28^{Aab}$	$5.35 \pm 0.31^{ABcd}$	$4.77 \pm 0.13^A$
abnormal	$4.97 \pm 0.35^{Aabc}$	$4.02 \pm 0.34^{Aa}$	$6.02 \pm 0.35^{Ac}$	$4.23 \pm 0.31^{Aab}$	$5.73 \pm 0.37^{Bbc}$	$4.99 \pm 0.15^A$
Overall mean	$4.52 \pm 0.18^{bc}$	$3.72 \pm 0.18^a$	$6.01 \pm 0.18^d$	$4.19 \pm 0.16^{ab}$	$5.02 \pm 0.18^c$	$4.67 \pm 1.62$
Colour (0-2)						
<5 years	$1.15 \pm 0.14^{Ba}$	$0.95 \pm 0.13^{Ba}$	$1.40 \pm 0.13^{Ba}$	$1.13 \pm 0.13^{Ba}$	$1.47 \pm 1.14^{Ba}$	$1.22 \pm 0.06^C$
6-10 years	$0.95 \pm 0.13^{ABa}$	$1.00 \pm 0.13^{Ba}$	$1.20 \pm 0.13^{ABa}$	$0.79 \pm 0.12^{ABa}$	$1.05 \pm 0.15^{ABa}$	$1.00 \pm 0.06^B$
>11 years	$0.70 \pm 0.13^{ABa}$	$0.90 \pm 0.13^{ABa}$	$1.20 \pm 0.13^{ABa}$	$0.75 \pm 0.12^{ABa}$	$1.00 \pm 0.13^{ABa}$	$0.91 \pm 0.06^B$
abnormal	$0.50 \pm 0.14^{Aa}$	$0.50 \pm 0.13^{Aa}$	$0.78 \pm 0.14^{Aa}$	$0.54 \pm 0.12^{Aa}$	$0.82 \pm 0.15^{Aa}$	$0.63 \pm 0.06^A$
Overall mean	$0.93 \pm 0.08^b$	$0.95 \pm 0.08^{ab}$	$1.27 \pm 0.08^a$	$0.89 \pm 0.07^a$	$1.18 \pm 0.08^a$	$1.03 \pm 0.64$
Semen pH						
<5 years	$6.51 \pm 0.09^{ABb}$	$6.46 \pm 0.09^{Ab}$	$6.05 \pm 0.09^{Aa}$	$6.71 \pm 0.09^{Ab}$	$6.72 \pm 0.09^{Ab}$	$6.49 \pm 0.04^{AB}$
6-10 years	$6.40 \pm 0.09^{Ab}$	$6.45 \pm 0.09^{Abc}$	$6.00 \pm 0.10^{Aa}$	$6.75 \pm 0.09^{Ac}$	$6.61 \pm 0.09^{Abc}$	$6.45 \pm 0.04^A$
>11 years	$6.70 \pm 0.09^{ABb}$	$6.69 \pm 0.09^{ABb}$	$6.05 \pm 0.09^{Aa}$	$6.99 \pm 0.08^{Ab}$	$6.80 \pm 0.09^{ABb}$	$6.67 \pm 0.04^{BC}$
abnormal	$6.94 \pm 0.10^{Bb}$	$6.78 \pm 0.10^{Bb}$	$6.25 \pm 0.10^{Aa}$	$6.89 \pm 0.09^{Ab}$	$7.04 \pm 0.10^{Bb}$	$6.79 \pm 0.04^C$
Overall mean	$6.53 \pm 0.05^b$	$6.56 \pm 0.05^b$	$6.03 \pm 0.05^a$	$6.82 \pm 0.05^c$	$6.71 \pm 0.05^{bc}$	$6.55 \pm 0.50$

Overall mean in a row represents values of all the apparently healthy buffalo bulls. Values in each row with different small letters and in each column with different capital letters are statistically different ( $P < 0.05$ )

Volume of semen showed no significant differences between age groups. However, it was relatively high in adult bulls, followed by old bulls (Table 2). YOUNIS (1996) and NORDIN et al. (1990), 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 (Table 2) was in accordance with the findings of POSWAL et al. (1977) in Murrah buffalo, EL-WISHY (1978) in Iraqi buffalo and GUPTA et al. (1978) in Surti buffalo bulls. However, higher volume in summer has been reported in Surti and Murrah (REDDY et al., 1983), Egyptian (SHALASH, 1984), Nili-Ravi (YOUNIS, 1996) and in Italian buffalo bulls (TEREZINHA et al., 1991). 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 SETTERGREN (1994). This suggests that semen volume does not depend on testicular status.

### *Semen colour and pH*

The colour of semen studied was actually the thickness of the semen together with colour of pigment. The numerical values allotted to colour for interpretation during the present study revealed an overall mean of  $0.94\pm 0.66$  (milky white colour, Table 2). Similarly, JAINUDEEN et al. (1982) in Swamp, and HEUER et al. (1987) and NAZIR (1988) in Nili-Ravi buffalo bull reported milky-white coloured semen. The whitish or milky colour of semen, reported to give an estimated concentration of 500,000 - 1,000,000 sperms/mm<sup>3</sup> (SETTERGREN, 1982), was also indicated from present findings ( $1.00\pm 0.50 \times 10^6/l$ ). The present study showed a better ( $P<0.05$ ) colour during autumn (between milky to creamy) and in bulls of young age - an indication of good sperm concentration ( $r=0.85$ ,  $P<0.001$ ).

Overall mean of semen pH observed during the present study ( $6.55\pm 0.50$ , Table 2) was close to the findings of ALEXANDER et al. (1971) in buffalo bulls, who reported semen pH of 6.51. TEREZINHA et al. (1991) and YOUNIS (1996) who reported it to be  $6.26\pm 0.05$  and  $6.38\pm 0.19$  in buffalo bulls. GOMES (1991) reported a range of 6.82-6.93 in buffalo bulls. 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 ( $P<0.05$ ) in adult than in older bulls (Table 2) and was comparable with the findings of TEREZINHA et al. (1991) and YOUNIS (1996), who have reported relatively lower pH in adult bulls. The pH during the present study was low ( $P<0.05$ ) in autumn ( $6.03\pm 0.05$ ) while it was high ( $P<0.05$ ) in winter

( $6.82 \pm 0.04$ ). This was in accordance with the findings of YOUNIS (1996), who also reported low pH during autumn. TEREZINHA et al. (1991) reported relatively 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 ( $r = -0.37$ ,  $P < 0.001$ ).

Semen pH during the present study was also lower ( $P < 0.05$ ) in autumn than in other seasons in abnormal bulls, but was higher ( $P < 0.05$ ) than healthy bulls (Table 2). A pH of above 7.00 has been reported by SETTERGREN (1994) 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 ( $2.65 \pm 1.14$ ) observed during the present study was close to 2.93 and 2.53, reported for buffalo bulls (HEUER et al., 1982; VYAWANARE et al., 1989) respectively. However, YOUNIS (1996) observed it to be  $1.88 \pm 0.07$  over a 6-month period in Nili-Ravi buffalo bulls. The variation in the two studies 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 ( $P < 0.05$ ) in adult than in older bulls (Table 3) and was comparable to the findings of YOUNIS (1996) of higher ( $P < 0.05$ ) mass activity ( $2.18 \pm 0.11$ ) in adult than young ( $1.93 \pm 0.13$ ) and old ( $1.53 \pm 0.10$ ) bulls. SAEED (1988) also observed higher mass activity in adult (3.49) bulls. The higher mass activity in adult bulls was probably due to higher sperm concentration in these bulls, and low sperm abnormalities, as has also been stated by DHAMI and KODAGALI (1988). Higher ( $P < 0.05$ ) mass activity in autumn and spring than in humid summer and winter (Table 3) was in line with the findings of YOUNIS (1996) of higher ( $P < 0.05$ ) mass activity in autumn than in summer in Nili-Ravi buffalo bulls. GILL et al. (1974) and BAJWA et al. (1982) also observed lower mass activity in winter (3.06 and 2.23, respectively). HEUER et al. (1987) and NAZIR (1988), however, reported no effect of seasons on mass activity 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, and that this may be related to low sperm concentration in winter (Table 3).

The progressive motility observed during the present study ( $56.89 \pm 0.65$  %) was lower than reported in Swamp (JAINUDEEN et al., 1982) and Murrah breed buffaloes (VYAWANARE et al., 1989;

SURYAPRAKASAM and RAO, 1993) ( $67.00 \pm 7.00$ ,  $73.95 \pm 5.21$  and  $69.00 \pm 0.86\%$ , respectively). However, it was close to the findings of YOUNIS (1996) in Nili-Ravi buffalo bulls ( $60.45 \pm 0.48$ ). 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.

Table 3. Comparison of semen volume, colour and pH between buffalo bulls of different ages in various seasons of the year, and in abnormal bulls

Age groups	Summer		Autumn	Winter	Spring	Overall mean
	Dry	Humid				
Mass activity (0-5 grades)						
<5 years	$2.89 \pm 0.22^{Bab}$	$2.30 \pm 0.21^{ABa}$	$3.00 \pm 0.21^{Bab}$	$2.54 \pm 0.20^{Bab}$	$3.16 \pm 0.22^{Bb}$	$2.78 \pm 0.09^C$
6-10 years	$3.15 \pm 0.21^{Ca}$	$2.80 \pm 0.21^{Ba}$	$3.25 \pm 0.21^{Ba}$	$2.50 \pm 0.19^{Ba}$	$3.00 \pm 0.23^{Ba}$	$2.94 \pm 0.09^C$
>11 years	$2.15 \pm 0.21^{ABa}$	$2.40 \pm 0.21^{Ba}$	$2.65 \pm 0.21^{ABa}$	$1.79 \pm 0.19^{ABa}$	$2.45 \pm 0.21^{ABa}$	$2.28 \pm 0.09^B$
abnormal	$1.28 \pm 0.23^{Aa}$	$1.60 \pm 0.22^{Aa}$	$1.84 \pm 0.23^{Aa}$	$1.29 \pm 0.20^{Aa}$	$2.00 \pm 0.24^{Aa}$	$1.60 \pm 0.10^A$
Overall mean	$2.73 \pm 0.12^{ab}$	$2.50 \pm 0.12^a$	$2.97 \pm 0.12^b$	$2.28 \pm 0.11^a$	$2.87 \pm 0.13^b$	$2.65 \pm 1.03$
Progressive motility (%)						
<5 years	$55.50 \pm 2.44^{ABa}$	$57.89 \pm 2.51^{Aa}$	$57.63 \pm 2.51^{Ba}$	$52.95 \pm 2.33^{Ba}$	$52.00 \pm 2.44^{ABa}$	$55.20 \pm 1.09^B$
6-10 years	$61.50 \pm 2.44^{Bb}$	$60.29 \pm 2.65^{Aab}$	$62.00 \pm 2.44^{Bb}$	$52.70 \pm 2.23^{Ba}$	$59.70 \pm 2.44^{Bab}$	$59.50 \pm 1.09^B$
>11 years	$54.50 \pm 2.44^{ABa}$	$57.50 \pm 2.44^{Aa}$	$60.75 \pm 2.44^{Bb}$	$51.45 \pm 2.23^{Ba}$	$60.00 \pm 2.44^{Bab}$	$56.84 \pm 1.07^B$
abnormal	$48.16 \pm 3.05^{Aab}$	$51.18 \pm 3.22^{Ab}$	$35.28 \pm 3.13^{Aab}$	$34.79 \pm 2.71^{Aa}$	$46.75 \pm 2.97^{Aab}$	$43.23 \pm 1.35^A$
Overall mean	$57.16 \pm 1.41^{ab}$	$58.56 \pm 1.46^b$	$60.13 \pm 1.42^b$	$52.37 \pm 1.30^a$	$57.16 \pm 1.41^{ab}$	$56.89 \pm 0.65$
Sperm concentration ( $10^6/\mu\text{l}$ )						
<5 years	$1.10 \pm 0.11^{Ba}$	$0.87 \pm 0.10^{Ba}$	$1.12 \pm 0.10^{Aa}$	$1.03 \pm 0.10^{Ba}$	$1.24 \pm 0.11^{Ba}$	$1.07 \pm 0.04^C$
6-10 years	$1.12 \pm 0.10^{Ba}$	$0.99 \pm 0.10^{Ba}$	$1.19 \pm 0.10^{Aa}$	$0.85 \pm 0.09^{ABa}$	$1.08 \pm 0.10^{ABa}$	$1.05 \pm 0.04^{BC}$
>11 years	$0.78 \pm 0.10^{ABab}$	$0.92 \pm 0.10^{Bab}$	$1.18 \pm 0.10^{Ab}$	$0.65 \pm 0.09^{Aa}$	$0.98 \pm 0.11^{ABab}$	$0.90 \pm 0.04^B$
abnormal	$0.56 \pm 0.11^{Aa}$	$0.56 \pm 0.10^{Aa}$	$0.81 \pm 0.10^{Aa}$	$0.53 \pm 0.09^{Aa}$	$0.86 \pm 0.11^{Aa}$	$0.66 \pm 0.04^A$
Overall mean	$1.00 \pm 0.06^{ab}$	$0.93 \pm 0.06^{ab}$	$1.16 \pm 0.06^b$	$0.85 \pm 0.05^a$	$1.15 \pm 0.06^b$	$1.00 \pm 0.50$

Overall mean in a row represents values of all the apparently healthy buffalo bulls. Values in each row with different small letters and in each column with different capital letters are statistically different ( $P < 0.05$ )

The non-significant difference observed in progressive motility in healthy groups accorded with the findings of NORDIN et al. (1990) in Swamp buffalo bulls. However, YOUNIS (1996) reported lower ( $P < 0.05$ ) motility in old than in young and adult bulls, which he related to senile changes in them and which appears to be true. The lower ( $P < 0.05$ ) motility in winter observed during the present study (Table 3) was in line with lower motility in winter in the Murrah breed reported by SINGH et al. (1992). However, MOHAN et al. (1977) and GILL et al. (1974) reported higher motility in winter (75 and 65%, respectively) in buffalo bulls, while SAEED et al. (1990) reported no effect of seasons on sperm

motility. 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. SETTERGREN (1994) also reported very low mass activity and less than 50% motility from various types of abnormal bulls (hypoplastic, *Pasteurella* infection of testes etc.). VEERAMACHANENI et al. (1986) also reported a decrease in motility with an increase in testicular lesions.

### *Sperm concentration*

Sperm concentration observed during present study ( $1.00 \pm 0.50 \times 10^6/\mu\text{l}$ ) was close to the findings of JAINUDEEN et al. (1982) in Swamp ( $0.97 \times 10^6/\mu\text{l}$ ), HEUER et al. (1982) in Nili-Ravi ( $1.06 \times 10^6/\mu\text{l}$ ) and RAHMAN et al. (1991) in Surti ( $0.94 \times 10^6/\mu\text{l}$ ) and Murrah ( $1.05 \times 10^6/\mu\text{l}$ ) buffalo bulls. EL-WISHY (1978), RAIZADA et al. (1988), NAZIR (1988) and TEREZINHA et al. (1991) reported higher sperm concentration (1.65, 2.90, 1.15 and  $1.33 \times 10^6/\mu\text{l}$ ) 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  $\times 10^6/\mu\text{l}$ .

Sperm concentration observed during the present study was lower ( $P < 0.05$ ) in old than in young bulls. However, YOUNIS (1996) 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 (Table 3) was similar to the higher sperm concentration during autumn and/or spring, reported by other researchers ( EL-WISHY, 1978; ZAFAR et al., 1988; DUMITRESCU et al., 1988; YOUNIS, 1996). Higher sperm concentration during summer has, however, also been reported (GUPTA et al., 1978). 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 (Table 3). SETTERGREN (1994), VEERAMACHANENI et al. (1986) and AHMAD et al. (1988) also reported lower sperm concentration from bulls showing inflammatory, degenerative and hypoplastic conditions of testes. ROSSI et al. (1975) related the decrease in sperm concentration with severity of testicular lesions.

### *Correlation*

Volume of semen showed no correlation with sperm concentration ( $r=0.06$ ) and was in line with the findings of YOUNIS (1996), KHOKHAR et al. (1987) and IGBOELI et al. (1987). FIELDS et al. (1979), however, observed a negative correlation between semen volume and sperm concentration ( $r=-1.3$ ) in cattle bulls. Present findings also showed no correlation of semen volume with other semen parameters, including mass activity ( $r=0.06$ ) and motility ( $r=-0.02$ ). This suggests that in buffalo bulls, semen volume is not a good predictor of sperm concentration, or of mass activity and motility.

Semen pH during the present study showed a significant ( $P<0.001$ ) negative correlation with colour ( $r=-0.38$ ), and sperm concentration ( $r=-0.37$ ). This suggests that pH and colour (thickness,  $r=0.85$ ,  $P<0.001$ ) of the semen is a good indicator of sperm concentration. It has been observed that pH depends upon sperm concentration and semen with good colour (thicker) and that concentration has an acidic pH (WACOL, 1991; BARNABE et al., 1992; YOUNIS, 1996). Mass activity and motility also showed a significant ( $P<0.001$ ) negative correlation with pH ( $r=-0.37$ ,  $r=-0.17$ , respectively), which indicates that the lower the pH the better the semen quality.

### **Conclusions**

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.

### **References**

- AHMAD, M., M. LATIF, M. AHMAD, M. H. QAZI, M. SAHIR, M. ARSLAN (1984): Age-related changes in body weight, scrotal size and plasma testosterone levels in buffalo bulls (*Bubalus bubalis*). *Theriogen.* 22, 651-656.
- AHMAD, M., N. AHMAD, M. ANZAR, I. H. KHAN, M. LATIF, M. AHMAD (1988): Postmortem studies on infertile buffalo bulls: Testicular histology. *Vet. Rec.* 122, 229-231.

- AHMAD, N. (1994): Clinical and experimental studies of reproductive functions in the ram and male goat with special reference to the use of diagnostic ultrasound. Ph.D Thesis. Department of Large Animal Med. & Surgery, Royal Vet. College, University of London. London.
- ALEXANDER, F. C. M., R. ZEMJANIS, E. F. GRAHAM, M. L. SCHMEHL (1971): Semen characteristics and chemistry from bulls before and after vasectomy. *J. Dairy Sci.* 54, 1530-1533.
- ANONYMOUS (1996): SPSS, 7.5.1. release, standard version. Sterling Technologies, Inc., 444 N, Michigan Avenue, Chicago, IL 60611.
- BAJWA, M. A., I. H. SHAH, M. TAHIR, N. AHMAD (1982): Seasonal effect on seminal attributes in Nili-Ravi buffalo bulls. *Pakistan J. Anim. Sci.* 4, 35-44.
- BARNABE, V. H., R. C. BARNABE, R. P. APPUDA, J. A. VISINTIN, M. T. L. FREITAS (1992): Seasonal behaviour of semen collection by electroejaculation from buffaloes raised in SAO Paulo State (Southeast Brazil). 12<sup>th</sup> Int. Reproduction. Vol. 4, 484. The Hague, Netherlands.
- BHATTACHARYA, P. (1968): Buffaloes. In: *The Artificial Insemination of Farm Animals*, 4<sup>th</sup> ed. (Perry, E. J., Ed.). Rutgers University Press. N.J. pp. 159-195.
- BHOSREKAR, M. (1980): Studies on buffalo semen. Seasonal variation in semen characteristics. *Indian Vet. J.* 57, 806-810.
- DHAMI, A. J., S. B. KODAGALI (1988): Seminal characteristics and their interrelationships in Surti buffalo. *Indian Vet. J.* 65, 61-64.
- DUMITRESCU, I., P. RISTEA, R. CHIVU (1988): Cytological and biochemical indices in buffalo semen and its freezing ability in different season. *Lucrari Stiintifice, Institutul Agronomic, "Nicolae Balcesca" Buersti Seria D. Zootchnie* 31, 49-55.
- EL-WISHY, A. B. (1978): Reproduction performance of Iraqi buffaloes. Seasonal variation in sexual desire and semen characteristics. *Zuchthygiens* 13, 28-32.
- ERB, R. E., F. N. ANDREWS, J. H. HILTON (1942): Seasonal variation in semen quality of the dairy bull. *J. Dairy Sci.* 25, 815-826
- FIELDS, M. J., W. C. BURNS, V. WARNICK (1979): Age, season and breed effects on testicular volume and semen traits in young beef bulls. *J. Anim. Sci.* 48, 1299-1304.
- GILL, R. S., P. C. GANGWAR, O. P. TAKKAR (1974): Seminal attributes in buffalo bulls as affected by different seasons. *Ind. J. Ani. Sci.* 44, 415--418.
- GOMES DE CASTRO, T. A. M., E. MOOSE, J. G. MANNARELI, F. GOMES DE CASTRO (1991): Physical and biochemical characteristics from Murrah buffalo bulls semen. *Proceedings of Third World Buffalo Congress III*, 571-578. Varna, Bulgaria.
- GUPTA, H. C., M. C. S. NASIR, S. G. NAIK, R. K. SRIVASTAVA (1978): Effect of age and season on certain seminal characteristics of Surti buffalo bulls. *Indian J. Dairy Sci.* 31, 245-252.
- HEUER, C. H. BADER, M. A. BAJWA (1982): Sperm morphology of the Nili-Ravi buffaloes. *Pakistan Vet. J.* 2, 155-160.
- HEUER, C., M. N. TAHIR, H. AMJAD (1987): Effect of season on fertility of frozen buffalo semen. *Anim. Reprod. Sci.* 13, 15-21.
- IGBOELI, G., L. N. NWAKALOR, B. I. ORJI, G. I. ONUORA (1987): Seasonal variation in the semen characteristics of Muturu (*Bos bubalis*) bulls. *Anim. Reprod. Sci.* 14, 31-38.
- JAINUDEEN, M. R., T. A. BONGSO, S. DASS (1982): Semen characteristics of Swamp buffalo (*Bubalus bubalis*). *Anim. Reprod. Sci.* 4, 213-217.
- JAVED, M. T. (1998): Study on buffalo testes with special reference to scrotal circumference, hormonal profile, sperm morphology and associated lesions. Ph.D. Thesis. University of Agriculture. Faisalabad, Pakistan.
- KHOKHAR, B. S., M. SINGH, K. C. CHAUDHRY (1987): Transaminases in cattle and buffalo semen in relation to fertility and seminal characteristics during moderate and colder seasons. *Anim. Reprod. Sci.* 13, 177-182.

- LI, Q., Y. ZHANG, Z. LI (1988): Seasonal changes in plasma testosterone, oestradiol-17 beta, progesterone and cortisol concentrations and the relationship of these hormones to libido and semen quality in Guanzhang male dairy goats. *Acta Vet. et Zootech. Sinica* 19, 224-230.
- MALIK, M. A., M. AHMAD, A. H. GILANI, R. A. CHAUDHRY (1974): Studies on the morphology and abnormalities of buffalo spermatozoa. *Pak J. Agric. Res.* 11, 41-46.
- MOHAN, G., M. L. MADAN, M. N. RAZDAN (1977): Composition of Murrah buffalo bull semen during winter and summer months in India. *Trop. Agri.* 54, 21-28.
- NAZIR, M. (1988): Semen evaluation and sperm morphology - monography on reproductive pattern of Riverine buffaloes and recommendations to improve their reproductive performance at small farmer level. PARC, Islamabad.
- NORDIN, W., M. HILIMI, T. A. BONGSO (1990): Semen characteristics related to age in growing Swamp buffalo (*Bubalus bubalis*). *Buffalo J.* 6, 161-166.
- POSWAL, M. L., H. K. SAXENA, G. W. SEIFER (1977): Effect of seasons on seminal attributes of Red Dane bulls. *Indian Vet. J.* 54, 163-166.
- RAHMAN, A., J. C. DUTTA, C. K. RAIKONWAR, K. C. NATH (1991): Studies on semen characteristics of buffalo bulls. *Livestock Adviser* 16, 27-32.
- RAIZADA, B. C., A. SATTAR, M. D. PANDEY (1988): A comparative study of freezing buffalo semen in two dilutors. *Proc. II World Buffalo Congress.* New Delhi, India.
- RAO, A. R. (1984): Infertility in the male. *Buffalo Bulletin* 3, 9-10.
- REDDY, N. C., C. L. R. GUPTA, T. G. RADHAKRISHNA (1983): Effect of seasons on the quantity of buffalo semen. *Indian J. Anim. Reprod.* 3, 113.
- ROSSI, G. L., R. KADERLI, H. KONIG, H. KUPFERSCHMIED (1975): Correlations between changes in the genital tract, total number of spermatoocytes per ejaculate and the pathology of the internal organs of bulls at the artificial insemination centre at Neuchatel. II. Statistical studies on spermatoocyte numbers and testicular lesions in 159 Simmental bulls. *Schweizer Arch. für Tierheilkunde* 117, 425-437.
- SAEED, A. (1988): Studies on morphology of buffalo bull semen of different age groups. Thesis. University of Agriculture. Faisalabad, Pakistan.
- SAEED, A., R. A. CHAUDHRY, I. H. KHAN, N. U. KHAN (1990): Morphology of semen of buffalo bulls of different age groups. *Proc. II World Buffalo Congr., Vol. 3.* December 12-16, 1990. New Dehli, India.
- SALISBURY, G. W., N. L. VANDEMARK, J. R. LODGE (1978): *Physiology of Reproduction and Artificial Insemination of Cattle*, 2<sup>nd</sup> ed. W. H. Freeman and Co. San Francisco.
- SETTERGREN, I. (1994): Bull fertility examination. XII<sup>th</sup> FAO/SIDA International Postgraduate Course on Animal Reproduction, III. Veterinary College. Upsala.
- SETTERGREN, I. (1982): Bull fertility examination. FAO/SIDA International Postgraduate Programme on Animal Reproduction. Stockholm, Sweden.
- SHALASH, M. R. (1984): Some aspects of mating behaviour in male buffalo and camel under the Egyptian environment. *Cong. 10<sup>th</sup> Int. Anim. Reprod. and A. I. University of Illinois.* Urbana-Champaign. pp. 272.
- SINGH, R.S ., N. S. TOMAR, K. C. SHARMA, K. B. SHARMA (1992): Studies on acrosomal abnormalities of cattle and buffalo spermatozoa in relation to other semen characteristics and fertility. *Indian Vet. J.* 69, 267-268.
- SODERQUIST, L., L. JANSON, M. HAARD, S. EINARSSON (1992): Factors affecting the variation in sperm morphological abnormalities in Swedish dairy A. I. bulls. *Proceedings 12<sup>th</sup> International Congress on A. R. August 23<sup>rd</sup> - August 27<sup>th</sup> 1992.* The Hague, Netherlands.
- SURYAPRAKASAM, T. B., A. V. NARSIMHA RAO (1993): Studies on seminal characteristics of multiple breed A. I. bull. *Indian Vet. J.* 70, 629-632.

- TEREZINHA, A. P., M. GOMES, D. CASTRO, M. ERALDO, M. G. JOCELIM, J. C. D. G. FERNANDO (1991): Physical and biochemical characteristics from Murrah buffalo bulls semen. 3<sup>rd</sup> Buffalo Cong. Bulgaria, Symp. IV. pp. 571.
- VEERAMACHANENI, D. N. R., R. S. OTT, E. H. HEATH, K. MCENTEE, D. J. BOLT, J. E. HIXON (1986): Pathophysiology of small testes in beef bulls relationship between scrotal circumference, histopathologic features of testes and epididymides, seminal characteristics, and endocrine profiles. Amer. J. Vet. Res. 47, 1988-1999.
- VYAWANARE, R., R. A. S. CHAUHAN, S. P. NEMA, M. L. POSWAL (1989): Studies on seminal attributes, enzyme leakage and preservability of buffalo semen. Indian Vet. J. 66, 1128-1132.
- WACOL (1991): Proceedings of the Bull evaluation workshop held on 21 March 1991. University of Sydney. Sydney, Australia. pp. 16-36.
- WIERZBOWSKI, S., M. N. TAHIR (1980): Sexual efficiency of the buffalo bulls used in A. I., Vol. III, pp. 179.
- YOUNIS, M. (1996): Studies on semen quality, freezability and fertility of buffalo bulls during low and peak breeding seasons. Ph.D Thesis. Department of Animal Reproduction, University of Agriculture. Faisalabad, Pakistan.
- ZAFAR, A. H., N. IQBAL, S. K. SHAH (1988): Monograph on reproductive pattern of riverine buffalo and recommendations to improve their reproductive performance at small farmer level. PARC, Islamabad, Pakistan.

Received: 1 March 1999

Accepted: 18 April 2000

---

**JAVED, M. T., A. KHAN, R. KAUSAR: Utjecaj starosti i godišnjeg doba na neke pokazatelje sjemena Nili-Ravi bivolskih (*Bubalus bubalis*) bikova. Vet. arhiv 70, 83-94, 2000.**

**SAŽETAK**

Istraživanje je izvedeno na 16 bivolskih bikova (*Bubalus bubalis*) podijeljenih u 4 dobne skupine, uključujući 3 skupine zdravih životinja u dobi do 5, između 6 i 10 i starijih od 11 godina, i od jedne skupine bikova starih između 6 i 10 godina s lošom kvalitetom sjemena. Istraživanje je trajalo jednu godinu tijekom 5 godišnjih doba: suho ljeto, vlažno ljeto, jesen, zima i proljeće. Prosječni volumen sjemena je bio  $4,67 \pm 1,62$  ml i nije pokazivao značajne razlike među dobnim skupinama, ali je bio veći ( $P < 0,05$ ) u jesen. Kiselost sjemena je bila  $\text{pH } 6,55 \pm 0,50$  i bila je niža ( $P < 0,05$ ) u skupini 6 do 10-godišnjih nego u skupini bikova starijih od 11 godina i u bikova s ne kvalitetnim sjemenom. pH je bio niži ( $P < 0,05$ ) u jesen, a viši ( $P < 0,05$ ) zimi. Vrijednost boje bila je viša ( $P < 0,05$ ) u bikova do 5 godina starosti. Bila je, međutim, niža ( $P < 0,05$ ) u skupini bikova s lošom kvalitetom sjemena u odnosu na bikove s normalnom kvalitetom sjemena, te je bila niža tijekom suhog ljeta. Opća aktivnost sjemena bila je  $2,65 \pm 1,03$  i bila je niža ( $P < 0,05$ ) u bikova starijih od 11 godina. Bila je, međutim, niža ( $P < 0,05$ ) u skupini bikova s lošim sjemenom u odnosu na bikove s kvalitetnim sjemenom, te je bila viša ( $P < 0,05$ ) u suhom ljetu nego u proljeće. Ukupna pokretljivost spermija je bila  $56,89 \pm 0,65\%$  i nije pokazivala značajne razlike među bikovima s normalnim sjemenom, ali je bila niža ( $P < 0,05$ ) u skupine s ne kvalitetnim sjemenom. Ukupna pokretljivost spermija bila je niža ( $P < 0,05$ ) zimi, nego u vlažnom ljetu i jeseni. Ukupna koncentracija spermija je bila  $1,00 \pm 0,50$  milijun/ml i bila je viša ( $P < 0,05$ ) u bikova do 5 godina starosti, dok je bila niža ( $P < 0,05$ ) u skupini bikova s ne kvalitetnim sjemenom, kao što je bila niža ( $P < 0,05$ ) i zimi. Korelacija pH sjemena je bila negativna ( $P < 0,001$ ) s bojom ( $r = -0,38$ ), s koncentracijom spermija ( $r = -0,37$ ), s općom aktivnosti ( $r = -0,37$ ) i progresivnom pokretljivošću ( $r = -0,17$ ). Koncentracija spermija je bila u pozitivnoj korelaciji ( $P < 0,001$ ) sa masovnom aktivnosti ( $r = 0,86$ ) i progresivnom mobilnosti ( $r = 0,16$ ).

**Ključne riječi:** sjeme, sperma, volumen, pH, boja, masovna aktivnost, pokretljivost, koncentracija spermija, bivolski bik, *Bubalus bubalis*, Pakistan

---