# STUDIES ON SEMEN QUALITY OF YOUNG, ADULT AND OLD BUFFALO BULLS DURING LOW AND PEAK BREEDING SEASONS

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## ABSTRACT

The semen quality during the low (May-July) and the peak (September-November) breeding seasons in 18 Nili-Ravi buffalo bulls varying in age from 3 to 15 years was studied. Ejaculatory volume was higher ($P<0.05$) in adult (3.03 ± 0.13 ml) and old (2.86 ± 0.13 ml) than young (2.51 ± 0.11 ml) bulls and during the low (3.44 ± 0.11 ml) than the peak (2.57 ± 0.90 ml) breeding season. Mass activity of semen collected from bulls of 3 age groups differed significantly and was higher ($P<0.05$) in the peak (2.27 ± 0.09) than the low (1.49 ± 0.08) breeding season. Sperm concentration did not differ among bulls of three age groups and between the two seasons. Total sperms per ejaculate were higher ($P<0.05$) in semen collected from audlt (3.49 ± 0.22 billions) and old (3.11 ± 0.23 billions) than young bulls (2.77 ± 0.18 billions) and in the low (3.38 ± 0.11 billions) than the peak (2.87 ± 0.17 billions) breeding season. Higher ($P<0.05$) percentage of motile spermatozoa was seen in ejaculates collected from young (60.69 ± 0.77) and adult (62.50 ± 0.78) than old (58.16 ± 0.76) bulls and during the peak (62.35 ± 0.66) than the low (58.56 ± 0.59) breeding season. Percentage of dead spermatozoa was higher ($P<0.05$) in semen collected from old (14.57 ± 0.74) than young (13.96 ± 0.98) and adult (12.04 ± 0.67) bulls and in the low (14.30 ± 0.57) than the peak (12.74 ± 0.75) breeding season. Ejaculates collected from old bulls (14.29 ± 1.03%) had higher ($P<0.05$) number of abnormal spermatozoa than those from young 13.02 ± 0.85%) and adult (12.22 ± 0.58%) bulls and in the low (13.74 ± 0.61%) than the peak (11.94 ± 1.61%) breeding season.

## INTRODUCTIN

The Nili-Ravi buffalo is the major dairy animal in Pakistan. Animals of this species are generally considered as seasonal breeder, and the maximum breeding activity occurs during autumn and winter while the lowest in summer (Heuer *et al.*, 1987). This seasonality of reproduction plays a major role in lowering the reproductive performance of animals of this species during summer. Buffalo bulls are comparatively more susceptible to heat stress due to their poor heat regulation mechanism than the females (Akhtar, 1988). Adverse effects of high ambient temperature during summer on the testicular size, libido and semen quality have been reported in sheep and goats (Ahmad, 1994), cattle (Soderquist *et al.*,1992) and buffaloes (Nazir, 1988; Barnabe *et al.*, 1992). The present paper describes physical characteristics of semen collected from young, adult and old Nili-Ravi buffalo bulls during the low (May to July). and the peak (September to November) breeding seasons.

## MATERIALS AND METHODS

### Experimental Animals

In this study 18 Nili-Ravi buffalo bulls with clinically normal reproductive tract and varying in age from 3 to 15 years were used. Depending on their age, these bulls were divided into 3 groups viz. young (3-4 years), adult (6-8 years) and old (12-15 years), with six bulls in each group.

### Feeding and Management

The experimental bulls were kept under naturally prevailing climatic conditions at the Semen Production Unit, Qadirabad, District Sahiwal, Pakistan. This study was carried out during the low (May to July, 1995) and the peak breeding season (September to November, 1995) of buffaloes. The climatic data for this period, recorded at the Cotton Research Station, Sahiwal, is given in Table 1.

The experimental bulls were housed individually in pens with open space, having sufficient cross ventilation and protection against heat during summer. Each bull was fed good quality seasonal green fodder at the rate of 35 to 65kg and 2 to 3kg of concentrate (cottonseed cake and wheat bran) daily.

### Semen Collection and Evaluation

Using an artificial vagina two ejaculates from each bull were collected once a week, with an interval of 10 minutes between the two ejaculates. Immediately after collection, each ejaculate was evaluated at 37°C for physical characteristics i.e. physical appearance,

ejaculatory volume, mass activity, sperm concentration, total sperm per ejaculate, pH and percentages of individually motile, dead and morphologically abnormal spermatozoa and acrosome morphology.

The volume and colour of ejaculates were determined directly from graduated collecting vials. Sperm concentration was determined with improved Neubauer counting chamber (Weber, Limited), as described by Hafez (1987).

In order to estimate the percentage of individual motile spermatozoa, a small drop of the fresh semen was diluted with a drop of 2.9 per cent sodium citrate solution and examined under bright field microscope (x 400). The spermatozoa showing progressive forward movement were considered motile, wile those showing circulatory movements or those oscillating at one place were taken as immotile.

The percentage of dead spermatozoa was determined by the eosin-nigrosin differential staining technique (Hancock, 1951). Two slides were made from each sample and at least 100 spermatozoa were counted in random fields per slide. Spermatozoa that absorbed the eosin, either partially or completely, were considered to be dead whilst those that did not absorb the stain at all were assumed to have been alive. At the same time the percentage of morphologically abnormal spermatozoa and those with abnormal acrosomes was assessed. The pH of each sample was measured with a digital pH meter (Strana, 5002).

**Statistical Analysis**

The mean values ($\pm$ SEM) of various parameters of semen quality for bulls of three age groups and for the low (May - July) and the peak (September - November) breeding seasons were calculated. In order to see the magnitude of variation in semen characteristics among bulls of three age groups and the two seasons, the data were subjected to analysis of variance using factorial experiment under completely randomized design (Snedecor and Cochran, 1989). Duncan's multiple range test (Duncan, 1955) was applied for multiple mean comparison, where necessary.

## RESULTS AND DISCUSSION

**Physical Appearance**

The ejaculates collected from experimental bulls appeared creamy or creamy-white in appearance and their consistency was thin during the low and thick during the peak breeding season. Jainudeen *et al.* (1982), Heuer *et al.* (1987) Nazir (1988) and Bhosrekar *et al.* (1992) also made similar observations for the buffalo bull semen of various breeds.

**Ejaculatory Volume**

Overall mean ejaculatory volume of 2.80 $\pm$ 0.07 ml, recorded in the present investigation, is in agreement with 2.9 and 2.08 ml reported by Jainudeen *et al.* (1982) and Nazir (1988), respectively. Higher ejaculatory volume of 4.1 ml recorded in Iraqi buffaloes by El-Wishy (1978) reflects breed differences. Ejaculatory volume of semen samples collected from adult and old bulls was significantly higher than those from young bulls, the difference between bulls of the former two groups was non significant (Table 2). Nordin *et al.* (1990) also reported that ejaculatory volume increased with advancing age of bulls, the highest volume was obtained at 8 to 10 years of age. At this age, bulls had larger testicular size and higher libido than younger bulls which results in increased ejaculatory volume (Anzar *et al.*, 1988; Bhosrekar *et al.*, 1992).

Significantly higher ($P<0.05$) ejaculatory volume was recorded during the low than the peak breeding season (Table 2). Similar findings have been reported for Iraqi (El-Wishy, 1978) and Murrah buffalo bulls (Reddy *et al.*, 1983). Ahmad (1987) also observed the lowest ejaculatory volume (2.40 $\pm$ 0.13 ml) for samples collected from Nili-Ravi buffalo bulls during autumn (August to October). The values for winter, spring and summer seasons were 2.92 $\pm$ 0.02, 3.03 $\pm$ 0.13 and 2.91 $\pm$ 0.31 ml, respectively. However, Nazir (1988) found the same ejaculatory volume during the low and the peak breeding seasons in the Nili-Ravi buffaloes. These discrepancies may be attributed to differences in managemental conditions at different farms. Besides season, ejaculatory volume is also influenced by the age of bulls and the frequencies of their use, and the method of semen collection (Saji, 1978).

**Mass Activity**

Overall mean mass activity of 1.88 $\pm$ 0.07 was recorded in the present study. Heuer *et al.* (1987) recorded mass activity of 2.01 for buffalo bull semen, which supports our findings. The mass activity of ejaculates collected from young, adult and old bulls averaged 1.93 $\pm$ 0.13, 2.18 $\pm$ 0.11 and 1.53 $\pm$ 0.10, respectively (Table 2). All the three age groups differed significantly from one another ($P<0.05$). Saeed (1988) recorded the best mass activity in young (2.95) and adult (3.49) bulls which supports the findings of this study. Mass activity depends upon the number of total, and percentage of live, spermatozoa in the semen samples and can be increased by pre-collection stimulation.

Higher mass activity ($P<0.05$) was noted for

ejaculates collected during the peak than those during the low breeding season. These findings are in agreement to those of Dumitrescu *et al.* (1988) who reported higher mass activity in winter than in summer. However, Nazir (1988) observed similar mass activity for samples collected during the low (2.33 ± 0.36) and the peak (2.06 ± 0.47) breeding seasons. This was apparently due to special managemental measures adopted during hotter months of the year.

**Sperm Concentration**

In the present study, an overall mean sperm concentration of 1.12 ± 0.04 billions/ml was recorded, which is comparable to 1.06 billions/ml reported by Heuer *et al.* (1987). There was no difference in the sperm concentration of semen samples collected from young, adult and old bulls (Table 2). Similar results were reported by Jainudeen *et al.* (1982) and Rahman *et al.* (1991). However, Nordin *et al.* (1990) reported that sperm concentration increased with the age of bulls. Sperm concentration is a highly variable parameter of semen quality and a wide range of sperm concentration (0.60 to 2.68 billions/ml) among bulls of three age groups was recorded in the present study. Perhaps this explains the discrepancies between our results and those of Nordin *et al.* (1990).

The effect of season on the sperm concentration revealed higher value for ejaculates collected during the peak than those collected in the low breeding season (Table 2), however the difference was non significant. Similar findings have been reported by Nazir (1988). Ahmad (1987) recorded relatively higher sperm concentration during summer (1103 ± 71.8 x $10^6$/ml) than during autumn (984 ± 15.2 x $10^6$/ml). However, the data were not analyzed statistically.

**Total Spermatozoa per Ejaculate**

Overall mean value for total spermatozoa per ejaculate was 3.13 ± 0.12 billions in the present study, which is in close line to 3.18 ± 0.52 billions reported by Jainudeen *et al.* (1982). Total spermatozoa per ejaculate in semen samples collected from adult and old bulls were higher ($P<0.05$) than those of young bulls (Table 2). Similar findings were reported previously by Heuer *et al.* (1987).

Seasonwise effect showed that the semen collected during the low breeding season had higher ($P<0.05$) number of total spermatozoa per ejaculate than that collected in the peak breeding season. This might be due to higher ejaculatory volume observed during the low than the peak breeding season. Heuer *et al.* (1987) made similar observations.

**pH (Hydrogen Ion Concentration)**

In the present study, overall mean pH of semen samples collected from 18 bulls was 6.26 ± 0.05. Terezinha *et al.* (1991) found the pH of 6.38 ± 0.19 for ejaculates collected from buffalo bulls. The buffalo semen is said to be slightly acidic, with the pH varying from 6.51 to 6.97 (Nazir, 1988). The pH of semen from bulls of three age groups did not differ. Similarly, Terezinha *et al.* (1991) recorded no variation in pH of semen from young, adult and old bulls.

The pH of the semen was higher ($P<0.05$) during the low than the peak breeding season. This might be due to higher sperm concentration noted in the peak than the low breeding season. Dense and thick semen samples are known to have lower pH than thin samples (Shalash, 1972). Terezinha *et al.* (1991) found that the effect of season on pH of the buffalo bull semen was significant.

**Motile Spermatozoa**

Overall mean motile spermatozoa in semen samples collected from 18 bulls was 60.45 ± 0.48 per cent. The number of motile spermatozoa in the buffalo bull semen, ranging from 57.9 ± 8.0 to 75.17 ± 4.92 per cent, have been recorded by various workers (Gupta *et al.*, 1978; Nordin *et al.*, 1990; Bhosrekar *et al.*, 1992).

Semen samples from young and adult bulls had higher ($P<0.05$) percentage of motile spermatozoa than old bulls (Table 2). Bhosrekar *et al.* (1992) found that the percentage of motile spermatozoa in semen samples was higher in bulls of 3-10 years of age and declined thereafter.

Semen collected during the peak breeding season had higher motility of spermatozoa ($P<0.05$) than that collected during the low breeding season (Table 2). These findings confirm an earlier report by Heuer *et al.* (1987). According to Ahmad (1987), the sperm motility for ejaculates collected from Nili-Ravi buffalo bulls during summer, autumn, winter and spring seasons averaged 67.0 ± 0.7, 65.6 ± 0.8, 60.2 ± 1.2 and 60.5 ± 0.2 per cent, respectively.

**Dead Spermatozoa**

In the present investigation, overall mean percentage of dead sperm was 13.52 ± 0.48, which is in agreement to 12.1 per cent reported by Rahman *et al.* (1991) in Murrah buffalo bulls. A significant effect of age of bulls on the percentage of dead spermatozoa was observed, the values being higher in old than young and adult bulls (Table 2). These findings are supported by thos of Gupta *et al.* (1978). Cook *et al.* (1994)

Table 1: Mean values of ambient temperature, relative humidity and day length recorded at Cotton Research Station, Sahiwal during the low and the peak breeding seasons.

| Months/seasons | Ambient Temperature (°C) | Relative humidity (%) | Day length (hrs.) |
|---|---|---|---|
| Low breeding season | | | |
| May | 34.0 (23-41) | 82.0 (80-83) | 13.00 |
| June | 37.0 (26-43) | 84.0 (78-91) | 14.00 |
| July | 33.0 (28-44) | 83.0 (75-87) | 13.30 |
| Mean | 34.90 | 82.00 | 13.45 |
| Peak breeding season | | | |
| September | 30.0 (22-37) | 81.0 (76-83) | 12.00 |
| October | 27.0 (13-34) | 78.0 (72-80) | 11.30 |
| November | 19.0 (09-32) | 71.0 (66-79) | 10.30 |
| Mean | 24.40 | 76.00 | 11.45 |

Values within parenthesis show ranges

found that increased number of dead spermatozoa in semen might be due to internal testis insult which was higher in old bulls because of age factor.

Significantly higher number of dead spermatozoa were found during the low than the peak breeding season. Previous studies have shown that ejaculates collected during summer had higher number of dead spermatozoa than those collected in winter (Ahmad *et al.*, 1987; Ahmad *et al.*, 1991; Barnabe *et al.*, 1992). The incidence of higher dead spermatozoa in the summer season seems to be due to impairment of spermatogenesis because of environmental and nutritional stress. A combination of these factors can further augment this condition.

**Morphologically Abnormal Spermatozoa**

The overall mean percentage of morphologically abnormal spermatozoa in ejaculates collected from 18 bulls was 12.97 $\pm$ 0.48. Similarly, Malik *et al.* (1974) reported 11.91 per cent abnormal spermatozoa in adult Nili-Ravi buffalo bulls. Higher percentages of abnormal sperm, 18.2 to 23.7 per cent, have been reported for Egyptian (Younis *et al.*, 1980) and Nili-Ravi buffalo bulls (Ahmad *et al.*, 1987). These discrepancies seem to be due to differences in management and nutritional status, in addition to age and seasonal influences, of the breeding bulls.

Age of bulls had a significant effect on the occurrence of abnormal spermatozoa in their semen. The highest abnormal sperm percentage was recorded in ejaculates collected from old bulls and the lowest in those from adult bulls (Table 2). Similarly, Ahmad *et al.* (1987) found 13.00 $\pm$ 3.70, 13.00 $\pm$ 0.90 and 20.90 $\pm$ 4.80 per cent abnormal spermatozoa in young, adult and old bulls, respectively. According to Settergren (1994), 10 per cent sperm abnormalities were found in semen samples collected from young and adult cow bulls whereas 20 per cent in those of old bulls.

In the present study, abnormal spermatozoa were higher in the low than the peak breeding season (Table 2). These findings are supported by Heuer *et al.* (1987) who found higher sperm abnormalities (14.2%) in the low than (10.8%) in the peak breeding season. Higher level of sperm abnormalities during hot summer season might be due to the heat stress experienced by bulls, as elevated environmental temperature impairs testicular function. Body temperature of buffalo bulls is elevated during summer season due to poor thermoregulatory mechanism in this species (Ahmad *et al.*, 1987; Nazir, 1988; Bhosrekar *et al.*, 1992). Ahmad (1987) reported the lowest sperm abnormalities (5.4 $\pm$ 0.1%) in autumn; the values for winter, spring and summer seasons were 6.4 $\pm$ 0.2, 6.9 $\pm$ 0.4 and 6.0 $\pm$ 0.9 per cent, respectively. However, the data were not analyzed statistically.

The results of the present study showed that the semen quality of the Nili-Ravi buffalo bulls was low during the low breeding season. Ambient temperature coupled with relative humidity and the photoperiod appear to be the main climatic components regulating the seasonal influence on the reproductive functions of the seasonal breeders. It is difficult to apportion any single climatic component as being solely responsible

Table 2: Mean values ( ± SEM) of various parameters of semen quality collected from buffalo bulls of three age groups during the low and the peak breeding seasons.

| Parameters | Low breeding season | Peak breeding season | Overall mean |
|---|---|---|---|
| Ejaculatory volume (ml) | | | |
| Young | 2.86 ± 0.09 | 2.39 ± 0.14 | 2.51 ± 0.11b |
| Adult | 3.90 ± 0.21 | 2.67 ± 0.14 | 3.03 ± 0.13a |
| Old | 3.56 ± 0.19 | 2.67 ± 0.16 | 2.86 ± 0.13a |
| Mean | 3.44 ± 0.11A | 2.57 ± 0.90B | 2.80 ± 0.07 |
| Mass Activity | | | |
| Young | 1.61 ± 0.17 | 2.25 ± 0.17 | 1.93 ± 0.13b |
| Adult | 1.72 ± 0.13 | 2.64 ± 0.09 | 2.18 ± 0.11a |
| Old | 1.14 ± 0.10 | 1.92 ± 0.13 | 1.53 ± 0.10c |
| Mean | 1.49 ± 0.08A | 2.27 ± 0.09B | 1.88 ± 0.07 |
| Sperm concentration (x $10^9$) | | | |
| Young | 1.01 ± 0.08 | 1.21 ± 0.11 | 1.11 ± 0.07a |
| Adult | 1.10 ± 0.80 | 1.23 ± 0.08 | 1.16 ± 0.06a |
| Old | 1.03 ± 0.08 | 1.13 ± 0.11 | 1.08 ± 0.07a |
| Mean | 1.05 ± 0.05A | 1.29 ± 0.06A | 1.12 ± 0.04 |
| Total sperm/ejaculate (x $10^9$) | | | |
| Young | 3.62 ± 0.42 | 3.03 ± 0.33 | 2.77 ± 0.18b |
| Adult | 2.97 ± 0.23 | 2.87 ± 0.27 | 3.49 ± 0.22a |
| Old | 3.54 ± 0.26 | 2.73 ± 0.30 | 3.11 ± 0.23a |
| Mean | 3.38 ± 0.11A | 2.87 ± 0.17B | 3.13 ± 0.12 |
| pH | | | |
| Young | 6.66 ± 0.11 | 6.37 ± 0.16 | 6.34 ± 0.09a |
| Adult | 6.31 ± 0.11 | 6.01 ± 0.09 | 6.16 ± 0.07a |
| Old | 6.37 ± 0.12 | 6.18 ± 0.13 | 6.27 ± 0.09a |
| Mean | 6.33 ± 0.06A | 6.18 ± 0.77B | 6.26 ± 0.05 |
| Sperm motility (%) | | | |
| Young | 59.44 ± 0.80 | 61.94 ± 1.29 | 60.69 ± 0.77a |
| Adult | 60.00 ± 0.99 | 65.00 ± 0.90 | 62.50 ± 0.78a |
| Old | 56.22 ± 1.07 | 60.11 ± 0.91 | 58.16 ± 0.76b |
| Mean | 58.56 ± 0.59A | 62.35 ± 0.66B | 60.45 ± 0.48 |
| Dead sperm (%) | | | |
| Young | 16.01 ± 0.98 | 11.90 ± 2.00 | 13.96 ± 0.98b |
| Adult | 13.27 ± 0.78 | 10.80 ± 1.36 | 12.04 ± 0.67b |
| Old | 13.61 ± 0.01 | 15.52 ± 0.98 | 14.57 ± 0.74a |
| Mean | 14.30 ± 0.57A | 12.74 ± 0.75B | 13.52 ± 0.48 |
| Total abnormal sperm (%) | | | |
| Young | 14.34 ± 1.23 | 11.69 ± 1.13 | 13.02 ± 0.85b |
| Adult | 13.76 ± 0.91 | 11.18 ± 0.58 | 12.22 ± 0.58b |
| Old | 13.64 ± 1.16 | 12.93 ± 1.34 | 14.29 ± 1.03a |
| Mean | 13.74 ± 0.61A | 11.94 ± 1.61B | 12.97 ± 0.48 |

Values with different lower case letters in a column and upper case letters in a row for each parameter differ significantly ($P<0.05$).

for seasonal variations in the reproductive activity of animals at a certain locality (Ahmad, 1994). In the tropical and the subtropical regions of the world, high ambient temperature (Edgar, 1963) and relatively humidity (El-Wishy and El-Sawaf, 1971) have been shown to adversely affect the semen quality of rams and goats.

In the area where this study was conducted, there was relatively more variation in the ambient temperature between the two seasons than the day length or relative humidity (Table 1). It appears that the reproductive activity of the Nili-Ravi buffalo bulls kept under Pakistan climatic conditions is probably influenced more by the ambient temperature than by the day length or relative humidity. The combined effect of high ambient temperature and increased relative humidity perhaps more adversely affects the reproductive performance of the males of this species than does the individual climatic parameter. However, there is no experimental evidence. According to Ahmad (1987), management of breeding bulls over the year should be improved to eliminate nutritional variation effects, as fodder used at flat rates for feeding over the year are not of the same nutrient composition.

It is well known that the male reproductive activity is under the infleunce of male sex hormones, including LH and testosterone. In the buffalo bulls, the blood testosterone concentrations are shown to be higher during the breeding (0.17-23.0 ng/ml) than the non-breeding season (0.15-2.21 ng/ml, Brown *et al.*, 1991). Thus, it seems that the low quality of semen during the low breeding season in the Nili-Ravi buffalo bulls observed in this study was due to low levels of LH and testosterone during that season. However, the possible effect of quality and quantity of fodder available during the low breeding season cannot be ruled out. This study was carried out during summer and autumn seasons only. Further studies on the secretory profiles of LH and testosterone over a 12 month period may be undertaken to explain seasonal differences in the reproductive functions of the Nili-Ravi buffalo bulls.

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