

## **Breeding soundness of stud bulls**

S. P. Shaha, M. G. S. Alam\*, M. Khatun and J. U. Ahmed<sup>1</sup>

Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh

### **Abstract**

Four bulls of mixed breeds (Sahiwal × Zebu, Sindhi × Zebu, Jersey × Zebu and Holstein-Friesian × Zebu) were examined physically and their semen was evaluated in three seasons: I (Rainy): May - November, II (Winter): December - February, III (Summer): March- April. The semen volume, sperm motility, sperm concentration and percentage of dead sperm ranged from 4.1 to 7.6 ml, 56.6 to 76%, 1010.3 to 1290.7 × 10<sup>6</sup>/ml and 18.4 to 24.7%, respectively. All these attributes varied significantly ( $p < 0.05$ ) between breeds and seasons. The variations of semen quality between breeds were not significant ( $p > 0.05$ ). It is suggested that the Holstein - Friesian × Zebu crossbred bull produces better quality semen than others. (*Bangl. vet.* 2008. Vol. 25, No. 2, 51-61)

### **Introduction**

Breeding soundness refers to a bull's ability to get cows pregnant. Although 20-40% of bulls may have reduced fertility, few are completely sterile (Kastelic *et al.*, 2000). Sub-fertility in bulls delays pregnancy, prolongs calving interval, reduces calf weaning weight and increases culling rate of females. Multiple sire breeding groups and low breeding pressure may mask sub-fertility, but single-sire mating groups and artificial insemination (AI) increase the importance of bull fertility.

In Bangladesh, cross breeding for upgradation of cattle was started in 1935 with a view to improve the local cattle stock (Ahmed and Islam, 1987), al with no systematic sexual health control programme. Because of low uptake of AI in smallholdings, production per animal has remained very low. To overcome this, a programme should be undertaken for improvement of cattle in Bangladesh, especially indigenous cattle. If pregnancy does not result from the minimum number of services, it constitutes an economic loss to the farmer. AI must be combined with a health control programme, which must take care of sub-fertility problems. AI continues to be the most effective weapon in the control of venereal and other diseases. Single bull in AI centre can produce 100000 doses of semen per year. A single collection of semen may yield over a thousand doses, which may be widely distributed over a long period.

Extremely low reproductive rates in tropical location are often blamed on female cattle, although it may be assumed that climate and poor health may influence the fertility of bulls, especially semen quality and libido (Bloom, 1978). In Bangladesh, comprehensive work has not been done with regard to fertility of cattle by AI. The present study was undertaken to evaluate bulls for breeding soundness.

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\* Correspondence: *E-mail*:- mgsalam52@yahoo.com

## Materials and Methods

Four cross-bred bulls (Sahiwal × Zebu, Sindhi × Zebu, Jersey × Zebu and Holstein-Friesian × Zebu) were examined for breeding soundness. The age was determined from the records maintained at the Government and Bangladesh Agricultural University (BAU), Mymensingh AI Centres, and varied from 5 to 10 years. Bulls were maintained under stall feeding and fed approximately 8 kg roughage and 2 kg concentrate per day with free access to drinking water. The bulls were allowed to exercise daily for one hour. Meteorological information was obtained and the year was divided into three seasons (Table 1).

Table 1. Average temperature, humidity and rainfall recorded by the Meteorological Department of BAU (1988-89)

Seasons	Temperature °C	Humidity (%)	Rainfall (mm)
I Rainy (May-November)	27.8	84.4	299.0
II Winter (December-February)	20.8	77.3	5.8
III Summer (March-April)	26.3	74.3	86.1

### *Examination of bulls*

Before introduction to the collection schedule, the bulls were examined physically and for integrity of testes and reproductive tracts as described by Herman *et al.* (1994). The sexual behaviour of the bulls was observed, including libido, erection, mounting and grasping of the dummy, protrusion of penis and thrust and ejaculation. The scrotum was inspected with respect to size, shape and freedom from skin disease. The scrotal circumference was measured by the method of Chenoweth and Ball (1981).

### *Semen collection*

The semen was collected twice a week by means of artificial vagina (Arthur *et al.*, 1982). Before collection, warm water was put into the space between the two tubes. Sterile soft petroleum jelly was smeared over the inner surface. The internal temperature of artificial vagina was checked to ensure that it was not above 50°C. The artificial vagina was held upright to flow the ejaculate into graduated tube which was attached with the latex extension cone.

### *Semen examination*

Two ejaculates were collected as described by Almaquist (1978) and Herman *et al.* (1994).

#### a) Volume and macroscopic examination

The volume, colour and opacity of the semen and presence of any foreign substances were checked according to Comhaire *et al.* (1992); Mortimer (2000). The

density of the ejaculate reflects sperm concentration, which was expressed with "D". The sperm concentration was determined by a haemocytometric method (Elliott, 1978). The samples of semen with concentration above  $1000 \times 10^6/\text{ml}$  were considered as good.

#### b) Mass activity and motility of sperm

A drop of undiluted semen was placed on a warmed ( $37^\circ\text{C}$ ) slide without cover slip and examined with low power objective ( $10\times$ ) as described by Herman *et al.* (1994). The mass activity was graded as :

- 0 = No mass activity
- + = Slow wave motion
- ++ = Rapid wave motion with formation of eddies at the end of waves.
- +++ = Eddies

Motility was evaluated in a small drop of semen under cover slip with higher magnification ( $100\times$ ). Sperm moving forward were included in the motility count while sperm moving in circles or backward or showing pendulating movement were excluded (Herman *et al.*, 1994).

#### c) Sperm concentrations

The concentration of sperm per cubic millilitre for each ejaculate was measured by direct cell count method (Bane, 1952; Elliott, 1978).

#### d) Semen pH and sperm viability

The pH of the semen was determined using indicator paper strips. The live and dead sperm were counted by East Green-Eosin blue stain (Barth and Oko, 1989; Herman *et al.*, 1994; Salisbury *et al.*, 1978).

## Results and Discussion

Breeding soundness evaluation is a practical method to eliminate bulls with unsatisfactory breeding potential. This evaluation was conducted on every bull 30 to 60 days before each breeding period to allow time to replace unsatisfactory bulls. Producers observed bulls with cows in oestrus to determine whether bulls showed the desire and ability to mate successfully. The bulls were all free from venereal diseases. The health of the bulls was good, as suggested by the Theriogenology Society in the United States of America (Kastelic and Thundathil, 2008).

Sexual behaviour of all the bulls was satisfactory. The scrotal sac was normal and no skin lesion was detected. The mean scrotal circumference was  $37.12 \pm 1.62$  cm. Observation of mating performance and behaviour and semen evaluation are traditionally used to assess bulls for suitability for breeding (Parkinson, 2004). Britol *et al.* (2004) found scrotal circumference was a good predictor of sexual maturity in

*Bos indicus* bulls. Chacón *et al.* (2002) observed that scrotal circumference is positively related to body condition in Brahman bulls in tropics and commented that nutrition may be a major factor affecting seasonal variations in male reproductive parameters, especially testicular size. Santos *et al.* (1998) in a study of Zebu bulls found that those fed high levels of concentrates had higher body weight and scrotal circumference than those fed low levels, but there was no difference in semen turbidity, motility and sperm concentration. As scrotal circumference increases, age at puberty decreases and productivity is improved in daughters (Engelken, 2008).

No pathological lesion was detected in the prepuce and penis during inspection. The testicles were freely movable inside the scrotal sac and felt elastic on superficial and deep palpation. Caput epididymis and corpus epididymis of each bull appeared normal; the seminal vesicles were symmetrical, lobulated and normal in consistency on rectal examination. The ampullae were symmetrical and had no signs of inflammation. The inguinal rings of all bulls allowed easy entrance of one or two fingers at rectal examination. Barth (2007) reviewed the common abnormalities of the accessory sex glands.

Male fertility is commonly defined in terms of the conventional semen profile, which describes the number of motile sperm in the ejaculate. Any mammalian ejaculate constitutes a heterogeneous population of sperm presenting three or four sperm subpopulations with specific patterns of movement, and this has been suggested to be a widespread phenomenon among mammalian ejaculates (Abaigar *et al.*, 1999; Quintero-Moreno *et al.*, 2003). The semen characteristics of bulls in our study are presented in Table 2.

#### Volume

Significantly ( $p < 0.05$ ) highest (7.6 ml) volume of semen was in Holstein-Friesian  $\times$  Zebu crosses and lowest (4.0 ml) in Sahiwal  $\times$  Zebu (Table 2). Sane *et al.*, (1994) found that the mean volume of the ejaculate in adult dairy and buffalo bulls was 5.4 to 6.5 ml and 1.5 to 3.7 ml (range : 0.5 to 6.0 ml), respectively.

Highest volume was in winter. Variations of semen volume among breeds were reported previously (Ahmed *et al.*, 1992). This is in agreement with Raja and Rao (1982). Repeated collection of semen will produce a large volume with low density. Volume increases with the advancement of age of the bull up to 4-5 years. Along with other parameters, semen volume and sperm concentration provide a gross estimate of reproductive performance of bulls (Alam and Hurtado, 1982). Season affects the characteristics of semen (Igboeli *et al.*, 1987). Djimde and Weniger (1986) reported seasonality in semen volume, the largest being collected in summer in crossbred cattle. But we observed higher semen volume in spring and winter (Fig. 1). December - February is winter in Bangladesh and cold temperature might influence semen production. Other workers did not find any seasonal variation on semen volume in *Bos taurus* (Koivisto *et al.*, 2008); *Bos indicus* (Chacon *et al.*, 2002); *Bubalus bubalis* (Koonjaenak *et al.*, 2007).

Table 2. Average volume, initial motility and sperm concentrations of bulls in various seasons

Semen characteristics	Sahiwal × Zebu			Sindhi × Zebu			Jersey × Zebu			Holstein-Friesian × Zebu		
	I	II	III	I	II	III	I	II	III	I	II	III
Volume of ejaculate	4.1 <sup>a</sup>	4.5 <sup>a</sup>	4.0 <sup>a</sup>	4.4 <sup>a</sup>	4.6 <sup>a</sup>	4.3 <sup>a</sup>	4.5 <sup>a</sup>	4.7 <sup>a</sup>	4.1 <sup>a</sup>	7.0 <sup>a</sup>	7.6 <sup>a</sup>	6.4
Sperm motility (%)	67.6 <sup>a</sup>	62.6 <sup>a</sup>	65.3 <sup>a</sup>	66.0 <sup>a</sup>	66.3 <sup>a</sup>	65.4 <sup>a</sup>	60.1 <sup>a</sup>	56.6 <sup>a</sup>	58.2 <sup>a</sup>	76.0 <sup>a</sup>	66 <sup>a</sup>	68.6 <sup>a</sup>
Dead sperm (%)	18.3 <sup>a</sup>	18.6 <sup>a</sup>	19.7 <sup>a</sup>	19.1 <sup>a</sup>	19.3 <sup>a</sup>	20.1 <sup>a</sup>	19.3 <sup>a</sup>	19.7 <sup>a</sup>	20.3 <sup>a</sup>	20.5 <sup>a</sup>	20.1 <sup>a</sup>	24.7 <sup>a</sup>
pH (Average)	6.4 <sup>a</sup>	6.4 <sup>a</sup>	6.4 <sup>a</sup>	6.4 <sup>a</sup>	6.4 <sup>a</sup>	6.5 <sup>a</sup>	6.4 <sup>a</sup>	6.4 <sup>a</sup>	6.5 <sup>a</sup>	6.1 <sup>a</sup>	6.2 <sup>a</sup>	6.8 <sup>a</sup>
Concentrations (×10 <sup>6</sup> /ml)	1133.4	1242.5	1260.1	1030.1 <sup>a</sup>	1110.3 <sup>a</sup>	1140.6 <sup>a</sup>	1180.8 <sup>a</sup>	1260.6 <sup>a</sup>	1290.7 <sup>a</sup>	1010.3 <sup>a</sup>	1059.8 <sup>a</sup>	1060.7 <sup>a</sup>

<sup>a</sup> means (p<0.05) <sup>b</sup> means (<0.05) <sup>c</sup> means no significant variation between breed (p>0.05)

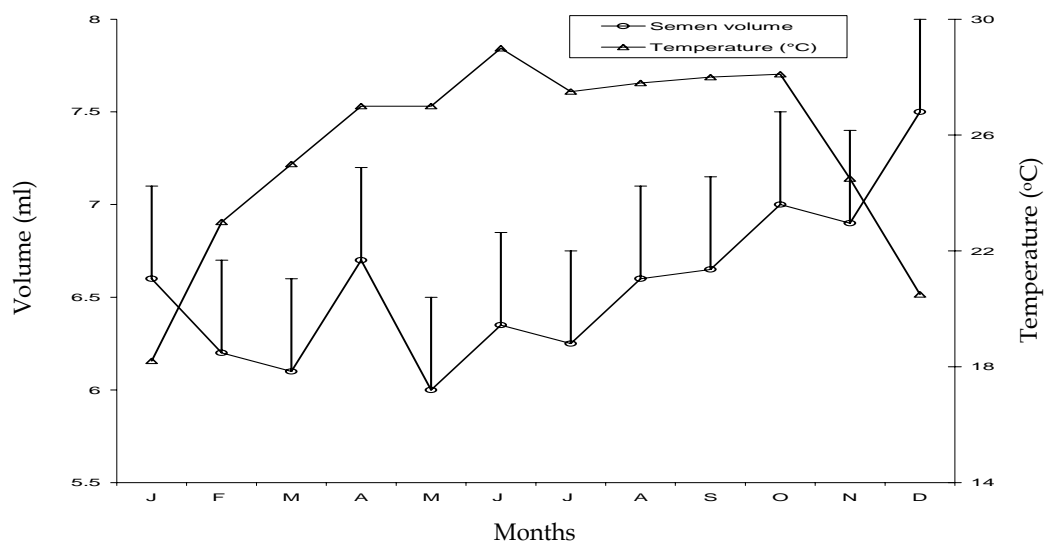


Fig. 1. Monthly variations of semen volume in AI bulls

### Motility

The highest percentage of motile sperm (66.2 - 76.0%) was in the Holstein-Friesian  $\times$  Zebu bulls and Jersey  $\times$  Zebu showed the lowest ( $p < 0.05$ ) percentage (56.6 - 60.1%). Motility of sperm is important for fertilization of oocytes and sustains embryonic development (Foote, 2003). Significant ( $p < 0.05$ ) seasonal variation was also evident. The highest motility was in rainy season and lowest in winter (Fig. 2). These results agree with those of Mukherjee and Banerjee (1980); Saxena and Tripathi (1981); Igboeli *et al.* (1987). But Ahmed *et al.* (1992) reported higher motility in November to March. This variation could be caused by the age of animals, climate and management. Significantly higher forward motility of sperm has been reported in *Bos taurus* and *Bos indicus* bulls in winter (Koivisto *et al.*, 2008).

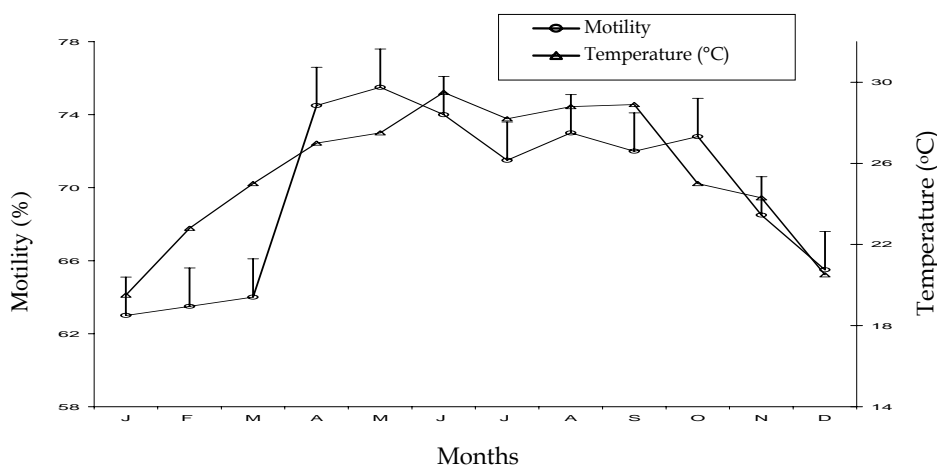


Fig. 2. Monthly variations of motility (%) in AI bulls

### Concentrations

The highest concentrations (1000 million/ml) of sperm were found in Jersey × Zebu bulls in summer, in agreement with Ahmed *et al.* (1992). But the concentration of sperm was lower in other breeds, in agreement with Djimde and Weniger (1986). Our results also agreed with Koivisto *et al.* (2008) where in *Bos indicus*, the highest concentration of sperm was in summer, but in *Bos taurus* the highest was in spring. The average concentration of sperm was higher during winter and lowest in rainy season (Fig. 3). Season had no effect on buffalo bull sperm concentration but it increased significantly with the age of the animals (Koonjaenak *et al.*, 2007).

### Live and dead sperm

The highest ( $p < 0.05$ ) percentage (24.7%) of dead sperm was found in summer in the Holstein-Friesian × Zebu (Table 2). The percentage of dead sperm in other breeds were 18.4 -19.8. Significantly major sperm defects were recorded in *Bos taurus* and *Bos indicus* (Koivisto *et al.*, 2008). There is significant ( $p < 0.05$ ) difference between Sindhi × Zebu and Jersey × Zebu. Fig. 4 shows that the percentage of dead sperm was higher in summer and lowest in rainy season. This agrees with Kim *et al.* (1983); Tomar *et al.* (1985). Heat stress and other adverse climatic condition cause the highest percentage of dead sperm. In a study in buffalo, highest percentages of normal sperm were found in summer. This number decreased with age, and was affected by season (Koonjaenak *et al.*, 2007).

### pH

Average pH was 6.1 - 6.5. The highest pH was found in summer. These results are in accordance with Mukherjee and Banerjee (1980). Lowering of pH with lactic acid was demonstrated to immobilize bull sperm (Acott and Carr, 1984; Carr *et al.*, 1985). The pH of seminal plasma ranges from 6.7 to 7.4, which is common in the domestic species (Roberts, 1986) and has the potential to neutralize vaginal acid. The pH value did not differ significantly between breeds and season.

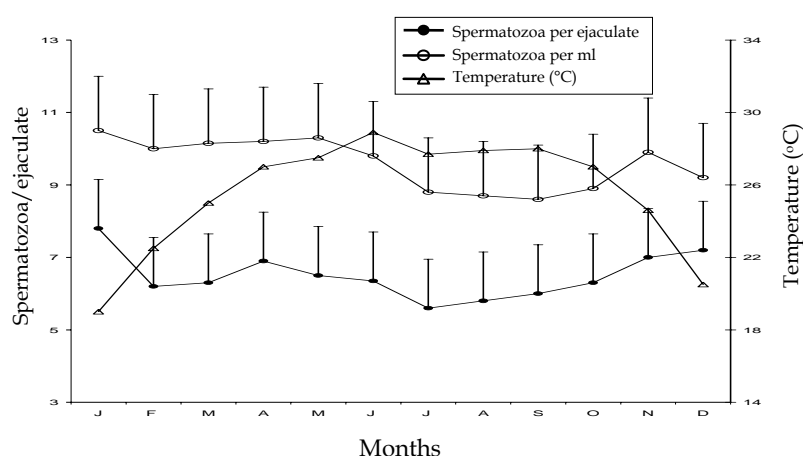


Fig. 3. Monthly variations of sperm output of AI bulls

Igboeli *et al.* (1987) found best quality semen during rainy season (April – August) and lowest in dry season (December – March). The authors suggested that lower temperature and abundant forage in the rainy season might influence semen production. Kim *et al.* (1983); Ibrahim *et al.* (1983) closely support the present findings. The variations obtained in the present study with many other reports might be due to variation in environment, climate and management. In domestic animals, some sperm defects are associated with subfertility or infertility, while other sperm defects affect fertility moderately when the recommended doses of sperm are used in AI (Andersson *et al.*, 1990). Sperm concentration decreases when bulls are utilized excessively. It has been reported that sperm concentration falls, with an increase of primary sperm abnormalities in testicular degeneration (Bloom, 1978). For insemination purposes the AI authority should provide 5-10 million sperm/dose (Den Daas *et al.*, 1998; Haard and Haard, 2000).

The breeding soundness evaluations used in Bangladesh are traditional, which grossly identify the abnormal bulls. Successful fertilization cannot be attributed solely to the number of normal sperm but more especially to their functional competence. Assessment of sperm function and fertility at molecular, cellular and whole-animal levels is needed to predict fertility of bulls that are producing apparently normal sperm. The tests employed for these assessments are flow cytometry in conjunction with fluorescent techniques, electronic cell counting, and computer-assisted image area analysis (Kastelic and Thundathil, 2008; Rodriguez-Martinez, 2008; Petrunkina *et al.*, 2007).

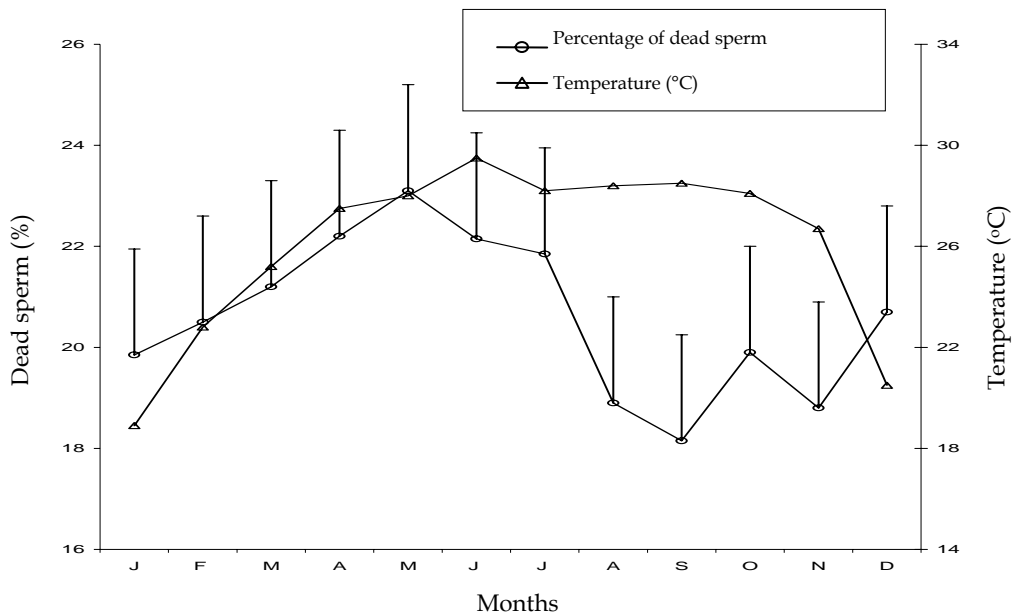


Fig. 4. Monthly variations of dead sperm (%) in AI bulls



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