

Effects of age, body weight, body condition and scrotal circumference on sperm abnormalities of bulls used for artificial insemination (AI) programme in Bangladesh

Md. Jalal Uddin Sarder*

Department of Animal Husbandry and Veterinary Science, University of Rajshahi, Rajshahi-6205, Bangladesh

*Email: jalalnusa@yahoo.com

Abstract: Impacts of age, body weight, body conditions, and scrotal circumference on sperm abnormalities of bulls used for artificial insemination (AI) in Bangladesh have been studied. During 1997-2002, 1390 ejaculates of semen from 71 bulls from Central Cattle Breeding Station and Dairy Farm, Savar, Dhaka, Rajshahi Dairy and Cattle Improvement Farm, Rajbarihat, and District Artificial Insemination Centre, Rajshahi, were collected and examined. Total head abnormalities, free loose head, mid-piece, tail abnormalities, proximal and distal cytoplasmic droplets, total tail abnormalities and total sperm abnormalities were recorded. Age of the bulls had significant effect on sperm abnormalities ($P < 0.01$) except for proximal cytoplasmic droplet. Similarly, body weight had significant effect ($P < 0.05$) except for the total head abnormalities, while body conditions had no significant effect on mid-piece, total tail abnormalities and total sperm abnormalities except for the total head abnormalities. Scrotal circumference had significant effect ($P < 0.05$) on all the sperm abnormalities. The present results demonstrate that the middle (8 to <10 yrs) and older (>10 yrs) bulls having body weight of <450 kg, very good body condition and 36 to <38 cm scrotal circumference had the lowest sperm abnormalities. The need for semen examination for morphological abnormalities of spermatozoa in bulls to be used for AI programme is strongly implicated by this study.

Key words: Bull sperm abnormalities, age, body weight, body conditions, scrotal circumference, artificial insemination (AI)

Introduction

A common saying that 'the bull is half of the herd' is taught in animal husbandry, and the reason behind the statement is that the sire is one of the parents of all the calves in the herd while a dam is a parent of one calf per season. Semen is the secretion of male reproductive glands containing the male gametes (spermatozoa) produced in the testis (Hafez, 1993). Among the reproductive traits, quality semen plays a major role in determining the fertility and reproductive efficiency of any livestock production. The abnormal sperm heads and tails originate in the testis and epididymis, respectively (Blom, 1950). Low reproductive efficiency of cattle is a problem to both dairy and beef cattle breeders. Since fertility is determined from the results of mating between male and female, a part of low breeding efficiency is due to the bull (Haque, 1968). The incidence of spermatozoa with abnormal morphology in fertile bulls was found to be 0-18% (Saacke, 1970; Rao, 1971) whereas Zemjanis (1970) reported that these abnormalities could be as high as 30-40%. Elliot (1978) recommended not tolerating more than 20% abnormalities of sperm head and/or mid-piece in routine artificial insemination (AI) practice. The information available on the semen characteristics of purebred and crossbred bulls is very meager (Rao & Rao, 1979; Abraham *et al.*, 1982). Abnormalities of the spermatozoa occur due to disorder of the semeniferous tubules, during ejaculation or in manipulation of the ejaculate including excessive agitation, over-heating to rapid cooling, mixture of

water, urine or antiseptic in the semen (Hossain *et al.*, 1990). In the process of evolving new types of crossbred cattle with different levels of exotic germplasm, the use of purebred and crossbred bulls in the breeding programme has become imminent. Spermatozoa with abnormal morphology are the most important factor that has direct bearing on fertility of bulls for successful AI programmes. Experiments reveal that the proportion of morphologically abnormal spermatozoa in the semen correlates negatively with fertility results (Shamsuddin *et al.*, 1993), whereas morphologically abnormal spermatozoa are unable to fertilize the oocytes (Shamsuddin & Rodriguez-Martinez, 1994). Crossbreeding of indigenous cows with exotic bulls has been accepted as an effective method for bringing about a quick genetic improvement of cows for increased milk production (Sultana, 1995). Recent studies have shown that 11% or more head, mid-piece or tail abnormalities and 18% or more total abnormalities of spermatozoa are associated with reduced fertility in bulls (Sarder, 2004). In this study impacts of age, body weight, body conditions and scrotal circumference on sperm abnormalities of bulls used in three AI centres in Bangladesh have been evaluated.

Materials and Methods

Experimental Bulls and parameters used: A total of 1390 ejaculates of semen collected from 71 bulls used for AI programmes in Bangladesh at Central Cattle Breeding Station and Dairy Farm, Savar, Dhaka, Rajshahi Dairy and Cattle Improvement Farm,

Rajabarihat and District Artificial Insemination Centre, Rajshashi, during 1997-2002. A total of 714 ejaculates of semen for head abnormalities and 676 for tail abnormalities were used. Bulls were classified into 4-5 groups according to their age, body weight, body conditions and scrotal circumference (Table-1). Age of the bulls was determined from the birth register and by examining their teeth. The body weight of each bull was recorded in kg using large weighbridge or calculated according to the Shaeffer's formula (McNitt, 1983): $L \times G^2 / 300 \times 2.2$ where L = body length in inch, and G = girth in inch. The body conditions of the bulls were scored into 1 to 4 scales (Nicholson & Butterworth, 1986). Measurements of the scrotal circumference were made at the area of the largest diameter of the scrotum using a cloth tape as described by Ott (1991).

Feeding and management of bulls: Stall-feeding was practiced regularly and concentrate feeds were given twice (mornings and evenings) daily. Concentrate feeds include chickpea, wheat bran, oil cake, rice bran, urea, molasses and salt. The green grasses like Para, Napier, maize and oats and in the form of silage were also supplied on the basis of year round availability. The bulls were also fed a balanced ration of 50% mixed concentrate hay supplemented with vitamins and minerals. All bulls were tested for fertility

Semen samplings: Semen was collected from the bulls using a standard artificial vagina at an interval of 3 to 4 days. The bull calves were selected, brought up in the farm and trained for semen collection from 18 months of age. Records such as the dates of collection, dates of culling and dates of death were maintained. Semen was sampled immediately after collection at a frequency of once in a month for each bull.

Staining techniques: A thin smear of fresh semen was prepared on a grease-free slide for the study of morphological abnormalities of sperm head after passing through Williams' stain. (Williams, 1920). The stain was prepared as follows: Stock solution-I contained 10 g basic Fuchsin and 95% alcohol. Stock solution-II consisted of saturated bluish eosin in 95% alcohol. Stock solution-III contained 10 ml stock solution-I and 170 ml 5% phenol. The final stain contained 25 ml stock solution-II and 50 ml stock solution-III. The stain was filtered before use. The smear was air-dried and fixed in flame. Such

information as bull ID and date of semen collection were marked on slide with the help of a permanent marker pen. Then the smear was treated with absolute alcohol for 3-4 min and washed with 0.5% chloramine for 1-2 min until it appeared fairly clear. Then it was washed in distilled water followed by rinsing in 95% alcohol and finally stained with Carbol-Fuchsin eosin for 8-10 min. After staining, the slides were washed in running tap water, dried off and examined under light microscope at 1000 \times .

Examination of sperm abnormalities: The normal sperm heads were free from any detectable abnormalities. Any head abnormalities in spermatozoa were detected and categorized according to Williams' (1920) classification. At least 500 spermatozoa from separate individual smears were examined for head abnormalities and microphotographs were taken. The morphology of sperm mid-piece and tail was studied after fixing with buffered Formol-saline at ambient temperature. The saline was prepared according to the method described by Hancock (1957) that consisted of 34.4 mmol di-sodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$), 18.7 mmol potassium di-hydrogen phosphate (KH_2PO_4), 92.6 mmol sodium chloride (NaCl), 1.54 mmol formaldehyde and distilled water to constitute 1000 ml.

One ml Formol-saline was taken in a sample glass tube and a small drop of fresh semen (10 μl) was mixed, shaken and marked with the information like bull ID, breed and date of semen collection. The sample was preserved in a refrigerator at 4 $^\circ\text{C}$ for later examination. The abnormalities of the smeared spermatozoa were observed under a phase contrast microscope at 1000 \times (Olympus CH-2, Japan), and some of them were selected for microphotographs. At least 200 spermatozoa from individual smears were examined for tail abnormalities.

Statistical analysis: Data were subjected to analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) using the computer programme SPSS (version 10.1). Interpretations were derived from the data categorized into total sperm abnormalities owing to age, body weight, body conditions and scrotal circumference of the bulls under study (Steel & Torrie, 1980).

Table 1. Bulls classified according to the parameters used.

Parameters	Groups				
	I	II	III	IV	V
Age (yrs)	<4	4 to <6	6 to <8	8 to <10	>10
Body weight (kg)	<450	450 to <550	550 to <650	>650	-
Body conditions	Average	Good	Very good	Excellent	-
Scrotal circumference (cm)	<34	34 to <36	36 to <38	>38	-

Results and Discussion

Head abnormalities, and mid-piece and tail abnormalities owing to the age, body weight, body conditions and scrotal circumference of the bulls under study have been presented in Plate 1 and Plate 2, respectively. Hence, the effects of different parameters on the incidence of sperm abnormalities are detailed in the following paragraphs. (Sharma *et al.*, 1991).

Age: Significant proportions of head and tail abnormalities were observed in bulls belonging to different age groups ($F_{4,492} = 5.591$; $P < 0.01$; Fig. 1). The semen of bulls <4 yrs of old contained the highest percentage of total head abnormalities of spermatozoa followed by 6-<8 yrs, 4-<6 yrs, 10 yrs and 8-<10 yrs age groups. There was no significant variation in the percentage of mid-piece and proximal cytoplasmic droplets abnormalities between the bulls under study. The highest mid-piece abnormalities of spermatozoa was found in 8-<10 yrs and the lowest in >10 yrs age

groups. The highest percentage of total tail abnormalities was recorded in 6-<8 yrs followed by 8-<10 yrs, <4 yrs, >10 yrs and 4-<6 yrs groups. The highest percentage of total sperm abnormalities (17.58%) was recorded in bulls of <4 yrs and the lowest (15.84%) in >10 yrs. Previous study showed that the age of bulls had highly significant effect on all the semen characteristics where the percentage of abnormal spermatozoa increased with growing age (Tomar *et al.*, 1985). Moreover, collection frequency, pre-collection sexual stimulation, feeding regime and climatic conditions can also influence the sperm abnormalities (Al-Hakim *et al.*, 1986). The present study indicated that age of the bulls has much bearing on the proportion of sperm abnormalities. The differences of sperm abnormalities, however, could also be due to variations in the breed, age and the secretory activities of the sex glands (Leon *et al.*, 1991).

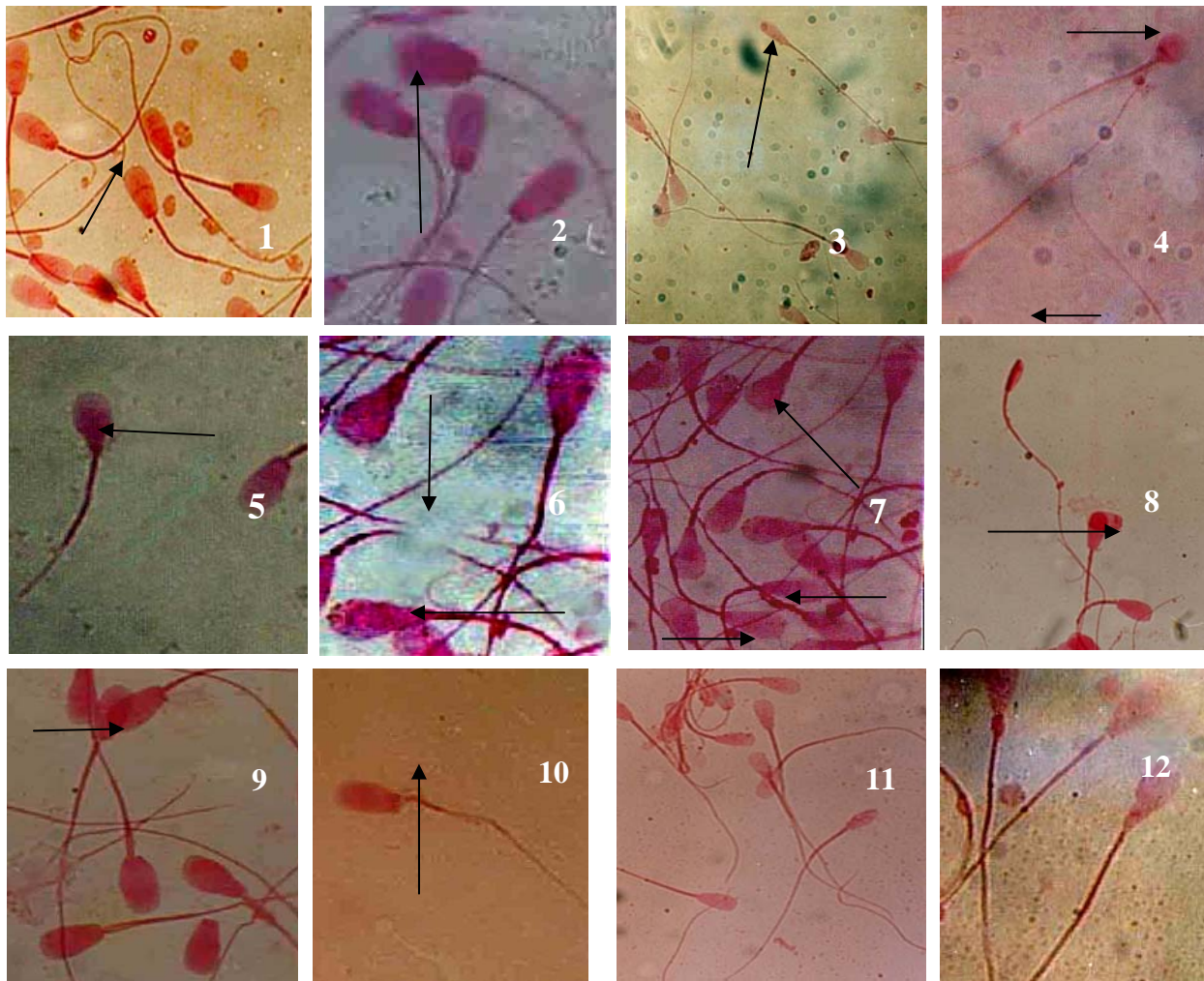


Plate 1. Microphotographs of normal and abnormal head morphology of bull spermatozoa (Williams' stain 1000x). (1) Normal (2) Big (3) Narrow (4) Round (5) Pear-shaped (6) Narrow at base (7) Constricted in lower portion (8) Undeveloped (9) Broad (10) Abaxial (11) Little and (12) Abnormal contour

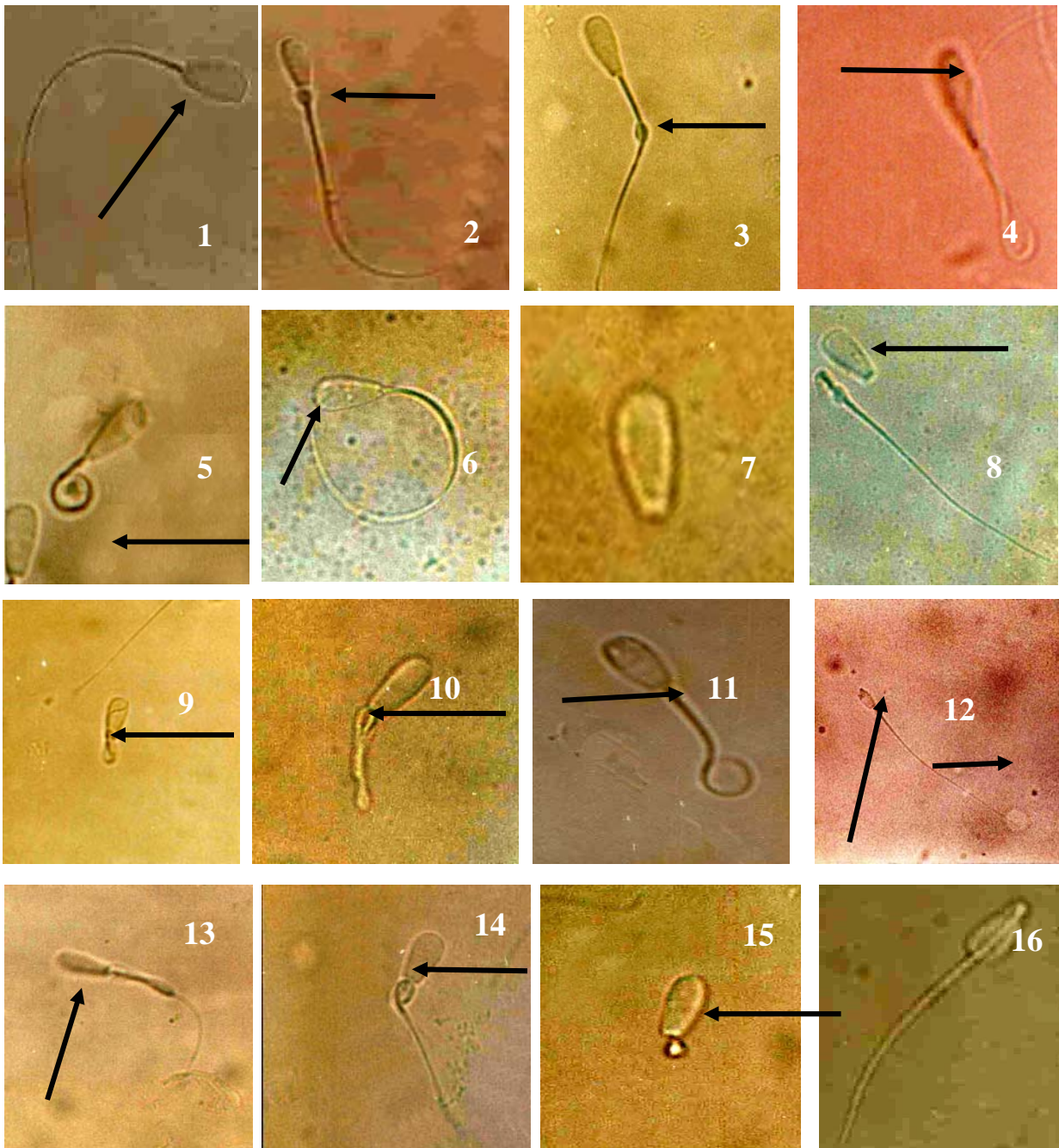


Plate 2. Microphotographs of normal and abnormal bull spermatozoa with respect to the mid-piece and tail (Phase contrast optics, 1000x). (1) Normal (2) Proximal cytoplasmic droplet (3) Distal cytoplasmic droplet (4) Simple bent (5) Coiled (6) Coil around the head (7) Free loose (8) Head detached from mid-piece (9) Mid-piece and tail defect (10) Double folded (11) Coiled in lower part (12) Abnormal acrosome (13) Folded (14) Mid-piece defect (15) Tailless mid-piece and (16) Tail broken at the neck

Body weight: The results on the sperm abnormalities due to body weight of the experimental bulls are presented in Fig. 2. The body weight was found to have a significant effect on the mid-piece and tail sperm abnormalities ($F_{3,493} = 2.643$; $P < 0.05$). The highest percentage of mid-piece abnormalities was observed in 450-<550 kg and the lowest in <450 kg weight groups. The highest proportion of total sperm abnormalities (17.07%) was recorded in bulls of >650 kg body weight

followed by 450-<550 kg, 550-<650 kg and <450 kg (abnormalities of 16.44%, 16.34% and 15.81%, respectively). Previous investigators (Saacke, 1970; Rao, 1971) observed 0-18% spermatozoa with abnormal morphology in fertile bulls whereas Zemjanis (1970) reported 30-40% total spermatozoon abnormalities in the semen of fertile bulls. Variations in the body weight and body size could give rise to differences in sperm abnormalities in bulls (Leon *et al.*,

1991; Sharma *et al.*, 1991). Sarder *et al.* (2001) demonstrated a significant effect of body weight on semen characteristics. These findings are in good agreement with that of the present study.

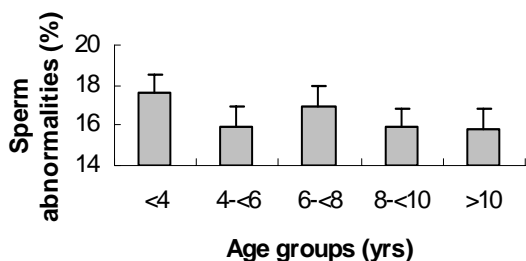


Fig. 1. Effect of age on total sperm abnormalities (mean±SD) in bulls used for AI.

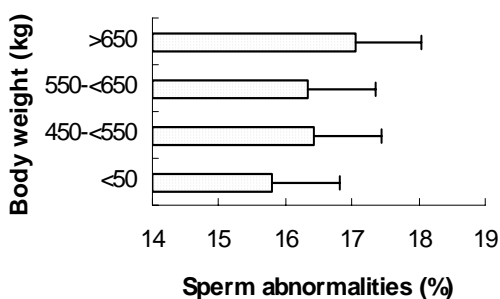


Fig. 2. Sperm abnormalities (mean±SD) in different body weight groups of bulls used for AI.

Body conditions: Fig. 3. shows a non-significant association of sperm abnormalities with the body conditions of the breeding bulls (Table 2). Notably the highest head abnormalities were recorded in bulls with excellent body condition, followed by average, very good and good body conditions. As has been reported earlier (Hossain *et al.*, 1990), abnormalities of the spermatozoa could occur due to disorder of the seminiferous tubules or germinal epithelium, which is directly or indirectly related to the body conditions of the bulls, the present results appear to fall within the normal variation range.

Scrotal circumference: The effect of scrotal circumference on the total abnormalities of spermatozoa in bulls is presented in Fig. 4. The over-all effect was statistically significant ($F_{3,493} = 3.293$;

$P < 0.05$), where the highest head abnormalities (6.04%) were recorded in <34 cm and the lowest (5.29%) in >38 cm scrotal circumference groups. The variations in the mid-piece abnormalities were non-significant between the groups. The highest proportion of mid-piece abnormalities was observed in 36-<38 cm and the lowest in <34 cm groups. The variations in the tail abnormalities were also found to be significant. The highest percentage of tail abnormalities was observed in >38 cm (6.64%) and the lowest in <34 cm (5.96%) groups. The highest percentage of total sperm abnormalities (17.00%) was recorded in >38 cm groups followed by <34 cm (16.76%), 34-<36 cm (16.39%) and 36-<38 cm (15.73%) groups. The sperm abnormalities occur during spermatogenesis either during spermatocytogenesis from spermatogonia to spermatids or during spermatogenesis from spermatids to spermatozoa (Blom, 1950). Previous reports suggest that young bulls with small scrotal circumference might have significantly lower fertility than the bulls with larger scrotum, and bulls with small testicles have semen with a higher percentage of sperm defects (Achnelt *et al.*, 1964). The present differences in sperm abnormalities between four groups of bulls, however, may not only be attributable to the variation of their scrotal circumference, but also to the differences in breed, age, body weight and body conditions coupled with the secretory activity of the sex glands (Leon *et al.*, 1991; Sharma *et al.*, 1991).

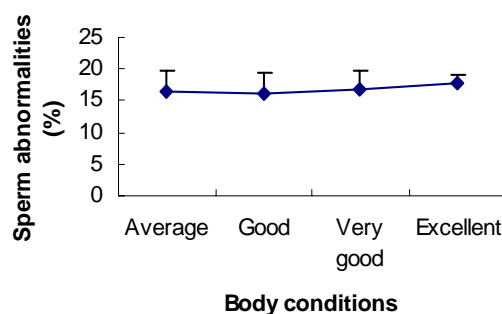


Fig. 3. Effect of body conditions on total sperm abnormalities (mean±SD) in AI bulls.

Table 2 Analysis of variance table showing the total sperm abnormalities due to the age, body weight, body conditions and scrotal circumference of 71 bulls used for AI programme.

Parameters	Sources of variation	Df	Sum of squares	Mean of squares	F-values
Age	Between groups	4	230.945	4.533	5.591**
	Within groups	492	5080.978	10.327	
Body weight	Between groups	3	84.088	28.029	2.643*
	Within groups	493	5227.835	10.604	
Body conditions	Between groups	3	45.683	15.228	1.426 ^{ns}
	Within groups	493	5266.682	10.682	
Scrotal circumference	Between groups	3	44.994	14.998	3.293*
	Within groups	493	5199.038	10.546	

Df = degrees of freedom; * = $P < 0.05$; ** = $P < 0.01$.

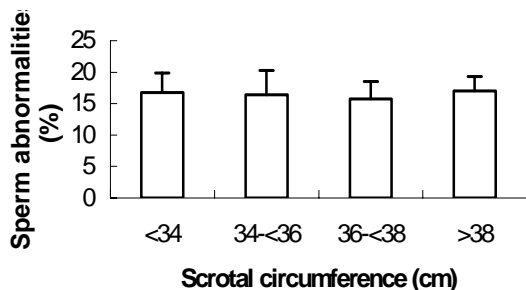


Fig. 4. Effect of scrotal circumference on total sperm abnormalities (mean±SD) in AI bulls.

The present results suggest that the bulls of 8 to >10 yrs, <450 kg body weight, having very good body condition and 36 to <38 cm scrotal circumference ensure the lowest percentage of sperm abnormalities. These findings are of immense importance for the selection of breeding bulls to be used for the AI programme in different farms in the country.

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