Vol. XI (1-2) 1974

STUDIES ON THE MORPHOLOGY AND ABNORMALITIES OF BUFFALO SPERMATOZOA

Muhammad Asghar Malik, Manzoor Ahmad, Abrar H. Gilani and Rashid Ahmad Chaudhry*

Two semen samples for detailed morphological studies were obtained from eight sexually normal buffalo bulls of Nilli-Ravi breed with a gap of 25 days between these samples. During this period regular biweekly collections from these bulls were also made.

The differences between bulls and within each bull was found to be non-significant so far as volume, concentration and the size of the normal spermatozoa was concerned. The overall average length of buffalo bull spermatozoa was 75.4±0.3 μ. The average sizes of head, mid and main piece and tail were 8.3, 4.25 μ, 12.3 u and 54.8 μ, respectively. The percentage occurrence of pathological spermatozoa in buffalo bull semen varied significantly among the various bulls under study. The range of abnormal spermatozoa was 7.5 to 18.5 and 4.3 to 14.3 per cent in stained and unstained smears, examined under ordinary and phase-contrast microscopes, respectively. The highest frequency of abnormal spermatozoa was because of head and tail defects. The abnormalities of neck, mid and main pieces were of very low frequency. The defective heads were; narrow at the base, pear shaped, giant, dwarf, free and double heads. The tail abnormalities included, tails around the head, coiled, free and double tails.

The percentages of abnormal cells recorded under phase contrast microscope (9.2±2.1) and ordinary microscope (11.9±2.8) in the semen of the buffalo bulls were within limits which had been described as normal for sexually normal and healthy breeding cow bulls.

* Faculty of Animal Husbandry, University of Agriculture, Lyallpur.

INTRODUCTION

Since the male animal is used to serve a large number of females every year, its reduced and ceased fertility causes serious economic losses to the livestock industry. According to certain available estimates, 36—48 per cent of the bulls used in artificial insemination are slaughtered because of low breeding efficiency (Roman and Wilcox, 1960).

The productive efficiency of male animals varies in degree. Any inherited anatomical defect or testicular disease modifies the quality and quantity of semen. Evaluations on the alterations in the structure of spermatozoa can be helpful in predicting the fertility of a breeding animal. Comprehensive studies on the morphology of buffalo bull spermatozoa are lacking and the relationships of spermatozoa abnormalities to the breeding efficiency of this species are almost absent. The present investigation was, therefore, undertaken to study the morphology of normal buffalo bull spermatozoa and to observe the various types of abnormalities which existed. The percentage of these defects in different samples of semen used in this study were also estimated.

MATERIAL AND METHODS

The buffalo bulls (Bos bubalus) used in this study were those of Nili-Ravi breed, maintained at Artificial Insemination Centre, Lyallpur, Gujrat, Sialkot, Sheikhupura and Jhang. Two semen samples for detailed morphological studies were obtained from each bull with a gap of 25 days between these samples. However, during this period regular biweekly collections from these bulls were also made for artificial insemination purposes. The semen after collection was immediately removed to the laboratory and placed in a water bath maintained at 37 °C. The physical characteristics like appearance, colour, consistency, sperm concentration and motility were also recorded along with detailed morphological examinations.

The morphological evaluation of spermatozoa was carried out on stained smears using ordinary microscopy. The semen smears were stained with William's Stain, washed with distilled water and air dried. Following Comstock *et al.* (1943), 500 spermatozoa were counted from each stained smear, under the oil immersion lens and percentage of abnormal cells in each sample was calculated.

For the micrometery of the spermatozoa, the length and the breadth of the whole stained cell was measured. Separate measurement of head, neck,

middle piece, main piece and tail were also recovered using the occular and the stage micrometer. To work out the average length and breadth of the whole cell and parts thereof, 200 cells from each stained smear were counted. Abnormal spermatozoa were not included in these measurements.

For the phase contrast microscopy, a drop of freshly collected semen was added to 1 to 2 ml of buffered normal saline solution. After placing a cover slip on a small drop of the diluted semen, it was sealed on slides with canada balsam to prevent drying. In order to calculate the percentage of abnormal cells, 200 cells were counted from each sealed slides using the phase contrast microscope. The various types of abnormalities encountered during these examinations were classified according to Lagerlof (1934).

RESULTS AND DISCUSSION

In order to evaluate the morphology of spermatozoa, a study on the semen samples of eight buffalo bulls of Nili–Ravi breed maintained at different Artificial Insemination Centres in the Punjab was undertaken. The results obtained are discussed as under :

Semen Volume. The volume of semen per ejaculate varied from 1.80 to 4.50 ml, with an average of 3.20 ml. The difference in volumes of semen within each bull and between bulls was non-significant. Ahmed *et al.* (1964) reported the average volume of semen per ejaculate to be 3.4 ml, which ranged from 2—6 ml in Nili–Ravi bulls, involving a much larger number of semen samples. The sperm concentration of these ejaculates ranged from 1,000 to 2,000 million per ml.

Micrometery. The heads of the spermatozoa of the buffalo bulls under study had an average length and breadth of 8.3, and 4.25, respectively. These measurements in different samples varied from 8.2 to 8.5 and 4.21 to 4.32, respectively. It was observed that the normal head of the buffalo spermatozoa had a specific rectangular shape and had no resemblance with the cow bull spermatozoa. It was, therefore, easily distinguishable.

It was recorded that the main and mid pieces of these spermatozoa on an average measured 12.3 μ with range from 12.0 to 12.6 μ. Guha *et al.* (1959) found the average length of the same parts as 12.5 μ in case of Murrah buffalo of India. The length of these parts was greater in bovine than in the buffalo.

The average length of the tail of the spermatozoa of these bulls was found to be 54.8 μ, with a range of 54.7 to 55.0 μ. Guha *et al.* (1959) reported

the average size of the tail of the spermatozoa of Indian Murrah bulls to be 54.6 μ. The tail of bovine spermatozoa on an average was 50.3 μ as reported by Mac-Gregor (1941) and 50 μ according to Rikmenspoel (1957). This indicates that the length of the tail of buffalo spermatozoa is larger than that of bovine spermatozoa. In this study the overall length of buffalo bull spermatozoa was 75.4±0.3 μ. From the available information it was concluded that although the average length of head and main and mid pieces of bovine spermatozoa was greater than the buffalo spermatozoa, yet the overall length of buffalo spermatozoa was greater than bovine spermatozoa because of its larger tail.

Morphological Studies (Under Ordinary Microscope)

The smears prepared from semen samples were stained with Williams's stain and examined under oil immersions lens. A significant difference was found in the total abnormal cells between experimental bulls ($P<0.05$). The overall average of pathological spermatozoa were 11.911±2.765 per cent, out of which the incidence of pathological heads was highest (6.2 per cent), than any other defect. The defective tails formed the next higher abnormal structure (3.8 per cent). The details of the abnormal cells recorded in the semen of the buffalo bulls are given below :

(a) The defective heads were 6.187±2.383 per cent, comprising narrow heads at the base, pear shaped, giant heads, dwarf heads, free heads and double heads.

(b) The enterior end of the mid-piece connecting the head termed as neck or implantation region had 0.36 per cent abnormalities which were either broken or had abaxial attachment.

(c) The mid piece defects were 0.49±0.15 per cent. The abnormalities included abaxial, broken, truncated and kinky appearances.

(d) The main piece defects were 0.200±0.165 per cent, defects were breakage and swelling.

(e) The tail defects were 3.862±0.910 per cent.

(f) The cytoplasmicdroplets, which were 0.625±0.240 per cent included proximal and distal placements.

The buffalo bulls of the present study possessing a low percentage of abnormal cells (average 11.9±2.8) had no history of impaired fertility. These studies agree with the findings of Lagerlof (1934) who gave a ratio of pathological sperms for normal fertile bulls as 10 to 11 per cent, whereas it was 35—50 per cent for a bull of reduced fertility.

Morphological Studies Under Phase Contrast Microscope)

The difference in the total abnormal cells among the buffalo bulls was significant ($P<0.05$). It was also recorded that some spermatozoal defects, like shape of head, neck attachment and cytoplasmic droplets were more clear when examined through this procedure. The range of abnormal spermatozoa in these studies was from 4.3 to 14.3 per cent with an average of 9.2 per cent. The following average results were recorded from the various types of defects :

Head abnormalities	..	4.97 per cent
Neck abnormalities	.	0.62 " "
Proximel cytoplasmic droplet	..	0.56 " "
Mid piece abnormalities	..	0.34 " "
Main abnormalities	..	0.19 " "
Tail abnormalities	.	2.48 " "
Total abnormalities		9.16 " "

The percentage of abnormal cells recorded by the phase contrast microscopy and on stained smears was within a range which has been described as normal for sexually normal and healthy breeding cow bulls. In the present study no defects other than mentioned in the classification advanced by Lagerlof (1934) were detected.

LITERATURE CITED

Ahmed, M., G.K. Garlik, I.R. Khan, M. Ahmad, and I. Ahmad. 1964. An experiment of Artificial Insemination in West Pakistan. Agri. Pak. 2: 124—133.

Comstock, R.E., W.W. Green, L.M. Winters, and A.W. Norskog. 1943. Studies of semen and semen production. Minnesota Agri. Expt. Station Tech. Bull. 162.

Guha, S.R., D.P. Mukerjee, and A.P. Labhsetwar. 1959. A.I. in Farm Animals. Rutgers University Press New Jersey, 3rd Ed.

Lagerlof, N. 1934. Classification of abnormal spermatozoa. 8th FAO–Swedish International Post-graduate Veterinary Course in Animal Reproduction. Royal Veterinary College, Stockholm, 50, Sweden, 1:34.

MacGregor, R. 1941. The Domestic buffalo. Vet. Rec. 53, 433—450.

Rikmenspoel, R. 1957. Photo electric and Cinematographic measurement of motility of bull sperm cells. In Physiology of Reproduction and Artificial Insemination of Cattle. W.H. Freeman and Co. London.

Roman, J. and C.J. Wilcox. 1969. Tenure and reasons for disposal of A.I. Dairy sires. J. Animal Sci. 27(5): 139.