

## **Mensuration of spermatozoa from different levels of the reproductive tract of the buffalo-bull (*Bubalus bubalis*)**

par A. K. SHARMA, R. C. GUPTA

*Department of Veterinary Gynecology and Obstetrics  
Haryana Agricultural University Hissar, India.*

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**Summary.** The dimensions of spermatozoa from different levels of the reproductive tract were measured in 6 buffalo-bulls castrated under local anesthesia ; the same measurements were taken in the semen of 6 breeding buffalo-bulls. During transit from caput to cauda epididymis the sperm showed a significant increase in the length of head and main piece. However, a significant decrease was observed in middle piece length. In ejaculated sperm there was a significant decrease in total length and in head and main piece length.

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### **Introduction.**

Important maturation changes take place in the spermatozoa of most, and perhaps all, mammalian spermatozoa during transit through the epididymis ; during this transit, the spermatozoa acquire fertilizing ability. It has been determined that fertilizing ability increases significantly as the spermatozoa approach the cauda epididymis. Several other differences presumed to be associated with developing maturity have been reported, such as movement and eventual loss of the protoplasmic droplet (Rendez, 1926 ; Nicander, 1958), change in heat resistance (Young, 1931), response to cold shock (Lasley and Bogart, 1944), harmful alkaloids (Metalnikov, 1911), acids and alkalies (Yochem, 1930), evidence of increase in specific gravity (Lavon, Amir and Danon, 1966 ; Levine and Marsh, 1971) and significant decrease in spermatozoal dimensions from testes to ejaculate (Osman, 1973).

In spite of the great interest in this field, little is known of the changes in spermatozoal dimensions during transit through the reproductive tract of the buffalo. The present study investigates the changes in different spermatozoal dimensions occurring during transit through this tract.

### **Material and methods.**

The testes of 6 sexually mature buffalo-bulls were removed by the open method of castration under local anesthesia. The epididymis was separated from the testis in the laboratory and a small incision was made in the epididymal region (caput, corpus

and cauda). The fluid from each of these parts was mixed separately with buffered eosin nigrosin stain in a watch glass. Semen was also collected from 6 breeding buffalo-bulls on a local farm by artificial vagina ; a drop from each sample was mixed with buffered eosin nigrosin stain. The stained sperm suspension was directly smeared with a second slide on pre-cleaned slides and dried immediately.

The different dimensions of 32 stretched spermatozoa from each epididymal region (8 sperm  $\times$  4 stained slides per region) as well as from the ejaculated semen sample of each of the 6 bulls were measured to the nearest fraction of a micron using an ocular micrometer scale. A single observer randomly measured every segment and bull sample. The dimensions measured included total sperm length, length and greatest width of head, length of middle and main pieces. Tail length was computed by subtracting the head length from total sperm length. The result was expressed by the mean and standard error ; the differences in sperm dimension of the different reproductive tract levels as well as of the bulls were tested for significance using the methods of Duncan (1955) and Snedecor (1956).

## Results.

The measurements and their statistical analysis are shown in table 1. Total sperm length and length of head and main piece increased significantly from caput to cauda epididymis. However, middle piece length decreased significantly during transit through this region. Although a decrease in most sperm dimensions was observed from cauda epididymis to ejaculate, a significant decrease was noted only in the length of head and main piece. No statistically significant difference ( $P > 0.05$ ) was observed between spermatozoal dimensions in different reproductive tract levels of castrated bulls and those in ejaculates obtained from breeding bulls.

TABLE I

*Measurements (in  $\mu$ ) of spermatozoa from different reproductive tract levels and from buffalo-bull semen*

Reproductive tract levels	Total sperm length	Sperm head		Sperm tail		
		Length	Greatest Width	Total length	Middle piece length	Main piece length
Epididymis :						
Caput . . . . .	67.69	8.41 <sup>a</sup>	4.90 <sup>a</sup>	59.27	13.84	45.43
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.89	0.10	0.10	0.86	0.35	0.77
Corpus . . . . .	70.29 <sup>a</sup>	8.86 <sup>b</sup>	5.09 <sup>a</sup>	61.43 <sup>a</sup>	13.17 <sup>b</sup>	48.26 <sup>a</sup>
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.43	0.14	0.14	0.78	0.45	0.66
Cauda . . . . .	71.44 <sup>a</sup>	8.74 <sup>b</sup>	5.09 <sup>a</sup>	62.70	12.91 <sup>a</sup>	49.79
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.75	0.10	0.14	0.53	0.35	0.42
Semen . . . . .	69.35 <sup>a</sup>	8.43 <sup>a</sup>	4.92 <sup>a</sup>	60.92 <sup>a</sup>	13.11 <sup>ab</sup>	47.81 <sup>a</sup>
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.61	0.10	0.17	0.25	0.14	0.22

$\pm$  Standard error. Any two means having the same letter (a or b) do not differ significantly at the 5 p. 100 level.

## Discussion.

Several methods have been employed for measuring spermatozoa (Van Duijn, 1975), but the most routinely used method in light microscopy for measuring the linear dimensions of spermatozoa is by direct micrometry with a pre-calibrated micrometer eye-piece. Factors such as heredity, age, season, nutrition, staining method, preparation technique and optical system have also been shown to cause mensuration variation (Van Duijn, 1975). Besides these, individual and species variation and the site from which the spermatozoa are taken have also been reported to cause differences in spermatozoal measurements (Van Duijn, 1975).

In the present investigation, no statistically significant difference was observed in the dimensions of bull spermatozoa. However, significant changes ( $P < 0.05$ ) were observed in spermatozoal dimensions during transit through the epididymis. The length of the head increased and middle piece length decreased significantly from caput to cauda epididymis, a significant decrease in the length of the head and main piece was observed in the ejaculated sperm as compared to the sperm collected from the cauda epididymis (table 1).

The mensuration changes during transit through the epididymis may possibly be due to biochemical and physiological modifications occurring during this time. Increased formation of disulfide bonds (Prasad and Rajalakshmi, 1977) and aggregation of DNA and basic protein complexes (Fawcett, Anderson and Phillips, 1971) during transit from caput to cauda epididymis may be the factors responsible for greater head length. Decrease in middle piece length may be attributed to structural condensation of the organelles of that part. Since considerable variation in the chemical composition of epididymal fluid and electrolytes has been reported in different epididymal regions of rat (Howard, Johnson and Jessee, 1975), bull (Crabo and Gustafsson, 1964) and buffalo-bull (Sharma, Chaudhry and Gupta, 1977), it is believed that structural condensation may be caused by this variation in fluid and electrolytes. Dimensional changes in ejaculated spermatozoa may also be due to further structural condensation of the organelles because of the high degree of permeability in the exchange reactions taking place between the spermatozoa and the surrounding medium (Mann, 1964).

Similar changes have also been reported by Oivindis (1968) in the bull and by Osman (1973) in the buffalo-bull. The different dimensions of mature spermatozoa in buffalo semen are similar to those reported by Kodagali, Bhavsar and Deshpande (1973).

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**Résumé.** Les dimensions du spermatozoïde de Buffalo ont été mesurées à différents niveaux de l'épididyme chez 6 animaux et dans l'éjaculat de 6 mâles fertiles. Les testicules ont été prélevés sous anesthésie. Pendant le transit de la tête à la queue de l'épididyme on observe un allongement de la tête et du flagelle du spermatozoïde. Dans le sperme éjaculé, les dimensions régressent significativement ; au niveau de la pièce intermédiaire l'évolution est inverse.

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