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### ARTICLE ARTIKEL

## Changes in Mithun (*Bos frontalis*) spermatozoa during epididymal passage

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### ABSTRACT

Genital organs of 10 healthy, adult Mithun bulls (6-8 years old) that were slaughtered at the dwellings of tribal people for meat were collected. Immediately after collection, spermatozoa from 3 different regions of the epididymis, *i.e.* the head, body and tail, were obtained to study morphological changes of the spermatozoa during passage through these regions. The prevalence of proximal cytoplasmic droplets significantly decreased from the head to the tail of the epididymis. Conversely, the percentage of distal cytoplasmic droplets increased significantly from the head to the tail region. The incidence of tailless heads rose significantly from head to body and then reduced significantly in the tail region. The percentage of total head abnormalities did, however, not change markedly, but total mid-piece and tail abnormalities differed significantly between the three epididymal regions.

**Keywords:** epididymal passage, male reproduction, Mithun, sperm morphology.

## INTRODUCTION

The Mithun (*Bos frontalis*) is a semi-wild bovid<sup>13</sup>. The World Conservation Monitoring Cell lists this 'ceremonial ox' of northeastern mountainous regions of India as endangered. It plays an important role in the socioeconomic life of the tribal communities but is yet to be scientifically studied in detail<sup>4</sup>. The Mithun is usually kept as a free-ranging animal in forests. Tribal people slaughter the Mithun for its meat, which they consider to be precious.

The number of Mithuns has declined greatly in recent times, creating the need to identify fertile males to use as sires in order to conserve the species. In the bull, the most striking morphological change in the spermatozoa during their passage through epididymis is the migration of the cytoplasmic droplet from the proximal to the distal end of the mid-piece<sup>9,12</sup>. Other changes have also been described<sup>3,8</sup>. Knowing the morphological changes in spermatozoa during epididymal passage in the Mithun will help define semen quality in the species. Thus the present study examines morphological changes as the spermatozoa pass through the epididymis.

## MATERIALS AND METHODS

The present study was conducted in the National Research Centre on Mithun (NRCM), Indian Council of Agricultural Research (ICAR), Nagaland, India. Genital organs of 10 healthy, adult Mithun bulls aged 6-8 years were collected from the dwellings of tribal people when they sacrificed them during marriage ceremonies. The animals were found to be in good health and no physical abnormalities were noticed pre-or post-slaughter. Different parts of the external and internal genitalia were also free of gross pathological changes.

The study was conducted from January to mid June. Maximum and minimum temperatures varied between 21.0 and 9.4 °C in January and between 30.0 and 25.71 °C in June. Monthly rainfall was 9.4-289.5 mm and relative humidity 72.5-83.8%. Immediately after collection the genital organs were transported to the laboratory in normal saline solution at 37 °C. The epididymis was dissected from the testis and epididymal fluid was obtained by incision with a scalpel followed by gentle pressure on the central part of each of three regions of the epididymis, namely the head, body and tail. The epididymal fluid containing spermatozoa from these regions was suspended separately in buffered formal saline. The suspended fluid was further suitably diluted in buffered formal saline to facilitate the study of sperm characteristics, namely proximal cytoplasmic droplets, distal cytoplasmic droplets, tailless heads and mid-piece and tail abnormalities, using a phase contrast microscope. Sperm head abnormalities were studied under oil immersion ( $\times 100$ ) using a light microscope. The epididymal fluid suspension was used to prepare the thin smears stained with Carbol-fuchsin-eosin stain<sup>17,11</sup>. Hundreds of spermatozoa per region of each epididymis were examined. The Mann-Whitney test was used to test for differences between the right and left parts of the epididymis, while the Kruskal-Wallis test was used to test for differences between the head, body and tail of the epididymis.

## RESULTS

As the spermatozoa pass through the epididymis there is a successive and significant reduction in the prevalence of proximal cytoplasmic droplets between the head, body and tail, and droplets were virtually absent in the tail ([Table 1](#)). On the other hand, the prevalence of distal cytoplasmic droplets increased significantly in succession in the 3 epididymal regions. The occurrence of tailless heads rose significantly from head to body and then decreased significantly in the tail region ([Table 1](#)). Percentage of total sperm head abnormalities did not differ significantly between the 3 regions. Abnormalities of the mid-piece and tail were absent in the head and increased significantly from there to the body and further to the tail. No significant difference was found between the right and left parts of the epididymis.

**Table 1: Occurrence of sperm abnormalities (mean  $\pm$  SE) in different regions of the epididymis in Mithuns (*Bos frontalis*).**

	Head (%)	Body (%)	Tail (%)	P-value
Proximal cytoplasmic droplet	37.36 $\pm$ 2.22*	10.55 $\pm$ 0.61*	0.09 $\pm$ 0.09*	0.000
Distal cytoplasmic droplet	19.18 $\pm$ 0.92*	23.36 $\pm$ 1.91*	37.09 $\pm$ 0.09*	0.000
Tailless head	2.45 $\pm$ 0.67*	5.20 $\pm$ 1.60*	1.45 $\pm$ 0.40*	0.000
Total head abnormalities	1.45 $\pm$ 0.41	1.36 $\pm$ 0.41	1.67 $\pm$ 0.32	0.560
Total mid-piece abnormalities	0*	0.82 $\pm$ 0.33*	2.27 $\pm$ 0.45*	0.000
Total tail abnormalities	0*	0.09 $\pm$ 0.09*	1.45 $\pm$ 0.39*	0.001

\*Significant at  $P < 0.05$ , SE = standard error.

## DISCUSSION

The increase in the prevalence of distal cytoplasmic droplets from the head to the tail regions of the epididymis corresponds to the migration process of the cytoplasmic droplet from a proximal to distal position during epididymal transit. The significant decrease in proximal cytoplasmic droplet and significant increase in distal cytoplasmic droplet percentages in the spermatozoa from the head to the tail regions of the epididymis was also reported by earlier workers in bulls<sup>1</sup>, rams<sup>2</sup>, boars<sup>16</sup> and goats<sup>15</sup>. The finding in the current study that the prevalence of tailless spermatozoa is higher in the body of the epididymis than in the head or tail is similar to that of Rao<sup>14</sup>, who showed a higher prevalence in the distal part of the head compared to the tail. Since the neck region of spermatozoa is vulnerable and fragile, the sperm head can become detached from the mid-piece with the initiation of motility, which takes place at the base of the sperm head<sup>5</sup>. Spermatozoa acquire motility between the head and tail of the epididymis and hence the scope for detachment of the head of the spermatozoa could be greatest in the lower head or body, which might explain the significantly higher incidence of tailless heads in the body region. The significant reduction in the incidence of tailless heads in the tail could be attributed to the dissolution and liquefaction<sup>3</sup> or phagocytosis within the tail epididymis. In agreement with the current study showing no effect of epididymal passage on the prevalence of sperm head abnormalities, Igboeli<sup>10</sup> observed that the prevalence of sperm head abnormalities is also similar in the head and tail of the epididymis of domestic bulls. The present finding in respect of tail abnormalities is in general agreement with observations of earlier workers<sup>2,6</sup> that most of the tail abnormalities arise after the spermatozoa leave the testis. Cury and Vinha<sup>7</sup> also reported that the incidence of tail abnormalities increased with the passage of spermatozoa through the epididymis. The increase in the percentage of mid-piece and abnormalities in the tail compared to the body could be ascribed to the exposure of the spermatozoa to varying biochemical microenvironments during their passage through the epididymis that might influence the development of sperm tail abnormalities. The present study revealed that the changes in sperm characteristics during epididymal passage in Mithun bulls are similar to those previously recorded in bulls but with some variation.

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