Processing-related sperm morphological changes in Jersey bulls

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Abstract

The present study evaluated sperm morphological abnormalities in eight Jersey bulls maintained at the Livestock Development Board Sperm Station, Palampur, using Rose Bengal staining. Sixty-four ejaculates (eight per bull) were collected twice weekly and examined at four semen-processing stages: post-dilution, equilibration, post-thaw, and one-hour post-thaw incubation. Sperm abnormalities were classified as head, midpiece, and tail defects. Significant differences ($p < 0.05$) among defect categories were observed at equilibration, post-thaw, and one-hour post-thaw incubation, while at post-dilution the significant difference was between midpiece and tail abnormalities. Total abnormalities remained below 10% at all stages, indicating acceptable morphological quality throughout processing.

Keywords: Jersey bull, Morphological abnormalities, Midpiece, Head, Tail

Introduction

A standard component of breeding-bull fertility evaluation is assessment of semen quality. Sperm morphology is an important indicator of semen quality because structural defects may adversely affect sperm function and fertilizing potential. Recent studies have also shown that semen processing and cryopreservation can alter bull sperm functional and molecular characteristics (Kumar et al., 2015; Samuel King et al., 2022).

Sperm abnormalities are commonly classified according to their location in the head, midpiece, or tail. Head defects include abnormal head size or shape and double heads; midpiece defects include bent necks, abnormal insertion, and changes in midpiece thickness; and tail defects include short, broken, bent, coiled, or multiple tails. Evaluation of these abnormalities at successive processing stages is useful for identifying morphological changes associated with semen dilution, equilibration, freezing, thawing, and subsequent incubation (Hussain et al., 2016; Nain et al., 2026). Therefore, the objective of the present study was to evaluate processing-related changes in head, midpiece, and tail sperm morphological abnormalities in Jersey bull semen at post-dilution, equilibration, post-thaw, and one-hour post-thaw incubation stages.

Materials and Methods

The study was conducted at the Livestock Development Board Sperm Station, Palampur, India (32.6°N, 76.30°E; altitude 1290.8 m), using semen from eight Jersey bulls. Semen collection was carried out during the winter season, twice weekly over a four-week period, using the artificial vagina technique. A total of 64 ejaculates (eight from each bull) were collected. After collection, semen was diluted with Tris–citric acid–egg yolk extender and a smear was prepared immediately at the post-dilution stage. Extended semen was then equilibrated, frozen in straws, thawed, and examined immediately after thawing and after one hour of post-thaw incubation. Thawing was performed at 37°C for 30 s. Smears from each processing stage were stained with Rose Bengal solution (Rose Bengal 3 g, distilled water 99 ml, and formalin 1 ml). Head, midpiece, and tail abnormalities were examined and classified according to standard morphological criteria. This sequence enabled stage-wise assessment of processing-related sperm morphological changes.

Results

The percentages of head, midpiece, and tail abnormalities are presented in Table 1, percentage changes are shown in Table 2, and representative morphological defects are illustrated in Figure 1 (A–L). Total abnormalities increased from 8.20% at post-dilution to 9.58% after one-hour post-thaw incubation. The most frequently observed defects included distal midpiece reflex, coiled tails, detached heads, short and stump tails, Dag defects, proximal and distal cytoplasmic droplets, and microcephalic or macrocephalic heads. Less frequent defects included double heads, double tails, and thick midpieces (Figure 1). Significant differences among head, midpiece, and tail abnormalities were observed at equilibration, post-thaw, and one-hour post-thaw incubation.

Common abnormalities included distal midpiece reflex, coiled tails, detached (free) heads, short tails, stump tails, Dag defects, proximal and distal cytoplasmic droplets, and microcephalic or macrocephalic heads. Rare abnormalities such as double heads, double tails, and thick midpieces were also observed (Figure 1). A significant difference was noted among head, midpiece, and tail abnormalities at the equilibration, post-thaw, and one-hour post-thaw incubation stages of processing.

Table 1: Stage-wise Sperm Abnormalities in Jersey Bull Semen

Stage	Head	Midpiece	Tail	Total
Post-dilution	2.75±0.68^{A}	1.48±0.59^{AB}	4.01±0.60^{AC}	8.2±1.66
Equilibration	2.85±0.59^{A}	0.97±0.15^{B}	4.52±0.57^{C}	8.34±1.21
Post-thaw	2.85±0.61^{A}	0.85±0.12^{B}	5.4±0.78^{C}	9.1±1.33
1 hr post thaw incubation	2.93±0.67^{A}	0.98±0.19^{B}	5.66±0.62^{C}	9.58±1.35

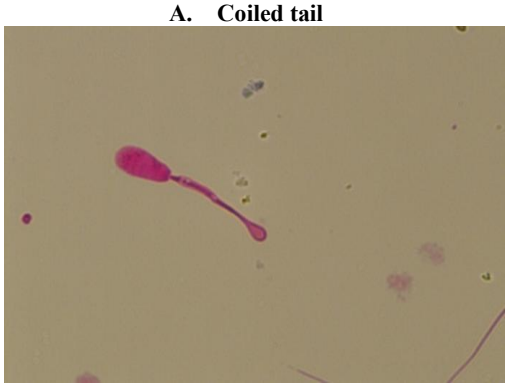
Values with different superscripts (A-C) within row differ significantly ($p<0.05$)

Table 2: Percentage Change in Sperm Abnormalities at Different Semen Processing Stages

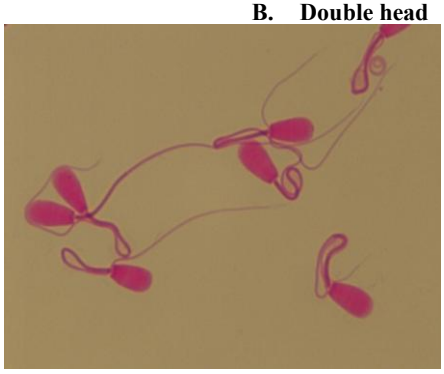
Type of Abnormality	Post-dilution (%)	Change to Equilibration (%)	Change to Post-thaw (%)	Change to 1 hr Post-thaw (%)
Head	2.75	+3.6	0.0	+2.8
Midpiece	1.48	-34.5	-12.4	+15.3
Tail	4.01	+12.7	+19.5	+4.8
Total abnormalities	8.20	+1.7	+9.1	+5.3

A. Coiled tail

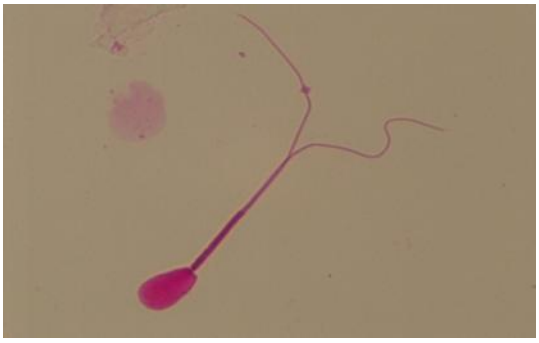
B. Double head



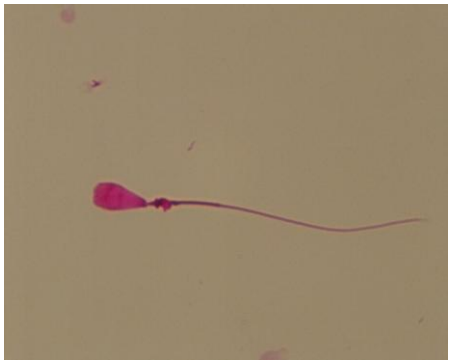
C. Distal midpiece reflex



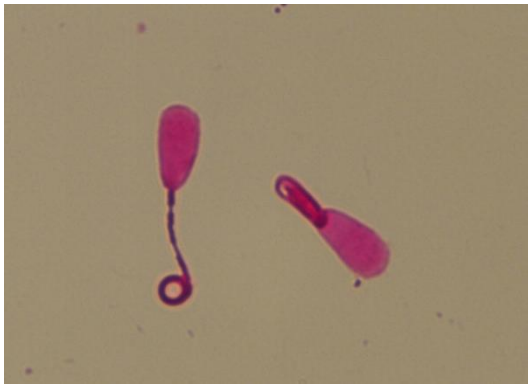
D. Bent tail



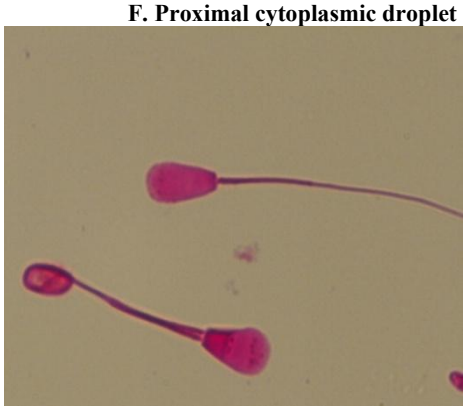
E. Double tail



F. Proximal cytoplasmic droplet



G. Coiled tail and dag defect



H. Coiled tail and thick midpiece

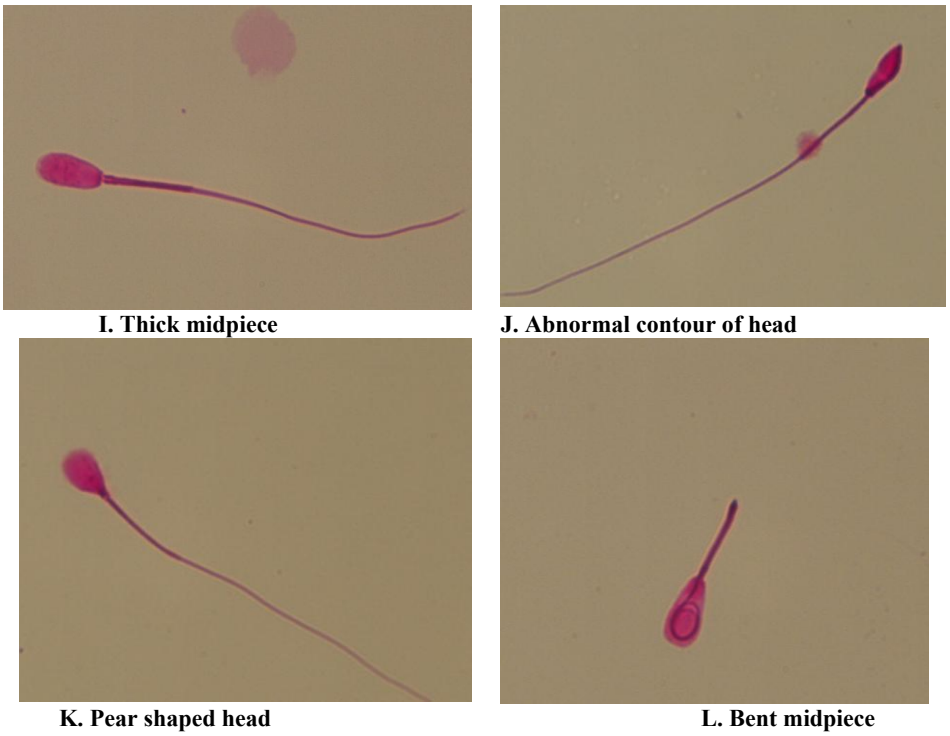


Figure 1. Microscopic views of structural abnormalities in Jersey bull spermatozoa

Discussion

The stage-wise pattern of sperm abnormalities was broadly comparable with previous reports showing changes in bull semen quality during dilution, cooling, and freezing (Hussain et al., 2016). In Jersey bulls, Kumar et al. (2015) also reported an increase in total abnormal sperm after freezing. In the present study, total abnormalities increased from 8.20% post-dilution to 9.58% after one-hour post-thaw incubation, with tail abnormalities showing the clearest increase across the processing sequence. Cryopreservation can affect sperm structural and functional integrity, and the equilibration stage may represent an important point of change in bull sperm quality (Arunkumar et al., 2022). Similarly, semen dilution conditions can influence post-thaw sperm function (Lone et al., 2022). The morphological abnormalities recorded in the present study remained below 10% at all stages, indicating that the observed processing-related changes were limited in magnitude under the conditions used. Recent work further emphasizes the value of stage-wise assessment of bull sperm quality during cryopreservation (Nain et al., 2026).
Sperm morphology is also sensitive to sample handling and storage conditions, emphasizing the need for standardized assessment procedures when comparing processing stages (Gunn and Baker, 2024). In the present study, the principal changes were observed in tail and overall abnormalities after cryopreservation and incubation, supporting the use of stage-wise morphological evaluation to monitor processing-related changes in Jersey bull semen.

Conclusion

Stage-specific changes in head, midpiece, and tail abnormalities were observed during semen processing, with the most evident changes occurring after cryopreservation and one-hour post-thaw incubation. Total abnormalities remained below 10% throughout the processing sequence. Thus, the study demonstrates processing-related changes in sperm morphology in Jersey bulls and highlights the importance of evaluating morphology at successive semen-processing stages, particularly after thawing and incubation.

Author's Contribution

SG, MS- Conceived and designed the experiments. SG- involved in conducting the staining procedure, wrote the manuscript. MS- Edited the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical standards

Experiments comply with the current laws of the country in which they were performed.

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