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Does the morphological abnormalities in semen of Jersey bulls vary significantly during different stages of processing?

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Abstract
Present study was undertaken to record the sperm abnormalities in eight Jersey bull maintained at Livestock development Board Sperm station, Palampur using Rose Bengal staining. A total of sixty four ejaculates, eight each from eight mature Jersey bulls, twice in a week, were collected and evaluated for morphological abnormalities at four stages of semen processing viz, post-dilution, equilibration, post thaw and 1 hr post thaw incubation. Sperm abnormalities were classified into head, midpiece and tail. Significant difference ($p<0.05$) was observed between head, midpiece, tail abnormalities at equilibration, post thaw and 1hour post thaw incubation. However, at post-dilution stage midpiece and tail abnormalities differed significantly. Since the different abnormalities of head, midpiece and tail were in the permissible limit this making ejaculates suitable for cryopreservation and its usage for artificial insemination at farmer's doorsteps.

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

1. Introduction
Standard method for assessing the fertility of breeding bulls, other than directly evaluating their ability to produce a pregnancy, is by examination of semen. Morphologic abnormalities of sperm have relationship to fertility as increased morphological abnormalities leads to fertilization failure. Sperm abnormalities have been associated with male infertility and sterility [1] and their evaluation is a fundamental component for analysis of semen quality. Structure and morphology of sperm have a major impact on rate of fertilization, early embryonic development and pregnancy rate in artificial reproduction [2]. Traditionally morphological abnormalities have been classified by location of defect (head, midpiece, tail), or its site of origin (primary, secondary, tertiary). Sperm abnormalities were classified according to their effect on fertility: major defect include most abnormalities of the head and midpiece, proximal cytoplasmic droplets and single abnormalities present in high percentage, whereas minor defects include looped tails, detached sperm heads and distal cytoplasmic droplets [3]. Head abnormalities include large, small, tapered, pyriform, round and amorphous heads, head with small cap area and double head. Midpiece defects include bent neck, asymmetrical insertion of the midpiece into the head, a thick or irregular midpiece, an abnormally thin midpiece, as well as combination of the both whereas, tail abnormalities short, multiple, broken and bent tails, coiled tail or combination of both. According to MSP, sample containing a total abnormality of more than 20% and head and midpiece abnormality (alone) of 7% should not be used. The analysis of morphological sperm abnormalities in the bull ejaculate is a common veterinary practice prior to the sale of the bull, natural service and storage of frozen semen [1].

2. Materials and Methods
Present study was conducted at Livestock Development Board Sperm Station Palampur, India (32.6°N, 76.3^{0}E and altitude 1290.8 m) on semen from eight Jersey bulls. Semen was collected twice a week by the artificial vagina method over a period of 4 weeks. Total 64 ejaculates (eight from each bull) were collected at four stages of semen processing i.e. immediately after dilution with Tris citric acid egg yolk extender, post-equilibration, post-thaw, and 1 hr post thaw incubation on for morphological abnormalities. Smears from semen samples were prepared and stained using Rose Bengal stain (Rose Bengal 3 gm, Distilled water 99 ml and Formalin 1ml).

The head, mid-piece and tail abnormalities were analysed and classified according to given reference [4].

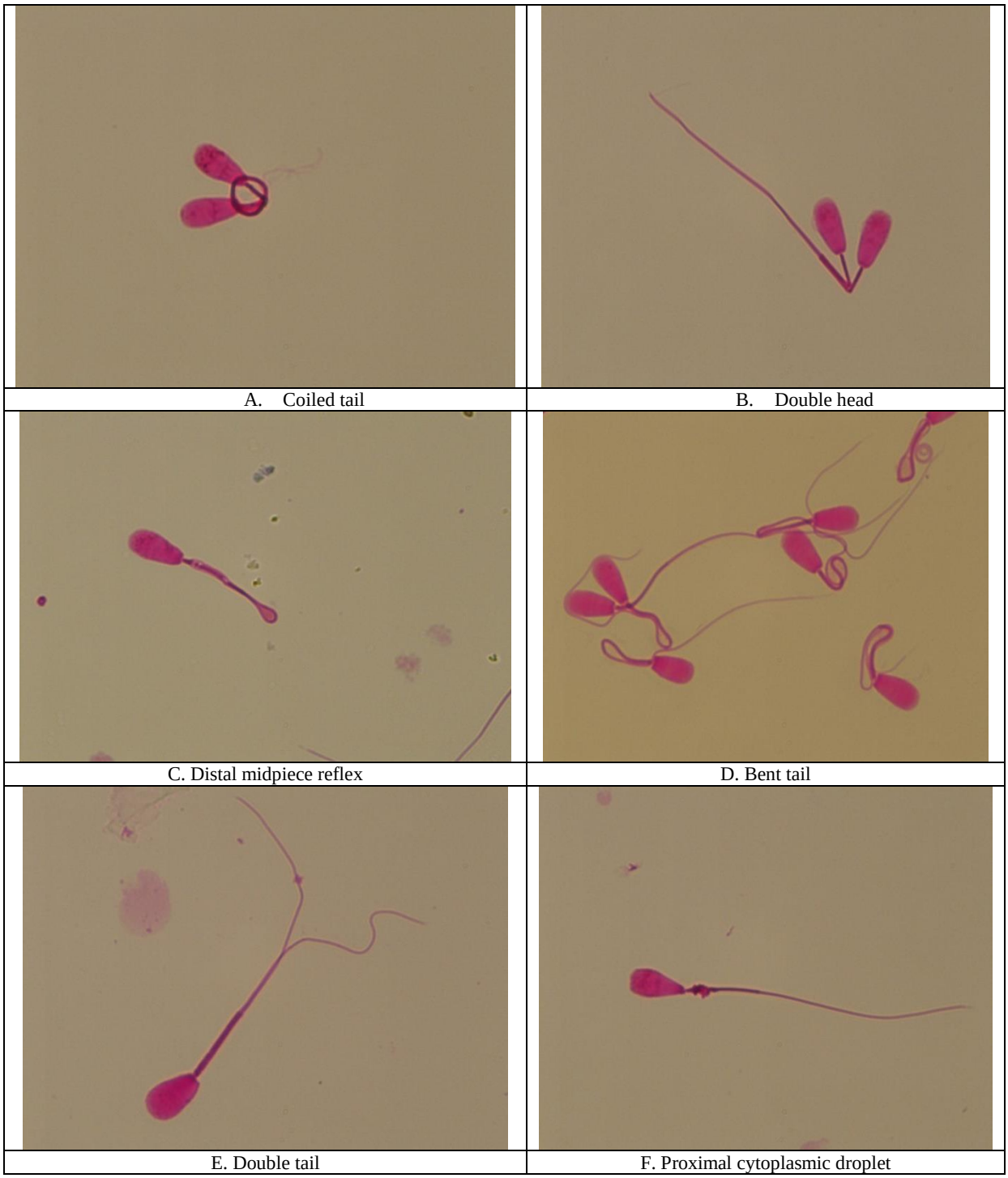
3. Results

Percentage of head, midpiece and tail abnormalities are shown in Table 1 and various abnormalities recorded have been shown in Figure 1 (A-L). A considerable variation in percent abnormalities were observed during post-dilution, equilibration, post thaw, 1hr post thaw incubation of 8 Jersey bulls. Total abnormalities observed in semen of Jersey bulls were within the permissible limits. The common abnormalities observed in tested samples were distal midpiece reflex, coiled tail, free heads, short tail, stump tail, dag defect, proximal / distal cytoplasmic droplet and microcephalic / macrocephalic heads. Rare morphological abnormalities observed include double head, double tail and thick midpiece (Figure 1). Significant difference was observed between head, midpiece and tail abnormalities at various stages of semen processing viz. equilibration, post thaw and 1 hr post thaw incubation.

Table 1: Morphological abnormalities at different stages of semen processing

Stages	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

$^{A, B, C, D}$ Values with different superscripts within row differ significantly ($p<0.05$)



A. Coiled tail

B. Double head

C. Distal midpiece reflex

D. Bent tail

E. Double tail

F. Proximal cytoplasmic droplet

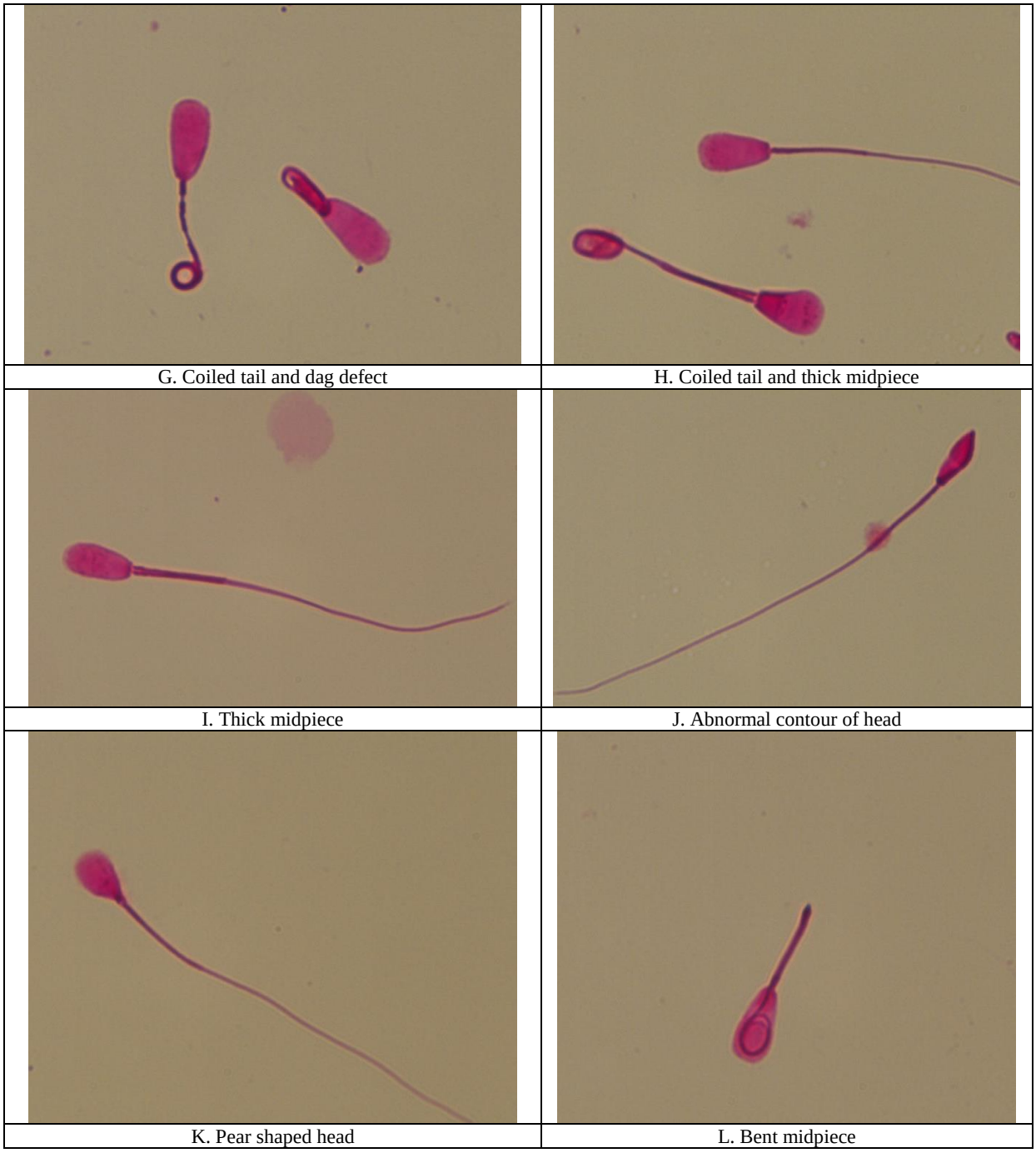


Fig 1: Different morphological abnormalities present in semen of Jersey bulls

4. Discussion

The results of the present findings were in close agreement to [5] for morphological abnormalities at post-dilution stage, cooling and freezing stages respectively. However, in contrast to our finding higher morphological abnormalities in Jersey bull during post thaw stage have been reported [6]. Results of the present study revealed lower abnormalities than those reported [7], where total head (5.45%), mid-piece (1.19%) and tail abnormalities (6.19%) were 5.45%, 1.19% and 6.19% respectively. Sperm abnormalities may be attributed to method of semen collection, temperature shock and technique employed [8]. After dilution and cooling, certain changes take place in the spermatozoa leading to further deterioration in the semen quality which may be attributed to loss of certain vital intracellular components in the dilution medium [9]. The increase in morphological abnormalities at post-equilibration stage could possibly be due to sudden exposure of the semen to a higher room temperature of 4^0C while smearing and staining or the coiling of tail as a result of cold shock. Plasma membrane integrity reflects sperm viability and cryopreservation damages it [10]. Freezing and thawing procedures result in destabilization of the plasma membrane and intracellular ice crystal formation that decreases the number of viable spermatozoa [9]. Cryopreservation process reduces sperm viability by 50-60 per cent [11]. Further, incubation of semen causes a progressive fall in sperm viability significantly correlated with incubation time [12].

Sperm morphology is a vital semen parameter and is considered to be a reliable index of the quality of spermatogenesis and subsequent fertility. Sperm morphology affects fertility because sperm have to be in a certain shape to be able to penetrate an egg. Sperm movement is affected due to the defects in the sperm neck and tail; while defects in the sperm head can affect the sperm's ability to bind and fertilize

the egg. Abnormally shaped sperm negatively affects fertility by preventing transport through the cervix and prevents sperm from adhering to the ovum. Insemination with high sperm head abnormalities >10% can reduce the success of fertilization [13], which subsequent can decrease the success rate of artificial insemination [7]. In order to achieve maximum pregnancy rate, a minimum number of 10 million motile sperms should be inseminated and at least 70 per cent of them should be morphological normal [14]. Conclusively, the sperm abnormalities in the Jersey bull are in permissible limit making it suitable for use in artificial insemination.

5. References

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