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Sperm Morphological Abnormalities in Fresh Semen of Marwari Stallion

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ABSTRACT: When it comes to predicting future fertility, sperm morphology might even be more significant than motility or count. Sperm with an irregular shape cannot fertilise an egg. Spermatozoa may have a combination of abnormalities. The present study was carried out on six healthy Marwari horses with a semen collection frequency of twice a week. Total 36 (6 per animal) ejaculates were collected using artificial vagina. After collection, semen samples were evaluated grossly, followed by microscopic evaluation. The assessment of sperm morphologies in fresh equine semen was conducted using the eosin and nigrosin staining technique. Morphological abnormalities in the head, mid-piece and tail were 1.42±0.12%, 1.44±0.14% and 1.56±0.10%, respectively and total sperm morphological abnormalities were 4.42±0.23%. Morphological sperm abnormalities (head, mid-piece and tail) along with total sperm morphological abnormalities exhibited a non-significant (P>0.05) difference among Marwari stallions.

Keywords: Equine semen, fertility, Marwari stallion, sperm morphology, sperm abnormalities.

INTRODUCTION

An important aspect of every breeding soundness assessment is an examination of spermatozoa morphology (Knobil and Neill 1998). The structure and form of a spermatozoon are referred to as sperm morphology. An oval, smooth head and a discernible acrosomal cap characterise typical sperm. Normally, there are no abrupt bends or breaks in the long sperm tail (Bjorndahl *et al*., 2010; WHO, 2010). Sperm morphology evaluation is still a crucial component of the standard semen analysis process. Increased aberrant sperm forms are referred to as teratozoospermia. While some claim that sperm morphology predicts the success of assisted reproduction (Kruger *et al*., 1988), others doubt its applicability due to the wide variations in morphology assessment (Centola, 1996; Centola and Jeyendran 2009; Rothman and Reese 2009; Bjorndahl, 2010; Centola, 2011). Spermatozoa with abnormal shapes can have many different forms, such as two heads, a very large circular head, a tapering head, a crooked head, a mid-piece with a pseudo droplet defect and a corkscrew defect and a tail with curls and kinks. A number of reasons, such as structural deformities, malnourishment, genetic flaws, chronic sickness and environmental variables, may culminate in an unknown cause of spermatozoa abnormality (Siddique *et al*., 2011). Head, mid-piece and tail abnormalities are the three categories of sperm malformations. Acrosomal defects, duplicate heads, elongated or tapered heads, small or large heads and amorphous heads defects that do not fit into any of the previously listed categories—are examples of head defects. Bent necks, thick or thin necks and off-centre tail insertion into the mid-piece are examples of mid-piece imperfections. Short tails, coiled tails, bent tails (bent at any point along the tail length) and coiled tips of the tail are examples of tail deformities. In rare circumstances, all sperm can show gigantic heads, pinheads or uniformly round heads with no acrosomal cap (Niederberger, 2011). The only treatment available when acrosomes are lacking is assisted reproduction, especially ICSI. However, varicocele, extremes in temperature and, seldom, genetic disorders can induce sperm morphological anomalies (Nagler and Grotas 2009; Oates and Lamb 2009; Niederberger, 2011). Morphological abnormalities can also result from exposure to environmental and toxic substances (Katz, 1991; Rothmann and Reese 2009). For example, a varicocele can make the heads of sperm taper more than usual. On the other hand, testicular injuries, toxins, or genetic problems can lead to sperm with duplicate heads, round heads, or pin-headed bodies (Katz, 1991; Nagler and Grotas 2009; Oates and Lamb 2009; Poch and Sigman 2010; Niederberger, 2011). Fertilisation is feasible even in cases of severe teratozoospermia (Poch and Sigman 2010); nevertheless, even with assisted reproductive techniques, pregnancy rates in these cases are low (Sigman *et al*., 2009). It is crucial to keep in mind that, while isolated elevated aberrant forms without other

abnormal parameters are likely clinically inconsequential, a consistently high percentage of abnormal forms can be clinically meaningful (Niederberger, 2011).

MATERIALS AND METHODS

Animals

Table 1: Identification of horses with their age.

Sr. No.	Marwari stallion (identification)	Age (months)
1.	Mohit	140
2.	139	86
3.	Dogger	78
4.	167	52
5.	170	51
6.	175	50

Six adult Marwari horses aged between 50 and 140 months being maintained at the Equine Production Campus, ICAR-National Research Centre on Equines, Bikaner, Rajasthan, India, under uniform and healthy conditions were used in this study (Table 1). Total 36 ejaculates were collected during the breeding season in the early morning before feeding. Semen samples were collected with frequency of twice a week using Colorado model artificial vagina (AV) with a mare in oestrus as a dummy. Just after the collection of semen, certain seminal parameters for each horse were recorded.

Semen Collection and Processing. After collection, each semen sample was examined macroscopically or grossly to determine its consistency and colour. Seminal pH was recorded using a digital pH metre (ERMA Inc., Japan). To obtain gel-free semen, the sample was filtered through sterile gauze into a prewarmed, graduated vial. Various seminal attributes like gel, gel-free and total semen volumes were recorded as previously mentioned by Kumar *et al.* (2019); further assessments and microscopic evaluation of fresh semen were carried out following gross examination, such as sperm concentration. In the present study, morphological abnormalities in the fresh semen of Marwari stallion are focused.

Evaluation of Sperm Morphology. The assessment of sperm morphology was conducted on a sample of fresh semen by creating stained smears using eosin-nigrosin. The smears were then viewed under a microscope with 1000X magnification and oil immersion (Nikon Instech Co. Ltd., Kanagawa, Japan).

The subsequent procedures were executed to create the eosin-nigrosin stain for evaluating the count of viable and non-viable sperm as well as the morphology of sperm:

1. To make a 3% sodium citrate solution, 3 gm of sodium citrate (dehydrate) was dissolved in 100 ml of distilled water.
2. A mixture of 1 gm of eosin B and 5 gm of nigrosin stain powders was dissolved in 100 ml of a 3% sodium citrate (dehydrate) solution.
3. The pH of the staining solution was altered to 7.0 by adding a few drops of 0.1 M NaH_2PO_4 and thereafter filtering the mixture. The staining solution was kept at ambient temperature for future use.

To make a smear, a single droplet of semen was carefully poured onto a clean glass slide that had been prewarmed. This droplet was then mixed with another droplet of eosin-nigrosin stain using a glass rod. Semen samples were examined at 100X oil immersion via a high-power microscope to count abnormal spermatozoa with abnormalities in their head, mid-piece and tail. Three smears were made and each smear was analysed for abnormalities in 200 spermatozoa. These abnormalities included an abnormal head, a detached head, a proximal or distal cytoplasmic droplet, an aberrant mid-piece, a bent or coiled tail and premature sperm cells.

Statistical Analysis. The collected data underwent statistical analysis using the SPSS/PC computer programme (version 26.0) following the established protocols given by Snedecor and Cochran (1994). The statistical analysis utilised analysis of variance (ANOVA) and Duncan's new multiple range test (DNMRT).

RESULTS AND DISCUSSION

Morphological Anomalies of Sperm in Freshly Ejaculated Semen. The evaluation of sperm morphological abnormalities was conducted using eosin-nigrosin stain under a microscope, as shown in Fig. 1. High-fertility stallions frequently have a proportion of normal sperm above 60% and a low incidence of abnormalities (<5%), especially in the mid-piece and acrosome areas (Samper *et al.*, 2007). According to Pereira *et al.* (2017), morphological abnormalities, particularly those affecting the head and tail, might obstruct spermatozoa's ability to reach and fertilise the egg by impeding their progressive motility. According to Morrell *et al.* (2008), the morphological abnormalities included narrow heads (1-3%), heads with narrow bases (1.5-5%), pear-shaped heads (3.6-7%) and proximal cytoplasmic droplets (7-27%). Few traceable literatures are available regarding detailed morphological sperm abnormalities of Marwari horses.

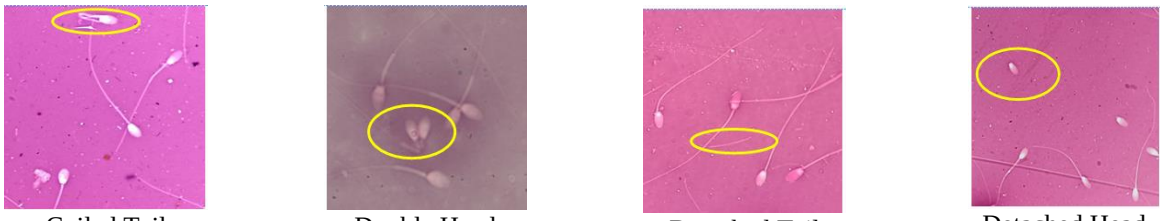

Fig. 1. Microphotograph showing sperm morphological abnormalities (eosin-nigrosin, 1000X).

Head Abnormalities. Sperm head abnormalities in fresh semen of Marwari horses were noted from 1.17±0.40 to 1.67±0.21% with a mean of 1.42±0.12%. A non-significant (P>0.05) difference was observed among horses for head abnormalities of sperm. In contrast, Kumar (2019) recorded a significant difference (P>0.05) among Marwari stallions for head abnormalities, with a slightly higher average value of 1.8±0.08%. Findings of the present study were also against Jhamb (2021), who declared a significant difference among Marwari horses for abnormalities of the sperm head in fresh semen. However, Kumar (2017) also recorded a non-significant difference (P>0.01) among Marwari stallions for sperm head abnormalities, with a higher mean value of 1.65±0.10%.

Mid-Piece Abnormalities. Sperm mid-piece abnormalities in fresh semen of Marwari horses were noted from 1.00±0.37 to 1.83±0.31% with a mean of 1.44±0.14%. A non-significant (P>0.05) difference was observed among horses for mid-piece abnormalities of sperm. Kumar (2017) recorded a higher average mid-piece abnormalities of 2.28±0.12% with a non-significant difference (P>0.01) among Marwari stallions. Observations of this study were dissimilar to those of Jhamb (2021), who declared a significant difference among Marwari horses for abnormalities of sperm mid-piece in fresh semen. Kumar (2019) recorded a significant difference (P>0.05) among Marwari stallions for mid-piece abnormalities with a higher average value of 2.73±0.09%, which is against the present findings.

Tail Abnormalities. Sperm tail abnormalities in fresh semen of Marwari horses were noted from 1.17±0.40 to 1.83±0.17% with a mean of 1.56±0.10%. A non-significant (P>0.05) difference was observed among horses for tail abnormalities of sperm. Similarly, Jhamb (2021) and Kumar (2017) observed a non-significant difference among Marwari stallions for tail abnormalities of sperm. However, Kumar (2017) recorded a greater average value of 3.93±0.15% (P>0.01). Contrary to the current investigation, Kumar (2019) observed a significant difference (P<0.05) for tail abnormalities among Marwari stallions, with a higher average value of 2.67±0.1%.

Total Morphological Abnormalities. Total sperm morphological abnormalities in fresh semen of Marwari horses were noted from 3.33±0.61 to 5.33±0.33% with a mean of 4.42±0.23%. A non-significant (P>0.05) difference was observed among horses for total morphological abnormalities of sperm. A non-significant difference was noted among stallions by Soni (2016); Kumar (2017); Kumar (2018); Kumar (2019); Jhamb (2021), which is similar to the current study. A lower mean value of 2.07±0.27% was observed by Soni (2016). The mean figure that Kumar (2017) reported was 6.78±0.52%, which was higher. Kumar (2018) found a very high mean value of 15.16±0.97% as compared to the present study. In contrast to the current values, Kumar (2019); Jhamb (2021) found high mean values of 7.19±0.15% and 8.8±0.14%, respectively.

CONCLUSIONS

The present study concludes the morphological abnormalities of sperm present in the fresh semen of Marwari stallions during the breeding season, including abnormalities in the sperm head, mid-piece and tail, along with total sperm morphological abnormalities. All the morphological abnormalities exhibited a non-significant difference (P>0.05) among Marwari stallions. The effects of environmental factors like weather and breeding season and the effects of age and frequency of semen collection on the morphological abnormalities of sperm should be studied to know the factors influencing sperm morphological abnormalities of Marwari stallion.

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Conflicts of Interest. None.

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