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Research Paper

Effect of different Buffalo Sperm Motility Patterns on Plasma Membrane Integrity and Morphology

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ABSTRACT

There are different types of motility patterns in human but in case of buffalo but there is no literature about if there is any effect of different types of buffalo sperm motility patterns on freezability and fertilizing capability of semen. Therefore, the study has been designed to know the effect of ejaculates from different types of motility patterns in buffalo semen on freezability and fertilizing capability by sperm morphology and sperm plasma membrane integrity. We collect the semen and divide them into 3 categories (Normal, Slow and Fast non-directional motile sperm) on the basis of sperm motility patterns, then cryopreserved it and assessed sperm morphology and plasma membrane integrity and found slow motile sperm have lowest abnormalities than fast non-directional and normal motile sperm.

Keywords: Buffalo, Plasma membrane integrity, Morphology, Sperm, HOST

Artificial insemination is first generation assisted reproductive technology (Parkinson *et al*. 2019). The India has first ranking in bovine population (DAHD, annual report 2020-21) and also first in milk production (NDDB report 2021-22). India has a largest A.I network and largest Bovine breeding network. The overall AI coverage in Bovine in India is only around 30% with an overall average conception rate of 33-36% (DAHD, annual report 2020-21). The reasons behind the low conception rate are failure to provide quality of semen at farmer doorstep (Vishwanath *et al.* 2003), absence of mechanisms to ensure use of semen from certified semen stations, and poor control over AI technicians are few limitations that need immediate redressal. As per minimum standard protocol for bovine frozen semen (MSP, 2022 GOI) the acceptance level of abnormal sperm in frozen semen should be <20% and the integrity of sperm plasma membrane should be >40%. The integrity of plasma membrane is essential for successful fertilization; therefore, our aim of the study was to assess the effect of sperm morphology and sperm plasma membrane integrity on different types of sperm motility patterns.

MATERIALS AND METHODS

Ethics statement

This article does not contain any studies with human or animal subjects.

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Experimental Location and design

The proposed work was carried out in the Semen Freezing Laboratory of Central Institute of Research on Buffaloes, Hisar.

Semen collection

The semen was collected by artificial vagina in the early hours of the morning before feeding, and from each buffalo bull, two ejaculates were taken on the day of collection, and the interval between two collections was 30 minutes.

Estimation of sperm concentration

The sperm concentration (million/mL) in the freshly collected semen was determined by using an AccuCell bovine photometer (IMV, L'Aigla, France) at 530 nm wavelength. For this, semen samples (40 µL) were diluted with normal saline 0.9% w/v (3960 µL) using an automatic dilutor. The absorbance of the diluted semen was measured by using an AccuCell bovine photometer to get the sperm concentration (million/mL) of the ejaculate.

Semen dilution and individual sperm motility

The sixty semen ejaculates of Murrah bulls which had a minimum of 70 % sperm motility were classified into three categories (normal, slow and fast non directional motile spermatozoa) on the basis of sperm motility pattern and considered for cryopreservation. The passed ejaculate of fresh semen of all three category (normal, slow and fast motile ejaculates) were evaluated for plasma membrane integrity and morphology.

Freezing of semen

Semen sealing and filling was done mechanically by using an integrated system of IMV Technologies as per Dalal *et al.* (2019), then subjected to equilibration for 4-6°C for 4 hours. After equilibration, the freezing was done by vapour static method as per Kumar *et al.* (2018).

Post-thaw evaluation

After 24 h of cryopreservation, the semen straws were thawed at 37°C for 30 seconds, and sperm motility was estimated immediately. The ejaculates that had a minimum of 50 % sperm motility were selected for incubation at the same temperature and every 30 min interval, sperm motility was checked. The incubation was continued for 120 min. and at the end of the incubation period, those ejaculates had a minimum of 20 % sperm motility were finally considered passed/qualified ejaculates, and the rest of the ejaculates were considered rejected/failed ejaculates. Further, the passed ejaculates of all categories (Normal, Slow, and Fast nondirectional motile sperm) were evaluated for sperm morphology and plasma membrane integrity.

Sperm morphology

To evaluate the sperm morphology, eosin-nigrosin staining was performed in cryopreserved semen samples as per Singh *et al.* (2013). A semen straw from each sample was thawed at 37°C, and one small drop of thawed semen was placed on the corner of a clean, grease-free pre-warmed slide. After that, one small drop was placed of each eosin and nigrosin stain over the semen drop. A thin smear was prepared by spreader slide at a 30-degree angle to disperse the semen suspension over the slide length and fixed with air drying. A total of 200 spermatozoa were evaluated for each sample in different fields at 100X objective under a phase-contrast microscope and percentage of abnormal spermatozoa (bent tail, coiling of tail over mid piece, proximal protoplasmic defect and eccentric thickening of acrosome) was determined.

Sperm plasma membrane integrity

To assess the functional integrity of the sperm plasma membrane, the hypo-osmotic swelling test was performed Jeyendran *et al.* (1984). For the test, 500 µL hypo-osmotic solution (0.735g sodium citrate dihydrate and 1.351g fructose in 100 mL of double distilled water, 100mOsm/L) was mixed with 50µL of frozen-thawed semen and for the control group, 500µL PBS mixed with 50µL frozen-thawed semen and incubated for 60 min at 37ºC. After incubation, 40µL of 2 % eosin solution was added to increase visibility. A drop of diluted semen was placed on a clean sterilized dry glass slide and covered with a cover slip. A total of 200 spermatozoa were counted in different fields at 100x objective under a phase-contrast microscope and the percentage of spermatozoa positive to HOST (having coiled tails) was determined. The percentage of curled tail spermatozoa in PBS was deducted from that

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of the HOST to get the true HOST-reactive sperm. The sperm with a coiled tail after incubation were considered to have an intact plasma membrane.

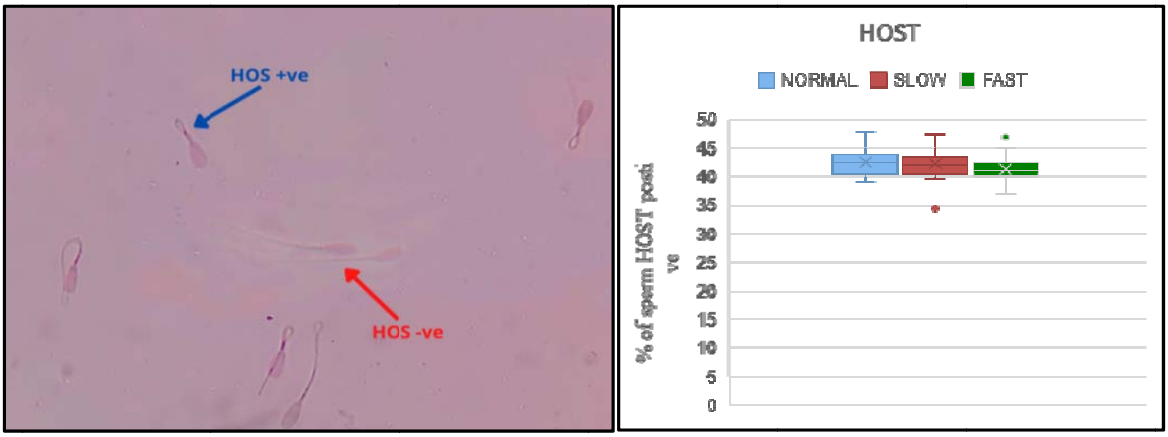
RESULTS

Effect of different types of motility patterns on sperm plasma membrane integrity

The results of sperm plasma membrane integrity are presented in Table 1. The sperm plasma membrane integrity of all three categories (20 samples in each category) in post thawed ejaculates was evaluated by HOST to determine the effect of sperm motility patterns on the sperm membrane (Fig. 1A and B). It was found there was no significant difference ($p>0.05$) between all these three categories. However, the percent of sperm plasma membrane integrity in all three categories was under recommended level.

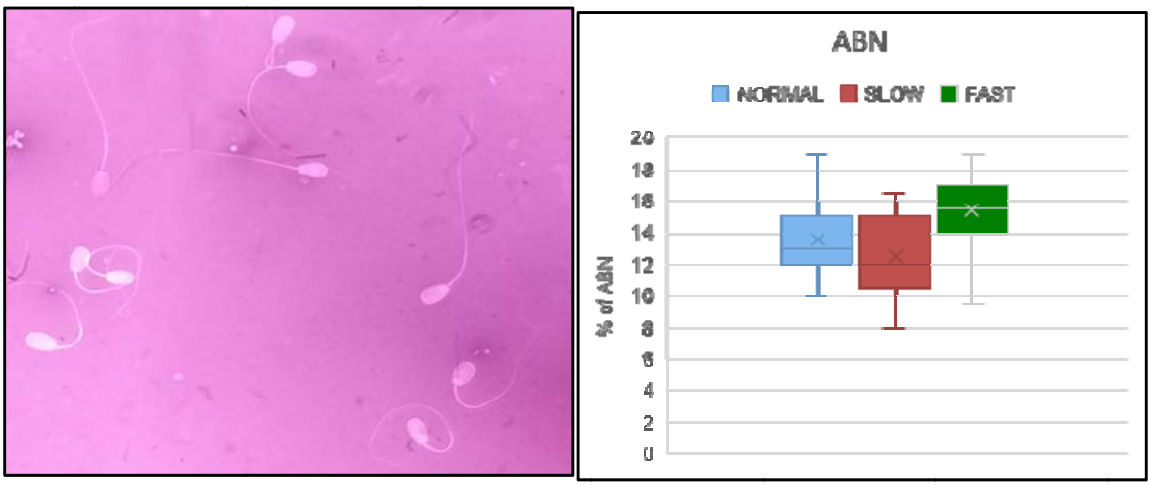
Effect of different types of sperm motility patterns on sperm morphology

The results of sperm morphology (abnormalities) are presented in Table 1. The sperm morphology in cryopreserved samples of all three categories (20 samples in each category) were evaluated by eosin nigrosin staining. The sperm abnormalities in fast non directional motile sperm were significantly higher ($P<0.05$) than slow motile sperm. While in normal motile sperm the sperm abnormalities were between slow and fast non directional. However, the percentage of sperm abnormalities in all these three categories was under the recommended level (Fig. 2 A and B).



(A) **(B)**

Fig. 1: Showing **(A)** spermatozoa with curled tail shows HOST positive **(B)** results of HOST in all three categories



(A) **(B)**

Fig. 2: Showing **(A)** picture of sperm morphology and **(B)** results of sperm morphology in all three categories

Table 1: Showing the results of sperm plasma membrane integrity and sperm abnormalities in different types of sperm motility patterns

Sperm motility patterns	Intact sperm plasma membrane (%)	Sperm abnormalities (%)
Normal motile sperm	42.47±0.63	13.52±0.60[a]
Slow motile sperm	42.36±0.66	12.5±0.59[ab]
Fast and non-directional motile sperm	41.26±0.56	15.39±0.54[a]

DISCUSSION

Due to the clinical importance of presence or absence of progressive motile spermatozoa, WHO has categorized sperm motility into four types fast progressively motile, slow progressively motile, non-progressively motile, and immotile (grade a, b, c, or d) (WHO, 2021). Sperm motility is an essential parameter for achieving successful conception because, sperm motility is only way to reach ejaculated spermatozoa up to the female reproductive tract and at the site of fertilization (Vijayaraghavan *et al.* 2003). The results of HOST were non comparable in all three categories but lies under the minimum recommended level. Considering the recommended level of (MSP, GOI 2022) all three categories have desirable integrity of plasma membrane. Morphologically abnormal sperm can reduce rates of fertilization and embryonic development (Thundathil *et al.* 2001). Comparatively, a lower number of sperm with abnormalities was in slow motile sperm than in fast nondirectional motile sperm. The present study is in agreement with the findings of Nirmal *et al.* (2020) who obtained average abnormal sperms ranging from 5.14 to 13.57 % with an overall average value of 7.66 %. The recommended maximum number of frozen-thawed sperm with abnormal sperm is <20% (MSP, 2022).

CONCLUSION

In this studied we found that slow motile sperm have lowest sperm abnormalities than normal and fast nondirectional motile sperm, while sperm plasma membrane integrity was almost similar in all these three categories. Might be slow motile sperm have a higher conception rate due to low sperm abnormalities in slow motile sperm.

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