Semen quality assessment in Ongole (*Bos indicus*) bulls – insights from ejaculate volume, progressive motility, live sperm count, sperm abnormalities and host

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ABSTRACT

Three Ongole bulls (designated as L-606, L-815 and L-838) aged 9, 5 and 5 years, respectively, housed at Livestock Research Station, Lam, Guntur, Andhra Pradesh were selected for the study. Semen was collected weekly once for six consecutive weeks, which equated to 18 ejaculates from three bulls (@ six ejaculates per bull) using the standard artificial vagina method. In neat semen, for bulls L-606, L-815 and L-838 mean ejaculate volume was 7.41 ± 0.68, 5.16 ± 0.49 and 5.13 ± 0.46 ml, progressive sperm motility percentage was 94.5 ± 0.18, 91.6 ± 0.66 and 92.1 ± 0.79, live sperm count was 91.5 ± 0.71, 84.66 ± 1.42 and 88.83 ± 1.90, sperm abnormalities were 6.53 ± 0.31, 7.6 ± 0.64 and 7.16 ± 0.48 and the HOST values were 84.0 ± 0.89, 77.0 ± 2.75 and 74.5 ± 1.58 respectively. Notably, bull L-606 exhibited significant variations in ejaculate volume, progressive motile sperms, live sperm count and HOST reactive sperms compared to the other bulls under study. Nevertheless, the observed sperm abnormalities were within acceptable thresholds, indicating the sound reproductive health of the three experimental bulls.

Keywords: Ongole bull; age; neat semen; ejaculate characteristics; artificial insemination

INTRODUCTION

Artificial insemination (AI) has emerged as a cornerstone method aimed at enhancing productivity and genetic advancements in cattle (Novianti et al 2020). The pivotal role of AI heavily relies on the availability of high-quality, either fresh or well-cryopreserved semen, with nearly 95 per cent of AI procedures relying on cryopreserved semen (Raheja et al 2018). In India, the Ongole bull stands as a prominent indigenous breed, renowned for its draught capabilities and resilience against tropical diseases (Ahmad et al 2019). AI has proven to be the most widely used assisted reproductive technology (ART), aiding in the preservation and propagation of indigenous breeds. When it comes to the selection of superior bulls for artificial insemination-related aspects, it is of utmost importance to have a thorough understanding of the characteristics of neat semen in cattle bulls (Armstrong 2022). The bull reproduction potential through the quantity and quality of semen produced is related to the scrotal circumference size (Anwar and Jiyanto 2019, Jiyanto and Anwar 2019). In this context, the present study was designed to investigate certain semen quality parameters such as volume, progressive motility, live sperm count, sperm abnormalities and HOST in Ongole bulls.

MATERIAL and METHODS

The current study involved three healthy Ongole bulls, aged between 5 to 9 years, maintained at the Livestock Research Station, Lam, Guntur, Andhra Pradesh. These experimental bulls received a well-

balanced diet in accordance with the standard guidelines and underwent regular deworming and vaccination. Semen was collected weekly once over a period of six consecutive weeks from each experimental bull by standard artificial vagina method. A total of 18 ejaculates from three bulls (@ six ejaculates per bull) were utilised for this study. The assessment of neat semen was conducted using the following criteria

Ejaculate volume (ml): The volume of each ejaculate was measured in millilitres (ml) by direct visualization with naked eye on the graduated centrifuge semen collecting tube.

Progressive sperm motility (%): Progressive sperm motility of the neat semen was expressed in percentage and evaluated as per the procedure described by Dhurvey et al (2012).

Live spermatozoa count (%): Live spermatozoa count of the neat semen was expressed in terms of percentage and evaluated as per the procedure described by Kondracki et al (2017).

Sperm abnormalities (%): To evaluate sperm abnormalities, Eosin-Nigrosin staining method was adopted as given by Hancock (1951).

Hypo-osmotic swelling test (HOST): The hypo-osmotic swelling test was performed according to the method described by Perumal et al (2014).

Statistical analysis

The data obtained for various neat semen parameters were analyzed statistically by one way analysis of variance (ANOVA) between the bulls followed by the Duncan post hoc test to ascertain the significant difference between the bulls (Snedecor and Cochran 1994).

RESULTS and DISCUSSION

The data on characteristics of neat semen in Ongole bulls are given in Table 1.

The mean ejaculate volume for three experimental bulls, namely L-606, L-815 and L-838, was 7.41 ± 0.68, 5.16 ± 0.49 and 5.13 ± 0.46 ml respectively. The overall mean ejaculate volume was 5.90 ± 0.39 ml exhibiting significant variation among the three experimental bulls. Several factors could be responsible for the observed variation in ejaculate volume in this study. These included the age of the bull, body weight, nutritional status, level of physical activity, season, frequency and the method used for semen collection (Pal et al 2020). Notably, the bull L-606, aged over 9 years exhibited a significantly greater ejaculate volume in comparison to the other bulls. One plausible reason for this phenomenon could be attributed to the age dependent phenomenon, wherein the scrotal circumference (SC) of the bull tends to develop and increase with age.

The overall mean progressive sperm motility recorded was 92.7 ± 0.44 with a range of 90-95 per cent. L-606, L-815 and L-838 bulls were recorded with the mean progressive sperm motility of 94.5 ± 0.18, 91.6 ± 0.66 and 92.1 ± 0.79 per cent respectively. The progressive sperm motility of bull L-606 was significantly higher than other bulls. In this study, a greater proportion of sperm exhibited progressive movement. Sperm motility plays a central role in both natural fertilization and clinical sperm selection as the key parameter reflecting the fertility potential of individual sperm (Gaffney et al 2011, Raveshi et al 2021), whereas, lower levels as reported by Novianti et al (2020) in Madura (66.9 ± 4.82), Bali (67.76 ± 4.26) and Simmental (65.9 ± 4.45) bulls of Indonesia might suggest potential problems that could hinder sperm from reaching and fertilizing an egg.

Table 1. Characteristics of neat semen in Ongole bulls (mean ± SE)

Bull number	Ejaculate volume (ml)	Progressive motility (%)	Sperm concentration (millions/ml)	Live sperm count (%)	Sperm abnormalities (%)	HOST (%)
606 (n = 6)	7.41 ± 0.68^{a}	94.5 ± 0.18^{a}	1,516.67 ± 70.31^{a}	91.5 ± 0.71^{a}	6.53 ± 0.31^{a}	84.0 ± 0.89^{a}
815 (n = 6)	5.16 ± 0.49^{b}	91.6 ± 0.66^{b}	1,138 ± 85.26^{b}	84.66 ± 1.42^{b}	7.6 ± 0.64^{a}	77.0 ± 2.75^{b}
838 (n = 6)	5.13 ± 0.46^{b}	92.1 ± 0.79^{b}	1,214 ± 119^{b}	88.83 ± 1.90^{ab}	7.16 ± 0.48^{a}	74.5 ± 1.58^{b}
Overall mean (n=18)	5.90 ± 0.39	92.7 ± 0.44	1,289.67 ± 64.52	88.33 ± 1.03	7.1 ± 0.28	78.5 ± 1.42

Means with different superscripts within columns differ significantly at 5% LoS

The overall mean live sperm count (%) recorded was 88.33 ± 1.03 with a range of 80 to 94 per cent. Among the bulls studied (L-606, L-815 and L-838), their respective mean live sperm counts were 91.5 ± 0.71, 84.66 ± 1.42, and 88.83 ± 1.90. Notably, bull L-606 exhibited a significantly higher live sperm count as compared to the other two bulls. The observed increase in live sperm count in bull L-606 might be attributed to age-related adaptability to environmental conditions, potentially affecting secretory activities, as suggested by Sugulle et al (2006).

The mean sperm abnormalities for bulls L-606, L-815, and L-838 were found to be 6.53 ± 0.31, 7.6 ± 0.64, and 7.16 ± 0.48 respectively. The overall mean sperm abnormalities were 7.1 ± 0.28 per cent, ranging between 6.5 to 7.6 per cent and exhibited no significant variation among the three experimental bulls. The mean abnormal sperm count observed in the current study aligns with the finding of Krishna (2021) who reported that mean abnormal sperm count was 7.74 ± 0.18 per cent in Ongole bulls. It is justifiable to speculate that the differences noted in various studies could be associated with factors like age, breed, season, changes in environmental conditions, testicular conditions and variations in spermatogenesis as reported by Krishna (2021). Nonetheless, the sperm abnormalities documented in the current study were within the acceptable thresholds, signifying the sound reproductive health of these experimental bulls.

The overall mean value of HOST recorded was 78.5 ± 1.42 per cent and ranged between 70 to 86 per cent. In L-606, L-815 and L-838 bulls the mean HOST values were 84.0 ± 0.89, 77.0 ± 2.75 and 74.5 ± 1.58 per cent respectively. The mean HOST value of the bull L-606 was significantly higher than other bulls. The sperm plasma membrane plays a crucial role in various physiological processes, including capacitation, zona binding and the acrosomal reaction (Priyanto et al 2015). This correlation suggests that mature bulls may exhibit superior levels of host-reactive sperm when provided with optimal feeding and management conditions. This phenomenon was observed in all the three experimental bulls, with particular emphasis on the notable case of bull L-606, which displayed a higher proportion of host-reactive sperms.

In a completely separate study conducted by Keerthana et al (2022), it was observed that the serum testosterone levels were higher in adult breeding bulls as compared to the younger counter parts. Budhiyadnya et al (2021) observed that serum testosterone increased with age in Pesisir cattle which in turn positively correlated with libido in bulls. Chacur et al (2013) observed that in Simmental bulls the quality of semen showed high correlation with testosterone.

CONCLUSION

The study showed that the semen quality of all three experimental bulls was influenced by factors such as age, breed, frequency and method of semen collection, optimal feeding, hormonal and managemental factors. However, the bull L-606 exhibited significant variations in ejaculate volume, progressive motile sperms, live sperm count and HOST reactive sperms as compared to the other bulls under study. Nevertheless, there were no significant differences observed among bulls in their mean sperm abnormalities. Also, the age-related increase in testosterone levels in the adult bull might have an influence on the semen attributes. Consequently, the semen from these bulls was deemed suitable for efficient cryopreservation and could be utilized in future artificial breeding programmes. Also, the data generated as part of this experiment can be utilized as baseline reference values for future studies as the data on Ongole breed on these lines is scanty.

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