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Baseline fresh semen characteristics of Murrah buffalo (*Bubalus bubalis*) bulls and their implications for semen processing and fertility prediction

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Abstract

Evaluation of fresh semen quality is a fundamental prerequisite for successful artificial insemination and cryopreservation programmes in livestock. Fresh seminal attributes reflect the functional status of the testes, epididymis, and accessory glands and directly influence sperm survival during processing and subsequent fertility. In Murrah buffalo bulls, systematic characterization of fresh semen parameters under organized semen station conditions remains limited despite their widespread use in dairy breeding programmes. The present study established baseline fresh semen characteristics of Murrah buffalo bulls by evaluating ejaculate volume, seminal pH, sperm concentration, mass motility, sperm viability, and morphological abnormalities. The overall mean ejaculate volume, pH, concentration, mass motility, live sperm percentage, and abnormal sperm percentage were 3.25 ± 0.18 ml, 6.75 ± 0.02 , 1023.47 ± 28.50 million/ml, 3.36 ± 0.08 (0-5 scale), $88.44 \pm 0.41\%$, and $5.69 \pm 0.27\%$, respectively. These values indicate excellent seminal quality with high membrane integrity and minimal structural defects. Functional parameters were interrelated, with higher motility and viability associated with lower morphological abnormalities. The findings provide reference standards for semen evaluation, bull selection, and cryopreservation planning in Murrah buffalo breeding programmes.

Keywords: Fresh semen, Murrah buffalo, seminal attributes, motility, viability, morphology, fertility

1. Introduction

Artificial insemination is widely recognized as one of the most impactful technologies for genetic improvement of livestock populations. The efficiency of artificial insemination programmes depends largely on semen quality, which determines fertilization success, conception rate, and herd productivity. Assessment of fresh semen immediately after ejaculation forms the cornerstone of semen evaluation because it provides direct information regarding reproductive health, spermatogenic efficiency, and functional competence of spermatozoa.

Buffaloes contribute substantially to global milk production, particularly in tropical and subtropical regions. Among buffalo breeds, Murrah buffaloes are extensively utilized in breeding programmes due to their superior milk yield and adaptability. However, buffalo spermatozoa are known to be relatively sensitive to environmental stress and cryogenic injury, resulting in comparatively lower post-thaw fertility than cattle (Andrabi, 2008) ^[1]. Consequently, optimal fresh semen quality is critical to ensure satisfactory cryosurvival and fertility.

Fresh semen parameters such as ejaculate volume, pH, sperm concentration, motility, viability, and morphology provide valuable insight into reproductive physiology. Ejaculate volume reflects accessory gland secretions, sperm concentration indicates testicular output, motility signifies mitochondrial function and energy metabolism, viability indicates membrane integrity, and morphology reveals structural normalcy of spermatozoa. Each parameter contributes uniquely to fertilization success (Amann and Hammerstedt, 1993) ^[2].

Although several studies have reported isolated semen traits in buffalo bulls, comprehensive baseline profiling under controlled semen station conditions remains insufficient. Establishing

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standardized reference values is important for routine quality control, bull selection, and prediction of freezability. Therefore, the present study aimed to characterize fresh semen attributes of Murrah buffalo bulls and to discuss their physiological significance and implications for fertility.

2. Materials and Methods

2.1 Experimental animals and management

The study was conducted at a biosecure frozen semen station housing healthy Murrah buffalo bulls. Four sexually mature bulls aged 3-6 years and weighing approximately 500-600 kg were selected. Animals were maintained under uniform feeding, housing, and management conditions to minimize environmental variation. Bulls received a balanced ration consisting of concentrate mixture, hay, green fodder, mineral mixture, salt, and ad libitum water.

Routine vaccination, deworming, and herd health protocols were followed. Only clinically healthy bulls free from reproductive disorders were included.

2.2 Semen collection

Semen was collected twice weekly using an artificial vagina maintained at 42-45 °C. One false mount was provided to stimulate libido before collection. Immediately after ejaculation, semen samples were transferred to graduated tubes and maintained in a water bath at 34-37 °C to prevent cold shock prior to evaluation.

2.3 Evaluation of fresh semen parameters

2.3.1 Volume

Ejaculate volume was measured directly from the graduated collection tube and expressed in milliliters.

2.3.2 Seminal pH

Semen pH was measured immediately using a digital pH meter.

2.3.3 Sperm concentration

Concentration was determined using the hemocytometer method following dilution with saline-eosin solution. Sperm numbers were counted in designated chamber squares and expressed as million sperm/ml.

2.3.4 Mass motility

A drop of undiluted semen was observed under low magnification on a warm stage (37 °C). Collective swirling motion was graded on a 0-5 scale.

2.3.5 Viability

Live-dead counts were performed using Eosin-Nigrosin staining. Unstained sperm were considered viable.

2.3.6 Morphological abnormalities

Smears were examined under oil immersion. Head, midpiece, and tail defects were recorded according to standard classification.

2.4 Statistical analysis

Data were expressed as mean $\pm$ standard error. Differences among bulls were analyzed using analysis of variance.

3. Results and Discussion

3.1 Ejaculate volume

The mean ejaculate volume of Murrah buffalo bulls was 3.25 $\pm$ 0.18 ml, with no significant difference among bulls ($p >$

0.05). Ejaculate volume primarily reflects the secretory activity of accessory sex glands and determines the quantity of seminal plasma available for buffering, nutrient supply, and sperm transport. The present value agrees closely with reports in Murrah buffaloes by Vyawanare *et al.* (1989) ^[3], Rao and Sreemanarayana (1996) ^[4], Shukla and Mishra (2005) ^[5], Saini *et al.* (2017) ^[6], Ram *et al.* (2017) ^[7], and Ravimurugan *et al.* (2020) ^[8]. Similar ranges were also documented by Bhakat and Raina (2001) ^[9] and Bhakat *et al.* (2011) ^[10].

Conversely, comparatively lower volumes have been reported by Sahu and Pandit (1997) ^[11], Ramajayan *et al.* (2023) ^[12], and Kumar *et al.* (2023) ^[13], suggesting that ejaculate volume may be influenced by factors such as age, management, nutrition, frequency of semen collection, and environmental conditions rather than breed alone. The consistency of volume across bulls in the present study indicates uniform accessory gland function and standardized management at the semen station.

3.2 Seminal pH

The overall mean seminal pH was 6.75 $\pm$ 0.02, and no significant variation was observed among bulls ($p >$ 0.05). Seminal pH is an important determinant of sperm metabolism, enzyme activity, and membrane stability. Slightly acidic pH is considered optimal for maintaining sperm viability and preventing premature metabolic exhaustion.

The values recorded in this study are comparable with those reported by Kumar *et al.* (1993) ^[14], Sansone *et al.* (2000) ^[15], Bhakat *et al.* (2015) ^[16], Saini *et al.* (2017) ^[6], and Kaka *et al.* (2016) ^[17]. The narrow range of variation observed suggests consistent seminal plasma composition and stable physiological conditions across animals.

3.3 Sperm concentration

The mean sperm concentration was 1023.47 $\pm$ 28.50 million/ml, with no significant difference among bulls ($p >$ 0.05). Sperm concentration reflects testicular spermatogenic efficiency and epididymal maturation and is one of the most economically important parameters for semen processing, as it determines the number of insemination doses obtainable per ejaculate.

The present findings are in agreement with the ranges reported by Vale (1994) ^[18], Bhakat *et al.* (2011) ^[10], and Bhakat *et al.* (2015) ^[16]. Slightly higher concentrations have been documented by Saini *et al.* (2017) ^[6], Ram *et al.* (2017), and Ramajayan *et al.* (2023) ^[12]. Such variations across studies may arise due to differences in age, season, environmental conditions, nutritional status, and individual variability. The high concentration observed in the current study indicates efficient spermatogenesis and good reproductive health of the bulls.

3.4 Mass motility

Mass motility averaged 3.36 $\pm$ 0.08, with no significant inter-bull variation ($p >$ 0.05). Mass motility provides a rapid assessment of collective sperm vigor and is closely associated with progressive motility and fertilizing potential. Motility depends largely on mitochondrial ATP production and structural integrity of the flagellum.

The present values were comparable to, or higher than, those reported by Shukla and Mishra (2005) ^[5], Ram *et al.* (2017) ^[7], and Kumar *et al.* (2019) ^[19], and were superior to those documented by Kumar *et al.* (1993) ^[14], Bhakat *et al.* (2015) ^[16], and Ramajayan *et al.* (2023) ^[12]. The relatively higher motility observed may be attributed to good management

practices, proper nutrition, and standardized semen collection procedures at the station. Variations in mass motility reported across studies highlight the dynamic influence of environmental and managerial factors on sperm activity.

3.5 Percentage live spermatozoa

The mean live sperm percentage was $88.44 \pm 0.41\%$, with no statistically significant difference among bulls ($p > 0.05$). Viability reflects plasma membrane integrity and functional competence of spermatozoa. Intact membranes are essential for maintaining osmotic balance, metabolic exchange, and resistance to stress during handling and cryopreservation. The present findings closely align with those reported by Shukla and Mishra (2005) [5], Ram *et al.* (2017) [7], Saini *et al.* (2017) [6], and Upadhyay *et al.* (2021) [20]. The high proportion of viable sperm indicates excellent semen quality and suggests good potential for subsequent processing and freezing.

3.6 Percentage abnormal spermatozoa

Morphological abnormalities averaged $5.69 \pm 0.27\%$, with no significant variation among bulls ($p > 0.05$). Morphological integrity is an indicator of efficient spermatogenesis and epididymal maturation. Abnormal spermatozoa often exhibit impaired motility and reduced fertilizing capacity, and higher

abnormality percentages have been associated with lower conception rates.

The abnormality rate observed in the present study was comparable to Singh *et al.* (2020) [21] and lower than the values reported by Ram *et al.* (2017) [7] and Upadhyay *et al.* (2021) [20]. The low abnormality percentage ($< 10\%$) indicates minimal spermatogenic defects and confirms the structural soundness of spermatozoa in these bulls.

3.7 Overall fertility implications

Collectively, the fresh semen characteristics observed in the present study moderate ejaculate volume, optimal pH, high sperm concentration, vigorous motility, high viability, and minimal abnormalities indicate excellent seminal quality. The absence of significant differences among bulls further suggests uniform management and consistent reproductive performance.

Functional interrelationships were evident, as ejaculates with higher motility tended to exhibit greater viability and fewer abnormalities, supporting earlier findings that these parameters jointly determine fertilizing competence (Watson, 2000; Medeiros *et al.*, 2002) [22, 23]. Therefore, the superior fresh semen quality observed here is likely to contribute positively to semen freezability and field fertility outcomes.

Table 1: Mean $\pm$ SE of Neat semen attributes (Volume, pH, mass motility, concentration, live sperm percentage, abnormal sperm percentage) of each bull

Semen attributes	Volume (ml)	pH (0-14)	Mass Motility (0-5)	Concentration (Million/ml)	Live Sperm (%)	Abnormal Sperm (%)
Bull no.	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
MB1023	3.84 ± 0.37	6.79 ± 0.04	3.29 ± 0.19	1000.13 ± 35.43	88.75 ± 0.61	5.75 ± 0.37
MB9672	3.30 ± 0.22	6.74 ± 0.04	3.22 ± 0.14	940.50 ± 32.18	87.88 ± 1.06	5.38 ± 0.26
MB0835	3.35 ± 0.40	6.73 ± 0.05	3.41 ± 0.17	1014.75 ± 83.80	88.44 ± 0.90	5.63 ± 0.68
MB9675	2.50 ± 0.33	6.74 ± 0.03	3.53 ± 0.12	1138.50 ± 45.93	88.69 ± 0.76	6.00 ± 0.78

Table 2: Total Mean $\pm$ SE of Neat semen attributes (Volume, pH, concentration, mass motility, live percentage, abnormal percentage)

Semen Attributes	Mean $\pm$ S.E.
Volume (ml)	3.25 ± 0.18
pH (0-14)	6.75 ± 0.02
Concentration (Million/ml)	1023.47 ± 28.50
Mass Motility (0-5)	3.36 ± 0.08
Live Sperm (%)	88.44 ± 0.41
Abnormal sperm (%)	5.69 ± 0.27

4. Conclusion

The present study establishes baseline reference values for fresh seminal characteristics of Murrah buffalo bulls maintained under organized semen station conditions, demonstrating optimal ejaculate volume (3.25 ± 0.18 ml), physiological pH (6.75 ± 0.02), high sperm concentration (1023.47 ± 28.50 million/ml), vigorous mass motility (3.36 ± 0.08), high viability ($88.44 \pm 0.41\%$), and minimal morphological abnormalities ($5.69 \pm 0.27\%$). These attributes indicate efficient spermatogenesis, adequate accessory gland function, intact plasma membrane integrity, and overall structural and functional competence of spermatozoa. The combination of high motility and viability with low abnormality rates suggests strong fertilizing potential and enhanced resilience to handling and cryogenic stress, thereby supporting good inherent freezability of semen. Collectively, these parameters serve as reliable predictors for bull selection, ejaculate screening, and quality control in semen stations, ultimately improving processing efficiency and fertility outcomes. Integration of these baseline traits with post-thaw

and field fertility evaluations may further strengthen predictive models for reproductive performance in Murrah buffalo breeding programmes.

Conflict of interest

Not available

Financial support

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