PHYSICAL ATTRIBUTE OF NEAT SEMEN AND OXIDATIVE STRESS PARAMETERS OF SEMINAL PLASMA OF JAFFARABADI BUFFALO BULLS

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ABSTRACT

The present study was undertaken during summer season on four mature Jaffarabadi buffalo bulls at the Cattle Breeding Farm, JAU, Junagadh, from March to June, 2021. A total of 24 semen ejaculates (6 ejaculates/bull) collected by AV method were evaluated for physical characteristics and oxidative stress parameters, viz., lipid peroxidation (LPO/MDA) and GST enzyme activity in seminal plasma using standard procedures and assay kits on an auto-analyzer. The mean values of fresh semen characteristics observed were ejaculate volume 4.03±0.38 ml, sperm concentration 1464±82.05 x106/ml, mass motility score 3.79±0.08, individual motility 90.00±0.52%, live sperm 92.04±0.59%, total sperm abnormalities 4.50±0.22%, intact acrosome 87.08±0.58%, and HOS reacted sperm 83.00±0.49 %. Among all the parameters studied, only the sperm concentration and sperm morphological abnormalities varied significantly (P<0.05) between bulls. The mean values of LPO and GST activities in fresh seminal plasma were 6.84±1.76 µM MDA and 18.64±1.34 nmol CDNB /ml/min, respectively, without significant variation between bulls. Overall, the semen picture and oxidative markers were within the normal physiological range of the species.

Key words: Jaffarabadi bulls, Physical attributes, Oxidative markers, Semen, Seminal plasma.

INTRODUCTION

Jaffarabadi is a riverine heaviest buffalo breed of India with native in the Gir forest of Saurashtra region of Gujarat. They are considered the important dairy species for sustainable development due to their ability to utilize low-quality roughage and convert it into higher economic returns. The lactation yield ranges from 2150 to 2340 kg with a fat % of 6.8-8.5 (Nivsarkar et al., 2000). Out of the total buffalo population of 87.74 Lakhs in Gujarat, the Jaffarabadi buffaloes in Saurashtra are 19.26 lakhs (21.95%, Buffalopedia, 2021). They are currently being developed as purebred buffaloes at Cattle Breeding Farm, JAU, Junagadh concerning the production of superior germplasm for improvement of breeds in the state of Gujarat. AI is one of the assisted reproductive techniques (ARTs), which is the first-generation biotechnological advancement that has made a profound contribution to the genetic improvement of livestock. In addition, AI gives livestock owners an unprecedented opportunity to introduce proven genetics for accelerated improvements. This has led to a subtle increase in the milk yield of buffaloes in India.

The seminal traits of bulls reflect the status of testicular function and hormonal interrelationship. Correct determination of the number of sperm in the ejaculate is extremely important as it is highly variable. Accurate prediction of fertilizing potential of semen is an extremely useful means for successful exploitation of the production potential of sires (Hafez and Hafez, 2000). However, no single test or combinations of tests have been proved to be totally reliable for the accurate prediction of semen quality in relation to fertility. The correlations of these physical characteristics with fertility are highly variable and relatively poor (Rodriguez-Martinez, 2000; Shelke and Dhami, 2001; Tiwari et al., 2009; Ghodasara et al., 2016). It is also known that the semen of buffalo bull is equipped with certain antioxidants such as enzymatic and non-enzymatic, which protect the sperm from reactive oxygen but it is proved to be highly insufficient in protecting the sperm motility, viability, plasma membrane, acrosome and DNA integrity from oxidative stress (Kumar et al., 2011). Therefore, this study was planned to evaluate the physical attributes and oxidative stress markers of semen of Jaffarabadi buffalo bulls in its home tract, Gujarat.

MATERIALS AND METHODS

The study was undertaken on four sexually mature Jaffarabadi buffalo bulls, 4 to 6 years old, at the Department of Veterinary Gynaecology and Obstetrics of the College in collaboration with the Cattle Breeding Farm, JAU, Junagadh (India), during hot summer from March to June 2021. Junagadh is located at 21.52o N 70.47oE at the foothill of mountain Girnar. It has an average elevation of 107 meters (351 ft) from mean sea level, and has a tropically hot and humid climate with three distinct seasons. It records annual rainfall of 1000 to 1200 mm. The bulls selected were healthy, free from

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diseases, in good libido, clinically normal and maintained under identical managemental conditions.

As per the MSP of Government of India (2000), each bull was fed @ 0.8 kg concentrate, 1.6 kg dry fodder and 4.16 kg of green fodder per 100 kg body weight, and mineral mixture supplement @ 60 gm per day/head. The bulls were having free access to clean drinking water throughout the day. Exercise in rotatory exerciser, bathing and grooming were carried out daily as a routine practice.

Semen was collected in the morning hours twice a week from the bulls by using a sterile artificial vagina (IMV, Danish model). Immediately after collection, the ejaculates were brought to the laboratory and kept in water bath at 34°C for assessing volume, sperm concentration, mass activity, individual motility, sperm viability, sperm abnormalities, hypo-osmotic swelling test, and acrosome integrity as per standard procedures (Tomar, 1984). Soon after evaluation, the freshly collected semen samples were diluted with andromed extender @ 80 million sperm/ml and centrifuged at 3000 rpm for 10 min to obtain seminal plasma, which was stored at -20°C until assessed for the lipid peroxidation and glutathione-S-transferase activities. The peroxidative membrane damage was determined in terms of malondialdehyde (MDA) produced by using the standard kit procured from Sigma Aldrich (Saint Louis, USA). Similarly, the Glutathione-S-Transferase (GST) activity was estimated with 1-chloro-2,4,-dinitrobenzene (CDNB) as substrate using ELISA kits supplied by HiMedia Lab. Pvt. Ltd., Mumbai as per the procedure and instructions of manufacturer.

The data obtained for various parameters were analyzed by one-way ANOVA and Duncan's post-hoc test was used to determine significant differences between the bulls (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Physical Attributes of Neat Semen

The mean semen ejaculate volume recorded among four Jaffarabadi buffalo bulls under study varied non-significantly from 2.65±0.27 to 5.32±1.15 ml with an overall mean of 4.03±0.38 ml, while sperm concentration varied significantly (P<0.01) from 1031.33±78.17 to 1813.17 ±182.70 million/ml, with an overall mean of 1464.08±82.05 million/ml (Table 1). The recorded overall mean values of both the parameters were in agreement with those reported earlier by Rana and Dhami (2003), Patel et al. (2012), Parmar et al. (2020) and Bhave et al. (2020) in the bulls of the same breed. However, Dhami et al. (2001), Shelke and Dhami (2001) and Dhami and Shelke (2005) recorded relatively lower sperm count in Jaffarabadi bull semen. Ejaculate volume and sperm concentration are of significance in production of AI doses. The variation in ejaculate volume and sperm concentration within the breed is attributed to a difference in individuality, environmental conditions/season, managemental practices followed and the influence of the handler.

The mean mass activity score of four Jaffarabadi bulls varied non-significantly from 3.67±0.21 to 3.83±0.17, with an overall mean of 3.79±0.08. Similarly, the mean individual sperm motility also varied insignificantly from 88.33±1.05% to 90.83±0.83%, with an overall mean of 90.00±0.52% (Table 1). Shelke and Dhami (2001), Rana and Dhami (2004), Patel et al. (2012), Parmar et al. (2020) and Bhave et al. (2020) however recorded somewhat lower values in the same breed than the present findings. The reason behind variation in sperm motility may be due to seasons, nutrition, techniques of collection, handling of semen and subjective variation in skill and experience of evaluation.

The mean live sperm percent in the semen of four Jaffarabadi bulls differed non-significantly from 90.67±1.05% to 93.17±0.87%, with an overall mean of 92.04±0.59%, while the mean total sperm abnormalities varied significantly (P<0.05) between 3.67±0.42% and 5.33±0.21%, with an overall mean of 4.50±0.22% (Table 1). Similar observations were recorded by Ghodasara et al. (2016) and Parmar et al. (2020) in Jaffarabadi buffalo bull semen; while lower values of sperm viability and higher sperm abnormalities were found by Shelke and Dhami (2001), Dhami et al. (2001), Rana and Dhami (2003), Dhami and Shelke (2005) and Patel et al. (2012) in the same breed. The variation in live and abnormal sperm percentage is attributed to individual bull variation, time adopted for semen processing, stain used and its temperature, etc. apart from inherent quality. Male fertility depends upon morphologically normal spermatozoa present in the ejaculated semen. A maximum of 15-20 % of total abnormalities of sperms do not affect much fertility of male animals, however above 30 % of total abnormalities affect fertility. Most workers agree that semen of fertile bull should not have total sperm abnormalities more than 20 %, and of the head 4%, midpiece 8 %, tail 2 % and free heads 6 %.

The acrosome is a cap-like structure covering the front side of the spermatozoa. It contains the necessary enzymes for fertilization. A higher percentage of sperm with intact acrosome is indicative of good quality semen samples. Similarly, the hypo-osmotic swelling test investigates whether the spermatozoon membrane is functional or not. A higher percentage of HOS-positive sperm is indicative of better sperm plasma membrane integrity. In the present study, the overall mean percent acrosomal integrity and HOS reactivity of sperm was 87.08±0.58% and 83.00±0.49 %, and it varied non-significantly between four bulls from 84.67±1.02% to

88.33±1.17%, and 81.50±0.85% to 83.67±1.26%, respectively (Table 1). Similar findings were recorded for both the traits by Ghodasara et al. (2016) and Parmar et al. (2020) in Jaffarabadi buffalo bulls. Percentage of acrosome integrity and/or HOS positive spermatozoa reported by Shelke and Dhami (2001), Dhami et al. (2001), Rana and Dhami (2003), Dhami and Shelke (2005) and Patel et al. (2012) in Jaffarabadi were however quite lower than present observations.

Oxidative Stress Parameters of Semen

Oxidative stress is a condition associated with an increased rate of cellular damage in the body induced by oxygen and oxygen-derived oxidants, i.e., reactive oxygen species (ROS). The role of oxidative stress in infertility and methods for counteracting its impact on reproductive tissue with antioxidants is still in their infancy. Oxidative stress (OS) is a result of the imbalance between ROS and antioxidants in the body. Sperm damage causes cellular enzymes like Glutathione-S-Transferase (GST) to leak into seminal fluid. GSTs are 24 kDa enzymes which are glycosylated proteins present on the sperm acrosome and required for sperm oocyte interaction during fertilization. GSTs are essential sperm antioxidant enzymes involved in cell protection against oxidative stress and toxic chemicals, preserving sperm function and fertilizing ability.

Table 2: Oxidative biochemical parameters of neat semen of Jaffarabadi buffalo bulls

(Mean ± SE)

Bull No.	Lipid Peroxidation (MDA, µM)	Glutathione-S-Transferase (nmol CDNB/ml/min)
1	5.73±0.39	18.33±0.6
2	7.09±1.09	21.44±2.25
3	6.74 ±0.03	17.50±0.66
4	7.81 ±0.25	17.29±1.84
Overall	**6.84±1.76**	**18.64±0.11**

Bull to bull variation was non-significant in both the parameters

The overall mean MDA concentration and GST activity recorded in the freshly diluted sperm-free seminal plasma samples of Jaffarabadi buffalo bulls were 6.84±1.76 µM, and 18.64±0.11 nmol CDNB/ml/min, respectively. These values varied insignificantly between four bulls from 5.73±0.39 to 7.81±0.25 µM and 17.29±1.84 and 21.44±2.25 nmol CDNB/ml/min, respectively (Table 2). Similar values of MDA were reported by Selvaraju et al. (2009), Dhami et al. (2020) and Chaturvedi et al. (2021) in buffalo bull semen. However, the higher levels of MDA than the present ones were reported by Dhami et al. (2021) in Murrah and Surti buffalo semen. Kumar et al. (2014) reported that the activity of glutathione-s-transferases increases in post-thaw semen than in fresh semen ejaculate of buffalo. A significantly (P<0.05) higher MDA production in buffalo bull sperms during LPO could be due to more unsaturated fatty acids in their spermatozoa than in other species. Lipid peroxidation arises as a consequence of excessive production of ROS and impaired antioxidant defense mechanisms making sperm more susceptible to ROS-induced damage.

CONCLUSION

The present study conducted during hot summer months on fresh semen of Jaffarabadi bulls in their native tract revealed normal physico-morphological seminal characteristics and oxidative stress parameters, *viz.*, lipid peroxidation (LPO/MDA) and glutathione-s-transferase (GST) enzyme activity in seminal plasma. Significant (P<0.05) bull variation was seen only in the sperm concentration and sperm morphological abnormalities suggesting better adaptability of bulls to the hot climate of the region.

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Conflict of Interest: None.

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Table 1: Physical characteristics of neat semen ejaculates of Jaffarabadi buffalo bulls (Mean±SE)

Bull No.	Ejaculate Volume (ml)	Sperm Concentration (M/ml)	Mass Activity (0-5 scale)	Individual Motility (%)	Sperm Viability (%)	Sperm Abnormalities (%)	Acrosome Integrity (%)	HOS Reactivity (%)
1	4.08±0.42	1031.33±78.17^{a}	3.67±0.21	90.00±1.29	91.83±1.01	4.50±0.22^{ab}	87.33±0.88	83.17 ±0.79
2	2.65±0.27	1813.17±82.70^{a}	3.83±0.17	90.83±0.83	93.17±0.87	4.50±0.56^{ab}	88.33±1.17	83.67 ±0.95
3	5.32±1.15	1611.67±116.99^{a}	3.67±0.21	88.33±1.05	90.67±1.05	5.33±0.21^{b}	84.67±1.02	81.50 ±0.85
4	4.07±0.56	1400.17±60.83^{ab}	3.83±0.17	90.83±0.83	92.50±1.69	3.67±0.42^{a}	88.00±1.18	83.67 ±1.26
Overall	**4.03±0.38**	**1464.08±82.05**	**3.75±0.09**	**90.00±0.52**	**92.04±0.59**	**4.50±0.22**	**87.08±0.58**	**83.00±0.49**
	NS	**	NS	NS	NS	*	NS	NS

Means with different superscripts within column differ significantly between bulls at *P<0.05 /** P<0.01 level, NS Non-significant.