RESEARCH ARTICLE

Physico-Morphological Characteristics and Oxidative Markers of Fresh Semen of Gir Bulls

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Abstract

The present investigation was undertaken on four mature Gir bulls (4-6 years) maintained at University farm, Junagadh for 4 months during hot summer season. Semen (n=6 ejaculate/bull) was collected using artificial vagina once a week from each bull. The fresh ejaculates were evaluated for physical attributes and oxidative stress markers in the seminal plasma. The overall mean values recorded based on 24 ejaculates for ejaculate volume, sperm concentration, mass motility (0-5 scale), individual motility, sperm viability, sperm abnormality, plasma membrane integrity (HOST reactive sperm), and acrosome integrity were 6.20 ± 1.27 mL, 1316.83 ± 155.07 million/ mL, 3.75 ± 0.20, 84.83 ± 0.94 %, 87.58 ± 1.07 %, 9.63 ± 0.83 %, 79.58 ± 1.01 % and 91.21 ± 0.52 %, respectively. There was a significant P<0.001) difference in ejaculate volume, sperm concentration and sperm abnormality between bulls. The levels of oxidative stress parameters, *viz*. lipid peroxidation and glutathione-S-transferase (GST) activity in the seminal plasma were 5.51 ± 0.26 µmol/mL and 16.30 ± 2.14 nmol/ mL/min, respectively. Individual motility, viability, HOST reactive spermatozoa, and acrosomal integrity were positively correlated, suggesting that any one or two attributes like motility or HOS test can predict the semen quality. The semen quality generally was in normal physiological range including oxidative markers even during heat stress.

Keywords: Correlations, Gir bull semen, Oxidative biomarkers, Physico-morphological attributes.

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Introduction

Gujarat is the cradle of India's white revolution. Gujarat state is well known for different cattle breeds, *viz*., Gir, Kankrej, and Dangi. Many farmers in state maintain 2-3 female cattle, breed them through AI, and improve them. The Gir is a famous milch cattle breed of India having native in Gir forest of Saurashtra region of Gujarat. The Gir animals are famous for their tolerance to stress conditions and resistance to various tropical diseases (Gaur *et al*., 2003). The highest recorded peak yield of Gir cows was 22.9 liters/day in an organized herd with a standard 305 lactation yield of 4538 liters. Under field condition highest standard lactation yield of 5956 kg was recorded. Gir cows produced 9989 liters of milk in their productive life and had recorded mean productive and herd life of 4.5 and 9.4 years, respectively, with an average number of 4.25 lifetime lactations (Patbandha *et al*., 2020).

The testicular function is reflected by semen quality of the ejaculates. Predicting fertilizing potential of semen as accurately as possible 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 this. The correlations of the physical characteristics of semen with fertility are relatively low (Rodriguez-Martinez, 2000; Shelke and Dhami, 2001; Chaudhary et al., 2017). It is also known that the antioxidants systems in bovine semen is insufficient to protect the sperm from reactive oxygen, affecting the sperm motility, plasma membrane, acrosome, and DNA integrity (Kumar *et al.,* 2011, 2014). Studies on oxidative stress markers in Gir bull semen are meagre (Patel *et al.,* 2020; Chaturvedi *et al.,* 2021). Therefore, this study was planned to evaluate the physical attributes and oxidative stress markers of semen of Gir bulls in its native tract, Gujarat.

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MATERIALS AND METHODS

The study was undertaken on four sexually mature healthy Gir bulls, 4 to 6 years old, maintained identically at the Cattle Breeding Farm, JAU, Junagadh (India), during hot summer months from March to July 2021. Junagadh is located at 21.52° N 70.47°E at the foothill of mountain Girnar at 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 under study were fed as per the MSP of Government of India (2000), @ 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 and had free access to clean drinking water throughout the day. Daily bathing and grooming was carried out as a routine, and physical exercise in the rotatory exerciser a day before semen collection was practiced.

Semen was collected in the morning hours once a week from the bulls by using sterile artificial vagina (IMV, Danish model). Immediately after collection, the ejaculates were kept in a water bath at 34°C to assess volume, sperm concentration, mass activity, individual motility, sperm viability, sperm abnormalities, hypo-osmotic swelling test, and acrosome integrity as per standard procedures (Hafez and Hafez, 2000). Soon after evaluation, the freshly collected semen samples were diluted with Andromed extender @ 50 million sperm/ mL and part of it was centrifuged at 750 x g for 10 min to obtain seminal plasma, which was stored at -20°C until analyzed for the lipid peroxidation and glutathione-S-transferase (GST) activities. The lipid peroxidation damage was determined in terms of malondialdehyde (MDA) produced using the standard procedure and kits (Sigma Aldrich, USA). Glutathione-S-Transferase (GST) activity was estimated with 1-chloro-2,4,-dinitrobenzene (CDNB) as substrate using the standard procedure and kits procured from Hi-Media Lab Pvt Ltd., Mumbai, India.

The data for various parameters were expressed as Mean ± SEs, and analyzed by standard one-way ANOVA and Duncan's post-hoc test to determine significant differences at $p<0.05$ between the bulls.

RESULTS AND DISCUSSION

The observations on mean values of 24 semen ejaculates obtained from four Gir bulls for various physico-morphological attributes are presented in Table 1.

Ejaculate Volume and Sperm Concentration

In Gir bulls, semen ejaculate volume ranged between 2.5 and 13.0 mL with an overall mean of 6.20 ± 1.27 mL, while sperm concentration varied from 810 to 1874 million/ mL with an overall mean of 1316.83 ± 155.07 million/ mL. The mean values of both the parameters varied significantly ($p<0.001$) amongst the bulls. Sperm concentration was statistically similar and higher ($p<0.001$) in three bulls than in bull No. 2. Ejaculate volume was the highest in bull No. 2 with the lowest sperm concentration, and vice versa was the case for bull No. 4 (Table 1). This is a normal physiological mechanism to compensate the total sperm output per ejaculate. Similar findings were also observed in Gir bulls by Chaudhary *et al.* (2017), Das *et al.* (2017), Dhami *et al.* (2017), Chikhaliya *et al.* (2018), Bhave *et al.* (2020). In contrast, Shelek and Dhami (2001) reported lower ejaculate volume as 4.84 ± 0.16 mL, while Rana and Dhami (2004) reported comparable sperm count, but higher ejaculate volume of 7.03 ± 0.44 mL in Gir bulls.

A highly significant ($p<0.01$) negative correlation of ejaculate volume with sperm concentration/ mL ($r= -0.638$) found in the present study (Table 2) was in agreement with Ray and Ghosh (2013) in Sahiwal bulls, Bhavsar (2014) in Kankrej bulls and Dhami *et al.* (2018) and Patel *et al.* (2020) in Gir bulls. Ray and Ghosh (2013) revealed that the ejaculate volume had a significant positive correlation with mass activity ($r=0.485$), whereas in this study, it has a negative correlation with mass activity ($r=-0.307$). The non-significant correlations of ejaculate volume with other *semen parameters* observed in the present study were also reported by Swain and Singh (2004) in Sahiwal bulls and Bhavsar (2014) in Kankrej bulls. Ejaculate volume and sperm concentration are breed characteristics, which depend upon the scrotal size and weight, reproductive health of bull, age of bull, frequency and method of semen collection, the pooled volume of semen, nutrition of bull, season, and management (Ijaz *et al.,* 2009; Patel *et al.,* 2020). This may be a reason for the variation observed by different workers for the ejaculate volume in the same breed.

Mass Motility and Individual Sperm Motility

The mean mass motility (score 0-4) and individual sperm motility recorded among four bulls varied from 3.17 ± 0.17 to 3.83 ± 0.17, and 83.00 ± 2.58 to 87.17 ± 0.95 %, with overall means of 3.75 ± 0.20 and 84.83 ± 0.94 %, respectively. The values of these traits did not differ significantly between bulls (Table 1). These findings concurred with the observations of Chikhaliya *et al.* (2018), Dhami *et al.* (2018), and Pathak *et al.* (2018) in Gir bulls, however, Shelke and Dhami (2001) and Rana and Dhami (2004) in Gir bulls, and Ray and Ghosh (2013) in Sahiwal bulls recorded lower mass activity and individual sperm motility in fresh ejaculates. The difference in the mass motility of the spermatozoa has been accredited to factors like age of the bull, season, collection frequency, degree of stimulus provided, and type of thrust (Shelke and Dhami, 2001; Pathak *et al.*, 2018). The sperm motility can also be affected by the experience of the technician, improper handling procedure, contaminated glassware, chemicals present over the fingers, cold or hot test tubes, glass slides, microscope stage, rapid drying or cooling of glass slides, prolongation during collection and examination period, etc. (Pal *et al.,* 2020).

Table 1: Physical characteristics of neat semen of Gir bulls (Mean ± SE)

Bull No.	Ejaculate Volume (ml)	Concentration (million/ml)	Mass Motility (0-4 score)	Individual Motility (%)	Sperm Viability (%)	Sperm Abnormality (%)	HOST Reactive Sperm (%)	Acrosome Intact Sperm (%)
1	6.93 ± 0.49bc	1420.00 ± 121.47b	3.83 ± 0.17	87.17 ± 0.95	90.17 ± 1.14	8.17 ± 0.60a	82.00 ± 1.06	91.00 ± 0.63
2	9.45 ± 1.13c	880.00 ± 39.98a	3.17 ± 0.17	85.50 ± 1.38	88.50 ± 0.99	9.17 ± 0.48b	80.33 ± 0.84	90.17 ± 0.70
3	4.53 ± 0.52ab	1358.33 ± 151.18b	3.67 ± 0.21	83.00 ± 2.58	86.17 ± 1.82	9.33 ± 0.42b	77.33 ± 2.06	91.00 ± 0.93
4	3.88 ± 0.53a	1609.00 ± 85.55b	3.83 ± 0.17	83.67 ± 1.41	85.50 ± 1.71	9.17 ± 0.60b	78.67 ± 2.36	92.67 ± 0.80
Overall	6.20 ± 1.27	1316.83 ± 155.07	3.75 ± 0.20	84.83 ± 0.94	87.58 ± 1.07	9.63 ± 0.83	79.58 ± 1.01	91.21 ± 0.52
P value	0.0001	0.0009	0.0555	0.3227	0.1232	0.0008	0.2692	0.1723

Means with different superscripts within column differ significantly at p<0.001 level.

In the present study, mass motility of semen was significantly (p<0.05) and positively correlated with individual sperm motility (r=0.491) and HOST (r=0.476), and had non-significant negative correlation with sperm abnormality, while individual sperm motility had highly significant positive correlations with viability and HOST (r=0.877, 0.791, p<0.01) and significant positive correlation with mass motility and acrosomal integrity (r=0.491, 0.487, p<0.05) (Table 2). Similar correlations have been reported earlier by Chikaliya *et al*. (2018) and Pathak *et al*. (2018) in Gir bulls, and Ray and Ghosh (2013) in Sahiwal bulls.

Sperm Viability and Morphology

The mean sperm viability of 4 bulls varied non-significantly from 85.50 ± 1.71 to 90.17 ± 1.14, %, but the sperm abnormality varied significantly (P<0.05) from 8.17 ± 0.60, to 9.33 ± 0.42%, with overall means of 87.58 ± 1.07 % and 9.63 ± 0.83 %, respectively (Table 1). Similar findings were also observed by Chikhaliya *et al*. (2018) and Pathak *et al*. (2018) in Gir bulls, however, Shelke and Dhami (2001) and Dhami *et al*. (2018) recorded lower mean sperm viability in Gir bulls, and Ray and Ghosh (2013) in Sahiwal bulls. The variation in per cent sperm viability and abnormalities observed in different studies might be due to environmental factors, age, season, temperature shock, state of maturation, collection frequency, etc. Studies on physico-morphological attributes of spermatozoa help in identifying good quality ejaculates. Sometimes many structural abnormalities can occur in the spermatozoa due to faulty spermatogenesis caused by heredity, diseases, bad environmental conditions and improper semen handling techniques. Accurate morphological screening of the semen ejaculates allows elimination of bulls with low fertility before the cryopreservation of semen, thus contributing to a major savings for AI enterprises.

The correlation study revealed that the sperm viability had highly significant (p<0.01) positive correlations with HOST (r=0.721) and individual sperm motility (r=0.791) and significant (p<0.05) positive correlation with acrosomal integrity (r=0.448). However, the sperm abnormality did not correlate significantly with any parameters (Table 2). Similar correlations have also been reported earlier in Gir and Sahiwal bull semen (Shelke and Dhami, 2001; Chikhaliya *et al*., 2018; Pathak *et al*., 2018). Ray and Ghosh (2013) reported a significant (p<0.05) negative correlation of sperm abnormality with mass activity and HOST and a highly significant (p<0.01) negative correlation with viability and acrosomal integrity in Sahiwal bulls.

Sperm Plasma Membrane and Acrosome integrity

The overall mean HOST reacted spermatozoa was 79.58 ± 1.01 % with a range of 70 to 85 %, and acrosomal integrity was 91.21 ± 0.52 % with a range of 84 to 96 %, among the four bulls, which did not differ significantly (Table 1). Similar findings were observed by Ray and Ghosh (2013) in Sahiwal bulls, and Patel *et al.* (2020) and Chaturvedi *et al.* (2021) in Gir bulls, while Bhave *et al.* (2020) recorded very low HOS reactive sperm (55.13 ± 0.005 %), and Dhami *et al.* (2018) reported higher HOST reacted spermatozoa (82.54 ± 0.91 %) in Gir bulls.

Table 2: correlation among the physical attributes of neat semen in Gir bulls

Attributes	Ejaculate Volume	Sperm Concen-tration	Mass Motility	Sperm Motility	Sperm Viability	Sperm Abnor-mality	HOS Reactivity
Sp Concentration	-0.638**	1					
Mass Motility	-0.307	0.121	1				
Individual Motility	0.281	-0.349	0.491*	1			
Sperm Viability	0.236	-0.333	0.304	0.791**	1		
Abnormality	-0.261	-0.174	-0.093	-0.176	-0.239	1	
HOS Reactivity	0.257	0.482	0.476*	0.877**	0.721**	-0.120	1
Acrosomal Integrity	-0.212	0.016	0.400	0.487*	0.448*	-0.198	0.430*

* Significant at p<0.05 level, ** Significant at p<0.01 level

The HOST reactive spermatozoa had highly significant ($p<0.01$) positive correlations with individual motility and viability and significant ($p<0.05$) positive correlations with mass motility and acrosomal integrity, while acrosomal integrity had significant positive correlations with individual motility and viability (Table 2). The present correlation findings concurred well with those reported by Ray and Ghosh (2013) in Sahiwal, and Chikhaliya *etal.* (2018), Dhami *et al.* (2018), Patel *et al.* (2020) and Chaturvedi *et al.* (2021) in Gir bulls.

Lipid Peroxidation (LPO) and Glutathione-S-Transferases (GST)

The overall mean malondialdehyde (MDA) concentration of freshly diluted seminal plasma was 5.51 ± 0.26 µmol/mL and it varied in-significantly between 4.73 ± 0.68 and 5.82 ± 0.17 µmol/mL among the four bulls. The corresponding overall mean Glutathione-S-Transferases (GST) activity was 16.30 ± 2.14 nmol/mL/min and it varied significantly from 10.0 ± 1.56 to 19.6 ± 0.46 nmol/mL/min among the four bulls. The finding on MDA level noted under summer heat stress in the present study was quite lower than that reported by Patel *et al.* (2020) and Chaturvedi *et al.* (2021) even during high breeding season in Gir bulls.

Malondialdehyde (MDA) was negatively and significantly associated with individual motility and viability. In the present study, bulls with higher sperm motility had lower MDA concentration on lipid peroxidation which was in harmony with Dorji *et al.* (2015) and Kumar *et al.* (2014), who reported significantly better sperm motility with MDA levels to be non-significantly and significantly lower and higher GST activity in bull semen. Patel *et al.* (2020) and Chaturvedi *et al.* (2021) also found significant negative correlations of MDA levels with sperm motility, viability and HOS reactivity and a positive correlation with sperm abnormalities in Gir and Murrah bull semen.

CONCLUSION

The present study conducted during hot summer months on fresh semen of Gir bulls in their native tract revealed normal physico-morphological seminal attributes 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 ejaculate volume, sperm concentration, and sperm abnormalities suggesting better adaptability of Gir bulls to hot climate of the native region.

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