The Pharma Innovation Journal 2023; SP-12(8): 379-384

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(8): 379-384
© 2023 TPI
www.thepharmajournal.com
Received: 21-04-2023
Accepted: 22-05-2023

Kaviraj Airani
M.V.Sc. Scholar, S/o Megharaj Airani, Belur, Karnataka, India

Renukaradhya GJ
Assistant Professor, Department of Veterinary Gynaecology and Obstetrics, Veterinary College (KVAFSU), Hebbal, Bengaluru, Karnataka, India

Sahadev A
Professor and Head, Department of Veterinary Gynaecology and Obstetrics, Veterinary College (KVAFSU), Hebbal, Bengaluru, Karnataka, India

Naveenkumar S
Associate Professor and Head, Department of Animal Genetics and Breeding, Veterinary College (KVAFSU), Hebbal, Bengaluru, Karnataka, India

Sathisha KB
Associate Professor (I/C), Livestock Research and Information Centre, (Sheep)(KVAFSU), Nagamangala, Karnataka, India

Ningaraju K
Assistant Chief Technical Officer ICAR- NDRI, SRS. Adugodi, Bengaluru, Karnataka, India

Corresponding Author:
Kaviraj Airani
M.V.Sc. Scholar, S/o Megharaj Airani, Belur, Karnataka, India

Seminal attributes and motion kinematics of Gir bulls in their non-breeding tract

Kaviraj Airani, Renukaradhya GJ, Sahadev A, Naveenkumar S, Sathisha KB and Ningaraju K

Abstract
The present study was undertaken with the objective of establishing the normal spermiogram in Gir bulls and sperm motion kinematics using CASA in their non-breeding tract. About 60 semen ejaculates from three Gir bulls in four months period were collected by AV method and colour of the ejaculates was creamy to milky white and consistency with mean value of 2.80±0.08 (out of 4). The observed overall mean ejaculate volume (mL), mass motility (0-5 scale), individual motility (%), sperm concentration (10^6/mL), sperm viability (%), sperm abnormality (%), HOST reacted (%), acrosome integrity and post thaw motility were 4.91±0.22, 3.40±0.06, 82.40±0.45, 900±34.90, 88.27±0.20, 11.87±0.19, 87.27±0.34, 90.80±0.28 and 57.03±0.51, respectively. The sperm kinematics parameters *viz*., VCL, VSL and VAP were 119.90±1.68,76.11±1.55 and82.30±1.37 in fresh semen and 100.70±1.58, 60.64±1.50 and 67.53±1.34 µm/sec inpost thaw values, respectively and are similar to those of native tract.

Keywords: Gir bull, spermiogram, CASA, HOST, kinematics

1. Introduction
Gir is an internationally recognized milch breed of India, which originated in the Gir forest of Saurashtra district Gujarat and are renowned for their ability to withstand stressful situations and their resistance to several tropical diseases (Gaur *et al*., 2003) [18]. The enhancement of morphometric measurement and motion kinematics of a spermatozoon due to the recent development of digitalized microscopic evaluation of spermatozoa using Computer Assisted Semen Analysis technology (CASA) opened up new opportunities in the spermiogram assessment (Yaniz *et al.*, 2015) [53]. In recent years, Gir cattle are reared and also bulls have been used for frozen semen doses production in their non-breeding tract. Sufficient information is not available about the Gir breed's semen traits, fertility and semen production efficiency outside its native tract. Hence, in view of these facts the present study was undertaken to establish the normal spermiogram in Gir bulls.

2. Materials and Methods
The present study was conducted at Central Frozen Semen Production and Training Institute (CFSPTI), Government of India, Hessaraghatta, Bengaluru during the period from August 2022 to October 2022 and procedures followed were in accordance with minimum standard protocol. Three sexually active Gir bulls aged around 3-7 years were selected and semen was collected twice weekly using artificial vagina (IMV technologies, France) maintained at 42 ºC. Atotal of 60 ejaculates were visualized with naked eye for colour (milky, thick milky, creamy and watery) and consistency were graded in 0-4 scale (0-translucent, 1- nonviscid, 2- slightly viscid, 3-viscid and 4-thick viscid) as described by Barth (1997) [4]. Samples with 2-4 grades were used for further processing and the samples having abnormal colour and consistency were discarded. Volume (mL) of the ejaculates was recorded by noting the semen in the collection tube immediately after collection (Pal *et al*., 2020) [32].
Mass activity (0-5 scale) was evaluated as per the method described by Ghodasara *et al*. (2016) [19]. Semen sample was immediately diluted with (1:100 with 0.9% normal saline) and concentration of the spermatozoa (Millions/mL) was determined using a calibrated photoelectric colorimeter (Accucell Bovine Photometer, IMV Technologies, France) with dilutor (Hamilton microlabs ®500B). Initial motility (%) of the neat semen after initial dilution was recorded using phase contrast microscope at 45X (Olympus, Tokyo, Japan) on 0-100 scale. Sperm viability (%) was assessed with eosin-nigrosin (S. D. Fine chemical limited,

Boisar, Maharastra, India) stained semen smears under oil immersion lens of phase contrast microscope (Campbell *et al*., 1953) [8]. Similarly the smears stained with Rose Bengal stain were subjected to determine sperm morphology and the total sperm abnormalities based on head, mid-piece and tail abnormalities and intact acrosome and acrosomal abnormalities were determined using geimsa stain (Merck specialties private limited, Mumbai) and expressed in percentage.

Sperm plasma membrane integrity of neat and frozen thawed semen was assessed by hypo-osmotic swelling test (HOST) as per the method described by Shalini *et al*. (2018) [46]. The frozen straws were subjected for post thaw assessment of semen sample after 24 hours of freezing such as post thaw motility, post thaw HOST and motion kinematics (VAP, VCL & VSL) was determined by CASA (Hamilton Thorne Biosciences, IVOS II, IMV Technologies, France). Descriptive statistics' for various parameters of semen quality was estimated as per Snedecor and Cochran (1994) [49]. The difference between bulls in various parameters was analyzed by ANOVA, followed by Tukey's test using Graph Pad Prism software version 5.0.

3. Results and Discussion

3.1 Fresh seminal characteristics

The colour of semen in the present study was found to be creamy (83.33%), thick milky (6.67%) and milky white (10.00%). The overall mean of consistency was 2.80±0.08 with a range from 2-4 (Slightly viscid to full viscid). The colour and consistency of semen observed is in agreement with those obtained by Sonar *et al*. (2016) [51] and Shelke and Dhami (2001) [47] in Gir, and also corroborates with Pathak (2008) [34] in Sahiwal, Bhavsar (2014) [6] in Kankrej bulls, Colour can be used as one of the important criteria among the other seminal traits for primary screening of semen ejaculates and variations in the colour of ejaculates may be partially due low sperm concentration (Kapadiaya *et al*., 2018) [24]. The overall ejaculate volume among the bulls ranged from 2.80 - 10.80 mL with an overall mean of 4.91±0.23 mL (Table1). Similarly, Sonar *et al.* (2016) [51] reported the mean ejaculate volume of 4.99±0.26 mL in Gir bulls whereas, Kedia *et al* (2014) [27] and Sannat *et al* (2015) [44] recorded 4.40±0.24 mL and 4.7±0.31 mL, respectively in Tharpakar bull. However, it is lower compared to values reported by Rana and Dhami (2004) [38] (7.03±0.44 mL), Chikhaliya *et al*. (2018) [13] (5.80±0.41) mL and Prajapati *et al*. (2022) [35] (6.20±1.27) mL in Gir bulls. Volume is an important inherited, individual and family character (Sane *et al*., 1994) [43]. The variations in semen volume recorded within a breed and among different breed/species and even the same male at different time of collection (Pal *et al*., 2020) [32], age (Ahmad *et al.* 2003) [1], season, testosterone level (Javed *et al.*, 2000) [21], genetics, breed (Rao and Sreemannarayana, 1996) [39], testicle size and management.

3.1.1 Concentration of neat semen

Accurate determination of spermatozoa per milliliter of semen is crucial and can be used as an early indicator of semen quality in semen utilized for cryopreservation (Shelke and Dhami, 2001) [47]. The sperm concentration (10^6/mL) among the bulls in the present study ranged between 510-1483, with overall mean of 900±34.90 (Table 1). Sonar *et al.*(2016) [51] reported in Gir bulls similar values of mean sperm concentration of 895.33±82.68 × 10^6 /mL However, previous studies among Gir bulls reported the meansperm concentration of 1267.63±106.32× 10^6/mL (Chikhaliya *et al*., 2018) [13], 1268.00±85.00 × 10^6/mL (Sannat *et al*., 2015) [44] and 1316.83±155.07 × 10^6 /mL (Prajapati *et al.*, 2022) [35] which were higher than the present study.Nevertheless, Chowdhury*et al*. (2013) [14] reported lower mean sperm concentration (736.00±53.00 × 10^6/mL) in Gir bulls compared to the values obtained in the present study. The variations may be due to the factors that influence sperm concentration like breed, scrotal size, libido, sexual rest, frequency of ejaculation (Kumar, 1979) [28], management, nutrition, physiological status of bulls and genetics, differences in age (Ahmad *et al*., 2003) [1], environment and season of collection (Sarder, 2007) [45].

3.1.2 Mass activity of sperms

Mass activity has been a crucial factor in the acceptance or rejection of the ejaculate for further processing and usage in artificial insemination and it has been positively correlated with the sample's fertility, freez ability and keeping quality (Bhoite *et al*., 2005) [7]. In the present study, the mass activity (0-5 scale) among the bulls ranged from 3-4 with an overall mean value of 3.40±0.06 (Table1). Similar values of3.33±0.11 and 3.44±0.09 were reported by Rana and Dhami (2004) [38] and Chaudhary *et al.* (2017) [12], respectively in Gir bulls. However, earlier studies reported the higher values of 3.88±0.07 and 3.75±0.20 (Chikhaliya *et al.*, 2018: Prajapati *et al.*, 2022) [13, 35] in Gir, 3.83±0.10 in Kankeraj bulls (Kapadiya *et al*., 2018) [24]. Pal *et al*. (2020) [32] opined that improper handling techniques, contaminated glassware (soap residue), chemicals on the fingers, cold or hot test tubes, glass slides, microscope stages, quick drying or cooling of glass slides, lengthening of the collection and examination period, etc. have an impact on the sperm motility.

3.1.3 Initial sperm motility

Initial sperm motility is a crucial factor in determining the quality of the neat semen and can provide an accurate picture of the semen's potency (Rana and Dhami, 2004) [38]. The per cent of initial motility of Gir bulls in the present investigation ranged from 73.00 - 90.00 with an overall mean of 82.40±0.45per cent (Table1) which is in close proximity with the values of Chikhaliya *et al.* (2018) [13] and Prajapati *et al.* (2022) [35] in Gir bulls, in Sahiwal bulls (Lodhi *et al*., 2008) [29], in Amrithmahal bulls (Iliger, 2014) [20]. However, the recorded values in the current study were higher than those reports of Sonar *et al.* (2016) [51], Chaudhary *et al*. (2017) [12] and Bhave *et al*. (2020) [5] in Gir bulls and Sannat *et al*. (2015) [44] in Sahiwal bull. The factors responsible for variations in the values observed by different authors are attributed to different agro climatic conditions, breed, season, age (Ahmad *et al*., 2003) [1], testosterone level management, physiological status of bulls, genetic factors and handling of semen (Javed *et al*., 2000) [21].

3.1.4 Live Spermatozoa per cent in neat semen

The samples containing 50-90 per cent live spermatozoa, showed no change in fertilizing potential, whereas sperm samples with less than 50 per cent live spermatozoa were of doubtful fertility (Pal *et al*., 2020) [32]. In the present study, the per cent live spermatozoa of all bulls ranged from 85 - 92 with overall mean of 88.27±0.20(Table1). The findings are in close agreement with Chikhaliya *et al.* (2018) [13] (85.83±1.94) and Prajapati *et al.* (2022) [35] (87.58±1.07) in Gir bulls, Patel

and Siddiquee, (2013) [33] (90.58±0.20) in Kankrej. The variation in live sperm count has been attributed to age, frequency of collection, and breeding bull of season (Singh *et al*., 2000b) [50].

3.1.5 Intact acrosome in neat semen

The capacity of spermatozoa to fertilize is positively connected with the integrity of the sperm acrosome membrane (Srivastava *et al*., 2013) [52]. The mean per cent intact acrosome in the current study among the bulls was 90.80±0.28 and ranged from 86.00 - 96.00 (Table1). The values are in close agreement with the findings of Chikhaliya *et al.* (2018) [13]. However, the values recorded in the present study were found to be higher than the reported values of 84.80±0.89, 84.42±0.77 by Rana and Dhami, (2004) [38] and Sonar *et al*. (2016) [51], respectively in Gir bull. The difference in observations by different authors might be due to temperature (Chandra *et al*., 1999) [11], age of the bull (Javed *et al*., 2000) [21], season, environment, breed and genetic factor (Andrabi *et al*., 2002) [2] and different protocols used for study (Farooq *et al*., 2013) [16].

3.1.5 Hypo Osmotic Swelling Test (HOST) in neat semen

According to Lodhi *et al*. (2008) [29], spermatozoa's capability to undergo hypoosmotic swelling is a sign of membrane integrity and consistent functional activity (Rota *et al*., 2000) [42]. The overall mean per cent HOST reacted spermatozoa in the present work was 83.35±0.38 and ranged from 76.00 - 93.00(Table1). The earlier studies recorded similar values like 84.00±0.84 (Sonar*et al*., 2016) [51] in Gir and 85.25±0.00 (Lodhi *et al*., 2008) [29] in Sahiwal bulls. Further, Chikhaliya *et al.* (2018) [13] and Prajapati *et al.* (2022) [35] have recorded lower values of 60.12±1.19 and 79.58±1.01, respectively in Gir bulls. The variations observed in different studies might be attributed to season (Kale*et al*., 2000) [23], mass activity, progressive motility, total sperm with intact acrosome and individuality of animal (Prasad *et al*., 1999) [37].

3.1.6 Total sperm abnormalities in neat semen

Abnormal spermatozoa have an influence on male fertility. The percentage of sperm abnormalities in neat semen of Gir bulls recorded in the present study was ranged from 09 - 15 with overall mean of11.87±0.19 (Table 1). These findings are in congruence with the findings of Shelke and Dhami (2001) [47] (12.78±0.91%)and Chikhaliya *et al.* (2018) [13] (10.87±0.41%) in Gir and Ray and Ghosh (2013) [40] (9.97±0.28%)in Sahiwal bulls. The mean percentage of total sperm abnormalities reported in the current study was found to be lower than the findings of 15.96±0.44 and 22.50±1.40 per cent in Gir bulls by Sonar *et al.* (2016) [51] and 19.87±0.54 by Rana and Dhami (2004) [38] in Tharparkar (Kedia *et al.*, 2014) [27]. Nevertheless, Prajapati *et al.* (2022) [35] reported the mean per cent of 9.63±0.83 in Gir bulls; Patel and Siddiquee (2013) [33] recorded 4.24±0.11 per cent in Kankrej and were lower than those values obtained for Gir bulls in the present investigation. However, the variation in per cent sperm abnormalities observed in different studies might be due to environment, age, season, temperature shock and collection frequency (Prajapati *et al.*, 2022) [35].

3.2 Post thaw seminal characteristics

3.2.1 Post thaw seminal parameters

The mean per cent post thaw motility among bulls in the present study ranged between 52.00 - 68.00, with overall mean of 57.03±0.51(Table 2). The present findings are in line with values of 56.00±0.67 (Chaturvedi *et al*., 2021) [10] in Gir and 56.83±0.34 (Patel and Siddiquee, 2013) [33] per cent in Kankrej bulls. However, previous studies reported lower values of 53.81±0.61(Sonar *et al*., 20160 and 47.08±1.10 per cent (Chaturvedi *et al*., 2021) [51, 10] in Gir bulls, 51.16±1.08 in Tharparkar (Kedia *et al*., 2014) [27]. Karmur *et al*. (2002) [25] reporteda decreased sperm motility nearly by 7.78 per cent due to equilibration and 36 per cent due to freezing. Other factors which also affect post thaw motility are thawing temperature and time (Bhosrekar *et al*., 1986) [8], diluters (Pramanik and Raina, 1998) [36], method of glycerol addition (Arancibia *et al*., 1987) [3] and equilibration time (Dhami and Sahni 1993) [15].

3.2.2 Hypo Osmotic Swelling Test (HOST) in frozen semen

In the current study, the overall mean of HOST per cent in post thaw semen was 56.15±0.43 and ranged from 51 – 65 (Table 2). The present findings are in close conformity with the values of55.81±1.72 (Chowdhury*et al*., 2013) [14], 55.71±1.33 (Sonar *et al*., 2016) [51], 55.13±0.00 (Bhave *et al*., 2020) [5] and 55.08±1.10 (Chaturvedi in *et al*., 2021) [10] in Gir bulls, 54.39±2.54 in Tharparkar (Kedia *et al*., 2018) [27]. In contrast to above findings, Shukla *et al.* (2012) [48] reported higher values of 57.71 per cent in Sahiwal bulls. The differences in HOS positivity may be due to the different sugars added to the extender, osmolarity and electrolytes (Jayendran *et al*., 1984) [22].

3.3 Computer Assisted Sperm Analysis (CASA)

Progressive motility and velocity parameters like VCL, VSL, and VAP can be useful in predicting the fertility of bull semen among the several sperm motion characteristics evaluated by CASA (Kathiravan *et al*., 2011) [26].

3.3.1 Sperm kinematics using CASA in fresh semen

In the present study the overall mean value of VCL, VSL and VAP of sperm was 119.90±1.68, 76.11±1.55 and 82.30±1.37µm / sec, (Table 3) respectively. However, earlier studies among Gir bulls observed values VCL, VSL and VAP of 88.62±1.66, 50.01±1.25, 44.51±1.35 µm/s (Pathak, 2008) [34] and 87.74±0.88, 43.17±0.65, 33.74±0.65 µm/s, respectively (Chaturvedi *et al.*, 2021) [10], Similarly, Galmessa *et al.* (2014)[17]in Sahiwal bulls reported overall mean values of 95.02±2.73, 61.62±15.07 and 32.18±2.91 µ/s, for VCL, VSL and VAP respectively and these values were lower than those values obtained for Gir bulls in the current study. The variations in the results among different authors may be due to variation in the breed/species of bulls, initial semen quality and software and model of CASA machines used during assessment (Pathak, 2008) [34].

3.3.2 Sperm kinematics using CASA in post freeze semen

The overall mean value of VCL, VSL and VAP in the present study was 100.70±1.58, 60.64±1.50 and 67.53±1.34µm/sec, respectively (Table 3). However, overall mean value of velocity parameters in the present study was found to be higher than the reported values of 73.68±1.03 (VCL), 30.89±0.63 (VAP), 23.98±0.65 (VSL) µm/s in Gir bulls (Chaturvedi *et al*., 2021) [10]. Similarly, 169.20±4.20 (VCL) 89.20±1.90 (VAP), 69.90±1.60 (VSL)µm/s in Kangayam bulls (Rengarajan, 2004) [41] these values higher than those values obtained for Gir bulls in the present study. However, there was no significant difference between bulls in the

present study with respect to the velocity parameters of sperm in frozen semen. The variations in the results among different authors may be due to variation in the breed of bulls used, freezing method, thawing technique and software and model of CASA machines used during assessment (Pathak, 2008) [34].

Table 1: Mean values for characteristics of fresh semen in Gir bulls (n = 60 ejaculates)

Bull No	Bull-01	Bull-02	Bull-03	Overall
Volume	4.82±0.33	4.48±0.33	5.44±0.47	4.91±0.22
Mass activity	3.40±0.11	3.20±0.09	3.55±0.06	3.40±0.06
Sperm concentration	919±56.80	790±54.40	992±34.90	900±34.90
Initial motility	81.80±0.80	82.65±0.69	82.60±0.88	82.40±0.45
Live and dead sperms	88.10±0.35	88.60±0.39	87.90±0.34	88.27±0.20
Sperm abnormality	12.20±0.30	11.65±0.35	11.75±0.33	11.87±0.19
Acrosome integrity	90.45±0.48	91.05±0.47	90.90±0.52	90.80±0.28
Host	84.05±0.58	84.00±0.65	82.00±0.68	83.38±0.37

Note: Mean± SE bearing different superscripts are significantly different at $p<0.05$

Table 2: Mean values for characteristics of post thawed semen in Gir bulls (n = 60 ejaculates)

Bull No	Bull-01	Bull-02	Bull-03	Overall
Post thaw motility	56.90±0.83	57.40±0.95	56.80±0.94	57.03±0.51
Post thaw Host	56.30±0.66	56.80±0.92	55.35±0.65	56.15±0.43

Note: Mean ±SE bearing different superscripts are significantly different at $p<0.05$

Table 3: Mean values (±SE) of velocity parameters of fresh and frozen thawed semen evaluated under CASA in Gir bulls (n = 60 ejaculates)

Sperm Velocity parameters		Bull 1	Bull 2	Bull 3	Total
VCL (µm/sec)	Fresh	119.70±3.10	123.60±2.72	116.50±2.82	119.90±1.68
	Frozen thawed	101.90±2.37	101.80±3.22	98.41±2.65	100.70±1.58
VSL (µm/sec)	Fresh	77.35±2.99	78.99±1.94	71.99±2.90	76.11±1.55
	Frozen thawed	61.08±2.36	61.17±3.03	59.67±2.46	60.64±1.50
VAP (µm/sec)	Fresh	82.05±3.36	84.87±2.73	79.97±2.94	82.30±1.37
	Frozen thawed	70.00±2.17	66.18±2.60	66.42±2.19	67.53±1.34

Note: Mean±SE bearing different superscripts are significantly different at p<0.05

4. Conclusion

Based on the results, it can be concluded that the seminal attributes of Gir bulls fall within the range established for other Indian breeds of cattle. Assessment of sperm motion characteristics under CASA have been found to be more efficient, accurate, time saving and precise means of qualitative evaluation of sperms motion kinematics. The study revealed that the Gir bulls perform well in non- native breeding tract and adapt well to local environmental conditions of Bengaluru.

5. Acknowledgment

The first author is highly thankful to Commissioner Department of Animal Husbandry and Veterinary Services, Government of Karnataka for deputing me for higher education and also to Dr. B. Arunprasad, Joint commissioner, Dr. M. Bhaskar, Deputy Commissioner and Dr. Atulya M, Assistant Commissioner, CFSP&TI Hesaragatta, Bengaluru for the timely permission and cooperation to carry out research.

6. Conflict of Interest

The authors have no clash of interest.

7. Reference

1. Ahmad M, Asmat MT, Rehman NU, Khan MZ. Semen characteristics of Sahiwal bulls in relation to age and season. Pakistan Veterinary Journal. 2003;23:202-206.
2. Andrabi SMH, Naheem S, Khan LA, Ullah N. Semen characteristics of crossbred (Friesian X Sahiwal) bulls at livestock research station, National agricultural Research Centre, Islamabad. Pakistan Veterinary Journal. 2002;22(4):181-187.
3. Arancibia EA, Hube WA, Jara MC, Veer OC. Effect of sudden temperature changes in thawed semen on the percentage of sperm motility and spermatozoa with normal acrosome in mini tubes. Animal Breeding and Genetics. 1987;55(5):2797.
4. Barth AD. Evaluation of potential breeding soundness of the bull. In: Current Therapy in Large Animal Theriogenology. Edt. Youngquist RS, W B Saunders. Philadelphia. 1997, 222-236.
5. Bhave KG, Jawahar KTP, Kumarasamy P, Sivakumar T, Joseph C, Shirsath T, *et al*. Genetic and non-genetic factors affecting semen production and quality characteristics of Gir cattle breed under semi-arid climate. Veterinary World. 2020;13(8):1714.
6. Bhavsar. Comparative efficacy of extenders for cryopreservation of Kankrej bull semen and pregnancy rate. M. V. Sc. Thesis, S.D.A.U., Dantiwada, India. 2014c.
7. Bhoite UY, Sutar DA, Ulmek BR. Effect of season and period on semen characteristics of two and three breed Gir crosses. Indian Journal of Animal Research. 2005;26(1):43-45.
8. Bhosrekar MR, Purohit JR, Pande AB, Mangurkar BR. Effect of collection regime and equilibration period on the quality and freez ability of semen of crossbred bulls. Indian Journal of Animal Science. 1986;56(1):58-59.
9. Campbell RG, Hancock JL, Rothscild L. Counting live and dead spermatozoa. Journal of Experimental Biology. 1953;30:44.
10. Chaturvedi D, Dhami AJ, Chaudhari DV. Interrelationships among Sperm Quality Parameters,

Cryocapacitation Status, Oxidative Markers and CASA Traits of Fresh and Frozen-thawed Semen of Gir and Murrah Bulls. Indian Journal of Veterinary Science and Biotechnology. 2021;17(4):54-60.
11. Chandra M, Srivastava VK, Shukla AK. Study of effect of temperature on semen quantity (volume) and quality (motility) using fuzzy approach. Buffalo Journal. 1999;15(1):105-113.
12. Chaudhary PJ, Dhami AJ, Chaudhari DV, Hadiya KK, Patel JA. Comparative Study of Gir Cattle and Surti Buffalo Bulls Semen under Middle Gujarat Climate. Indian Journal of Veterinary Science and Biotechnology. 2017;13(1):56-61.
13. Chikhaliya PS, Ahlawat AR, Solanki GS, Raval RJ, Vala KB and Verma AD. Physical seminal attributes of Gir bull semen. International Journal of Current Microbiology and Applied Sciences. 2018;7(7):1152-1159.
14. Chowdhury S, Das S, Gupta T, Sana D, Bose S. A comparative study with Murrah buffalo and indigenous Gir spermatozoa to hypo osmotic swelling test. Animal Medical Research. 2013;3(2):159-164.
15. Dhami AJ, Sahni KL. Evaluation of different cooling rates, equilibration periods and diluents for effects on deepfreezing, enzyme leakage and fertility of taurine bull spermatozoa. Theriogenology. 1993;40:1269-80.
16. Farooq U, Ijaz A, Ahmad N, Rehman H, Zaneb H. Investigations on semen quality and freezability of Cholistani breeding bulls - A preliminary study from Cholistan desert of Pakistan. Journal of Animaland Plant Science. 2013;23(2):359-363.
17. Galmessa U, Mohanty TK, Raina VS, Gupta AK. Post-thawed and fresh spermatozoa motion characteristics of Sahiwal bulls under computer-assisted semen analyzer (CASA). International Journal of Livestock Production. 2014;5(4):65-70.
18. Gaur GK, Kaushik SN, Garg RC. The Gir cattle breed of India: characteristics and present status. Animal Genetics Resources Information. 2003;33:21-29.
19. Ghodasara SN, Gajbhiye PU, Ahlawat AR, Murthy KS. Evaluation of fresh semen quality and predicting the number of frozen semen doses in Jaffrabadi buffalo bull. Buffalo Bulletin. 2018;35(1):65-72.
20. Iliger MR. Studies on semen production potential of Amritmahal bulls. M.V.Sc. Thesis, KVAFSU, Bidar, India, 2014.
21. Javed MT, Khan A, Ali M. Influence of season on seminal plasma testosterone and oestrogen in healthy and abnormal buffalo bulls and their relationship with other semen parameters. Veterinarski Archives. 2000;70(3):141-49.
22. Jayendran RS. Van der van HH, Perez-Palaez M, Grabo BG, Zaneveld LJD. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to the other semen characteristics. Journal of Reproduction and Fertility. 1984;70:219-228.
23. Kale MM, Manik RS, Tomer OS. *In-vitro* assessment of crossbred buck fertility. Indian Journal of Animal Science. 2000;70(1):25-29.
24. Kapadiya PS, Nakhashi HC, Chauhan PM, Sutaria TV, Suthar BN, Sharma VK. Studies on certain physical and biochemical attributes of Kankrej bull semen. International Journal of Current Microbiology and Applied. Science. 2018;7:4574-4581.
25. Karmur SD, Rana CM, Dhami AJ, Panchani GV. Hypo-osmotic swelling test in relation to motility of fresh and frozen thawed murrah buffalo semen. Indian Journal of Dairy Science. 2002;55(6):363-365.
26. Kathiravan P, Kalatharan J, Karthikeya G, Rengarajan K, Kadirvel G. Objective Sperm Motion Analysis to Assess Dairy Bull Fertility Using Computer-Aided System - A Review. Reproduction in Domestic Animals. 2011;46(1):165-172.
27. Kedia NK, Tiwari RP, Mishra GK, Poyam MR, Pandey AK, Nair AK and Sahasrabudhe SA. Characterestics and freezability of Tharparkar bull semen. Indian Journal of Animal Science. 2014;84(4):382-388.
28. Kumar S. Effect of successive ejaculations oncytomorphology and biochemistry of bull and buffalo semen. M.V.Sc. Thesis, G. B. Pant University of Agriculture and Technology, Pantnagar, India, 1979c.
29. Lodhi LA, Zubair M, Qureshi ZI, Ahmad I, Jamil H. Correlation between hypo-osmotic swelling test and various conventional semen evaluation parameters in fresh Nili-Ravi buffalo and Sahiwal cow bull semen. Pakistan Veterinary Journal. 2008;28(4):186-188.
30. Maxwell WMC, Watson PF. Recent progress in the preservation of ram semen. Animal Reproduction Science. 1996;42(1):55-56.
31. Mishra SR, Kundu AK, Mahapatra APK. Effect of ambient temperature on membrane integrity of spermatozoa in different breeds of bulls. The Bioscanning. 2013;8(1):181-183.
32. Pal AS, Singh V, Patel A. Studies on Seminal Attributes of Neat Semen of Hariana Bulls. International Journal of Current Microbiology and Applied Science. 2020;9(1):1742-1749.
33. Patel BR, Siddiquee GM. Physical and morphological characteristics of Kankrej bull semen. Veterinary World. 2013;6(7):405-408.
34. Pathak V. Studies on seminal characteristics and freezability of Sahiwal and Red Sindhi Bull. M.V.Sc Thesis. Indira Gandhi Krishi Vishwavidyalaya, Raipur, India, 2008.
35. Prajapati SG, Vala KB, Singh VK, Solanki GB, Havda BP. Physico-Morphological Characteristics and Oxidative Markers of Fresh Semen of Gir Bulls. Indian Journal Veterinary Science and Biotechnology. 2022;18(4):104-108
36. Pramanik PS, Raina VS. Refrigerator preservation of buffalo semen in various extender Ind. Journal of Dairy Science. 1998;51(6):375-79.
37. Prasad JK, Kumar S, Mohan G, Shanker U, Agarwal SK. Hypo-osmotic swelling test (HOST) and its response in fresh and freeze thawed semen. Indian Journal of Animal Science. 1999;69:766-769.
38. Rana CM, Dhami AJ. Physical attributes intact acrosome, HOS test and freez ability of semen of Gir and Jafarabadi bulls. Indian Veterinary Journal. 2004;81:406-410.
39. Rao AVN, Sreemannarayana O. Seminal traits and frozen semen production in relation to age in Murrah bulls. Indian Veterinary Journal. 1996;73:526-30.
40. Ray K, Ghosh BB. Semen ejaculates characteristics, *in vitro* fertility and their interrelationships in Sahiwal bull semen, Iranian Journal of Applied Animal Science. 2013;3(3):483-489.
41. Rengarajan K. Effect of different cooling rates on the post-thaw motility of bovine spermatozoa assessed using

computer assisted semen analyzer (CASA). MVSc thesis, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India, 2004c.
42. Rota A, Penzo N, Vincenti L, Mantovani, R. Hypo-osmotic swelling as a screening assay for testing *in-vitro* fertility of bovine spermatozoa. Theriogenology. 2000;53(7):1415-1420.
43. Sane CR, Luktuke SN, Kaikini AS, Hukeri VB, Deshpande BR, Velhankar DP. Artificial insemination of cattle. In: Reproduction in Farm Animals (Theriogenology). Edt. Sane CR, Hukeri VB, Marathe MR. Varghese Publishing House, USA. 1994, 524-89.
44. Sannat C, Nair A, Sahu SB, Sahasrabudhe SA, Kumar A, Gupta AK, *et al*. Effect of species, breed, and age on bacterial load in bovine and bubaline semen. Veterinary World. 2015;8(4):461.
45. Sarder MJU, Joarder OI, Ali MS, Imam MH. Influence of genetic group, season and age on their semen characteristics of breeding bull. Bangladesh Journal of Genetics Biotechnolology. 2007;1:51-57.
46. Shalini I, Antoine D, Kantharaj S, Murugavel K, Raju MS. Testicular volume and seminal attributes in an infertile Labrador. Indian Veterinary Journal. 2018;95(12):11-13.
47. Shelke VB, Dhami AJ. Comparative evaluation of physic morphological attributes and freez ability of semen of Gir cattle (*Bos indicus*) and Jafarabadi buffalo (*Bubalus bubalis*) bulls. Indian Journal of Animal Science. 2001;71(4):319-324.
48. Shukla MK, Saha S, Rathore KS, Mishra GK, Siddqui MU, Saxena SK. Comparative study of plasma membrane integrity of spermatozoa by using HOS medium and distilled water and their relation with post thaw motility. Indian Journal of Animal Reproduction. 2012;33(2):7-9.
49. Snedecor GW, Cochran WG. Statistical Methods. Edn.14, Oxford and IBH Publishing house, New Delhi, India, 1994.
50. Singh SP, Pandit RK, Bhadoriya HB S. Sexual behavior and seminal characteristics in Jersey, Sahiwal and half-bred bulls. Indian Journal of Animal Science. 2000b;70(3):279-280.
51. Sonar BP, Tiwari MR, Poyam, Mishra GK, Pandey AK, Nair AK, Sahasrabudhe SA Characteristics and freezability of Gir bull semen. Indian Journal of Animal Science. 2016;86(3):264-272.
52. Srivastava N, Srivastava SK, Ghosh SK, Kumar A, Perumal P, Jerome A. Acrosome membrane integrity and cryocapacitation are related to cholesterol content of bull spermatozoa. Asian Pacific Journalof Reproduction. 2013;2(2):126-131.
53. Yaniz JL, Soler C, Santolaria P. Computer assisted sperm morphometry in mammals: A review. Animal Reproduction Science. 2015;156:1-12.