

SEMINAL CHARACTERISTICS AND THEIR INTERRELATIONSHIPS IN GIR BULLS

S.B. VALA, K.B. VALA*, G.B. SOLANKI¹, R.J. PADODARA², K.H. PARMAR

*Department of Veterinary Gynaecology and Obstetrics,
College of Veterinary Science and Animal Husbandry, Kamdhenu University,
Junagadh - 362 001, Gujarat, India*

Received: 14-11-2022

Accepted: 14-11-2022

ABSTRACT

The present investigation was undertaken on semen quality parameters and their interrelationships on three Gir bulls (5 years old) maintained at Cattle Breeding Farm, Junagadh. Semen ejaculates (n=30, 10/bull) were collected using artificial vagina (Danish model) once weekly and were soon evaluated for physico-morphological attributes. The ejaculate volume, sperm concentration, mass motility (0-5 scale), individual sperm motility, sperm viability, sperm abnormality, HOST reactive spermatozoa, and acrosome integrity were found to be 7.93 ± 1.72 ml, 1376.90 ± 55.21 million/ml, 3.70 ± 0.08 , 83.67 ± 0.93 %, 87.03 ± 0.74 %, 9.87 ± 0.52 %, 90.17 ± 0.47 % and 75.30 ± 1.07 %, respectively. There was a significant ($P < 0.01$) difference only in ejaculate volume and sperm concentration between bulls. The sperm concentration, sperm motility, viability, acrosome integrity and plasma membrane integrity were highly significantly and positively ($r = 0.512$ to 0.880 ; $P < 0.01$) interrelated and all of them had significant negative correlations with sperm abnormality ($r = -0.498$ to -0.827). The ejaculate volume did not show significant correlations with any of the other traits studied. The semen quality in general was within normal physiological limits of the breed.

Key words: Gir bull, HOS test, Pearson's correlations, Semen quality, Variation between bulls.

INTRODUCTION

The Gir cattle, a famous milch breed of India, has home tract in Gir hills and forests of Saurashtra region in Gujarat. This breed is known for its stress tolerance and resistance to various tropical diseases (Gaur et al., 2003). Highest recorded peak yield of Gir cows was 22.9 liters/day in an organized herd with standard 305 days lactation yield of 4538 liters (Patbandha et al., 2020). Gujarat is the cradle of India's white revolution through dairy cooperatives and is well known for different breeds of cattle, viz., Gir, Kankrej and Dangi. Majority of farmers keep 2-3 female cattle, and breed them through AI or natural service. To meet this demand, sires of superior germplasm are maintained at government and institute farms or semen stations for the purpose of providing good quality semen.

There is no single panacea test that can predict quality and fertility of semen ejaculate, hence evaluation of multiple physico-morphological attributes of spermatozoa helps 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 affecting ultimate fertility. Accurate morphological screening of the semen ejaculates allows elimination of bulls with low fertility, prior to the cryopreservation of semen, thus contributing to a major savings for AI enterprises ((Hafez and Hafez, 2000). The aim of this study was to evaluate the quality of semen ejaculates and their interrelationships in Gir bulls under their native climatic conditions of Gujarat.

MATERIALS AND METHODS

The study was undertaken on semen of three mature Gir bulls (5 years old) kept under identical management and nutritional practices at the Cattle Breeding Farm, JAU, Junagadh (India), during the period of January to July 2022. Junagadh is located at the foot hill of mountain Girnar at 21.52° N 70.47° E at an average elevation of 350 feet from mean sea level. It has a hot and humid tropical climate, with three distinct seasons, viz., winter, summer and monsoon, and records annually rainfall of 100 to 120 cm. The station faces adverse climatic conditions in the summer months with the temperature ranging from 28° C to 40° C, whereas, in the winter months the temperature ranges from 10° C to 25° C.

Semen ejaculates (n=30) from three mature Gir bulls were collected in AV at weekly interval and were evaluated for various physico-morphological attributes of neat semen such as ejaculates volume, color-

*Corresponding author, e-mail: drkkvala@gmail.com

¹Cattle Breeding Farm, Junagadh Agricultural University, Junagadh - 362 001, India

²Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh - 362 001, India

consistency, Sperm concentration, mass motility, individual sperm motility, vitality, morphology, acrosome integrity and plasma membrane integrity (HOST) adopting standard procedures (Tomar, 1984). The data were analyzed using one way ANOVA and Duncan's multiple range test to show the significant differences between bulls at $p < 0.05$. Pearson's correlations among the seminal attributes were worked out as per standard procedure using SPSS software.

RESULTS AND DISCUSSION

The findings on seminal attributes of a total of thirty semen ejaculates, obtained from three Gir bulls and their interrelationships observed are presented in Table 1 and 2, respectively.

Ejaculate Volume and Sperm Concentration

The mean ejaculate volume of 3 Gir bulls recorded was 7.93 ± 0.31 ml, while the mean sperm concentration was 1376.90 ± 55.21 million/ml, both of which varied significantly among the bulls (Table 1). Similar ejaculate volume has been recorded by Rana and Dhama (2004) in Gir bulls and Mostari et al. (2005) in Sahiwal bulls. However, many researchers reported somewhat lower mean ejaculate volume of 5.80 ± 0.41 to 6.69 ± 0.17 ml in Gir bulls (Chaudhary et al., 2017; Das et al., 2017; Dhama et al. 2017; Chikhaliya et al., 2018; Bhavsa et al., 2020), while Shelek and Dhama (2001) and Sonar et al. (2016) reported much lower ejaculate volume of 4.84 ± 0.16 ml, and 4.99 ± 0.26 ml, respectively, in Gir bulls. Moreover, comparable findings on sperm concentrations were also observed in Gir bulls by Shelke and Dhama (2001), Sannat et al. (2015), Chikhaliya et al. (2018), Pathak et al. (2018), and even in Kankrej bulls by Patel and Siddiquee (2013). However, lower sperm concentrations were observed by Sonar et al. (2016) and Das et al. (2017) with values of 835.33 ± 82.68 and 948 ± 18.8 million/ml in Gir bull semen. Ejaculate volume and sperm concentration are breed characteristics, which depend upon the scrotal size and weight, reproductive health of bulls, age of bulls, frequency and method of semen collection, nutrition of bull, season, management etc. (Ijaz et al., 2009).

Sperm Motility, Viability, Morphology

The mean values of mass motility score, individual sperm motility, sperm viability and total sperm abnormalities recorded in neat semen of Gir bulls were 3.70 ± 0.08 (0-5 scale), 83.67 ± 0.93 %, 87.03 ± 0.74 % and 9.87 ± 0.52 %, respectively. The values of these parameters did not differ significantly among the bulls (Table 1).

Comparable findings on mass motility score were also observed by Chikhaliya et al. (2018), Dhama et al. (2017) and Pathak et al. (2018) in Gir bulls' semen, and

Rajoria et al. (2011), Patel and Siddiquee (2013) and Pal et al. (2020) in Tharparkar, Kankrej and Harijana bulls, respectively. However, lower mass motility has been reported by Shelke and Dhama (2001) and Rana and Dhama (2004) in Gir bulls (2.96 ± 0.14 and 3.33 ± 0.11), Ray and Ghosh (2013) in Sahiwal bulls, and Pal et al. (2020) in Harijana bulls.

Present findings on individual sperm motility concurred with reports of Chikhaliya et al. (2018) and Pathak et al. (2018) in Gir bull semen, Patel and Siddiquee (2013), and Bhavsa (2014), in Kankrej bulls, and Rajoria et al. (2011) in Tharparkar bull semen. However, lower motility of 69.10 ± 0.75 to 80.21 ± 0.88 % was observed in Gir bulls' semen by Sannat et al. (2015), Sonar et al. (2016), Das et al. (2017), Dhama et al. (2017), and Bhavsa et al. (2020), and also in Harijana bulls by Pal et al. (2020), and in Sahiwal bulls by Ray and Ghosh (2013).

Sperm viability observed in Gir bulls under study was in agreement with Chikhaliya et al. (2018), Bhavsa (2014), Rajoria et al. (2011), Sharma et al. (2018) in Gir, Kankrej, Tharparkar and Harijana bulls, respectively. However, lower mean per cent sperm viability was reported by Shelke and Dhama (2001), Sonar et al. (2016) and Dhama et al. (2017) in Gir bulls, Pal et al. (2020) in Harijana bulls, Ray and Ghosh (2013) in Sahiwal bulls, and relatively higher values in Kankrej by Patel and Siddiquee (2013).

Comparable sperm abnormalities were also observed by Chikhaliya et al. (2018) in Gir bulls, Ray and Ghosh (2013) in Sahiwal bulls, and Rajoria et al. (2011) in Tharparkar bulls. Although, relatively higher per cent sperm abnormality was recorded by Sonar et al. (2016) in Gir bulls (15.96 ± 0.44 %), and lower by Patel and Siddiquee (2013) and Bhavsa (2014) in Kankrej bulls (4.24 ± 0.03 and 4.22 ± 0.15 %, respectively).

The difference in the semen quality and 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 (Tomar, 1984). The semen motility can also be affected by improper handling procedure, pH of extender, contaminated glass wares, chemicals, temperature of biotherm stage and glass wares, period between collection and evaluation, etc. (Pal et al., 2020).

Sperm Plasma Membrane and Acrosome Integrity

The means of HOS reacted sperm and sperm with intact acrosome observed in neat semen of Gir bulls under study were 75.30 ± 1.07 and 90.17 ± 0.47 %, respectively, which did not differ significantly among the bulls (Table 1). Similar findings on HOST were observed by Rajoria et al. (2011), Ray and Ghosh (2013) and Singh

et al. (2018) in Tharparkar, Sahiwal and Hariana bulls, respectively. However, lower HOS reacted spermatozoa were reported by Sonar et al. (2016) and Bhave et al. (2020) in Gir bulls (60.12±1.19 and 55.13±0.005 %), and Bhavsar (2014) in Kankrej bulls (68.82±0.47 %), while Dhama et al. (2017) reported higher HOS reacted spermatozoa as 82.54±0.91 % in Gir bulls. Further, the findings on acrosome integrity were in line with those reported by Chikhaliya et al. (2018) in Gir bulls, and Rajoria et al. (2011) in Tharparkar bull. However, lower acrosome integrity was reported by Patel and Siddiquee (2013), Ray and Ghosh (2013), Sonar et al. (2016) and Singh et al. (2018) in bulls of different cattle breeds. The difference in HOS reacted spermatozoa per cent and acrosome integrity may be due to variations in bull, season, inherent semen quality, testicular health, lab environment, osmolarity of test solution/stain used etc (Prasad et al., 1999).

Interrelationships among Seminal Attributes

In the present study, the ejaculate volume showed very low and non-significant negative correlations with all other sperm quality traits studied. However, the sperm concentration, mass motility, individual sperm motility, sperm viability, acrosome integrity and plasma membrane integrity were highly significantly and positively interrelated ($r= 0.512$ to 0.880 ; $P<0.01$) and all of them had significant negative correlations with sperm abnormality ($r= -0.498$ to -0.827) (Table 2).

The non-significant correlations of ejaculate volume concurred well with Swain and Singh (2004) in Sahiwal bulls and Bhavsar (2014) in Kankrej bulls. In contrast, Ray and Ghosh (2013), Bhavsar (2014) and Pathak et al. (2018) reported a highly significant ($p<0.01$) negative correlation of ejaculate volume with sperm concentration/ml, while Patel and Siddiquee (2013) found a positive correlation of ejaculate volume with sperm concentration/ml. Non-significant positive correlation of ejaculate volume with mass motility found in the present study was in agreement with Ray and Ghosh (2013) and Pathak et al. (2018) in Sahiwal and Gir bulls, respectively.

A non-significant negative correlation of mass motility with sperm abnormality observed concurred well with reports of Rajoria et al. (2011), Ray and Ghosh (2013), Chikhaliya et al. (2018) and Pathak et al. (2018) in zebu bulls. The significant positive ($p<0.05$) correlation of mass motility with individual motility observed was also in line with these reports. Further, the highly significant ($p<0.01$) positive correlations of individual sperm motility with sperm viability and HOST reactivity observed (Table 2) were also reported by these workers in Gir, Kankrej, Tharparkar and Sahiwal bulls. A significant ($p<0.05$) positive correlation of individual motility with acrosomal

integrity observed in Gir bulls was also reported by Rajoria et al. (2011) in Tharparkar bulls.

Table 2: Correlation coefficients (r) among various attributes of neat semen on Gir bulls

Quality attributes	Ejaculate volume	Sperm concentration	Mass motility	Individual sperm motility	Sperm viability	Sperm abnormality	Acrosome integrity
Sperm concentration	-0.331	1					
Mass motility	-0.211	0.540**	1				
Individual sperm motility	-0.303	0.649**	0.627**	1			
Sperm viability	-0.189	0.611**	0.643**	0.880**	1		
Sperm abnormality	0.336	-0.498**	-0.631**	-0.827**	-0.780**	1	
Acrosome integrity	-0.312	0.658**	0.621**	0.681**	0.709**	-0.685**	1
Plasma membrane integrity	-0.145	0.512**	0.399*	0.754**	0.838**	-0.655**	0.752**

n=30, *Significant at $p<0.05$, **Significant at $p<0.01$.

Significant positive correlations of sperm viability with sperm motility, plasma membrane integrity (HOS reactive sperm), acrosome integrity, and negative correlations with sperm abnormality and sperm concentration observed (Table 2) in the present study have also been reported by several workers (Patel and Siddiquee, 2013; Ray and Ghosh, 2013; Chikhaliya et al., 2018; Pathak et al., 2018) in different breeds of cattle. Ray and Ghosh (2013) reported significant ($p<0.05$) negative correlations of sperm abnormality with mass activity and HOST and highly significant ($p<0.01$) negative correlations with viability and acrosomal integrity in Sahiwal bulls. Rajoria et al. (2011) reported a significant ($p<0.05$) negative correlation of sperm abnormality with individual motility and highly significant ($p<0.01$) negative correlation with HOST in Tharparkar bulls. In the present study, highly significant positive correlations of HOS reactive spermatozoa noted with individual sperm motility, sperm viability, acrosome integrity, and negative correlation with sperm abnormality have also been reported by the said workers.

CONCLUSION

The findings of the study revealed that the semen quality of mature Gir bulls harvested during January to June was within normal physiological limits of the breed, and that the sperm concentration, sperm motility, viability, acrosome integrity and plasma membrane integrity were highly significantly and positively ($r= 0.512$ to 0.880 ; $P<0.01$) interrelated and all of them had significant negative correlations with sperm abnormality ($r= -0.498$ to -0.827), thus the quality of ejaculate can be predicted based on one or two simpler lab tests like sperm motility and/or HOST, rather than going for clumsy time consuming staining procedures.

ACKNOWLEDGEMENT

We gratefully thank the Research Scientist and Head of Cattle Breeding Farm, Junagadh for permitting us to utilize their bulls and lab facilities, and the Dean, Veterinary College, Junagadh and authorities of Kamdhenu University for the support and cooperation in this work.

REFERENCES

- Bhave, K.G., Jawahar, K.T.P., Kumarasamy, P., Sivakumar, T., Joseph, C., Shirsath, T., Deshmukh, P. and Venkataramanan, R. (2020). Genetic and non-genetic factors affecting semen production and quality characteristics of Gir cattle breed under semi-arid climate. *Vet. World*, 13(8): 1714.
- Bhavsar, J.A. (2014). Comparative efficacy of extenders for cryopreservation of Kankrej bull semen and pregnancy rate. M.V.Sc. Thesis, Sardarkrushinagar Dantiwada Agricultural University, Sardar Krushinagar, Gujarat, India.
- Chaudhary, P.J., Dhami, A.J., Chaudhari, D.V. and Parmar, S.C. (2017). Freezability of cattle and buffalo semen and association of fresh and frozen-thawed sperm quality parameters. *Intl. J. Curr. Microbiol. Appl. Sci*, 6(12): 1445-1454.
- Chikhaliya, P.S., Ahlawat, A.R., Solanki, G.S., Raval, R.J., Vala, K.B. and Verma, A.D. (2018). Physical Seminal Attributes of Gir Bull Semen. *Intl. J. Curr. Microbiol. Appl. Sci*, 7(7): 1152-1159.
- Das, S., Sana, D., Gupta, T., Chowdhury, S., Chakraborti, A., Biswas, J. and Sarkar, S. (2017). Seasonal variation in semen production parameters of Gir bulls reared under tropical climate. *Indian J. Appl. Res*, 38(2): 49-50.
- Dhami, A.J., Chaudhary, P.J., Chaudhari, D.V. and Hadiya, K.K. (2017). Comparative study of Gir cattle and Surti buffalo bulls semen under middle Gujarat climate. *The Indian J. Vet. Sci. & Biotech*, 13(1): 55-61.
- Gaur, G.K., Kaushik, S.N. and Garg, R.C. (2003). The Gir cattle breed of India- characteristics and present status. *Anim. Genetic Resources*, 33: 21-29.
- Hafez, E.S.E. and Hafez, B. (2000). Reproductive Cycle in Farm Animals, 7th Edn., Publ., Lippincott Williams and Wilkins, Philadelphia, USA, pp 55-67.
- Ijaz, A., Hussain, A., Aleem, M., Yousaf, M.S. and Rehman, H. (2009). Butylated hydroxyl toluene inclusion in semen extender improves the post-thawed semen quality of Nili-Ravi buffalo (*Bubalus bubalis*). *Theriogenology*, 71: 1326-1329.
- Mostari, M.P., Hasanat, M.S., Azmal, S.A., Monira, K.N. and Khatun, H. (2005). Effect of seasonal variation on semen quality and herd fertility. *Pak. J. Biol. Sci*, 8(4): 581-585.
- Pal, A.S., Singh, V. and Patel, A. (2020). Studies on Seminal Attributes of Neat Semen of Haryana Bulls. *Intl. J. Curr. Microbiol. Appl. Sci*, 9(1): 1742-1749.
- Patbandha, T.K., Sabapara, G.P., Savaliya, B.D., Dash, S.K., Parikh, S.S. and Ali, M. (2020). Physical characteristics and production performance of Gir cattle in India. *Intl. J. Livest. Res*, 10(8): 1-11.
- Patel, B.R. and Siddiquee, G.M. (2013). Physical and morphological characteristics of Kankrej bull semen. *Vet. World*, 6(7): 405-408.
- Pathak, P.K., Dhami, A.J. and Chaudhari, D.V. (2018). Seminal attributes, freezability and their interrelationships in zebu cattle and buffalo bulls from central Gujarat. *The Indian J. Vet. Sci. & Biotech*, 14(2): 1-8.
- Prasad, J.K., Satish Kumar, Greesh, M. and Bisht, G.S. (1999). Physio-morphological seminal attributes, sperm mensuration and enzymatic profile of crossbred bull semen. *Indian Vet. Med. J*, 23(4): 293-298.
- Rajoria, J.S., Prasad, J.K., Ghosh, S.K., Kumar, S., Singh, M., Ramteke, S., Kumar, A. and Kumar, A. (2011). Effect of season on physico-morphological and enzymatic profile of Tharparkar bull semen. XXVII Annual convention of ISSAR and national symposium, Mizoram, India, pp: 84.
- Rana, C.M. and Dhami, A.J. (2004). Physical attributes, intact acrosome, HOS test and freezability of semen of Gir and Jafarabadi bulls. *Indian Vet. J*, 81: 406-410.
- Ray, K. and Ghosh, B.B. (2013). Semen ejaculates characteristics, in vitro fertility and their interrelationships in Sahiwal bull semen. *Iranian J. Appl. Anim. Sci*, 3(3): 483-489.
- Sannat, C., Nair, A., Sahu S.B., Sahasrabudhe S.A., Kumar, A., Gupta, A.K. and Shende, R.K. (2015). Effect of species, breed, and age on bacterial load in bovine and bubaline semen. *Vet. World*, 8(4): 461.
- Shelke, V.B. and Dhami, A.J. (2001). Comparative evaluation of physico-morphological attributes and freezability of semen of Gir cattle (*Bos indicus*) and Jafarabadi buffalo (*Bubalus bubalis*) bulls. *Indian J. Anim. Sci*, 71(4): 319-324.
- Singh, V., Kumar, A., Yadav, C.L., Patel, A., Yadav, B. and Saxena, A. (2018). Effect of season on physico-

-
- morphological attributes of cryopreserved Haryana bull spermatozoa. *The Indian J. Vet. Sci. & Biotech*, 14(1): 47-51.
- Sonar, B.P., Tiwari, R.P., Poyam, M.R., Mishra, G.K., Pandey, A.K., Nair, A.K. and Sahasrabudhe, S.A. (2016). Characteristics and freezability of Gir bull semen. *Indian J. Anim. Sci*, 86(3): 264-272.
- Swain, A.K. and Singh, S.V. (2004). Enzymatic profile in relation to physical parameters of Sahiwal bull semen. *Indian J. Anim. Sci*, 74(6): 594-596.
- Tomar, N.S. (1984). *Artificial Insemination and Reproduction of Cattle and Buffalo*. 3rd Edn. Saroj Prakashan, Allahabad, India.