

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.910.381>

Correlative Studies on Physio-morphological Characteristics of Gir Bull Semen during Summer and Winter Seasons

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A B S T R A C T

Keywords

Gir breeding bulls,
Semen quality,
Winter, Summer

Article Info

Accepted:
26 September 2020
Available Online:
10 October 2020

The objective of the present study was to investigate the seasonal effect on neat semen quality of Gir bulls. In the winter, a total of 27 ejaculates were collected and in the summer, 59 were collected from three healthy bulls. Ejaculates were collected on a weekly basis using the artificial vagina method (42–45°C). Meteorological data were recorded daily during the experimental period. Semen samples were evaluated for ejaculated volume, initial motility, mass motility, sperm livability, hypo-osmotic swelling test (HOST), acrosomal integrity and sperm abnormalities. During winter season, initial motility, mass motility, sperm viability, HOST and sperm acrosomal integrity was significantly ($p<0.05$) lower compared to the summer season, while, ejaculate volume and sperm concentration was higher during summer. The season had no significant effect on sperm abnormalities. Mass motility was positively correlated with the initial motility ($r=0.309$), sperm viability ($r=0.600$), HOST ($r=0.512$), acrosomal integrity ($r=0.558$), sperm abnormalities ($r=0.027$). Whereas, initial motility was positively correlated with the mass motility ($r=0.309$), sperm viability ($r=0.127$), HOST ($r=0.149$), acrosomal integrity ($r=0.210$) and negatively correlated with sperm concentration ($r= -0.123$) and sperm abnormalities ($r= -0.450$).

Introduction

The Girbreed of cattle is a popular Indian milch breed. This breed is the native of Kathiawar consisting of Junagadh, Bhavnagar, Rajkot and Amreli districts of

Gujarat. Gir breed has been conserved and bred successfully in the countries like Argentina, Mexico, USA, and Venezuela. This breed is adapted in various geographical locations under varying climatic conditions in India. It is pertinent that the adaptation in different geographical locations certainly

affect the productivity of the breeding bulls, especially during heat stress in tropical environment. The impact of high ambient temperatures affects libido, testicular size, semen production and semen quality (Soderquist *et al.*, 1996). The information regarding the effect of seasons on semen characteristics in dairy bulls has been reported to be of a conflicting nature (Bhakat *et al.*, 2009) and some researchers have reported significant season effects (Rekwot *et al.*, 1987; Goswami *et al.*, 1991 and Singh and Raina, 2000), while others have found no seasonal effects (Mathur *et al.*, 2002; Helbig *et al.*, 2007) on semen quality parameters. The testicular temperature in most mammals is maintained at about 2–6°C below their body temperature for the production of morphologically normal and fertile spermatozoa (Waites, 1970).

Little information is available on seasonal variation in Gir semen quality production. The present study was carried out to evaluate the effect of winter and summer seasons on the morphological features of semen in Gir bulls and their correlation.

Materials and Methods

Selection of animals and management practices

Study was conducted on three healthy Gir bulls having three years of age with an average body weight of 350-400 kg available at the Frozen Semen Laboratory (FSL), Nagpur, India. Weekly ejaculates were collected in winter (December to February) and in summer (March to June) seasons in the early morning with sterilized artificial vagina (42–45 °C). The dry bulb temperature and wet bulb temperature was recorded on daily basis for calculating Temperature Humidity Index as per the formula described by McDowell (1972). Standard operational and management

practices were followed considering the Standard Operating Procedures (SOP) & Minimum Standard Protocol (MSP) of Government of India. All bulls were maintained at identical and optimal conditions of feeding and management practices throughout the period of study. In winter a total of 27 ejaculates were collected and 59 ejaculates were collected during the summer. Each ejaculate was processed to evaluate the ejaculated volume, initial motility, sperm concentration, mass motility, sperm livability, hypo-osmotic swelling test (HOST), acrosomal integrity and sperm abnormalities.

Semen analysis

Immediately after semen collection, ejaculated volume was recorded in a graduated glass centrifuge tube as per standard laboratory method. The initial motility was observed under a low power (10x) microscope on thermostatically controlled stage maintained at 37°C (Olympus BX41TF attached Progress CT3 camera with Progress capture Pro 2.6 software program made at Tokyo, Japan). The concentration of sperm (million/ml) in neat semen was determined by the Accucell bovine Photometer No. RS232, IMV Technology, France. The mass motility was measured from 0 to 5 scale based on the presence of vigour waves motions and swirls (Salisbury *et al.*, 1978). Eosin-nigrosin (EN) stain was evaluated as a percentage of live spermatozoa as indicated by Blom (1977). About 10 µl of neat semen was thoroughly mixed with 10 µl of stain eosin-nigrosin at 37°C and a thin smear was prepared. About 200 sperm cells were counted under oil immersion objective lens (100x) of phase contrast microscope in different fields and are classified as live sperm cells having colorless head portions and dead sperm cells with pinkish or red coloration. Live sperm percentage has been determined. The HOST was carried out as per

the method described by Jeyendran *et al.*, 1984. The HOST reactive spermatozoa (A) and non-reactive spermatozoa (B) are shown in Plate 1.

Giemsa staining technique was used for evaluation of the acrosomal integrity in accordance with the methodology defined by Hancock (1952). About 200 spermatozoa were counted under oil immersion light microscope for intact acrosome at the head of spermatozoa (Plate 2).

Statistical analysis

Data obtained from the study was analyzed using t-test, correlation coefficients and presented as means \pm SE with the aid of SPSS statistical software, version (SPSS 20.0).

Results and Discussion

The weekly dry bulb (Tdb) and wet bulb (Twb) temperature were recorded between December to February (winter) and between March to June (summer). Temperature Humidity Index (THI) was determined using the McDowell (1972) formula. Table 1 displays the typical Tdb, Twb and THI values.

The environmental stresses on the Gir bulls were classified as described by McDowell (1972), where the THI above 72 is considered as thermal stress. Therefore, the THI of 88.32 ± 1.61 in summer exhibited thermal stress on bulls and the THI of 70.21 ± 1.25 in winter shows that thermal stress was not on bulls. Table 2 presents the results concerning semen characteristics of Gir bull fresh semen. Table 3 indicates the correlation between the different characteristics of the semen.

Ejaculate volume

Ejaculate volume in the summer was significantly ($p<0.05$) higher at higher THI. Similar findings have been reported in the

Tharparkar bulls, Haryana bulls and Karan Fries bulls by Rajoriya *et al.*, (2015), Singh *et al.*, (2018) and Bhakat *et al.*, (2014). In contrast to the present results in Gir bulls and Karan Fries bulls by Das *et al.*, (2017) and Soren *et al.*, (2016) reported a higher ejaculate volume in winter than summer. The ejaculate volume is positively correlated to the initial motility ($r=0.160$), sperm concentration ($r=0.158$) and has a negative correlation with the mass motility ($r= -0.230$), sperm viability ($r= -0.248$), HOST ($r= -0.178$), acrosomal integrity ($r= -0.096$) and abnormalities of sperm ($r= -0.218$) as shown in table 3.

Initial motility

The significantly ($p<0.05$) higher initial motility was recorded during winter than in summer. Similar to our results in Gir bulls, Haryana bulls and Karan Fries bulls were reported by Das *et al.*, (2017), Singh *et al.*, (2018) and Soren *et al.*, (2016). Initial motility correlated positively with ejaculate volume ($r=0.160$), mass motility ($r=0.309$), sperm viability ($r=0.127$), HOST ($r=0.149$), acrosomal integrity ($r=0.210$) and negative correlation with sperm concentration ($r= -0.123$) and sperm abnormalities ($r= -0.450$) as shown in table 3.

Sperm concentration

Significantly ($p<0.05$) higher sperm concentration was observed during summer season than the winter season. Similar findings in Haryana bulls and Nilli Ravi buffalo bulls with Singh *et al.*, (2018) and Hameed *et al.*, (2017). In contrast to present findings Das *et al.*, (2017), Rajoriya *et al.*, (2015) and Soren *et al.*, (2016) reported the highest concentration of sperm during winter than summer season in Gir bulls, Tharparkar bulls and Karan Fries bulls respectively. Sperm concentration was positively correlated

with the ejaculate volume ($r=0.158$), mass motility ($r=0.107$), sperm viability ($r=0.368$), HOST ($r=0.009$), acrosomal integrity ($r=0.374$) and negative correlation with initial motility ($r=-0.123$), abnormalities of sperm ($r=-0.063$) as shown in table 3. Similar correlation was reported by Rajoriya *et al.*, (2015), significant positive correlation of sperm concentration with HOST ($p<0.01$) positive sperm in the summer season.

Mass motility

Mass activity of Gir bull semen was significantly ($p<0.05$) higher in winter than summer season. Similar findings were reported in Haryana bulls and Murrah buffalo bulls by Singh *et al.*, (2018) and Ram *et al.*, (2017). Mass motility positively correlated with initial motility ($r=0.309$), sperm concentration ($r=0.107$), sperm viability ($r=0.600$), HOST ($r=0.512$), acrosomal integrity ($r=0.558$), sperm abnormalities ($r=0.027$) and negative correlated with ejaculate volume ($r=-0.230$) as shown in table 3. Similar correlation was reported by Rajoriya *et al.*, (2015), Patel *et al.*, (1989) and

Rafiq, (2009) mass activity has been significantly ($p<0.01$) positively correlated with sperm concentration, initial progressive motility, live percentage and acrosomal integrity in both summer and winter season.

Sperm livability

Significantly ($p<0.05$) higher sperm livability during winter as compared with summer season. Similar findings with Singh *et al.*, (2018), Soren *et al.*, (2016) and Bhakat *et al.*, (2014) have been reported maximum sperm livability in Haryana bulls and Karan Fries bulls during the winter than summer season. In contrast to present finding by Rajoriya *et al.*, (2015) reported maximum sperm livability during summer than winter season in Tharparkar bulls. The viability of sperm was positively correlated with initial motility ($r=0.127$), sperm concentration ($r=0.368$), mass motility ($r=0.600$), HOST ($r=0.404$), acrosomal integrity ($r=0.441$), sperm abnormalities ($r=0.045$) and negative correlation with ejaculate volume ($r=-0.248$) as shown in table 3.

Table.1 Average environmental variables values during the experimental period

Season	Dry bulb temperature T_{db} (°C)	Wet bulb temperature T_{wb} (°C)	Temperature Humidity Index (THI)
Winter	22.12 ± 1.36	20.62 ± 0.96	70.21 ± 1.25
Summer	$35.00 \pm 1.49^*$	$31.28 \pm 0.77^*$	$88.32 \pm 1.61^*$

Table.2 Semen characteristics of fresh semen of Girbulls at different THI

Sr. No.	Semen Parameters	Winter season (THI 72.91) (N=27)	Summer season (THI 88.32) (N=59)
1.	Ejaculate volume (ml)	4.45 ± 0.28	$5.69 \pm 0.21^*$
2.	Initial motility (%)	$72.70 \pm 1.19^*$	65.37 ± 1.38
3.	Sperm concentration (million/ml)	879.25 ± 64.01	$1192.38 \pm 66.18^*$
4.	Mass motility (0-5 scale)	$3.40 \pm 0.09^*$	2.25 ± 0.08
5.	Sperm viability (%)	$77.59 \pm 1.26^*$	70.11 ± 1.31
6.	HOST (%)	$67.59 \pm 0.75^*$	58.03 ± 1.28
7.	Acrosomal integrity (%)	$75.14 \pm 1.11^*$	69.28 ± 1.06
8.	Sperm abnormalities (%)	4.70 ± 0.75	5.00 ± 1.50

* Represents significantly higher at 5% level

Table.3 Correlation among the various seminal characteristics of fresh semen of Gir bulls

	Ejaculate Volume	Initial Motility	Sperm Conc.	Mass motility	Sperm viability	HOST	Acrosomal integrity
Ejaculate Volume							
Initial Motility	0.160						
Sperm Concentration	0.158	-0.123					
Mass motility	-0.230	0.309	0.107				
Sperm viability	-0.248	0.127	0.368	0.600			
HOST	-0.178	0.149	0.009	0.512	0.404		
Acrosomal integrity	-0.096	0.210	0.374	0.558	0.441	0.347	
Sperm abnormalities	-0.218	-0.450	-0.063	0.027	0.045	0.115	-0.138

Plate.1(A) HOST reactive spermatozoa (B) HOST non-reactive spermatozoa

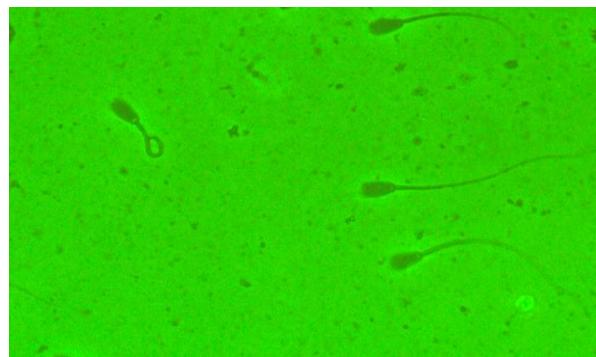


Plate.1(A) HOST reactive spermatozoa
(B) HOST non-reactive spermatozoa

Plate 2.Live spermatozoa with healthy acrosome were identified as light rose colored post acrozmomal area and pink acrozmomalarea in the sperm head



Plate 2.Live spermatozoa with healthy acrosome were identified as light rose colored post acrozmomal area and pink acrozmomalarea in the sperm head.

Hypo-osmotic swelling test (HOST)

Total HOST reactive spermatozoa significantly ($p<0.05$) maximum during winter season than summer.

Similar findings were recorded in the Haryana bulls, Tharparkar bulls and Karan Fries bulls by Singh *et al.*, (2018), Rajoriya *et al.*, (2015) and Soren *et al.*, (2016). HOST positively correlated with initial motility ($r=0.149$), sperm concentration ($r=0.009$), mass motility ($r= 0.512$), viability of sperm ($r=0.404$), acrosomal integrity ($r=0.347$), sperm abnormalities ($r=0.115$) and negatively correlated with ejaculate volume ($r= -0.178$) as shown in Table 3.

Similar correlation was reported by Rajoriya *et al.*, (2015) where, HOST positive spermatozoa were positively correlated with progressive motile ($p<0.05$), live percentage ($p<0.01$) and acrosomal integrity ($p<0.01$) in both the seasons in Tharparkar bull semen.

Acrosomal integrity

During winter, the acrosomal integrity was significantly ($p<0.05$) higher during winter as compared to summer season. Similar to our results finding on Haryana bulls and Karan Fries bulls reported by Singh *et al.*, (2018), Bhakat *et al.*, (2014) and Soren *et al.*, (2016).

Positively correlation with initial motility ($r=0.210$), sperm concentration ($r=0.374$), mass motility ($r= 0.558$), viability of sperm ($r=0.441$), HOST ($r=0.347$) and negative correlation with ejaculate volume ($r= -0.096$), sperm abnormalities ($r= -0.138$) as shown in Table 3.

Sperm abnormalities

In both winter and summer season there was no significant difference observed. Higher sperm abnormalities in summer than winter

season are observed with Singh *et al.*, (2018), Bhakat *et al.*, (2014) and Ram *et al.*, (2017) in Haryana bulls, Karan Fries bulls and Murrah buffalo bulls.

In contrast to present findings of Rajoriya *et al.*, (2015) higher sperm abnormalities in Tharparkar bulls in the winter than summer season. Sperm abnormalities positively correlated to the mass motility ($r= 0.027$), sperm viability ($r=0.045$), HOST ($r=0.115$) and negative correlation with ejaculate volume ($r= -0.218$), initial motility ($r= -0.450$), sperm concentration ($r= -0.063$), acrosomal integrity ($r= -0.138$) as shown in Table 3.

It is concluded that the semen quality adversely affect during high THI in Gir bull semen, however, the ejaculate volume and sperm concentration improves. The sperm abnormality parameter remains unaffected by high THI.

Correlation study concluded that, most of the semen quality parameters of Gir bulls positively correlated with each other and negatively correlate with ejaculate volume except initial motility and sperm concentration. The physio-morphological characteristics dynamically interact with each other.

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How to cite this article:

Karuna P. Bambole, J. P. Korde, Mahesh Gupta, K. B. Bahiram, Manoj Patil, Rajendra Nikhate, D. V. Patil and Sardar, V. M. 2020. Correlative Studies on Physio-morphological Characteristics of Gir Bull Semen during Summer and Winter Seasons. *Int.J.Curr.Microbiol.App.Sci.* 9(10): 3185-3191. doi: <https://doi.org/10.20546/ijcmas.2020.910.381>