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Comparative Study of Gir Cattle and Surti Buffalo Bulls Semen under Middle Gujarat Climate

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Abstract

This study was undertaken during the favourable breeding season of the year 2016-17 on healthy mature Gir cattle and Surti buffalo bulls, three each, at Sperm Station of the College. The ejaculates (9/bull, total 54) collected in the morning using artificial vagina were evaluated for routine seminal attributes, including acrosomal and plasma membrane integrity. The mean values of ejaculate volume, sperm concentration, mass activity (0-5 score), individual sperm motility, live sperm, abnormal sperm, intact acrosome and HOST reactive sperms observed in fresh semen of Gir cattle and Surti buffalo bulls were 6.20±0.42 and 3.34±0.23 ml (P<0.01), 1169.44±61.71 and 846.30±54.82 million (P<0.01), 3.44±0.09 and 3.42±0.08, 76.53±0.53 and 80.76±0.39 % (P<0.05), 81.00±1.32 and 84.73±0.78 % (P<0.05), 6.00±0.37 and 5.81±0.40 %, 95.59±0.35 and 95.54±0.25 % as well as 80.30±1.90 and 84.58±0.88 % (P<0.05), respectively. The variation between cattle and buffalo semen was significant for most of these traits. The variations between bulls within breed were however not significant. Significant correlations were observed only between mass activity and initial motility (0.62), and live and abnormal sperm (-0.41) in Gir bulls, and for ejaculate volume with sperm concentration (-0.56) and abnormal sperm (0.45), and between live and HOS reactive sperms (0.48) in Surti bulls.

Key Words: Gir bulls, Surti bulls, Fresh semen quality, Comparison, Correlations.

Introduction

Male fertility is an important factor in bovine reproduction since a single bull is generally bred to numerous cows/buffaloes. The importance of assessing semen quality is in establishing the relationship of that quality to fertility. Accurate prediction of fertility of each male or an accurate method for estimating fertilizing potential of semen are extremely useful means for successful exploitation of production potential of sires. However, no single test or combinations of tests have been proved to be totally reliable for accurate prediction of semen quality in relation to fertility. Most of the tests that are used for evaluation of semen are based on physical characters of spermatozoa. Semen characteristics such as sperm motility, viability, acrosome morphology, HOS reactivity etc. have been found to be significantly correlated with freezability and/or fertility of bovine semen (Bhavsar *et al.,* 1990; Belorkar *et al*., 1990; Dhami and Sahni, 1994; Shelke and Dhami, 2001; Raval and Dhami, 2006; Chaudhari *et al.,* 2014), and hence are currently being used as routine tests

for the assessment of semen quality. The object of this study was to compare the semen quality of Gir cows and Surti buffalo bulls and their interrelationships during favourable breeding season under middle Gujarat agro-climatic conditions.

Materials and Methods

The study was conducted during favourable breeding season from November to February of the year 2016-17 on three mature healthy Gir cattle and three Surti buffalo bulls, aged 4-6 years, maintained at Sperm Station of the College. All these bulls were in good health and under optimal veterinary care. They were maintained in nearly identical nutritional and managerial conditions throughout the period of study and were under regular weekly twice semen collection schedule using AV. For the present study, the ejaculates (9/bull, 27 per breed) were evaluated once in a week for routine physico-morphological attributes, including acrosome integrity and hypo-osmotic swelling (HOS) test.

The ejaculate volume, mass activity and individual motility were assessed as per Salisbury *et al.* (1978). The concentration of spermatozoa (million/ml) in the neat semen was determined by the digital-photometer (Accucell Photometer, IMV, France). The percentages of live as well as abnormal spermatozoa were estimated by differential staining technique using eosin-nigrosin stain. The morphological abnormalities were classified as of head, mid-piece and tail region. Acrosomal integrity was assessed using Giemsa stain. Hypo-osmotic swelling test was carried out using 150 mOsm citrate-fructose solution as per Jayendran *et al*. (1984). The data was analyzed statistically using CRD and 't' test and the breed-wise interrelationships among various traits were worked out (Snedecor and Cochran, 1994).

Results and Discussion

The findings (Mean ± SE) on physico-morphological attributes of fresh semen ejaculates of Gir and Surti bulls observed are given in Table 1. The colour of semen was found to be milky white to yellowish in Gir bulls and milky to creamy white in Surti bulls, which is the normal colour of cattle and buffalo bull semen.

Ejaculate Volume:

The ejaculate volume in Gir and Surti bulls varied from 4.00 to 8.00 and 2.00 to 5.00 ml with an overall mean of 6.20±0.42 and 3.34±0.23 ml, respectively, being significantly ($P<0.01$) higher in Gir bulls. Ejaculate volume together with sperm concentration and motility are of great importance in frozen semen production and wider application in AI industry. The present finding was in harmony with the reports of Dhami *et al.* (2001) and Chowdhury *et al.* (2013) in Gir bulls and Khawaskar *et al.* (2012) and Chaudhari *et al.* (2014) in Surti bulls. However, comparatively higher ejaculate volume than the present one has been reported by Rana and Dhami (2004) and lower one by Shelke and Dhami (2001) in Gir bulls. Similarly, the ejaculate volume obtained in Surti bulls was comparatively higher than that reported by earlier workers in Surti bulls (Kodagali *et al.,* 1972; Dhami and Kodagali, 1988; Orin *et al.,* 2016). The differences in seminal volume reported in different studies might be attributed to age, heath and genetic make-up of bulls, nutritional status, season, climatic conditions and individual variation, apart from collection methodology.

Sperm Concentration:

The sperm concentration per ml of semen in Gir and Surti bulls varied from 600 to 1700 and 600 to 1550 million with an overall mean of 1169.44±61.71 and 846.30±54.82 million, respectively. It was significantly ($P<0.01$) higher in Gir bulls. Sperm concentration per unit volume is vital in semen processing since the dilution rate relies on concentration of spermatozoa along with the initial motility and viability in given ejaculate. Better is the sperm count per unit volume of semen greater is the number of insemination doses that can be produced. The mean sperm concentration recorded in the study compared well with the values reported earlier from Gujarat in Gir (Shelke and Dhami,

2001; Dhami *et al.,* 2001), Kankrej (Patel and Siddiquee, 2012) as well as in Surti buffalo bulls (Kodagali *et al.,* 1972; Khawaskar *et al.,* 2012). However, Rana and Dhami (2004) reported higher sperm concentration, while Chowdhury *et al.* (2013) revealed lower one in Gir bulls than the present finding. Similarly, Dhami and Kodagali (1988), Chaudhary *et al.* (2014) reported much higher sperm concentration in Surti bulls. The sperm concentration in bovine and bubaline species fluctuates between breeds, age, libido, climate, testicular health, accessory sex glands function, frequency and method of collection, nutritional management etc., and in many circumstances there are individual variations within breed also.

Table 1: Physico-morphological attributes of fresh semen of Gir cattle and Surti buffalo bulls during breeding season (Mean ± SE)

Sr. No.	Seminal characteristics	Overall Mean ± SE Values	
		Gir bull semen (n=27)	Surti buffalo semen (n=27)
1	Ejaculate volume (ml)	6.20±0.42	3.34±0.23**
2	Density score (1-4)	3.00±0.05	3.04±0.09
3	Sperm concentration (million/ml)	1169.44±61.71	846.30±54.82**
4	Mass activity score (0-5)	3.44±0.09	3.42±0.08
5	Individual sperm motility (%)	76.53±0.53	80.76±0.39*
6	Live sperm (%)	81.00±1.32	84.73±0.78*
7	Abnormal sperm (%)	6.00±0.37	5.81±0.40
8	Acrosome intact sperm (%)	95.59±0.35	95.54±0.25
9	HOST reactive sperm (%)	80.30±1.90	84.58±0.88*

** P<0.05; ** P<0.01 between breeds*

Mass Activity and Individual Sperm Motility:

The mass activity scores (0-5 scale) of semen of individual bulls in Gir and Surti bulls varied from 2.5 to 4.5 with an overall mean of 3.44±0.09 and 3.42±0.08, respectively. These values were statistically similar. The individual sperm motility varied from 75 to 85 per cent with an overall mean of 76.53±0.53 and 80.76±0.39 per cent for Gir and Surti semen, respectively, being significantly (P<0.05) higher in Surti bulls (Table 1). There was no variation in mean initial sperm motility between individual Gir and Surti bulls. The mass activity of spermatozoa is a wholesome effect of sperm concentration and individual sperm motility. According to Patel *et al.* (2012) mass activity score is more closely related with seminal traits rather than fertility. The present findings were in accordance with many of the previous reports on Gir bulls (Dhami *et al.,* 2001; Rana and Dhami, 2004; Chowdhury *et al.,* 2013), as well as Surti (Dhami and Kodagali, 1988; Khawaskar *et al.,* 2012), Murrah (Dhami and Sahni, 1994), Mehsana (Patel *et al.,* 2012) and Tarai (Tiwari *et al.,* 2009) buffaloes. However, the relatively lower individual sperm motility of fresh semen in Surti bulls (Chaudhari *et al.,* 2014) has been documented. The individual sperm motility is an essential parameter for assessment of semen quality and can yield a reliable picture of semen potency, because it gives clue concerning acceptance or rejection of the ejaculate for advance processing, and it is positively correlated with keeping quality and freezability of semen sample (Shelke and Dhami, 2001; Rana and Dhami, 2004; Patel *et al.,* 2012). Sperm motility is essential during their transportation in oviduct and in oocyte penetration. However, a decrease in sperm motility is observed in disease condition, change of environment/season and temperature variations, and it swings between breeds, individuals, age groups and the evaluation technique employed.

Live and Abnormal Spermatozoa:

In fresh semen of Gir and Surti bulls, the percentages of live sperms varied from 75.00 to 95.00 and 80.00 to 95.00, and the percentages of abnormal sperms varied from 2.00 to 10.00 and 3.00 to 11.00, respectively. The overall mean percentages of live sperms were 81.00±1.32 and 84.73±0.78 ($P<0.05$); and those of abnormal sperms 6.00±0.37 and 5.81±0.40 in Gir and Surti bulls, respectively. The variation between cattle and buffalo was significant ($P<0.05$) only for live sperm (Table 1). These findings were comparable with the reports of Shelke and Dhami (2001) and Dhami *et al.* (2001) for Gir bulls, and of Dhami and Kodagali (1988), Khawaskar *et al.* (2012) and Orin *et al.* (2016) in Surti bulls for both live as well as abnormal sperm percentages. Patel and Siddiquee (2012) in Kankrej bulls, Raval and Dhami (2006) in triple crossbreds found higher sperm viability than the present observations. However, Rana and Dhami (2004) and Khawaskar *et al.* (2012) reported relatively lower values of live sperm and higher values of abnormal sperm in Gir and Surti bulls, respectively.

The overall segment-wise abnormalities of sperm head, midpiece and tail region recorded in fresh semen were 1.15±0.21, 1.89±0.32 and 2.96 ± 0.38% for Gir bulls and 1.46±0.28, 1.92±0.30 and 2.42±0.19% for Surti bulls, respectively. Patel and Siddiquee (2012) in Kankrej bulls and Raval and Dhami (2006) in triple crossbred bulls found higher sperm abnormality than the present one. Bhavsar *et al*. (1990) reported the comparable overall incidence of sperm head, midpiece, tail and total sperm abnormalities in Mehsana bulls, while Shukla and Mishra (2005) found higher values of tail and total sperm abnormalities (9.22±0.22 and 12.57±0.25 %) in Murrah bull semen However, Shelke and Dhami (2001) recorded comparable mean values of head and midpiece abnormalities, but the tail and total sperm abnormalities were much higher in Jafarabadi (22.18 ± 3.10%) and Gir bull (15.54± 1.09 %). It is now universally accepted that for semen sample to be used in AI should have more than 75 % initial motility and less than 20 % total sperm abnormalities. The segment-wise sperm abnormalities should not go beyond 5 %. This is because only the live and morphologically normal sperm can drift in the forward direction to reach the site of fertilization in the oviduct after being deposited in the reproductive tract of the female animal in estrus. The current observations are well within the acceptable limit for both these traits and even others in both the species.

Acrosomal Integrity:

The mammalian spermatozoa are impotent to fertilize the egg instantly after ejaculation. They require a period of incubation in the female reproductive tract in order to gain the capacity to fertilize the egg, known as “capacitation” (Yanagimachi, 1994). Binding to the zona pellucida arouses the spermatozoa to go through acrosome reaction in which the outer acrosomal membrane fuses with the overlying plasma membrane, this physiological exocytotic event results in the release of acrosomal hydrolytic enzymes which are crucial for the fertilization process. Spermatozoa which have lost their acrosome integrity spontaneously after ejaculation or induced by physical damages are unable to bind to oocytes, and consequently they are unable to fertilize the egg. In present study sperms with intact acrosome ranged from 91.00 to 98.00 and 92.00 to 97.00 % with a mean of 95.59±0.35 and 95.54±0.25% in Gir and Surti bulls, respectively, which did not vary significantly. These findings concurred with the observations of Raval and Dhami (2006), Khawaskar *et al.* (2012) and Singh *et al.* (2014) in different breeds of cattle and buffalo. However, Rana and Dhami (2004), Chaudhari *et al.* (2014) and Orin *et al.* (2016) found relatively lower values of sperms with intact acrosome in different bovine breeds. These variations reported in intact acrosome could be due to inherent quality of particular breed/bull, age, physio-pathological status, stain, staining technique & duration of staining used etc by different workers.

Hypo-Osmotic Swelling Test (HOST):

The hypo-osmotic swelling positive sperm in neat semen were in the range of 70.00 to 94.00 and 70.00 to 92.00% with an overall mean of 80.30±1.90 and 84.58±0.88% ($P<0.05$) for Gir and Surti bulls, respectively. Gir bull sperms were more fragile than the Surti buffalo sperms. These findings were in harmony with the reports of Lodhi *et al.* (1998), Shukla and Mishra (2005), Chaudhari *et*

al. (2014) and Orin *et al.* (2016) in different breeds of cattle and buffalo spermatozoa, while others (Rana and Dhami, 2004; Singh *et al.,* 2014) observed lower values of intact plasma membrane in fresh semen. Besides, significant (P<0.05) positive correlations among progressive motility, normal morphological spermatozoa, sperm viability and percentage of HOS test positive spermatozoa have been obtained by some of these researchers (Rana and Dhami, 2004; Raval and Dhami, 2006; Chaudhari *et al.,* 2014), and have inferred that HOS test could be a valuable method for routine evaluation of semen for AI and as an indirect / *in vitro* fertility assessment test for a given sample. The HOST is used as a predictor of an intact sperm plasma membrane. It is based on the principle that spermatozoa swelled in hypotonic media display coiled flagella when their plasma membrane remains intact (Jeyendran *et al*., 1984). Spermatozoa with damaged plasma membranes do not expand and no swelling or curling of the tails occur. The osmolality of the HOS solutions must be sufficiently low (100–150 mOsm/kg H_2O), to yield the highest effect without lysis of the sperm membrane, however the appropriateness of the HOS test as a extrapolative tool for *in vitro* fertility of bulls has not yet been established fully (Rota *et al.,* 2000).

Interrelationships among Various Seminal Attributes:

In Gir bulls, the physico-morphological attributes did not reveal significant interrelationship, except mass activity and individual sperm motility (0.62, P<0.01) and live sperm and abnormal sperm per cent (-0.41, P<0.05). In Surti bulls also, the ejaculate volume had significant correlations with sperm concentration (-0.56, P<0.01), and with abnormal sperm (0.45, P<0.05), and live sperm was significantly correlated with HOS reactive sperm (0.48, P<0.05), otherwise no significant interrelationships were noted among various seminal attributes. The present findings on interrelationships among physico-morphological attributes of semen in both cattle and buffalo bulls however contradicted the significant correlations reported by Belorkar *et al.* (1990), Dhami and Sahni (1994), Raval and Dhami (2006), Khawaskar *et al.* (2012), Chaudhari *et al.* (2014) and Orin *et al.* (2016) in cattle and buffalo semen. Further, Shelke and Dhami (2001) reported significant (P<0.01) positive correlations for mass activity of Gir and Jafarabadi bulls semen with initial motility, live spermatozoa and sperm concentration; initial motility with live sperm % and sperm concentration; and live sperm with sperm concentration. Tiwari *et al. (*2009) also found significant positive correlations for progressive motile sperm with sperm concentration, live spermatozoa and HOS reactive spermatozoa; sperm concentration with live sperm and HOS reactive sperm; and live sperm with HOS reactive sperm.

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Conflict of Interest: All authors declare no conflict of interest.

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