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Characteristics and freezability of Gir bull semen

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ABSTRACT

The present research was undertaken to study the characteristics of fresh and cryo-preserved semen of elite pure breed Gir *(Bos indicus)* bulls. The mean values of fresh seminal parameters in neat semen viz. seminal volume (ml), sperm concentration (millions/ml), progressive sperm motility (%), live sperm (%), intact acrosome (%), total morphological sperm abnormalities (%), hypo osmotic swelling (HOS %) and sperm penetration distance (SPD- mm) were 4.99 ± 0.26, 895.33 ± 82.68, 69.10 ± 0.75, 72.16 ± 0.64, 84.42 ± 0.77, 15.96 ± 0.44, 60.12 ± 1.19 and 31.32 ± 0.70, respectively. Sperm concentration, individual motility, live sperm, total sperm abnormalities and sperm penetration distance differed significantly between bulls. The semen was extended, filled and sealed in 0.25 ml straws maintaining 20 million spermatozoa/straw and cryo-preserved using programmable bio freezer (IMV). Cryo-preserved semen was assessed 24 h after freezing and immediately after thawing. Freezing significantly lowered progressive sperm motility (69.10 ± 0.75 vs 53.81 ± 0.61), intact acrosome (84.42 ± 0.77 vs 75.69 ± 1.10), HOST (60.12 ± 1.19 vs 55.71 ±1.33) and CMPT (31.32 ± 0.70 vs 27.97 ±0.72). Whereas, significantly higher percentages of sperm abnormalities (15.96 ± 0.44 vs 16.92 ± 0.57) were observed after freezing.

Key words: Freezability, Gir bulls, Semen characteristics

Fertility is a measure of reproductive success. In males, it can be defined as the ability of a bull to produce semen that will result in a successful pregnancy. No individual herd member bears as much responsibility for fertility as the herd sires (Barth 1997). It is rightly said that the "Bull is half of the herd" as the male is responsible for 50% of the genes present in his daughters. Several methods can be used to assess bull fertility. Prediction of fertility prior to breeding rather than post breeding could largely increase reproductive efficacy (Rodriguez-Martinez 2003). India is losing its wealth of genetic resources in domestic animals. Practices of injudicious cross breeding with exotic stock and blind acceptance of the western philosophy of good cow breeds are some of the major forces behind the negligence of the indigenous breeds that have adapted to local conditions over thousands of years. Each one of the indigenous breeds of cow has a unique pool of genes. Secondly, the indigenous breeds have capacities of adjusting productivity with the availability of food and changing climatic conditions. They remain resistant to a number of diseases peculiar to a particular region in which they were evolved. On the other hand, exotic breeds are productive under favorable, ideal and disease free conditions. Thus, exotic breeds will not be economically viable in the long run. Gir cows are amongst the hardiest of high yielders in the world. The Gir is a indigenous milch breed of India having stress, heat and disease tolerance (Kumar and Singhal 2006). Considering these features, the Indian Council of Agricultural Research, New Delhi have started projects on Gir cattle aim to undertake testing and selection of bulls for the genetic improvement and to provide superior germplasm for utilization in other development programmes (Gaur *et al.* 2003).

Cryopreservation is a non-physiological method that involves a high level of adaptation of biological cells to the osmotic and thermal shock that occur both during the dilution, cooling–freezing and during the thawing procedures (Holt 2000). Damage occurring during the freezing–thawing procedures affect mainly cellular membranes (plasma and mitochondrial) and in the worst case, the nucleus (Blesbois 2007). Therefore, such changes in the integrity of spermatozoa affect the viability and fertility. Little is known about semen characteristics and freezability of Gir bull semen, hence these aspects were studied in high pedigreed indigenous Gir bulls.

MATERIALS AND METHODS

The study was conducted on five Gir bulls of 4 to 5 years

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age maintained at Central Semen Station (CSS) Anjora, Durg Chhattishgarh, India. A total of 40 semen ejaculates from 5 bulls (8 ejaculates from each bull) were collected. All the bulls were maintained in identical feeding and management regimen according to minimum standard protocol (MSP) of Government of India. Semen from experimental bulls was collected once a week, in morning hours between 7.00 to 8.30 A.M. before feeding by using Artificial Vagina (40 cm long and 6.5 cm in diameter) maintained at 42–45°C in incubator as per procedure described by Singh *et al*. 2000. A bull of the same species was used as a dummy for semen collection. Two false mounts were provided to each bull before collection. Immediately after collection, the semen was kept at 37°C in a water bath placed inside the passbox. Evaluation for various macro (volume, colour and consistency) and microscopic (initial progressive motility, per cent live sperm, per cent total abnormal sperm, and per cent intact acrosome) characteristics were done as described by Salisbury *et al.* (1978). Semen was diluted in Tris diluent (Rasbech 1975) and freezing was carried out after equilibration under standard conditions (Graham *et al.* 1985). Post thaw progressive motility was assessed 24 h after freezing.

Fresh seminal characteristics: Ejaculate volume was recorded visually with the help of graduated semen collection tube in milliliters. Colour and consistency were recorded visually. Colour was recorded as creamy, milky, skim milky and watery, whereas consistency was recorded as thick viscid, slightly viscid, non viscid and translucent (Barth 1997).

The concentration of spermatozoa (million per ml) in fresh undiluted semen was determined by using calibrated spectrophotometer (Accucell - IMV, Technologies, France) and Dilutor (HAMLITON micro lab ®500B). The sperm initial motility was evaluated as per the procedure described (Ahmad 1994).

Differential staining technique using Eosin-Nigrosin stain (Campbell *et al.* 1953) was applied to estimate the percentage of live spermatozoa. The acrosomal integrity (per cent normal acrosome) based on acrosomal damage was studied in Giemsa stained smears according to method of Watson (1975). Sperm abnormalities were studied in semen samples diluted with phosphate buffered saline (1: 10) and smears were prepared gently and carefully on a clean grease free microscopic glass slide. The smears were then air dried. All the smears were stained with 3% Rose Bengal stain (Rose Bengal powder 3 gm, distilled water 99 ml, commercial formalin 1 ml) at pH 6.9, for 10 min at 37ºC. After staining, smears were washed in double distilled water and allowed to air dry and mounted with DPX. The stained slides were observed under oil immersion lens (100 ×). A total of 200 spermatozoa from 10 different microscopic field that showed different abnormalities of head, mid piece and tail were counted randomly and the mean results were expressed as per cent abnormalities.

The Hypo osmotic swelling test (HOST) was carried out as per the procedure described by Jeyendran *et al.* (1984). Sperm penetration distance (SPD) in **c**ervical mucus was carried out as described by Kremer (1965).

A sample of neat semen was processed in Accucell Bovine Photometer (IMV technologies France), so as to pack 20 million sperm per 0.25 ml straw. Filling and sealing of straws was done in an integrated system-4 (IS-4, IMV technologies France) under laminar air flow cabinet which was subsequently used for cryopreservation and post thaw evaluation. After extension, filling and sealing, the straws were transferred to the cold handling cabinet (IMV technologies, France) for equilibration at 4ºC for 4 hrs and then to a programmable bio-freezer (IMV technologies, France) for 8–10 min. so as to reach a temperature to –140ºC. The straws were then collected in the pre-cooled goblet and were immersed directly into the liquid nitrogen (–196°C) and stored.

Cryopreserved seminal characteristics: Post thaw semen was assessed at least 24 h after cryopreservation. Post thaw motility (PTM) was assessed as per method described by Sardar 2007. Other seminal characteristics viz. per cent intact acrosome, sperm abnormalities, HOST and CMPT for cryopreserved semen were carried as per the procedure described for fresh semen.

The data was analyzed statistically using standard procedure of ANOVA as per Snedecor and Cochran (1994). A paired 't' test was used to assess the freezability of the semen.

RESULTS AND DISCUSSION

Fresh seminal characteristics

Fresh seminal characteristics of five Gir bulls are presented in Table 1. No significant difference for ejaculate volume was found between these bulls while the colour varied from creamy to watery and consistency varied from thick viscid to translucent in all Gir bulls. The average concentration of spermatozoa, individual motility and per cent live sperm differed significantly ($P<0.01$) between the bulls. There was no significant difference in per cent intact acrosome and HOS positive sperm between bulls but there were significant difference ($P<0.05$) in per cent abnormal spermatozoa and sperm penetration distance (SPD) observed between bulls within breed.

Similar semen characterestics for average concentration of spermatozoa, per cent individual motility, per cent intact acrosome, HOS positive sperm and cervical mucus penetration in Tharparkar bulls, while lower values for per cent live sperm (67.23 ± 1.08), total sperm abnormalities (19.87 ± 0.54) and slightly higher findings related to per cent intact acrosome (79.65 ± 1.21) were reported (Kedia *et al*. 2014) in the same location. Initial motility, cervical mucus penetration, HOS positive sperm and cervical mucus penetration and percent intact acrosome were corresponding to the present study while, higher values for ejaculated volume (4.37 ± 0.43; 2.96 ± 0.26), sperm concentration (1212.81 ± 98.94; 1298.00 ± 116.08) and total sperm abnormalities (18.75 ± 1.61; 19.54 ± 1.41) were reported

Table 1. Fresh semen characteristics in Gir bulls (n=5)

Parameters	Bull No					Overall	Significance
	892	BP-943	MP-120	BP-902	BP-934		
Volume (ml)	5.39 ±0.60	3.95 ±0.68	5.07 ±0.52	5.84 ±0.48	4.73 ±0.51	4.99 ±0.26	NS
Sperm concentration (million/ml)	355.05 ±63.17^{c}	814.32 ±75.24^{b}	630.67 ±55.33^{b}	115.29^{b} ±795.83	100.74^{a} ±1880.8	82.68 ±895.33	**
Individual progressive motility (%)	64.00 ±2.67^{b}	69.50 ±1.16^{a}	69.5 ±1.17^{a}	71.00 ±1.00^{a}	71.50 ±0.76^{a}	69.10 ±0.75	**
Live sperm (%)	69.20 ±1.30^{b}	71.90 ±1.29^{ab}	69.40 ±1.03^{a}	75.10 ±1.23^{a}	75.20 ±1.25^{a}	72.16 ±0.64	**
Intact acrosome (%)	85.50 ±1.71	85.00 ±1.59	80.50 ± 2.01	85.90 ±1.53	85.20 ±1.54	84.42 ±0.77	NS
Total morphological sperm abnormalities (%)	16.60 ± 0.93^{a}	14.80 ±0.92^{b}	18.40 ±1.10^{a}	14.70 ±0.82^{b}	15.30^{b} ±0.77	15.96 ±0.44	*
Head abnormalities (%)	6.20^{b} ±0.61	6.50 ±0.45^{b}	9.00 ±0.63^{a}	6.30 ±0.47^{b}	6.70 ±0.49^{b}	6.94 ±0.27	**
Mid piece abnormalities (%)	5.20 ±0.49	4.00 ±0.58	4.20 ±0.51	3.80 ±0.48	4.80 ±0.55	4.40 ±0.23	NS
Tail abnormalities (%)	5.20 ±0.49	4.30 ±0.30	5.20 ±0.49	4.60 ±0.31	4.10 ±0.43	4.68 ±0.18	NS
HOS Response or positive (%)	59.70 ±2.14	59.40 ±3.44	55.10 ±3.30	61.60 ±1.59	64.80 ±1.74	60.12 ±1.19	NS
CMPD (Cervical mucus penetration distance) (mm)	28.10 ±1.71^{b}	31.70 ±1.66^{b}	29.00 ±0.94^{ab}	33.40 ±1.43^{a}	34.40 ±1.28^{a}	31.32 ±0.70	*

Means bearing different superscript (a, b, c) in row differ significantly. **, p≤0.01; *, P≤0.05; NS, Non significant.

in Sahiwal and Red Sindhi bulls semen in same semen production centre (Pathak 2008).

The seminal volume of Gir bulls in present study was in close agreement with Shelke and Dhami (2001) and Suryaprakasam and Rao (1993) for Jersey × Ongole and Hariana × Ongole. However, it is higher as compared to Sahiwal (Ramchandran *et al.* 2006), Ongole (Veeraiah *et al.* 1999), HF × Red Sindhi × Sahiwal (Singh and Pangaokar 1990) and Sahiwal and Red Sindhi (Pathak 2008). This value in present study is lower than Gir bulls (Rana and Dhami 2004), Ongole (Rao *et al*. 2010), and HF × Hariana bulls (Shrivastava and Kumar 2006). Significant difference in semen volume between bulls was reported in Red Sindhi bulls (Pathak 2008), bulls of Gir and Sahiwal (Ramchandran *et al.* 2006) breed.

Variation in semen volume reported by different workers might be due to degree of sexual excitement (Collins *et al*. 1951), skill of semen collector/attendant and temperature of artificial vagina, nutritional status of bulls (Mukherjee and Bhattacharya 1952), frequency of semen collection (Prabhu and Sharma 1954), diseases (Mixner 1959), age (Ahmad *et al.* 2003), season, testosterone level (Javed *et al.* 2000), genetics, breed (Rao *et al.* 1996), testicle size and management.

Same variations in colour and consistency in semen of Sahiwal and Red Sindhi bulls were reported by Pathak (2008). Shelke and Dhami (2001) also reported thick creamy yellow to milky white semen in Gir and Jafarabadi bulls. Creamy thin to milky thick semen was reported in Jersey pure breed (Thanawala 1985) bull semen. Variation in colour and consistency of semen between bulls appears to be a normal phenomenon.

The sperm concentration of Gir bulls is in close agreement with Jersey × Ongole and Hariana × Ongole (Suryaprakasam and Rao 1993) and it is lower as compared to Ongole (Rao *et al*. 2010), Gir (Rana and Dhami 2004), Sahiwal and Red Sindhi (Pathak 2008), HF × Sahiwal (Gupta *et al*. 1990), HF × H bulls (Shrivastava and Kumar 2006), HF × Red Sindhi × Sahiwal (Singh and Pangaonkar 1990) and Sahiwal bulls (Ramchandran *et al.* 2006). There was significant variation in sperm concentration between Gir bulls while Pathak (2008) reported that the concentration of spermatozoa did not differ significantly between bulls within Sahiwal and Red Sindhi breed. Such variations in observation by various workers may be due to difference in age (Ahmad *et al.* 2003), environment, season (Sardar 2007), breed, scrotal size, libido, sexual rest, frequency of ejaculation (Kumar 1979), management, nutrition, physiological status of bulls and genetics (Mathevon *et al.* 1998).

The individual motility of Gir bulls was in close agreement with that reported by Pathak (2008) in Red Sindhi bulls. However, the individual motility is found to be higher as compared to Sahiwal (Ramchandran *et al.* 2006), HF ×

Table 2. Post-thaw seminal characteristics of Gir bulls after 24 h of cryopreservation

Parameters	Bull No					Overall	Significance
	892	BP-943	MP-120	BP-902	BP-934		
Post thaw progressive motility (%)	53.75 ±1.25	53.13 ±1.31	52.14 ±1.01	54.44 ±1.55	55.00 ±1.29	53.81 ±0.61	NS
Intact acrosome (%)	75.25 ±2.46	76.87 ±2.39	72.14 ±2.51	75.22 ±2.22	77.70 ±2.51	75.69 ±1.10	NS
Total morphological sperm abnormalities (%)	19.50 ±1.84	14.62 ±0.63	18.71 ±1.61	15.78 ±0.92	17.50 ±1.17	16.92 ±0.57	NS
HOS Response or positive (%)	55.00 ±2.04	56.75 ±2.15	49.28 ±3.93	57.11 ±2.97	58.4 ±2.39	55.71 ±1.33	NS
CMPD (Cervical mucus penetration distance) (mm)	26.00 ±1.08	28.25 ±1.71	24.85 ±1.47	28.56 ±1.30	30.20 ±1.47	27.97 ±0.72	NS

NS, Non significant.

Red Sindhi × Sahiwal (Singh and Pangaonkar 1990) and Jersey × Ongole and Hariana × Ongole bulls (Suryaprakasam and Rao 1993). This value is lower than Ongole (Veeraiah *et al.* 1999, Rao *et al.* 2010), Gir (Rana and Dhami 2004), HF × Sahiwal (Gupta *et al*. 1990), HF (Shrivastava and Kumar 2006) and Sahiwal bulls (Pathak 2008). In contrast to present finding, Pathak 2008 reported that average individual motility did not differ significantly between bulls within Sahiwal and Red Sindhi breed. The difference in observations by various workers may be attributed to different agroclimatic conditions, breed, Season (Fiaz *et al*. 2010), age (Ahmad *et al.* 2003), testosterone level (Javed *et al*. 2000), management, physiological status of bulls and genetic factors (Mathevon *et al.* 1998). This could also be due to the variation in the seminal plasma composition, since it is known to affect the spermatozoan motility (Ganguly 1988).

The per cent live sperm of Gir bulls is in close agreement with that reported by Rana and Dhami (2004) in Gir bulls and Pathak (2008) in Sahiwal bulls. The per cent live sperm of Gir bulls is lower than that reported by Shelke and Dhami (2001) in Gir bulls, Veeraiah *et al.* (1999) and Rao *et al.* (2010) in Ongole bulls, Singh and Pangaokar (1990) in HF × Red Sindhi × Sahiwal bulls. While it was higher as compared to Sahiwal (Ramchandran *et al.* 2006) and Red Sindhi (Pathak 2008). Similar to the findings of present study, significant difference was reported in per cent live sperm between bulls of different genetic group Singh and Pangaonkar (1990) and Red Sindhi bulls (Pathak 2008). Nair (1997) also reported significant difference in per cent live sperm of HF × Hariana and HF × Jersey × Hariana bulls. While Pathak (2008) found no significant difference in per cent live sperm between Sahiwal bulls. These differences in observations may be attributed to age and season (Thongtip *et al*. 2008), breed and genetic reasons (Singh and Pangaonkar 1990).

The per cent intact acrosome of Gir bulls is in close agreement with Rana and Dhami (2004) and Pathak (2008) for Sahiwal and Red Sindhi bulls. However, per cent normal intact acrosome is lower than that reported by Shrivastava and Kumar (2006) in HF and F × H bulls and is higher than Sahiwal bulls (Ramchandran *et al.* 2006). The difference in observation by different workers may be due to season, environment, breed and genetic factor (Andrabi *et al.* 2002).

Shelke and Dhami (2001) also reported comparable sperm morphological abnormalities in Gir bulls. The per cent total sperm abnormalities in our study was higher as compared to Sahiwal (Ramchandran *et al.* 2006), Panganur bulls (Babu Rao *et al.* 1999), Ongole bulls (Veeraiah *et al.* 1999 and Rao *et al.* 2010) and lower as compared to Sahiwal and Red Sindhi bulls (Pathak 2008). Pathak (2008) observed no significant difference in per cent abnormal spermatozoa between bulls within Sahiwal and Red Sindhi breed. It has been accepted that semen of average quality should not contain more than 20 per cent abnormal sperm, while semen containing above 30% abnormal sperm would be considered as poor (Hafez 1993, Lagerlof 1934). The overall head, mid piece and tail abnormalities were found to be comparable with that reported by Mandal and Tyagi (2007). Variation obtained in the sperm abnormalities might possibly be due to agroclimatic conditions (Saxena and Tripathi 1985), nutrition (Andrabi *et al*. 2002), scrotal circumference, testicular size (Chacon 2001), age, breed, season (Soderquist *et al.* 1996 and Valakazi 2003) and different techniques employed for studying the estimation of abnormalities (Rao and Rao 1978).

In our study, per cent HOS positive sperm in Gir bull semen is higher than that reported by Shrivastava and Kumar (2006) in HF and F × H cross bulls. Similar to our finding, no significant difference between bulls was reported by Prasad *et al.* (1999a) and Pathak (2008), whereas Pant *et al*. (2002), Correa and Zavos (1994) and Shrivastava and Kumar (2006) reported significant difference between bulls. Pathak (2008) also observed no significant difference in HOS positive bulls between bulls within Sahiwal and Red Sindhi breeds. These differences may be due to bull (Prasad *et al*. 1999a), season (Kale *et al*. 2000), mass activity, progressive motility, sperm count, total sperm with intact acrosome (Prasad *et al*. 1999a) and total sperm abnormalities in semen of different breeds (Nur *et al.* 2005).

The sperm penetration distance travelled by freshly ejaculated spermatozoa of Gir bulls was comparable to that

reported by Prasad *et al.* (1999b) in F × H and F × J × H bulls, but was lower as compared to SPD of HF and F × H reported by Shrivastava and Kumar (2006) and Kumar and Devanathan (1996) in Jersey bulls. Similar to our study, Shrivastava and Kumar (2006) reported significant difference among the bulls, while Pathak (2008) observed no significant difference between bulls within Sahiwal and Red Sindhi breed. Differences in observations by various workers may be due to sperm abnormalities, sperm antibodies, age and quality of cervical mucus (Tang *et al*. 1999), mass motility, initial motility (Suttiyovin *et al.* 1995), kinetics and quality of progression of spermatozoa (Keel and Webster 1988) and per cent live sperms as they are known to affect the penetration of sperms in cervical mucus.

Cryopreserved seminal characteristics: In the present study, the average post thaw progressive motility, intact acrosome, per cent abnormal sperm, HOS positive sperm and sperm penetration distance (SPD) was 53.81 ± 0.61 (range 50 – 60) %**,** 75.69 ± 1.10 (range 63 – 89) %, 16.92 ± 0.57 (range 12 – 25) %, 55.71 ± 1.33 (range 29 – 72) % and 27.97 ± 0.72 (range 18–36) mm in 60 min, respectively (Table 2). There was no significant difference for post thaw evaluation namely, viz. progressive motility, per cent intact acrosome, per cent abnormal sperm, per cent HOS positive sperm and sperm penetration distance (SPD).

Kedia *et al*. (2014) reported similar findings of post thaw motility, per cent intact acrosome, sperm abnormalities, per cent hypo osmotic swelling reactive sperm and cervical mucus penetration on cryopreserved semen of Tharparkar bulls in same geographical location.

The average per cent post thaw motility of semen in Gir bulls is in close agreement with Pathak (2008) in Sahiwal and Red Sindhi bulls and Jersey × Red Sindhi bulls (Thakur *et al*. 2006). The post thaw motility (PTM) value in present study was lower than Jersey bulls (Thakur *et al*. 2006) while higher as compared to Frieswal bulls (Mandal and Tyagi 2007), HF × Jersey × Kankrej bulls (Raval *et al*. 2007), Gir bulls (Rana and Dhami 2004), HF and HF × H bulls (Shrivastava and Kumar 2006). Bhupal *et al*. (1993) reported that in identical environment, HF bull semen had better post thaw motility than that of Sahiwal bulls. Dhami *et al*. (1991) reported that post thaw motility did not vary in cattle and buffalo (45.23 ± 1.71 Vs 44.49 ± 1.99 per cent). Karmur *et al*. (2002) reported decline in sperm motility by 7.78% due to equilibration and 36.08% after freezing. Post-thaw motility can be affected by thawing temperature and time (Bhosrekar *et al*. 1986), dilutors (Belorkar *et al*. 1993 and Pramanik and Raina 1998), method used for glycerol addition (Arancibia *et al.* 1987 and Gilbert and Almquist 1978) and equilibration time (Belorkar *et al*. 1993 and Dhami and Sahni 1993). All these factors might have contributed to difference in observations by other workers for post thaw motility.

The per cent post thaw intact acrosome of Gir bulls was in close agreement with Sahiwal (Pathak 2008), HF (Sharma *et al*. 1990), Jersey × Red Sindhi and Jersey bulls (Thakur *et al*. 2006). However, the post thaw per cent normal acrosome was higher as compared to HF and HF × Hariana bulls (Shrivastava and Kumar 2006). This value is lower than that reported in Red Sindhi (Pathak 2008) and HF × Jersey × Kankrej bulls (Raval *et al*. 2007). Mourya and Tuli (2003) reported 20 to 35% damage in the acrosome of spermatozoa, which is in accordance to present study. However, Veeraiah *et al*. (1999) recorded 20 to 25 per cent sperms with acrosomal damage. Rana and Dhami (2004) reported 74.55±1.34 per cent intact acrosome after 48 h of refrigeration storage of Gir bull semen. Sharma *et al.* (1990) reported relatively low acrosomal integrity in post thawed semen. The post-thaw motility is the most frequently examined seminal characteristics. However, spermatozoa could be motile but not fertile, owing to the acrosomal damage. The damage to acrosome may occur during dilution, cooling, freezing and thawing processes (Tasseron *et al.* 1977).

The value of per cent abnormal sperm, in present study was in close agreement with that reported by Vyas *et al*. (1974) and Kumare (2004). This value was lower than crossbred bulls (Belorkar *et al.* 1993). A significant increase in total sperm abnormalities after freezing of buffalo semen (14.45±1.49% Vs 20.83±3.61%) was reported by Nath *et al*. (1991) whereas, about 9% increase (18.91% Vs 27.4%) was reported in total sperm abnormalities in frozen semen of Holstein Friesian bulls (Luthra and Mariony 1995).

The average post thaw HOS positive sperm is in close agreement with Sahiwal and Red Sindhi bulls (Pathak 2008). Percent HOS positive sperm in post thaw Gir semen is higher than that reported by Prasad *et al.* (1999a) and Rasul *et al.* (2000). These differences may be due to the different sugars, osmolarity and electrolytes (Jayendran *et al.* 1984). pH of semen diluents has also considerable effect on the activity and metabolism of spermatozoa (Steinbach and Foote 1967).

The average post thaw SPD in present study was in close agreement with Sahiwal and Red Sindhi bulls (Pathak 2008). Dev *et al*. (1996) reported that fertilizing capacity of spermatozoa significantly depend on its penetrating abilities into estrual cervical mucus and also reported a significant correlation between sperm penetration distance (SPD) values with sperm motility, live sperm count and normal sperm count of cryopreserved semen. Sperm penetration into cervical mucus has been successfully used as a test of fertility in human (Kremer 1965) and bull (Murase and Braun 1990) semen. Differences may be attributed to various post thaw cytomorphological characteristics which are known to affect SPD (Kumar and Devanathan 1996).

Freezability of Gir bull semen

In present study, there was significant ($P<0.01$) decrease in motility per cent, per cent intact acrosome, HOS per cent and CMPT - mm while there was significant ($P<0.05$) increase in abnormal sperm per cent after freezing. Kedia *et al*. 2014 reported almost parallel freezability pattern in motility post thaw motility, per cent intact acrosome, HOS

per cent (P<0.05) and CMPT and sperm abnormalities per cent (P<0.01) after freezing semen of Tharparkar bulls in the same laboratory.

Initial motility is an important attribute for acceptance or rejection of ejaculates for further processing and use in AI, and it has been found positively correlated with keeping quality, freezability and fertility of the semen sample (Belorkar *et al*. 1993). Belorkar *et al*. (1990) reported that better initial motility of semen results in better freezability/ preservability with higher live sperm and lower dead/ abnormal spermatozoa in crossbred bulls. Karmur *et al.* (2002) reported decline in sperm motility by 7.78% due to equilibration and 36.08% due to freezing. Saacke and White (1972) reported that the percentage of spermatozoa with normal acrosome remained higher after dilution, cooling or equilibration (73.2% ± 2.4 %) than after freezing and thawing (61.8 % ± 2.4 %; P< 0.05). The presence of an acrosomal cap is important in the fertilization process and has been highly related with fertility of frozen bull semen (Saacke and White 1972). Loss of plasmalemma over the entire acrosome, a marked projection in the anterior part of outer acrosomal membranes and extensive vesiculation and disruption of plasmalemma and outer acrosomal membranes are the common membrane defects in frozen-thawed bull sperm (Krogenaes *et al.* 1994). In bulls, this damage is evidenced by the loss in motility and acrosomal cap (O'Connor *et al.* 1981) and low fertility (Shannon and Vishwanath 1995). Morphological abnormalities of spermatozoa affects the freezability of semen (Pangaonkar and Sharma 1989) and fertility in AI bulls (Soderquist *et al.* 1991). Dilution of semen did not affect spermatozoa structure (Saacke and Marshall 1968 and Singh *et al*. 1991), but the sperm morphology was highly affected between equilibration period and 24 hours after freezing (Singh *et al*. 1991). A bull must have greater than 70% morphologically normal sperm to be classified as a satisfactory potential breeder (Chenoweth *et al.* 1994). A significant increase in total sperm abnormalities after freezing of buffalo semen (neat semen 14.45 ± 1.49 % Vs frozen semen 20.83 ± 3.61 %) was reported by Nath *et al*. 1991, whereas, about 9% (neat semen 18.91 Vs frozen semen 27.4 percent) increase in total sperm abnormalities in frozen semen of Holstein Friesian bulls was reported by Luthra and Mariony (1995). HOST is used as a complementary test for *in vitro* evaluation of frozen semen, due to its high accuracy. This is possible because the sperm suffer damages that lead to alterations in the plasma membrane and loss in viability during the cooling and freezing-thawing procedures (Watson 2000). Thus, hypo-osmotic swelling tests may be useful in assessing changes in the sperm membrane functional integrity during freezing thawing procedures (Revell and Mrode 1994). Dev *et al*. (1996) reported that fertilizing capacity of spermatozoa significantly depends on its penetrating abilities into estrual cervical mucus and also reported a significant correlation between sperm penetration distance (SPD) with sperm motility, live sperm count and normal sperm count of cryopreserved semen. Sperm velocity and density parameters have good correlation with sperm penetration in bovines (Murase and Braun 1990). SPD value was greatly influenced by cryopreservation of semen and showed decreasing trend when frozen semen was used instead of fresh semen in bovines (Matousek *et al*. 1989, Okuda *et al*. 1988, Prasad *et al*. 1999b). Kumar and Devanathan (1996) suggested that among three major parameters of semen quality i.e. post-thaw motility, normal spermatozoa and acrosome intact sperms, motility had more significant contribution for sperm progression distance (SPD) in estrual cervical mucus. Galli *et al*. (1991) reported linear correlation (P<0.01) between sperm penetration and acrosomal integrity (r = 0.53). Shrivastava and Kumar (2006) reported that sperm penetration distance travelled by the freshly ejaculated spermatozoa of HF and crossbred (F × H) was 45.06 ± 3.32 mm and 39.94 ± 2.98 mm, respectively and in frozen thawed semen of same breed, it was 19.69 ± 0.47 and 18.38 ± 0.59 mm in 60 min, respectively. Prasad *et al*. (1999b) reported SPD in fresh semen of F × H and three breed cross of F × J × H as 34.71 ± 2.51 and 29.92 ± 2.72 mm, respectively and SPD in frozen thawed semen as 13.75 and 10.83 mm, respectively.

There was significant variation in sperm concentration, individual motility, live sperm, abnormal spermatozoa and sperm penetration distance in fresh semen of Gir bulls. The pattern of freezing resulted in significant decline in post thaw motility, acrosomal integrity, HOST and CMPT and increase in sperm abnormalities in Gir bull semen.

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