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Characteristics and freezability of Tharparkar bull semen

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ABSTRACT

The study was undertaken to know the characteristics of various attributes of ejaculates collected from 6 elite pure Tharparkar bulls using artificial vagina in fresh and cryopreserved semen. The mean values for fresh seminal parameters, viz. seminal volume (ml), mass motility (0–5 scale), sperm concentration (millions/ml), individual motility (%), live sperm (%), intact acrosome (%), total sperm abnormalities (%), hypo osmotic swelling test (HOST -%) and cervical mucus penetration test (CMPT – mm) were 4.40 ± 0.24, 3.15 ± 0.12, 995.16 ± 70.99, 71.88 ± 0.99, 67.23± 1.08, 79.65 ± 1.21, 19.87± 0.54, 60.15 ± 1.76 and 31.56 ± 0.96, respectively. Semen volume and sperm concentration significantly differed between bulls within breed. The semen was cryopreserved after extension maintaining 20 million sperm/ straw, filling and sealing in 0.25 ml straw using programmable bio-freezer (IMV). Cryopreserved semen was assessed after 24 h freezing after thawing. Freezing significantly lowered motility (71.88 ± 0.99 vs 51.16 ± 1.08), intact acrosome (79.65 ± 1.21 vs 72.43 ± 1.42), HOST (60.15 ±1.76 vs 55.81 ±1.72) and CMPT (31.56 ± 0.96 vs 26.90 ± 0.91). Significantly higher percentages of sperm abnormalities (19.87 ± 0.54 vs 23.50 ± 0.82) were observed after freezing. Freezing resulted in significant decline in post thaw motility, acrosomal integrity, HOST and CMPT and increase in sperm abnormalities in Tharparkar bull semen.

Key words: Cryopreservation, Seminal attributes, Tharparkar bulls

India ranks first in the milk production in the world with an estimated milk production of about 100.9 million tonnes (GOI 2006–07). The contribution of the bull either through natural mating or AI represents half of the genetic composition of its progeny (Blezinger 1999). Demand of good quality semen is increasing due to increasing trend of artificial insemination (AI) in the field, resulting in increasing importance of elite bulls. Many cows can be inseminated with the semen of a bull (Hafez 1993) through AI. No single measurement of seminal quality is considered as a reliable criterion for predicting fertility that necessitates incorporation of many useful measurements of seminal characteristics (Faulkner and Pineda 1980). Evaluation and assessment of various seminal parameters are useful in contribution to improvement of the genetic quality of breeding herds. The quality of semen decreases after freezing. The information on seminal characteristics and effects of freezing of such bulls is totally meagre, hence, an attempt was made to study these aspects in indigenous Tharparkar bulls.

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MATERIALS AND METHODS

Semen ejaculates (48) from Tharparkar bulls (6; from each bull 8 ejaculates), 4 to 5 year-old maintained at Central Semen Station (CSS) Anjora, Durg (Chhattishgarh) were collected. As per Standard Disease Protocol of the semen station, all animals were periodically vaccinated against theileriosis, foot and mouth disease (FMD), haemorrhagic septicemia (HS) and black quarter (BQ). They were tested to detect the incidences of brucellosis, John's disease (JD) and tuberculosis (TB) and the positive reactors were suitably disposed off as per the mandate of the herd health management. The faecal samples and blood smears were also screened periodically for the detection of parasitic load and protozoan parasites, respectively. As a routine, all animals were dewormed once in a year before monsoon.

All the bulls were maintained in identical feeding and management regimes 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 AM before feeding by using artificial vagina (40 cm long and 6.5 cm in diameter) maintained at 42–45°C in incubator as per Singh *et al*. (2000). 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 (mass motility, initial progressive motility, per cent live sperm, per cent total abnormal sperm, and per cent intact acrosome) characteristics were done as per Salisbury *et al.* (1978). Semen was diluted in Tris diluents (Rasbech 1975) with 7% glycerol and freezing was carried out after equilibration under standard conditions (Graham *et al.* 1985). Post thaw progressive motility was assessed 24 h after freezing. If the progressive motility was equal or more than 50%, the semen was cryopreserved.

Fresh seminal characteristic: Ejaculated volume was recorded visually with the help of graduated semen collection tube in milliliters. The mass motility of the semen was recorded as per Salisbury *et al*. (1978). The concentration of spermatozoa (million per ml) in fresh undiluted semen was determined by using calibrated spectrophotometer and dilutor. The sperm initial motility was evaluated as per 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 (Watson 1975).

Sperm abnormalities: Each semen sample was diluted with PBS (1: 10) and smears were prepared gently and carefully on a clean grease free microscopic glass slide. The smears were air-dried. All the smears were stained with 3% Rose Bengal stain (Rose Bengal powder 3 g, distilled water 99 ml, commercial formalin 1 ml) at pH 6.9, for 10 min at 37°C. After staining smears were washed in double distilled water and allowed to air dry and mount with DPX. The stained slides were observed under oil immersion (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 Jeyendran *et al*. (1984). Cervical mucus penetration test was carried out according to Kremer (1965).

The sample of neat semen was processed in bovine photometer, to find out the concentration of sperm (million/ml), dilution grade, total extended volume and total number of straws that would contain 20 million sperms in a 0.25 ml straw. Following the initial evaluation, the semen sample was finally diluted with a calculated quantity of extender in sterilized conical flask so as to pack 20 million sperms / straw. Filling and sealing of straws was done in integrated system 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 and spread evenly over the freezing rack for equilibration at 4°C for 4 h. After equilibration the straws were cleaned and wiped on a filter paper. The racks along with straws were kept in a programmable bio-freezer for 8–10 min so the temperatures inside the straw reached to –140°C. The straws were then collected in the pre-cooled goblet and were immersed directly into the liquid nitrogen (–196°C) and stored. Post thaw semen straws were assessed at least 24 h after cryopreservation. Post thaw motility (PTM) was assessed as per Sardar (2007). Other percent intact acrosome, sperm abnormalities, HOST and CMPT for cryopreserved semen were carried as per the procedure described for fresh semen.

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

RESULTS AND DISCUSSION

Volume: The volume of semen differed significantly ($P<0.01$) between bulls (Table 1). The seminal volume of Tharparkar bull was in close agreement with that reported by Pathak (2008) in Sahiwal, Shelke and Dhami (2001) in Gir and Suryaprakasam and Rao (1993) in Jersey × Ongole.

Table 1. Fresh semen characteristics in Tharparkar bulls (n=6)

Parameters	Bull 7032	Bull 7110	Bull 6990	Bull 6957	Bull 7043	Bull 7097	Overall	Significance
Volume (ml)	4.05^{bc}±0.56	5.40^{ab}±0.39	3.60^{c}±0.34	5.78^{a}±0.66	4.35^{abc}±0.45	3.23^{c}±0.61	4.40±0.24	**
Mass motility (0–5 scale)	3.50±0.27	3.00±0.27	3.00±0.27	3.13±0.23	3.13±0.30	3.13±0.40	3.15±0.12	NS
Concentration (millions/ml)	1490.54^{a} ±284.36	747.46^{b} ±82.61	928.46^{b} ±158.49	898.35^{b} ±51.85	904.83^{b} ±107.74	1001.63^{b} ±170.17	995.16 ±70.99	*
Individual motility (%)	71.88±3.53	71.25±3.37	69.38±1.48	71.88±2.10	73.13±1.32	66.25±1.83	71.88±0.99	NS
Live sperm (%)	65.75±2.53	66.38±2.73	67.50±3.27	67.00±2.60	67.63±2.17	69.13±3.21	67.23±1.08	NS
Normal acrosome (%)	79.13±3.12	81.75±3.40	80.13±3.35	78.00±3.16	79.75±2.70	79.13±2.88	79.65±1.21	NS
Sperm abnormalities (total) {%)}	18.37±1.42	19.50±2.22	21.37±1.79	18.87±1.49	21.62±2.47	19.50±2.17	19.87±0.54	NS
Head abnormalities (%)	7.75±0.95	9.25±1.35	7.12±0.98	7.87±0.66	7.87±1.05	6.87±0.89	7.79±0.40	NS
Mid piece abnormalities (%)	6.50±0.86	6.12±0.76	7.75±1.11	6.00±0.94	7.87±1.02	7.37±1.52	6.93±0.42	NS
Tail abnormalities (%)	4.13±0.61	4.13±0.74	6.50±0.71	5.00±0.68	5.88±0.64	5.25±0.77	5.15±0.30	NS
HOST (%)	65.63±4.04	62.25±3.17	56.50±4.20	59.75±4.98	56.25±3.70	60.50±5.78	60.15±1.76	NS
CMPT (mm)	29.88±2.42	33.25±2.26	33.50±2.57	31.13±2.42	31.88±2.33	29.75±2.58	31.56±0.96	NS

However, it was higher as compared to Red Sindhi bulls (Pathak 2008), Sahiwal (Ramchandran *et al.* 2006), Ongole bulls (Veeraiah *et al.* 1999), Punganur bulls (Babu Rao *et al*. 1999) and HF × Red Sindhi × Sahiwal (Singh and Pangaonkar 1990). The present values were lower than HF × Hariana bulls (Shrivastava and Kumar 2006) and Gir bulls (Rana and Dhami 2004). The difference in volume between bulls might be due to variation in the androgen dependent accessory glands particularly those affected by testosterone concentration.

Mass motility: The mass motility did not differ significantly between bulls. The mass motility of Tharparkar bulls is in close agreement with that reported by Rao *et al.* (2010) in Ongole bulls, Pathak (2008) in Sahiwal and Red Sindhi bulls, Shrivastava and Kumar (2006) in crossbred bulls, Rana and Dhami (2004) in Gir bulls and Shelke and Dhami (2001) in Gir bulls. However the mass motility was higher as compared to Ramchandran *et al.* (2006) in Sahiwal and Suryaprakasam and Rao (1993) in Jersey × Ongole and Hariana × Ongole bulls. These values were lower than that reported by Shrivastava and Kumar (2006) in HF bulls and Hazarika *et al*. (1988) in Jersey and Sahiwal bulls.

Sperm concentration: The concentration of spermatozoa differed significantly ($P<0.05$) between bull within breed. High spermatozoa concentration might be due to individual genetic variation (Singh and Pangaonkar 1990). The sperm concentration of Tharparkar is in close agreement with that reported by Shrivastava and Kumar (2006) in HF and crossbred (HF×Hariana) bulls, Suryaprakasam and Rao (1993) in Jersey × Ongole and Hariana × Ongole and was higher as compared to Hazarika *et al.* (1988) and Gupta *et al*. (1984) in Jersey × Sahiwal bulls. These values were lower than that reported in bulls of different breeds, viz. Ongole (Rao *et al.* 2010), Sahiwal and Red Sindhi (Pathak 2008), Sahiwal (Ramchandran *et al.* 2006), HF × Hariana (Shrivastava and Kumar 2006), Gir (Rana and Dhami 2004, Shelke and Dhami 2001), Punganur (Babu Rao *et al.* 1999) and Ongole (Veeraiah *et al.* 1999).

Individual motility: The average individual motility did not differ significantly between bulls within breed. The individual motility of Tharparkar bulls was in close agreement with that reported by Pathak (2008) in Sahiwal and Red Sindhi bulls, Shrivastava and Kumar (2006) in crossbred (HF×Hariana) bulls, Sori *et al.* (2006) in Horo bulls and Rana and Dhami (2004) in Gir bulls. However the individual motility was higher as compared to Ramchandran *et al* (2006) in Sahiwal, Suryaprakasam and Rao (1993) in Jersey × Ongole and in Hariana × Ongole bulls. These values were lower than that reported by Rao *et al.* (2010) in Ongole bulls, Shrivastava and Kumar (2006) in HF bulls, Babu Rao *et al.* (1999) in Punganur bulls and Veeraiah *et al.* (1999) in Ongole bulls.

Percent live sperm: There was no significant difference in percent live sperm between bulls. The percent live sperm of Tharparkar bulls is in close agreement with that reported in bulls of Sahiwal and Red Sindhi (Pathak 2008), Sahiwal (Ramchandran *et al.* 2006) , Gir (Rana and Dhami 2004) and Jersey × Sahiwal (Hazarika *et al.* 1988). These values were lower than that reported in bulls of Ongole (Veeraiah *et al.* 1999, Rao *et al.* 2010), HF and HF × H (Shrivastava and Kumar 2006), Horo (Sori *et al.* 2006), Gir (Shelke and Dhami 2001) , Punganur (Babu Rao *et al.* 1999), Kankrej × HF (Patel *et al.* 1988, Patel *et al.* 1989), Kankrej × Jersey, HF × Sahiwal and Jersey × Sahiwal (Gupta *et al.* 1984, Gupta *et al.* 1990), Jersey × Sahiwal (Saxena and Tripathi 1978) and Tharparkar and Jersey (Tatte 1968).

Percent intact acrosome: No significant difference in percent normal acrosome was found between bulls within breed. The percent normal acrosome of Tharparkar bulls was in close agreement with that reported in bulls of Sahiwal and Red Sindhi (Pathak 2008) and Sahiwal (Ramchandran *et al.* 2006). These values were lower than that reported by Shrivastava and Kumar (2006) in HF and HF × Hariana bulls and Rana and Dhami (2004) in Gir bull semen. The difference in observation by different workers may be due to season, environment, breed and genetic factor (Andrabi *et al.* 2002).

Sperm abnormalities: No significant difference in percent abnormal spermatozoa was observed between bulls. It has been accepted that semen of average quality should not contain more than 20% abnormal sperm, while semen containing above 30% abnormal sperm would be considered as poor (Lagerlof 1934, Hafez 1993). The percent total sperm abnormalities in present study is in close agreement with that reported in Sahiwal and Red Sindhi bulls (Pathak 2008) and was higher as compared to bulls of Frieswal (Mandal and Tyagi 2007), Sahiwal (Ramchandran *et al.* 2006), Panganur (Babu Rao *et al.* 1999), Ongole (Veeraiah *et al.* 1999) and Kankrej × Jersey cross bulls (Patel *et al.* 1988). Comparable abnormalities were reported by Rana and Dhami (2004) in Gir and Jafarabadi bulls.

Hypo-osmotic swelling test (HOST): The spermatozoa which showed the characteristic tail swelling (HOST positive sperm) in fresh semen were 60.15 ± 1.76% (range 28 –78) in Tharparkar bull. No significant difference was observed between bulls within breeds. Our finding is in close agreement with Pathak (2008) in Sahiwal and Red Sindhi bulls, Ramchandran *et al.* (2006) in fresh semen of Sahiwal bulls.

In present study percent HOST positive sperm was higher than that of Shrivastava and Kumar (2006), Pant *et al.* (2002), Rasul *et al.* (2000) and Prasad *et al.* (1999b). The fertilizing capacity of spermatozoa bears positive association with this attribute. Significant difference between breed was reported by Prasad *et al.* (1999b), whereas Shrivastava and Kumar (2006), Pant *et al*. (2002) and Correa and Zavos (1994) reported significant difference between bulls and breeds. These differences may be due to bull (Prasad *et al*. 1999a), season (Kale *et al*. 2000), mass activity, progressive motility as motility partly depends on transports of compounds across membrane of spermatozoa (Jayendran *et al.* 1984), sperm count, total sperm with intact acrosome (Prasad *et al*. 1999a) and total sperm abnormalities in semen of different breeds (Nur *et al.* 2005).

Cervical mucus penetration test (CMPT): The sperm penetration distance (SPD) travelled by vanguard spermatozoa of fresh semen in Tharparkar bulls was 31.56 ± 0.96 (range 18 – 45) mm. No significant difference was found between bulls within breed. The sperm penetration distance travelled by freshly ejaculated spermatozoa of Tharparkar bulls was comparable to that reported by Pathak (2008) in Sahiwal and Red Sindhi bulls and Prasad *et al.* (1999a) in F × Hariana and F × J × Hariana bulls, but was lower as compared to SPD of HF and F × Hariana reported by Shrivastava and Kumar (2006) and Kumar and Devanathan (1996) in Jersey bulls and were higher to that reported by Dev *et al.* (1996) in buffalo semen.

Post thaw evaluation of cryopreserved semen: Bull wise cryopreserved seminal characteristics of 6 Tharparkar bulls are presented in Table 2.

Post thaw motility: The average post thaw motility was 51.16 ± 1.08 (range 35 – 65)% in Tharparkar bulls. There was no significant difference between bulls. The percent post thaw motility of Tharparkar bulls was in close agreement with that reported by Pathak (2008) in Sahiwal and Red Sindhi bulls, Raval *et al*. (2007) in HF × Jersey × Kankrej bulls, Rana and Dhami (2004) in Gir bulls. These values were lower than that reported by Thakur *et al*. (2006) in Jersey × Red Sindhi and Jersey bulls. However the post thaw motility was higher as compared Mandal and Tyagi (2007) in Frieswal bulls, Shrivastava and Kumar (2006) in HF and HF × Hariana bulls.

Percent intact acrosome: There was no significant difference between bulls on post thaw percent normal acrosome in Tharparkar bulls. The percent post thaw normal acrosome of Tharparkar bulls was in close agreement with that reported by Pathak (2008) in Sahiwal, Thakur *et al*. (2006) in Jersey × Red Sindhi and Jersey bulls and Shrivastava and Kumar (2006) in HF bulls. However the post thaw percent normal acrosome was higher as compared to Shrivastava and Kumar (2006) in HF × Hariana bulls. These values were lower than that reported by Pathak (2008) in Red Sindhi and Raval *et al*. (2007) in HF × Jersey × Kankrej bulls.

Percent abnormal sperm: The average post thaw percent abnormal sperm was 23.50 ± 0.82 (range 12 – 31)% in Tharparkar bulls. There was no significant difference between bulls. Dilution of semen did not affect spermatozoa structure (Saacke and Marshall 1968, Singh *et al*. 1991), but the sperm morphology was highly affected between equilibration period and 24 h after freezing (Singh *et al*. 1991). 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 various workers (Shetti *et al*. 1981, Hazarika *et al*. 1989 and Nath *et al*. 1991), whereas, Luthra and Mariony (1995) reported about 9% (neat semen 18.91 vs frozen semen 27.4%) increase in incidence of total sperm abnormalities in frozen semen of Holstein Friesian bulls.

Percent HOS positive sperm: There was no significant difference in overall percent HOS positive sperm in Tharparkar bulls. The average post thaw HOS positive sperm was in close agreement with that reported by Pathak (2008) in Sahiwal and Red Sindhi bulls.

Cervical mucus penetration test (CMPT): The average post thaw sperm penetration distance was 26.90 ± 0.91 (range 20 – 40) mm in Tharparkar bulls. There was no significant difference in overall post thaw sperm penetration distance between Tharparkar bulls. The average post thaw sperm penetration distance (SPD) was in close agreement with that reported by Pathak (2008) in Sahiwal and Red Sindhi bulls. 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 and 39.94 ± 2.98 mm respectively and in frozen thawed semen of same breed was 19.69 ± 0.47 and 18.38 ± 0.59 mm respectively. Prasad *et al*. (1999a) reported SPD in fresh semen of F × H and 3 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.

Freezability of Tharparkar bull semen

Present study on Tharparkar bull semen showed significant difference between fresh and frozen semen. There was significant decrease in motility% (71.88±0.99 vs 51.16±1.08; P<0.01), percent intact acrosome (79.65±1.21 vs 72.43±1.42; P<0.01), HOST% (60.15±1.76 vs 55.81±1.72; P<0.05) and CMPT - mm (31.56±0.96 vs 26.90±0.91; P<0.01) while there was significant increase in abnormal sperm morphology percent (19.87±0.54 vs 23.50±0.82; P<0.01) after freezing.

Cryopreservation is a non-physiological method that involves a high level of adaptation of biological cells to the osmotic and thermal shocks that occur both during the dilution, cooling–freezing and during the thawing

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

Parameters	Bull 7032	Bull 7110	Bull 6990	Bull 6957	Bull 7043	Bull 7097	Overall	Significance
Post thaw motility (%)	54.29±3.17	55.00±2.24	47.86±2.40	51.25±2.80	53.13±1.32	45.71±2.48	51.16±1.08	NS
Abnormal sperm (%)	22.57±1.84	21.33±1.76	24.57±1.85	23.63±1.94	24.75±2.27	23.67±2.70	23.50±0.82	NS
Normal acrosome (%)	72.43±3.81	75.33±4.24	72.00±3.59	70.63±2.92	73.63±2.92	70.83±4.66	72.43±1.42	NS
HOST (%)	61.43±4.21	59.17±1.05	53.00±4.00	52.75±4.94	49.75±3.89	61.33±4.32	55.81±1.72	NS
CMPT (mm)	26.43±2.55	28.00±1.57	28.14±2.95	26.75±2.47	26.75±2.07	25.33±1.94	26.90±0.91	NS

NS, Nonsignificant.

procedures (Holt 2000). Damage occurring during the freezing–thawing procedures affect mainly cellular membranes (plasma and mitochondrial) and in the worst case, the nucleus.

Abnormal morphology of spermatozoa affects the freezability of semen (Pangaonkar and Sharma 1989). Dilution of semen did not affect spermatozoa structure (Saacke and Marshall 1968, Singh *et al*. 1991), but the sperm morphology was highly affected between equilibration period and 24 h after freezing (Singh *et al*. 1991). A bull must have greater than 70% morphologically normal sperm to be classified as a satisfactory potential breeder (Elmore 1994, 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 various workers (Shetti *et al*. 1981, Hazarika *et al*. 1989 and Nath *et al*. 1991), whereas, about 9% (neat semen 18.91 vs frozen semen 27.4%) increase in incidence of total sperm abnormalities in frozen semen of Holstein Friesian bulls was reported by Luthra and Mariony (1995).

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%; $P< 0.05$). Decrease in acrosomal integrity after freezing was due to damage to acrosome during dilution, cooling, freezing, and thawing process (Tasseron *et al*. 1977).

HOST is used as a complementary test to *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) values 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 (Okuda *et al*. 1988, Matousek *et al*. 1989, Prasad *et al*. 1999b) attributed to cryoinjury. Kumar and Devanathan (1996) suggested that among 3 major parameters of semen quality i.e. post-thaw motility, presence of normal form of spermatozoa and acrosome intact sperms, motility had more significant contribution for sperm progression speed (SPD) in estrual cervical mucus.

Semen quality of Tharparkar bulls were found optimal for germplasm conservation and cryopreservation for future use through AI. Cryopreserved seminal characteristics implies that there was no significant difference in the post thaw motility percent, percent normal acrosome, percent total sperm abnormalities; percent hypo osmotic swelling of spermatozoa and cervical mucus penetration test between bulls. Based on the present findings of freezability pattern, it could be concluded that freezing resulted in significant decline in post thaw motility, acrosomal integrity, HOST and CMPT and increase in sperm abnormalities in Tharparkar bull semen.

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