The Pharma Innovation Journal 2023; SP-12(9): 107-111

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(9): 107-111

www.thepharmajournal.com
Received: 03-07-2023
Accepted: 07-08-2023

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Studies on seminal attributes and motion kinematics in Tharparkar bulls outside their breeding tract

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Abstract
The present study was carried out with the objectives to evaluate seminal attributes and to study the motion kinematics of Tharparkar breeding bull semen using computer assisted semen analyzer (CASA) outside its breeding tract. Semen was collected bi-weekly from three Tharparkar bulls for 10 weeks. The spermiogram revealed, the colour variation among the bulls was from milky to creamy white, consistency (0-4 scale) 2.57 ± 0.07, ejaculate volume 3.97 ± 0.16 mL, sperm concentration 1303 ± 51.29 million / mL, mass activity (0-5 scale) 3.48 ± 0.07, initial motility 81.50 ± 0.68%, sperm viability 86.72 ± 0.46%, total sperm abnormality 14.13 ± 0.20%, acrosomal integrity 92.82 ± 0.24% and HOST 86.78 ± 0.43 percent (80.00 to 92.00). The motion kinematics (µm/s) of neat semen was: VCL (Curvilinear velocity) 132.00 ± 1.74, VSL (Straight-line velocity) 74.21 ± 1.28 and VAP (Average path velocity) 87.14 ± 1.45. The post thawed semen characters revealed motility of 61.50 ± 0.49%, HOST 59.87 ± 0.60% and the motion kinematics (µm/s) was: VCL 111.10 ± 1.99, VSL 65.61 ± 1.28 and VAP 79.48 ± 1.48. Significant ($p<0.01$) reduction in seminal characters of post thawed semen was noticed. It was concluded that seminal attributes of Tharparkar bulls fall within the range established for other Zebu cattle and CASA found to be more efficient and precise means of qualitative evaluation of motion kinematics.

Keywords: Tharparkar, semen, CASA, motion kinematics

1. Introduction
Tharparkar is one among the most important dual-purpose breeds of India and are well adopted to harsh environmental conditions and much resistant to many tropical diseases with the ability of heat tolerance (Rajoriya *et al.*, 2014) [30]. Due to unplanned breeding and cross breeding programme, number of Tharparkar cattle population is rapidly decreasing, such that this breed is considered as "insecure" according to FAO expert panel (Rajoriya *et al.*, 2013) [31]. Artificial insemination (AI) is the most important and commonly implemented technology for breeding of cattle population (Gravance *et al.*, 2009) [10] and development of Computer Assisted Semen Analysis (CASA), that analyzes and records every sperm track characteristic has firmly improved the accuracy of semen evaluation (Verstegen *et al.*, 2002) [42].
For the conservation of germplasm of Tharparkar bulls, the knowledge of semen quality and its Freezability is much necessary and the studies related to seminal attributes and freezability in this species is inadequate and preliminary (Rajoriya *et al.*, 2014) [30]. Hence, the present study was planned to evaluate seminal attributes and to study the motion kinematics of Tharparkar breeding bull semen using CASA (Computer Assisted Semen Analyzer) outside its breeding tract.

2. Materials and Methods
Study was carried out at the Central Frozen Semen Production and Training Institute, Hesaraghatta, Bengaluru of Karnataka state between August and October 2022. Three adult Tharparkar breeding bulls aged 3-7 years, andrologically healthy with an acceptable semen quality were maintained in identical dietary and managerial circumstances during the study period.

2.1 Semen collection and evaluation
Semen from each study bull was collected weekly twice for 10 weeks period. Totally 60 ejaculates (20 from each bull) were available for further processing. Evaluation for various macroscopic characters like volume, colour - consistency (Barth, 1997; Mutha Rao, 2015) [5, 22] and microscopic parameters like Mass activity (Ghodasara *et al.*, 2018) [9], Individual motility

(Ahmad, 1984) [1], percent live sperms, percent total abnormal sperms (Shalini *et al*., 2018) [35], percent intact acrosome (Watson, 1975) [44] and Hypo-osmotic swelling test (HOST) (Shalini *et al*., 2018) [35] were carried out as described by different authors. Assessment of motion kinematics of neat semen was done under CASA (Hamilton Thorne Biosciences, IVOS II, Beverly, MA).

2.2 Post thawed semen evaluation
The same semen samples were processed for cryopreservation and post thaw motility, motion kinematics under CASA system and post thaw HOST were assessed after 24 hrs. The data collected from the experimentation was tabulated and statistical analysis was done accordingly.

3. Results and Discussion
3.1 Neat seminal characters
3.1.1 Colour and consistency
Colour is one of the criteria of seminal characteristics for preliminary screening of semen ejaculates and the variation in the colour might be ascribed partly to the low concentration of sperm (Kapadiya *et al*., 2018) [15]. Colour of semen in Tharparkar bulls was creamy in 85.50 percent of ejaculates, thick milky in 11.33 percent, milky white in 3.66 percent. The overall mean of consistency was 2.57±0.07 with a range of 2 - 3 (Slightly viscid to full viscid). The observations of the presented study are in agreement with those reported by Sonar *et al.* (2016) [39] in Gir, Pathak (2008) [26] in Red Sindhi and Kapadiya *et al*. (2018) [15] in Kankrej bulls. Whereas, Patel and Siddiquee (2013) [24] reported 100 percent creamy white ejaculates in Kankrej bulls.

3.1.2 Volume
Volume of an ejaculate may be a good indicator of fertility and it varies from breed to breed and influenced by a number of factors such as age, breed, weight and season. The overall mean of ejaculate volume in the current study was 3.97 ± 0.16 mL and ranged from 2.00 to 7.20 mL (Table 1). The ejaculate volume did not differ significantly among the bulls. The present findings are in agreement with Hossain *et al.,* (2012) [12] in Sahiwal, Sonar *et al.,* (2016) [39] in Gir and Kapadiya *et al*., (2018) [15] in Kankrej. However, the observations of the present study are at variance with the results reported by Prajapati *et al.* (2022) [28] in Gir and Patel and Siddiquee (2013) [24] in Kankrej. The variation may be due to the level of teasing before collection (Collins *et al*., 1951) [8], skills of collector, temperature of AV and collection frequency (Prabhu and Sharma, 1954) [27].

3.1.3 Sperm concentration
Sperm concentration combined with ejaculate volume helps in determining number of females that can be inseminated with optimal number of sperm cells (Hafez, 1993) [11]. The mean of sperm concentration (millions/mL) recorded in the present study was 1303 ± 51.29 (Table 1) and no significant difference noticed among the bulls. Similar results were reported by Premkumar *et al.* (2020) [29] in Hallikar bulls and Hossain *et al.* (2022) [13] in Red Chittagong. On the other hand, significant effect of bull on sperm concentration was reported by Chikhaliya *et al.* (2018) [7] in Gir, Patel and Siddiquee (2013) [24] in Kankrej and Pal *et al.* (2020) [23] in Hariana bulls. The difference in age group (Ahmad *et al.*, 2003) [2], environmental factor, breed, scrotal circumference, libido, sexual rest, frequency of collection (Kumar, 1979) [19] and season of study (Sardar, 2007) [34], have been known to influence the sperm concentration.

3.1.4 Mass activity
In order to facilitate semen transit through the cervix and utero-tubal junction, sperm motility is essential and much more important in actual penetration of the cumulus cells and Zona Pellucida of the ovum (Hafez, 1993) [11]. The mean mass activity in the presented study was 3.48 ± 0.07 in a scale of 0 to 5 (Table 1) and variations among the bulls was found to be significant (p<0.05). Bull-02 bull has shown the higher mean of mass activity (3.95 ± 0.05) than the Bull-01 (3.15 ± 0.08) and Bull-03 (3.30 ± 0.11). Similar analytical results were obtained by Shelke and Dhami (2001) [36] in Gir, Ray and Ghosh (2013) [32] in Sahiwal and Mannapur (2015) [21] in Amrithmahal bulls. However, non-significant variations between the bulls was observed by Singh *et al*. (2000) [38] in Sahiwal, Chikhaliya *et al.* (2018) [7] in Gir and Kapadiya *et al.* (2018) [15] in Kankrej bulls. Subjective nature of evaluation through naked eye and the equipment used always influence the observations (Premkumar *et al.*, 2020) [29].

3.1.5 Initial motility
Initial sperm motility is an important parameter whether to consider an ejaculate or not for further processing to use in AI and is positively correlated with keeping quality, freezability and fertility of the ejaculate (Shelke and Dhami, 2001) [36]. The present study revealed the mean individual sperm motility percent of 81.50 ± 0.67 with a range of 70 to 90 and there was significant variation among the bulls (p<0.05) (Table 1). Bull-02 has shown higher mean initial motility percent (85.75 ± 0.83) than the Bull-01 (80.00 ± 0.81) and Bull-03 (80.25 ± 0.99). This result is consistent with the earlier reports in Hariana (Pal *et al.*, 2020) [23], Gir (Prajapati *et al*., 2022) [28] and Sahiwal bulls (Ray and Ghosh, 2013) [32]. However, this study findings contradict with those reported in Tharparkar bulls (Kedia *et al.*, 2014) [18], Sahiwal and Red Sindhi (Pathak, 2008) [26] and Gir bulls (Chikhaliya *et al.*, 2018) [7]. The variance in the reported values of individual motility may be caused by the differences in handling of ejaculates, number of bulls studied and environmental factors (Bailey *et al.*, 2003) [4].

3.1.6 Live and total sperm abnormality
The overall mean percent live spermatozoa recorded in the present study was 81.55 ± 0.54 (Table 1) and it ranged from 81 to 96. There were no significant variations in viability among the bulls. Similarly, Prajapati *et al.* (2022) [28] in Gir, Kedia *et al*. (2014) [18] in Tharparkar and Pathak (2008) [26] in Sahiwal also reported non-significant difference in live sperm percent. The mean total sperm abnormalities recorded in the present study was 14.13 ± 0.218 percent and it ranged from 10.50 to 17.50 percent with non-significant (P>0.05) variations among the bulls investigated (Table 1). Similar observations were made by Kedia *et al.* (2014) [18] in Tharparkar, Prajapati *et al.* (2022) [28] in Gir and Patel and Siddiquee (2013) [24] in Kankrej bulls.

3.1.7 Percent intact acrosome
The presence of intact acrosome is very important to facilitate fertilization through acrosomal reaction and the major cause for the disturbance in acrosome integrity is sperm ageing or injury (Akhter *et al*., 2008) [3]. The mean percent of intact acrosomes recorded in the current study was 92.82 ± 0.243

with range of 89.00 - 97.00 (Table 1). These results in the present study are in close agreement with the reports of Rajoriya *et al.*, (2013) [31] in Tharparkar, Chikhaliya *et al.*, (2018) [7] in Gir and Kapadiya (2018) [15] in Kankrej who have reported 91.41 ± 1.16, 91.87 ± 0.32 and 92.88 ± 0.24 percent of intact acrosomes respectively. There were no variations in percent intact acrosomes among the bulls in the present study and parallel studies in Gir (Chikhaliya *et al.*, 2018; Prajapati *et al.*, 2022) [7, 28], Tharparkar (Kedia *et al*., 2014) [18] and Hariana bulls (Pal *et al.*, 2020) [23] also revealed no significant effect of bull on acrosome integrity.

3.1.8 HOST: The hypo osmotic swelling ability of spermatozoa has been reported as a sign of membrane integrity and normal functional activity which is not only essential for the maintenance of sperm motility but also for the induction for acrosome reaction and other event related to fertility (Lodhi *et al.*, 2008) [20]. In the present study the mean percentage of HOST reactive spermatozoa was 86.78 ± 0.432 and it varied from 80.00 to 92.00 (Table 1) with no significant variations among the bulls. These results in the present study are in close agreement with the reports in Gir (Prajapati *et al.*, 2022) [28], Tharparkar (Kedia *et al.*, 2014) [18] and Sahiwal breeds of bulls (Ray and Ghosh, 2013) [32].

Table 1: Mean values (± SE) and range for characteristics of fresh semen (n = 60 ejaculates)

Seminal attributes	Bull-01		Bull-02		Bull-03		Overall	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Volume (mL)	3.71 ± 0.22	2.0 - 6.0	4.05 ± 0.27	2.0 - 6.4	4.14 ± 0.32	2.0 - 7.2	3.97 ± 0.16	2.0 – 7.2
Mass activity	3.15 ± 0.08[a]	3.0 – 4.0	3.95 ± 0.05[b]	3.0 – 4.0	3.30 ± 0.10[a]	3.0 - 4.0	3.48 ± 0.07	3.0 – 4.0
Sperm concentration (millions/mL)	1248 ± 88.30	530.0 – 2305.0	1407 ± 83.12	650.0 - 2120.0	1254 ± 94.61	508.0 -2322.0	1303 ± 51.29	508.0 – 2322.0
Initial motility (%)	80.00 ± 0.81[a]	70.00 – 85.00	85.75 ± 0.83[b]	80.00 – 90.00	80.25 ± 0.99[a]	70.00 - 90.00	81.50 ± 0.68	70.00 – 90.00
Sperm viability (%)	86.60 ± 0.87	81.00 – 92.00	86.95 ± 0.87	82.00 – 96.00	86.60 ± 0.63	82.00 – 92.00	86.72 ± 0.46	81.00 – 96.00
Total Sperm abnormality (%)	14.18 ± 0.39	12.00 – 17.00	14.32 ± 0.38	10.50 – 17.50	13.90 ± 0.38	11.00 - 17.00	14.13 ± 0.20	10.50 – 17.50
Acrosomal integrity (%)	92.45 ± 0.51	89.00 – 96.00	93.10 ± 0.35	91.00 – 97.00	92.90 ± 0.40	90.00 – 96.00	92.82 ± 0.24	89.00 – 97.00
HOST (%)	87.10 ± 0.70	81.00 – 92.00	87.20 ± 0.79	80.00 – 92.00	86.05 ± 0.78	80.00 - 92.00	86.78 ± 0.43	80.00 – 92.00

Note: Mean± SE bearing different superscripts (a, b) within rows vary significantly at $p<0.05$

3.2 Post thawed seminal characters

Cryopreservation of semen in bovines involves crucial steps such as extension, cooling, freezing, storage and thawing of straws, which can cause both physiological and structural changes in the sperm (Bailey *et al.*, 2003) [4]. The overall mean percent post thaw motility in the present study was 61.50 ± 0.49 with the range of 55.00 to 70.00 (Table 2) and is in close agreement with those reported mean values of 57.85 ± 1.61 (Talluri *et al*., 2011) [41] in Ongole, 63.60 (Hossain *et al*., 2012) [12] and 57.21 ± 0.66 (Singh *et al*., 2000) [38] in Sahiwal bulls. Post thaw HOST is used as a corresponding test for *in vitro* evaluation of frozen thawed semen due to its high precision because the sperm undergoes damages that lead to alterations in the plasma membrane integrity and loss in viability during the cooling and freezing-thawing procedures (Watson, 2000) [43]. Thus, HOST is useful in assessing changes in the sperm membrane functional integrity during freezing thawing procedures (Revell and Mrode, 1994) [33]. The mean percent post thaw HOST recorded in the present study was 59.87 ± 0.60 with an overall range from 51.00 to 62.00 as shown in Table 2. The recorded values in the present study are similar to those reported values of 54.39 ± 2.54 (Kedia *et al.*, 2013) [17] in Tharparkar, 57.85 ± 1.61 (Talluri *et al.*, 2011) [41] in Ongole, 63.60 (Hossain *et al.*, 2012) [12], 57.21 ± 0.66 (Singh *et al.*, 2000) [38] in Sahiwal bulls and 55.08 ± 1.10 percent (Chaturvedi *et al*., 2021) [6] in Gir bulls. The variations observed by different authors may be due to the difference in the sugars used, osmolarity, electrolytes (Jayendran *et al*., 1984) [14] and pH of semen diluents (Steinbach and Foote, 1967) [40].

Table 2: Mean ± SE of characteristics of post thawed semen (n = 60 ejaculates)

	Mean	Range
Post thaw motility (%)	61.50 ± 0.49	55.00 – 70.00
Post thaw HOST (%)	59.87 ± 0.60	51.00 – 68.00

3.3 Motion kinematics of sperm

Among the different sperm motion characteristics assessed by CASA, forward motility and velocity parameters like curvilinear velocity (VCL), straight-line velocity (VSL) and average path velocity (VAP) are useful in predicting the fertility of bulls (Singh *et al.*, 2017; Kathiravan *et al.*, 2011) [16, 37]. In the present study the overall mean VCL, VSL and VAP of fresh semen among the bulls were 132.00 ± 1.74, 74.21 ± 1.28 and 87.14 ± 1.45 µm/s, and respective overall range of 96.40-162.60, 46.00-98.30 and 54.00-105.80 µm/s (Table 3). The mean values of velocity parameters recorded in the present study are higher than the reported values in Sahiwal (Pathak, 2008) [26] and Gir bulls Chaturvedi *et al*., 2021) [6].

Similarly, the post thawed semen curvilinear velocity (VCL), Straight-line velocity (VSL) and average path velocity (VAP) among the bulls was 111.10 ± 1.99, 65.61 ± 1.28 and 79.48 ± 1.48 µm/s and the range varied between 73.40-140.50, 40.50-86.20 and 50.50-98.20 µm/s respectively (Table 3). These values of velocity parameters in the present study were found to be similar to those in Jafarabadi and Mehsana buffalo bulls (Patel and Dhami, 2016) [25]. The variations in the results of velocity parameters may be due to variation in the breed of bulls, initial semen quality and software and model of CASA machines used during assessment (Pathak, 2008) [26].

Table 3: Velocity parameters (Mean ± SE) of fresh and post thawed semen (n = 60)

Velocity parameters	Fresh semen		Post thawed semen	
	Range	Mean	Range	Mean
VCL (µm/s)	96.40 – 162.60	132.00 ± 1.74	73.40 – 140.50	111.10 ± 1.99
VSL (µm/s)	46.00 – 98.30	74.21 ± 1.28	40.50 – 86.20	65.61 ± 1.28
VAP (µm/s)	54.00 – 105.80	87.14 ± 1.45	50.50 – 98.20	79.48 ± 1.48

4. Conclusion
It was concluded that the seminal attributes of Tharparkar bulls fall within the range established for other Indian breeds of cattle even outside the breeding tract. Assessment of sperm motion characteristics under CASA have been found to be more efficient, accurate, time saving and precise means of qualitative evaluation of sperms motion kinematics. This data contemplates further studies with a larger sample size that needs to be established along with the field trails utilizing the semen for assessing the conception rate to assess the fertility of Tharparkar bulls.

5. Conflict of interest
There is no competing interest among the authors.

6. Acknowledgement
The first author is grateful to the officers and staff of CFSP&TI, Hesaragatta, Bengaluru and the Department of Gynaecology and Obstetrics, Veterinary College, Bengaluru for their cooperation for the study.

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