International Journal of Bio-resource and Stress Management https://pphouse.org/ijbsm.php

Crossref

IJBSM February 2023, 14(2):298-305 Print ISSN 0976-3988 Online ISSN 0976-4038 Article AR3339

Research Article Natural Resource Management

DOI: HTTPS://DOI.ORG/10.23910/1.2023.3339

Effect of Non-Genetic Factors on Seminal Traits of Indigenous and Crossbred Bulls under Semi-arid Climatic Conditions

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ABSTRACT

The study was conducted at Post Graduate Institute of Veterinary Education and Research (PGIVER), Jaipur, Rajasthan, India during July–December 2020 to evaluate the effects of non-genetic factors on semen characteristics of bulls. The data of 4608 ejaculates during the period of 2017–2020 from Tharparkar and its cross with Holstein, Jersey and its cross with Sahiwal cows was maintained at Frozen Semen Bank (FSB), India. The Semen characteristics were recorded and stored in Information Network for Animal Productivity & Health (INAPH) application maintained at FSB and National Dairy Development Board (NDDB). The average semen volumes were estimated as 4.18±0.02 ml. Non-genetic factors *viz.* season, period, breed, ejaculation order and age of bulls showed significant effects on various semen parameters. Higher estimates of semen concentration, motility, Total Ejaculation Volume (TEV) and Post-thaw Motility (PTM) were recorded in Jersey Sahiwal crossbred bull whereas semen motility was observed as higher for Tharparkar bull. Seasonal analysis revealed that winter and autumn were favorable seasons for higher quantity and quality semen production. Age wise analysis presented significantly increased values with the increasing age except semen motility. Non-genetic factors under semi-arid climatic conditions revealed significant effects on semen characteristics of exotic, crossbred and indigenous genotypes and need to be accounted for quality semen production in a semen production centre. Present findings indicate that Jersey Sahiwal cross bulls were found better for quality semen production under semi-arid climatic condition than exotic and other crossbreed bulls.

KEYWORDS: Motility, post-thaw mortality, semen volume, sperm concentration

Citation* (VANCOUVER)*: Meena et al., Effect of Non-Genetic Factors on Seminal Traits of Indigenous and Crossbred Bulls under Semi-arid Climatic Conditions. *International Journal of Bio-resource and Stress Management,* 2023; 14(3), 298-305. HTTPS://DOI.ORG/10.23910/1.2023.3339.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 18th November 2022 RECEIVED in revised form on 28th January 2023 ACCEPTED in final form on 10th February 2023 PUBLISHED on 22nd February 2023

1. INTRODUCTION

India is blessed with a 192.49 million cattle, with an overall growth of 0.83% in the total cattle population, several of the indigenous breeds suffered decline mainly due to their becoming uneconomical (Anonymous, 2020). Several efforts in terms of national missions and plans have been initiated by the government of India (Anonymous, 2016), leading to the reduction in pace of decline in the cattle population particularly indigenous cattle from 9–6% from the previous census (Anonymous, 2020). Rajasthan ranks second in total livestock population however, in regards to total cattle population the state stands sixth andabout36% of total livestock is contributed by cattle. Cattle reproduction is affected by heat stress when body thermoregulatory mechanisms are unable to increase body heat loss and internal temperature increases above physiological limits (Chemineau, 1994). Rajasthan is majorly an arid region and there is inadequate availability of fodder and feed resources offering several challenges to reproduction and production potential of animals. Genetic variability in semen quality traits in cattle and their inter-relationships are getting due consideration targeting higher production (Berry et al., 2019) however, non-genetic factors need due consideration. Even though one bull can be used on thousands of females with frozen semen and AI, studies of bull fertility have received much less attention and the studies performed have generally been based on relatively small data sets (Olsen et al., 2021, Tiezzi et al., 2012, Carthy et al., 2015) as compared to cows in terms of their heritability estimation (Berry et al., 2014) as well as inclusion of cow fertility in the total merit index of many dairy cattle populations (Pryce et al., 2014). Fertility of animal is directly influenced by semen characteristics under the different climatic conditions (Biniovaet al., 2017). Optimum reproductive performances of both male and female populations are important factors for reducing the cost of production. Female fertility is included in most cattle breeding programmes, male reproductive traits are often ignored (Berry et al., 2011) particularly in developing countries like India. Number of insemination conception^{-1} can be reduced by providing the quality semen. High fertility of bull was observed in indigenous semen (Tomaret al., 1966) from ancient times still male fertility traits are not yet considered in the breeding programme, although nearly 50% of the inseminations are performed artificially and a large fraction of natural service sires is progeny or grand-progeny of AI sires (Bonfatti et al., 2013). High rate of infertility in males is a growing concern for enhancing the profitability of farmers as improvement of semen quality may allow breeding organizations to increase the success of artificial insemination (AI) (Christensen et al., 2005). Evaluation of non-genetic factors *viz.* thermal stress, change in feed, age (D'Andre et al., 2017; Boujenane and Boussaq, 2013; Suyadi et al., 2020), sexual development and maturity (Brito et al., 2002; Murphy et al., 2018), post-thaw motility (Senger, 1980, Dhami et al., 1992) on seminal traits of Indigenous and crossbred bulls will be of immense help to increase its productivity. Seasonal effects have been reported to affect the seminal traits (Tiwari et al., 2011, Orgal et al., 2012, Al-Kanaan et al., 2015). Also, studies on effects of non-genetic factors on seminal traits evaluation of cattle population are scanty (Chauhan et al., 2010, Sudheer, 2000). Keeping mind, the present study was planned to evaluate the effects of non-genetic factors on seminal traits of indigenous and crossbred bulls.

2. MATERIALS AND METHODS

The study was conducted at Post Graduate Institute of Veterinary Education and Research (PGIVER), Jaipur, Rajasthan, India during July–December 2020. Ejaculation data related to 32 bulls ofTharparkar and its cross with Holstein, Jersey and its cross with Sahiwal maintained at Frozen Semen Bank (FSB), Bassi, Jaipur, Rajasthan, India were collected over a period of 5 years (2017–2020). The FSB is located in the hot semi-arid region of India where the climate is generally dry and subtropical in characteristics with long extremely hot summers and short mild to warm winters. Bulls used in AI programme fulfill quality norms, also semen was collected and processed as per standard protocols. Bulls were prepared for collection by giving 2–3 false mounts followed by restraint. The gap between two ejaculates was 1/2–1 h depending on the bull. Preferably, semen was collected using bovine artificial vagina by veterinarian and trained staff following the norms of two ejaculates collection^{-1} and minimum two collections bull^{-1} week^{-1} for taking at least 90 collections and 180 ejaculates annually from each adult bull. After examination of sperm concentration and initial motility, semen samples were primarily maintained at 34°C. Sperm concentration was checked preferably by a digital photometer with auto dilutor. After freezing, the semen straws were stored in a separate container. Post-thaw motility of semen was examined at 24 h (after freezing). For a minimum concentration of 20 million dose^{-1}, minimum acceptable post-thaw motility was 50%. Semen doses below 50% progressive motility were discarded. Data available for the analysis during the period 2017–20 was collected from a database maintained at Frozen Semen Bank (FSB) Bassi, Jaipur. Datasets including ejaculation, volume, total extended Volume (TEV), motility, sperm concentration and post-thaw motility (PTM) were analyzed at Department of Animal Genetics and Breeding, PGIVER, Jaipur by using SPSS software. The season, period, age and number of ejaculations for various genotypes were considered as non-genetic factors in the present study. To know the effect of non-genetic factors, data was classified according to the season, period and age of bull. The data spread during the period of 2017–2020 were collected from INAPH and divided into 4 different levels. The season of semen ejaculations were divided into 4 levels. Statistical analysis was carried out using least squares and maximum likelihood analysis method for non-orthogonal data as described by Harvey (1960) using following model.

$Y_{ijklmn}=\mu + P_i + S_j + Ord_k + Br_l + c_m + e_{ijklmn}$……………….(1)

Y_{ijkl}=Observation on the lth individual in ith season, jth period and kth age group

μ=Overall population mean

P_i=Effect of ith period of semen collection

S_j=Effect of jth season of semen collection

Ord_k=Effect of kth order of ejeculation

Br_l=Effect of lth breed during semen ejeculation

c_k=Effect of lth age group during semen collection

e_{ijklmn}=Random error, NID

The statistical significance of various fixed effects in the least squares model was determined by 'F' test. For significant effects, the differences between pairs of levels of effects were tested by Duncan's multiple range tests as modified by Kramer (1957). The differences were considered significant, if

$(X_i - X_j) = \sqrt{2/(Cii+Cjj+Cij)} > σ\_eZpn\_2$…………………..(2)

where, X_i and X_j are the least squares means for ith and jth treatment and

Cii, Cjj and Cij are diagonal and off-diagonal elements in the inverse of coefficient matrix in the least squares normal equations,

Zpn_2 is ranged value in Duncan's table (0.05) at n_2 degrees of freedom,

P is number of means in the range chosen,

σ_e is standard deviation of error,

n_2 is degree of freedom for error.

3. RESULTS AND DISCUSSION

Ejaculation data of Tharparkar and its cross with Holstein, Jersey and Jersey cross Sahiwal was analyzed and details of dataset are presented in Table 1. Seminal traits were analyzed using ANOVA and results of ANOVA and LS Means with SE are presented in Table 1–2. Season, period, order of ejaculates and age at collection showed significant effect on semen volume, concentration, motility, post-thaw motility and TEV in Holstein, HF cross and Sahiwal bulls.

3.1. *Semen volume*

The overall least square means along with standard error of semen volume was observed as 4.18±0.02 ml for all bulls. It was found to be 4.33±0.10 ml for HF cross Tharparkar and 4.83±0.11 ml for Jersey cross Sahiwal. The mean value of ejaculate semen volume was highly variable (p<0.001) characteristic among Holstein and Tharparkar bull. Crossbred and exotic bulls showed higher semen volume as compared to Tharparkar. Selected non-genetic factors *viz.* season, period, age of bulls had significant effect (p<0.001) on semen volume of HF cross Tharparkar, Jersey cross Sahiwal, Jersey and Tharparkar bulls. Period of semen collection had significant effect on semen volume in bulls. The ejaculated volume of bulls increased significantly with the increasing age of bulls (27–72 months) but decreased again later (>72 months) older group. Deviation in semen volume might be observed in HF cross Tharparkar, Jersey cross Sahiwal, Jersey and Tharparkar bulls due to degree of sexual excitement and genetic factors. It may also be attributed to variation in climatic condition over the period. As summer stress affects normal reproductive function by reducing feed intake, inhibiting release or response to GnRH, FSH and LH. The reduced secretion of thyroxin and further reduction in feed intake may also be a reason for reduction in semen volume (Krishnan et al., 2017). Thermal stresses cause testicular degeneration abdominal scrotal thermo gram and hence lower the semen output (D'Andre et al., 2017). Variation in semen production between years may also be due to changes in feed, climatic condition, management practices and techniques. The present findings are in agreement with Boujenane and Boussaq (2013), who reported increase in semen production with age of bull. The similar pattern was observed by Suyadi et al. (2020) in Bali cattle. Ejaculation volume increased with age may be related to an increase in activity of the hypothalamic pituitary-testicular axis and the concurrent development of the testis and accessory glands with sexual maturity which is believed develop continuously up to 5 year post puberty in Holstein (Murphy et al., 2018). Also, scrotal circumference, scrotal shape and testicular size increase with age (Brito et al., 2002).

Table 1: General Description Data for semen collection from bulls

Breed	No. of bulls	No. of ejaculates	No. of Collection days	No. of Ejaculates week^{-1} bull^{-1}
Tharparkar Tharparkar	4	937	515	1.32
HF×Tharparkar Tharparkar	15	1649	1094	0.62
Jersey×Sahiwal	5	637	387	0.72
Jersey	8	1385	776	0.97

3.2. Semen concentration

Semen concentration was estimated as 1119.39±7.72 million ml^{-1}. The mean semen concentration was lower in HF cross Tharparkar bull as compared Jersey cross Sahiwal and Tharparkar bull. It was also observed that all non-genetic factors showed significant effect on sperm concentration of pure breed. Season of semen ejaculation had significant effect on semen concentration. Winter and autumn season showed favourable season for higher semen concentration and rainy season showed for lowest semen concentration in cattle. Period of semen collection had significant effect on semen concentration and it showed decreasing trend over the period. Highest semen concentration (1276.27±31.06 million) was found in Jersey cross Sahiwal and lowest semen concentration (870.25±26.88 ml) for HF cross Tharparkar. Order of semen ejaculation also had significant effect on semen concentration and highest semen concentration was observed in first ejaculation during semen collection. Crossbred and exotic bulls showed higher semen volume as compared to Jersey cross Sahiwal. Season of semen collection had significant effect (p<0.001) on semen volume of HF cross Tharparkar bulls. Winter and autumn season showed favorable season for higher semen production. Semen concentration varied from 1187.41±12.35 million ml^{-1} in first ejaculates to 1112.20±14.51 million ml^{-1} in second ejaculate. A significant effect of age of bull was observed on semen concentration and ejaculated semen concentration showed significantly decreased with the increasing age but increased in older group (>72 months). Similar to our finding seasonal variation was observed and winter was considered as favorable season for sperm cell concentration in Karan Fries bull (Bhakat et al., 2014). On the contrary, Tiwari et al. (2011) reported lower semen concentration in winter season for Murrah buffalo. The difference in semen concentration between seasons might be attributed to variation in ambient temperature and relative humidity. In the present finding lower concentration of sperm cell during summer and rainy season may be due to climatically stressful environment. These results were broadly-agreement with seasonal variation reported by Murphy et al. (2018) in Holstein Friesian bull. The significant difference in semen production between years may be due to changes in feed, climatic condition, management practices and techniques. Spermatogenesis had been shown to be susceptible to temperature variation. Similar to this finding, higher sperm concentration was also observed in first ejaculate in Holstein Friesian bull by Murphy et al. (2018). This might be primarily due to lower semen production decrease with the collection of multiple ejaculates on the same day. A significant effect of age of Bull was observed on semen concentration and ejaculated semen concentration showed significantly decreased with the increasing age but increased in older group (>72 months). Similar to this finding, low semen concentration associated with young Holstein Friesian bulls compared with older bulls corroborates with the findings of Murphy et al. (2018).

3.3. Semen motility

Semen motility was analyzed to assess the effect of season, period, age of bull and order of ejaculates and results of ANOVA have been presented in Table 2. The overall least square means of semen motility have been shown in Table 3. Semen motility was estimated as 67.75±0.17. Semen motility was estimated as 64.56±0.61 for Holstein cross Tharparkar, 67.09±0.70 for Jersey×Sahiwal, 69.08±0.55 for Jersey, 70.25±0.60 Tharparkar. All non-genetic factors had significant effect on semen motility. Least squares ANOVA found that season of semen ejaculates showed significant effect on semen motility. Lowest semen motility was observed in summer season and highest semen motility (69.41±0.64) was found in autumn. Period of semen collection showed erratic trend over the period. Highest motility (69.57±0.59) was found in the year of 2019 and lowest semen motility was observed as (65.67±0.59) in the year 2018. Highest semen motility was observed in third ejaculations during semen collection and it was estimated as 68.42±1.45. A significant effect of age of bull was observed on semen motility for pure exotic and crossbred cattle. High ejaculated semen motility was observed in early age and decreased in older group (>72 months). The ejaculated semen motility of bull was highest (68.38±0.70)

Table 2: ANOVA for Semen characteristics traits in Hf×Tharparkar, Jersy×Shaiwal, Jersy×Tharparkar

Source of variation	df	Volume	Concentration	TEV	PTM	Motility
Season of semen collection	3	148.91***	4380875.24***	53540.63***	48.84***	1849.75***
Period of semen collection	3	36.90***	1086685.24***	14631.26***	50.77***	2784.12***
Order of Ejaculates number	1	1.21	3305685.26***	9364.11***	13.84	170.98
Breed	2	207.14***	33996942.47***	128173.38***	32.63*	6361.58***
(Age) at collection (in Months)	2	301***	1943886.66***	64505.25***	0.96	254.22
Error		3.47 (4594)	248838.55 (4594)	1417.55 (4594)	11.043 (3623)	118.22 (4290)
R^2		0.141	0.099	0.099	0.014	0.090

Table 3: Least squares means with standard error of seminal traits in HF×Tharparkar, Jersy×Shaiwal, Jersy Tharparkar and its crossbred bull

Effect	Volume	Concentration	TEV	PTM	Motility
μ±S.E.	4.18±0.02 (4608)	1119.39±7.72 (4608)	52.57±0.58 (4608)	49.72±0.05 (3637)	67.75±0.17 (4304)
Season					
Winter (Jan–March)	4.52±0.10[a] (1003)	1192.75±27.37[a] (1003)	59.96±2.06[a] (1003)	49.82±0.20[ab] (818)	68.26±0.62[a] (951)
Summer (April–June)	3.77±0.09[b] (1411)	1072.15±25.73[b] (1411)	45.51±1.94[b] (1411)	49.49±0.19[b] (1059)	66.42±0.58[b] (1303)
Rainy (July–September)	3.95±0.09[cb] (1248)	1057.38±26.32[b] (1248)	47.58±1.98[cb] (1248)	49.57±0.20[b] (930)	66.89±0.60[b] (1141)
Autumn (October–December)	4.45±0.10[a] (946)	1155.26±28.13[b] (946)	57.23±2.12[a] (946)	50.01±0.20[a] (830)	69.41±0.64[a] (909)
Period					
2017	4.36±0.10[a] (1402)	1169.59±27.58[b] (1402)	57.56±2.08[a] (1402)	49.83±0.20[a] (1108)	67.27±0.63[a] (1315)
2018	4.21±0.09[a] (1318)	1120.61±26.04[b] (1318)	52.30±1.96[b] (1318)	49.80±0.19[a] (946)	65.67±0.59[b] (1203)
2019	3.92±0.09[b] (1303)	1096.60±25.96[b] (1303)	48.47±1.96[b] (1303)	49.99±0.19[a] (1126)	69.57±0.59[c] (1231)
2020	3.78±0.07[a] (1483)	1090.74±23.23[b] (585)	51.95±2.31[b] (585)	49.27±0.23[b] (457)	68.41±0.69[ac] (555)
Breed					
Tharparkar8	3.55±0.09[d] (937)	1200.78±26.52[d] (937)	49.48±2.00[c] (937)	49.77±0.19[ab] (831)	70.25±0.60[c] (895)
Hf× Tharparkar	4.33±0.10[a] (1649)	870.25±26.88[a] (1649)	40.17±2.02[a] (1649)	49.44±0.20[a] (1119)	64.56±0.61[a] (1482)
Jersy× Shaiwal	4.83±0.11[b] (637)	1276.27±31.06[b] (637)	69.75±2.34[b] (637)	49.81±0.23[a] (499)	67.09±0.70[b] (613)
Jersy	4.00±0.09[c] (1385)	1130.24±24.31[c] (1385)	50.88±1.83[c] (1385)	49.86±0.17[b] (1188)	69.08±0.55[c] (1314)
Order of Ejaculate					
First	4.21±0.04 (2773)	1187.41±12.35[a] (2773)	55.25±0.93[a] (2773)	49.52±0.09 (2157)	67.14±0.27 (2597)
Second	4.17±0.05 (1769)	1112.20±14.51[b] (1769)	51.08±1.09[b] (1769)	49.68±0.10 (1427)	67.67±0.32 (1648)
Third	4.15±0.23 (66)	1058.55±63.03[b] (66)	51.38±4.75[a] (66)	49.97±0.46 (53)	68.42±1.45 (59)
Age in month					
<48Months	3.49±0.11[a] (680)	1059.43±29.78[a] (680)	41.51±2.24[a] (680)	49.73±0.21 (599)	67.65±0.67 (646)
48–72 months	4.34±0.11[b] (561)	1178.17±30.68[b] (561)	57.38±2.31[b] (561)	49.75±0.22 (466)	68.38±0.70 (524)
>72 months	4.70±0.08[c] (3367)	1120.56±22.47[b] (3367)	58.81±1.69[b] (3367)	49.68±0.16 (2572)	67.21±0.51 (3134)

upper 48 months of age. Similar to the present findings, Tiwari et al. (2011) reported winter season as best season for motility in frozen semen production of Murrah buffalo bulls and summer season was unfavourable season and showed lowest sperm motility in bovine semen by Orgal et al. (2012). However, other workers have reported summer and autumn seasons as better seasons for semen production (Bhave et al., 2020; Rai and Dorji, 2021). The difference in semen motility between seasons might be attributed to variation in ambient temperature and relative humidity. It may be due to seasonal alteration of fatty acid composition and cholesterol concentration (Orgal et al., 2012).

3.4. Semen total extended ejaculation volume (TEV)

The overall least square means of total extended ejaculated semen for various breeds have been shown in Table 3. Semen total ejaculation volume ranged from 40.17±2.02 ml (HF×Tharparkar) to 69.75±2.34 ml in Jersey cross Sahiwal, 50.88±1.83 in Jersey, 49.48±2.00 in Tharparkar. Jersey cross Sahiwal (69.75±2.34) cattle showed higher total ejaculation volume. Least squares ANOVA found that non-genetic factors showed significant effects on semen total ejaculation volume (Table 2). Season of semen ejaculation had significant effect on total ejaculation volume. Winter and autumn season showed favourable season for higher semen total ejaculation volume and low semen total ejaculation volume was estimated in rainy and summer season. Season is known to affect normal reproduction process multidimensionally, like by reducing feed intake, by inhibiting the release or response to important reproduction hormones that is, GnRH, FSH and LH. LH is the important hormone, responsible for spermatogenesis. It is inhibited by increasing level of plasma corticosteroids (Clarke and Tilbrook, 1992) due to heat stress. During summer, thyroxin secretion declines leading to reduced intake of feed by the animal, subsequent metabolism and is responsible for reducing production of sperm (Zafar et al., 1988). Due to extreme heat stress bulls get physically exhausted and their reduced eagerness might result in higher reaction time and subsequently total time for successful ejaculation also increase, thus having an ultimate effect on production of sperms (Mandal et al., 2000).Total extended semen ejaculation volume showed decreasing trend over the period. Highest semen total ejaculation volume (57.56±2.08 ml) was found in the year of 2017 and lowest semen total ejaculation volume (48.47±1.96) ml for in the year of 2019 which is in congruent to the findings of Bhave et al., 2020; Rai and Dorji, 2021. Further the availability of semen at the earliest possible age from breeding bulls is not only economical but may increase productive life span and prove the bulls under progeny testing program (Dahiya and Singh 2013). Order of first ejaculation showed 55.25±12.35% higher TEV from 51.08±1.09 second order. The ejaculated semen total ejaculation volume showed significantly enhanced with the increasing age. The mean value of total extended volume was lower in exotic and crossbred bull as compared to indigenous bull. Sahiwal and its crossbreed bull showed higher total ejaculation volume as compared to exotic bull. It was also observed that all non-genetic factors showed significant effect on sperm total ejaculation volume.

3.5. Post-thaw motility (PTM)

Estimates of semen post-thaw motility ranged from 49.72±0.05% Jersey bull to 49.86±1.83% highest in crossbred bull and it showed very low variability due to minimum 50% criteria of initial motility for frozen the semen. It was also observed that all non-genetic factors showed significant effect on sperm post-thaw motility. A highly significant effect of season has been reported earlier (Gopinathan et al., 2021) while Rai and Dorji, 2021 reported a non-significant effect. In the present study, rainy season showed unfavourable effect on semen post-thaw motility. Decreasing trend was observed over the period for post-thaw motility. Highest post-thaw motility (49.86±0.17) was found in Jersey and lowest (49.44±0.20) for Holstein cross Tharparkar bred. Post-thaw motility showed very low variability for ejaculate orders and varied from 49.52±0.09 (first order ejaculate) to 49.68±0.10 (second order ejaculate). In terms of overall post-thaw motility or plasma and acrosomal sperm membrane integrity there were no significant differences between the three thawing methods evaluated (Muino et al., 2008). However, a number of studies have shown that thawing temperatures as high as 60–80°C could further improve post-thaw motility (Senger, 1980, Dhami et al., 1992). Although the use of such a high temperature is far from being a practical method of thawing the straws, especially in field conditions, it appears to cause a lower degree of cellular damage, yet the magnitude of this effect is still uncertain.

4. CONCLUSION

Non-genetic factors under semi-arid climatic condition had significant effects on semen characteristics of exotic, crossbred and indigenous genotype. Indigenous genotype produced better quality semen as compared to exotic and crossbred bulls in semiarid region. Winter and autumn seasons were reported favorable seasons for semen characteristics. Higher age of bulls presented better results in maximum semen parameters except the semen motility.

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