Evaluation of non-genetic factors on semen production potential of Surti bulls under semi arid conditions

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Abstract: Evaluation of non-genetic factors on semen production potential of bulls on 379 ejaculates from Surti maintained at Frozen Semen Bank (FSB), India for the period of two years from 2017-18 was analyzed. The Semen characteristics were drawn from Information Network for Animal Productivity & Health (INAPH) application maintained at FSB, Bassi, jaipur and National Dairy Development Board (NDDB). Data revealed that average semen volumes were 2.32±0.06 mL, Motility 69.11±0.38, Concentration 1027.80±28.82, Total Ejaculation Volume 27.72±0.17, Semen Concentration Per Dose 20.45±1.28, Post Thaw Motility 49.85±0.16 in Surti. Non genetic factors *viz.* season, period, breed, order and age of bulls showed a significant difference in the various semen parameters. Seasonal analysis revealed autumn and winter as favorable seasons for higher quantity and quality semen production with a significant difference. Also, significant difference in ejaculation order in descending order was reported. Surti may be considered as a preferred breed in the semi arid conditions of the state.

Keywords: semen volume, motility; SCPD; sperm concentration TEV and PTM;

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1. Introduction

Surti is a water buffalo breed also known as Deccani, Gujrati, Charator and Nadiadi (RajRas, 2022) native of Gujarat having average milk yield of 1600-1800 litres and milk fat content of about 8-10 percent. The animals are also commonly found in South Rajasthan extending over to Udaipur, Bhilwara, Rajsamand, Chittorgarh and Dungarpur districts in Rajasthan. Being medium in body size as compared to heavy breeds, consumes less feed, thrives well both on limited and without green, and produce milk with higher fat and SNF content and regularity in calving, maintained easily by landless, small and marginal farmers (CIRB, 2022). These animals are preferred by city milk producers, adaptability of this breed is increasing in this part of the country. Rajasthan ranks second in the country in terms of buffalo population (20th Livestock census, 2020) and increasing adaptability demands an urgent need to increase the number of good quality semen doses from best suited superior bulls like Surti. Conception rate of bovines by frozen semen are directly influenced by semen characteristics and these characteristics are affected by both genetic and non genetic factors under the different climatic conditions (Biniova *et al.,* 2017). Favorable reproductive performance of male and female cattle population are important factors for reducing the cost of production and needs attention. Number of insemination per conception can be reduced by providing the frozen semen of best quality. Also, fertility disorders were higher in exotic and cross-bred dairy cows as compared to indigenous dairy cows under sub-tropical conditions. Higher level of exotic inheritance showed reduction in bull fertility. On the other hand, high fertility of bull was observed in indigenous semen (Tomar *et al*. 1966). Keeping in view of the above economic importance of semen quality to achieve the optimum fertility under subtropical condition, estimation of semen characteristic in Surti bulls are the imperative requirement in reference to non-genetic factors *viz*. season, orders of ejaculates and age of bull.

2 Material and Method:

Data of 379 ejaculates of Surti maintained at Frozen Semen Bank (FSB), Bassi, Jaipur, Rajasthan, India were collected over a period of 2 years (2017-2018). Being located in the hot semi arid region of India , the region generally have dry and subtropical conditions having long extremely hot summers and short mild to warm winters. Bulls qualified all the quality norms as well as semen was collected and processed as per standard protocols. Preferably, semen was collected using bovine artificial vagina by veterinarian and trained staff following the norms of two ejaculates per collection and minimum two collections per bull per week 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 hours (after freezing). For a minimum concentration of 20 million per dose, minimum acceptable post thaw motility was 50%. Semen doses below 50% progressive motility were discarded. Datasets including ejaculation, volume, total extended Volume (TEV), motility, sperm concentration post thaw motility (PTM) and semen concentration per dose (SCPD) were analyzed at Department of Animal Genetics and Breeding, PGIVER, Jaipur by using SPSS software. Evaluation of effect of non-genetic factors, data was classified according to the season, period and age of bull. Statistical analysis was carried out using least squares and maximum likelihood analysis method for non-orthogonal data as described by Harvey (1987) using following model.

Y_{ijkl} = $\mu + P_i + S_j + c_k + e_{ijkl}$

Y_{ijkl} = Observation on the l^{th} individual in i^{th} season, j^{th} period and k^{th} age group

μ = Overall population mean

P_i = Effect of i^{th} season of semen collection

S_j = Effect of j^{th} period of semen collection

c_k = Effect of k^{th} age group during semen collection

e_{ijkl} = 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 test as modified by Kramer, 1957. The differences was considered significant, if

$$(X_i - X_j) = \frac{\sqrt{2}}{\text{Cii+Cjj+Cij}} > \sigma_e Zpn_2$$

where, Xi and Xj are the least squares means for i^{th} and j^{th} 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,

$\sigma_e$is standard deviation of error,

n_2 is degree of freedom for error.

3 Results and Discussion

Statistical and logical techniques were applied to analyze and evaluate the data and the findings illustrated in terms of effect of Season, order of ejaculates on semen volume, concentration, motility, post-thaw motility, TEV and SCPD in Surti bulls have been presented in tables below.

3.1 Semen Volume

The overall least square means along with standard error of semen volume was observed as 2.32±0.06 ml. Selected non genetic factors *viz*. season as well as order ejaculate presented significant effects ($p<0.001$) on semen volume while period, collection individual presented non-significant effect. Deviation in semen volume might be observed among indigenous bulls may be due to degree of sexual excitement and genetic factors. Variation in semen production between years may also be due to changes in feed, climatic condition, management practices and techniques when related as per age (Boujenane and Boussaq, 2013), who reported increase in semen production with age of bull. 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 yr post puberty (Murphy *et al.* 2018).

3.2 Semen concentration

Semen concentration was estimated as 1027.80±28.82 million / ml. Season of semen ejaculation had significant effect on semen concentration. Winter and autumn season showed favourable season for higher semen concentration. Period of semen collection had non significant effect on semen concentration and it showed increasing trend over the period. Order of semen ejaculation also had non-significant effect on semen concentration and highest semen concentration was observed in third ejaculation during semen collection. Autumn and winter season showed favorable season for higher semen production. Semen concentration varied from 911.56±46.50 million per ml in first ejaculates to 990.70±11.98 million per ml in second ejaculate. 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. Variation in ambient temperature and relative humidity particularly 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 thermogram and hence lower the semen output (Andre *et al.,* 2017). These results were broadly-agreement with seasonal variation reported by Murphy *et al.* (2018). 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.

3.3 Semen motility

Semen motility was analyzed to assess the effect of season, period and order of ejaculates and results of ANOVA have been presented in Table 1. The overall least square means of semen motility have been shown in Table 2. Semen motility was estimated as 69.11±0.38. All non-genetic factors had significant effect on semen motility. Least squares ANOVA found that season of semen ejaculates showed non-significant effect on semen motility. Lowest semen motility was observed in summer season and highest semen motility (70.29±1.82) was found in autumn. Period of semen collection showed decreasing trend over the period with Highest motility (69.38±1.70) was found in the year of 2017 and lowest semen motility was observed as (68.85±1.90) in the year 2018. Highest semen motility was observed in third ejaculations during semen collection and it was estimated as 70.99±3.89. 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. It may be due to seasonal alteration of fatty acid composition and cholesterol concentration (Orgal*et al.,* 2012). The significant difference in motility between years may be due to changes in management practices and techniques.

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 2. Semen total ejaculation volume ranged from 27.72±0.17 ml Least squares ANOVA found that non-genetic factors showed significant effect on semen total ejaculation volume (Table 1).In a similar trend, autumn and winter seasons showed favourable season for higher semen total ejaculation volume and low semen total ejaculation volume was estimated in rainy and summer season. Normal reproduction process are multidimensionally affected by season in terms of reducing feed intake, by inhibiting the release or response to important reproduction hormones that is, GnRH, FSH and LH. LH production is known to be 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). Total extended semen ejaculation volume showed erratic trend over the period with complete details in table 2. It was also observed that non-genetic factors *viz*. season, period and collection individual showed significant effect of on sperm total ejaculation volume.

3.5 Post Thaw Motility (PTM)

Estimates of semen post thaw motility ranged from were observed 49.85±0.16 in Surti bulls and it showed very low variability due to minimum 50 percent criteria of initial motility for frozen the semen. It was also observed that all non-genetic factors showed non- significant effect on sperm post thaw motility. Autumn was reported as the most favourable season while winter reported as the most unfavourable season for post thaw motility of semen. Increasing trend was observed over the period for post thaw motility. 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). Similarly, in the present study, post thaw motility showed very low variability for ejaculate orders and varied from 49.76±0.33 to 50.02±0.40 with highest at second order ejaculate.

3.6 Semen concentration per dose:

Average semen concentration per dose was reported as 20.45±1.28 with highest in autumn season and lowest in winter season with complete details in table 2. Increasing trend was observed in the period of ejaculation with highest in 2018 while erratic trend was observed in order of ejaculations. All the non genetic factors evaluated in the study were found to have non significant effect on semen concentration per dose.

4 Conclusion:

Surti bulls have presented potential and adaptability under subtropical conditions of the region in the present study. Inspite of erratic trends in the effects of non genetic factors on the semen characteristics of Surti bulls the present study reports suitability of Surti bulls in the study area.

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Table 1: ANOVA for Semen characteristics traits

Source of variation	df	Volume	motility	Concentration	TEV	SCPD	PTM
Season of semen collection	**3**	17.91***	78.81	2287836.73***	8973.77***	13.23	5.06
Period of semen collection	**3**	1.56	3.01	908976.35	2097.72**	41.28	14.60
Collection individual	**5**	1.49	69.71	1343306.36***	1903.90***	11.03	7.36
Order of Ejaculates number	**2**	9.72***	29.28	429146.16	44.65	0.95	2.26
Error		1.44(367)	5.66(304)	269873.75 (367)	506.79 (367)	11.55(283)	283(11.11)
R2		0.184	0.046	0.168	0.21	0.025	0.020

Table 2: Least squares means with standard error of different semen characteristics and its crossbred bull

Effect	Volume	Motility	Concentration	TEV	SCPD	PTM
μ ± S.E.	2.32±0.06 (379)	69.11± 0.38 (316)	1027.80±28.82 (379)	27.72±0.17 (379)	20.45±1.28 (295)	49.85±0.16 (295)
Season						
Winter (Jan. - March)	2.23±0.25 (73)^{abc}	68.95±2.02 (57)	1011.85±111.66 (73)^{a}	26.79±4.83 (73)a	19.41±0.94 (52)	49.14±0.92 (52)
Summer (April - June)	1.77±0.23 (165)^{b}	67.76±1.68 (131)	965.38±99.52 (165)^{ab}	18.63±4.31 (165)a	20.39±0.78 (118)	49.85±0.76 (118)
Rainy (July-September)	2.31±0.26 (66)^{a}	69.46±1.89 (59)	841.05±113.06 (66)^{a}	21.36±4.90 (66)a	20.68±0.87 (58)	50.02±0.85 (58)
Autumn (October-December)	2.96±0.25 (75)^{c}	70.29±1.82 (69)	1292.90±110.32 (75)^{b}	44.10±4.78 (75)b	21.33±0.84 (67)	50.39±0.82 (67)
Period						
2017	2.16±0.22 (229)	69.38±1.70 (206)	9906.79±95.46 (229)	21.91±4.13a (229)^{a}	19.44±0.79 (196)	49.25±0.77 (196)
2018	2.48±0.26 (150)	68.85±1.90 (110)	1148.80±113.73 (150)	33.53±4.92 (150)^{b}	21.47±0.88 (99)	50.45±087 (99)
Collection individual						
1	1.84±0.27 (76)	69.23±2.23 (46)	649.96±116.8 (76)^{a}	14.66±5.06 (76)^{a}	19.39±1.04 (41)	48.46±1.02
2	2.21±0.44 (9)	71.19±3.00 (8)	1273.14±191.82 (9)^{bc}	33.15±8.31 (9)^{ab}	20.93±1.37 (8)	50.19±1.34 (41)
3	2.53±0.31 (25)	67.42±2.26 (19)	816.65±135.27 (25)^{ab}	19.62±5.86 (25)^{ac}	20.09±1.06 (17)	50.08±1.04 (8)
4	2.46±0.24 (51)	69.39±1.74 (46)	1217.08±104.1 4(51)^{c}	35.96±4.51 (51)cb	20.34±0.80 (44)	50.20±0.79 (17)
5	2.49±0.23 (55)	67.34±1.64 (49)	1131.96±102.0 8(55)^{bc}	32.65±4.42 (55)^{bc}	20.81±0.77 (42)	50.36±0.75 (44)
6	2.38±0.20 (163)	70.11±1.48 (148)	1077.98±89.25 (163)^{ac}	30.28±3.86 (163)^{a}	21.16±0.68 (143)	49.80±0.66 (143)
Order of Ejaculate						
1	2.84±0.10 (230)^{a}	67.86±0.72(195)	911.56±46.50 (230)	29.00±2.01 (230)	20.51±0.34 (180)	49.76±0.33 (180)
2	2.44±0.12 (143)^{b}	68.49±0.87 (117)	90.13±55.61 (143)	28.30±2.41 (143)	20.34±0.41 (111)	50.02±0.40 (111)
3	1.68±0.51 (6)^{b}	70.99±3.89(4)	1181.70±220 (6)	25.85±9.54 (6)	20.51± 1.78 (4)	49.77±1.74 (4)