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**Research Article**

# EFFECT OF NON-GENETIC FACTORS ON SEMEN CHARACTERISTICS OF SAHIWAL AND HOLSTEIN CROSSBRED BULLS IN A SUBTROPICAL ENVIRONMENT

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## ABSTRACT

With an objective to evaluate the effect of non-genetic factors on semen characteristics of bulls, data of 6217 ejaculates from Holstein, Sahiwal and crossbred bulls, maintained at Frozen Semen Bank (FSB) V343+MX2, Bassi, Jaipur, Rajasthan 303301, India during the period of 2017-2020 was analyzed. The semen characteristics were recorded and collected in Information Network for Animal Productivity & Health (INAPH) application maintained at FSB and National Dairy Development Board (NDDB). The average semen volumes were 5.36±0.07 mL for Holstein crossbred, 5.24 ± 0.04 mL for pure Holstein and 4.18±0.06 mL for Sahiwal bulls. Non-genetic factors *viz*. season, period, breed, order and age of bulls showed a significant difference in the various semen parameters. Higher estimates of semen concentration, motility, Total Ejaculation Volume (TEV) and Post Thaw Motility (PTM) were recorded in Sahiwal bull whereas semen volume was observed as higher for Holstein crossbred bull. Seasonal analysis revealed winter and autumn as favorable seasons for higher quantity and quality semen production with a significant difference. Age wise analysis presented significantly increased values with the increasing age except semen motility. Present results indicate that indigenous Sahiwal bulls are better for quality semen production under subtropical environment than exotic and crossbreedbulls.

**Keywords:** Motility, PTM, Semen volume, Sperm concentration, TEV

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Conception rate of cows 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. Optimum reproductive performance of male and female cattle population are important factors for reducing the cost of production. Number of inseminations per conception can be reduced by providing the frozen semen of best quality. Fertility disorders were higher in exotic and crossbred dairy cows as compared to indigenous dairy cows under sub-tropical conditions (Dekka *et al.*, 2021). Similarly, 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.*, 2021). Therefore, high rate of infertility in males is a growing concern for enhancing the profitability of farmers. Keeping in view of the above economic importance of semen quality to achieve the optimum fertility under subtropical condition, estimation of semen characteristic in Sahiwal and HF crosses bulls are the imperative requirement in reference to non-genetic factors viz. season, orders of ejaculates and age of bull.

## MATERIAL AND METHOD

Ejaculation data related to a total of 33 bulls belonging to Holstein, Sahiwal and crossbred breeds 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 program fulfill quality norms, also semen was collected and processed as per standard protocols. Bulls were prepared for collection by giving two tothree false mounts followed by restraint. The gap between two ejaculates was half an hour to one hour depending on the bull. 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. Total extended semen volume (TEV) was estimated by multiplying the ejaculate volume with concentration of sperm per ejaculate on the basis of initial ejaculate volume, motility and sperm concentration as per Standard Operating Procedures (SOP) defined by Department of Animal Husbandry, Dairying and Fisheries, Government of India.Minimum 20 million motile sperm per dose is required to determine the TEV and SCPD. After freezing, the semen straws were

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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. 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, and age group ofvarious 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 the 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 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

$\mu$ = 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

For testing any significant differences between differentlevels of effects in the model Duncan's multiple range test (Kramer, 1957) was used.

## RESULTS AND DISCUSSION

Season, period, order of ejaculates and age at collection has significant effect on semen volume, concentration, motility, post-thaw motility and TEV in Holstein, Sahiwal and HF cross bulls.

### Semen Volume

The overall least square means along with standard error of semen volume was observed as 4.93±0.04 mL for all bulls. It was found to be 5.36±0.0.07 mL for HF,4.18±0.06 mL for Sahiwal and 5.24 ± 0.04 mL for HFcross (Table 2). The mean value of ejaculate semen volume was highly variable (P<0.001) characteristic among Holstein and Sahiwal bull (Table 2). Crossbred and exotic bulls showed higher semen volume as compared to Sahiwal. Selected non-genetic factors *viz*. season, period, age of bulls had significant effect (p<0.001) on semen volume of Sahiwal and Holstein 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 Holstein, Sahiwal and HF crossbred bull due to degree of sexual excitement (Collins *et al.*, 1951; Pound *et al.*, 2002) and genetic factors (Kumar *et al.*, 2015). It may also be attributed to variation in ambient temperature and relative humidity. Assummer 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 causes testicular degeneration,and hence affecting the volume of the semen (Andre *et al.*, 2017). Variation in semen production between years may also be due to changes in feed, climatic condition, management practices and techniques. Variations in semen productions with age has earlier been reported by various workers *viz*. Nugraha *et al.*, 2019; Putri *et al.*, 2019; Suyadi *et al.*, 2020. 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 to develop continuously up to 5 yr post puberty in Holstein (Murphy *et al.*, 2018). Also, scrotal circumference, scrotal shape and testicular size increase with age (Ahirwar *et al.*, 2018).

**Table 1**

**General Description Data for semen collection from bulls**

| Breed | Number of bulls | Number of ejaculates | No. of Collection days |
|---|---|---|---|
| Holstein | 13 | 3743 | 2256 |
| Sahiwal | 16 | 1470 | 963 |
| HF x Sahiwal | 4 | 1055 | 638 |

### Semen concentration

Semen concentration was estimated as 1173.73 ±93.94 million/ml. The mean semen concentration was lower in Sahiwal bull as compared to Holstein and crossbred bull. It was also observed that all non-genetic factors showed significant effect on sperm concentration ofpure breed (Table 2). Season of semen ejaculation had significant effect on semen concentration. During winter and autumn seasonshigher semen concentration was observed and the concentration was lowest during rainy season. Period of semen collection had significant effect

**Table 2. Least squares means with standard error of different semen characteristics in Holstein, Sahiwal and its crossbred bull**

| Effect | Volume | Concentration | TEV | PTM | Motility |
|---|---|---|---|---|---|
| μ ± S.E. | 4.93±0.04 (6217) | 1173.73±93.94 (6217) | 62.25±8.02 (6215) | 49.76±0.045091 | 67.62±0.135893 |
| **Season** | | | | | |
| Winter (Jan. - March) | 5.25±0.06[a] (1417) | 1265.80±14.38 (1417) | 72.81±1.20[a] (1417) | 49.91±0.09[a] (1156) | 68.28±0.31[a] (1349) |
| Summer (April - June) | 4.47±0.05[b] (1724) | 1133.90±13.33[b] (1724) | 53.14±1.11[b] (1722) | 49.75±0.08[a] (1418) | 66.58±0.28[b] (1649) |
| Rainy (July-September) | 4.70±0.05[b] (1971) | 1090.37±11.68[b] (1971) | 54.01±0.97[b] (1971) | 49.58±0.07[b] (1549) | 67.06±0.25[b] (1827) |
| Autumn (October-December) | 5.26±0.07[a] (1105) | 1204.86±16.05[c] (1105) | 69.03±1.34[a] (1105) | 49.78±0.10[ab] (968) | 68.54±0.34[a] (1067) |
| **Period** | | | | | |
| 2017 | 5.08±0.05[a] (1706) | 1272.48±15.23[a] (1706) | 70.71±1.27[a] (1706) | 50.06±0.09[a] (1500) | 68.39±0.32[a] (1654) |
| 2018 | 4.98±0.05[b] (1816) | 1221.20±12.69[b] (1816) | 65.86±1.06[b] (1814) | 49.91±0.08[ab] (1397) | 66.35±0.27[b] (1709) |
| 2019 | 4.63±0.05[c] (1802) | 1153.69±12.24[c] (1802) | 56.61±1.02[c] (1802) | 49.63±0.07[bd] (1508) | 68.52±0.26[a] (1683) |
| 2020 | 5.00±0.07[d] (893) | 1047.56±18.17[d] (893) | 55.82±1.52[c] (893) | 49.42±0.12[cd] (686) | 67.50±0.39[a] (846) |
| **Breed** | | | | | |
| Holestein | 5.24±0.04[a] (3707) | 1056.99±9.10[a] (3707) | 60.54±0.76 (3705) | 49.76±0.06[a] (2952) | 66.92±0.19[a] (3491) |
| Sahiwal | 4.18±0.06[b] (1458) | 1365.02±15.33[b] (1458) | 63.39±1.28 (1458) | 49.95±0.09[b] (1316) | 69.65±0.33[b] (1400) |
| HF* Sahiwal | 5.36±0.07[a] (1052) | 1099.19±15.96[a] (1052) | 62.82±1.33 (1052) | 49.56±0.10[a] (823) | 66.28±0.34[a] (1001) |
| **Order of Ejaculate** | | | | | |
| First | 5.06±0.03[a] (3857) | 1242.123±8.99[a] (3857) | 67.60±0.75[a] (3857) | 49.62±0.05[a] (3095) | 66.88±0.19[a] (3676) |
| Second | 4.79±0.04[b] (2360) | 1105.33±11.08[b] (2360) | 56.90±0.92[b] (2358) | 49.89±0.07[b] (1996) | 68.35±0.24[b] (2216) |
| **Age in Month** | | | | | |
| <48Months | 4.46±0.05[a] (2279) | 1194.88±12.60[a] (2279) | 58.07±1.05[a] (2278) | 49.89±0.08[a] (2019) | 68.83±0.27[a] (2182) |
| 48-72 months | 5.05±0.05[b] (2188) | 1126.03±11.67[b] (2188) | 61.20±0.97[a] (2188) | 49.92±0.07[a] (1739) | 67.72±0.25[b] (2056) |
| >72 months | 5.26±0.06[c] (1750) | 1200.29±13.64[a] (1750) | 67.48±1.14[b] (1749) | 49.45±0.09[b] (1333) | 66.30±0.29[c] (1654) |

on semen concentration and it showed decreasing trend over the period. Highest semen concentration (1365.02 ±15.33 million) was found in Sahiwal and lowest semen concentration (1056.99±9.10 ml) for HF. 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 Sahiwal. Season of semen collection had significant effect ($P<0.001$) on semen volume of Sahiwal and Holstein bulls. Winter and autumn season showed favorable season for higher semen production. Semen concentration varied from 1242.123±8.99 million per ml in first ejaculates to 1105.33±11.08 million per ml in second ejaculate. A significant effect of age of bull was observed on semen concentration and ejaculated semen concentration showed significantly decrease with the increasing age. Similar to our finding winter was considered as favorable season for sperm cell concentration in Karan Fries bull (Bhakat *et al.*, 2015). 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). A significant effect of age of bull was observed on semen concentration and ejaculated semen concentration showed significant decrease with the increasing age (>72 months). Low semen concentration associated with young Holstein Friesian bulls compared with older bulls corroborates with the findings of Murphy *et al.* (2018).

**Semen motility**

Semen motility was analyzed to assess the effect of season, period, age of bull and order of ejaculates and the overall least square means of semen motility have been shown in Table 2. Semen motility was estimated as 67.62±0.13. Semen motility was estimated as 66.92±0.19 for Holstein, 69.65±0.33 for Sahiwal and 66.28± 0.34 for HF X Sahiwal. 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 (68.28±0.31) was found in autumn. Period of semen collection showed erratic trend over the period. Highest motility (68.39± 0.32) was found in the year of 2017 and lowest semen motility was observed as (66.35±0.27) in the year 2018. Highest semen motility was observed in second ejaculations during semen collection and it was estimated as 68.35 ±0.24. 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 (68.83 ±0.27) below 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. 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. The significant difference in motility between years may be due to changes in management practices and techniques.

**Post Thaw Motility (PTM)**

Estimates of semen post thaw motility ranged from 49.56 in Sahiwal bull to 49.95 percent in crossbred bull 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 significant effect on sperm post thaw motility. The present study revealed the effect of season on PTM and the ejaculate collected during 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 (69.65±0.33) was found in Sahiwal and lowest (49.56±0.10) for Holstein crossbred. Post thaw motility showed very low variability for ejaculate orders and varied from 49.62±0.05 incase of first order ejaculate to 49.89±0.07 incase of second order ejaculate. A highly significant effect of season has been reported earlier (Gopinathan *et al*., 2021) while Rai and Dorji (2021) reported a non-significant effect. The effect of season on PTM could be attributed to change in melatonin and testosterone secretion. Season influences the hormonal secretion either through photoperiod and or through temperature humidity index during rainy season in male animals (Ponraj *et al.*, 2022).

## CONCLUSION

The present study revealed that non-genetic factors under subtropical condition have a significant source of variation for semen characteristics of exotic, crossbred and indigenous genotype. Therefore, effects of genotype and environmental factors need to be accounted for quality semen production in a semen production center. Indigenous genotype produces better quality semen as compared to exotic and crossbred bull under subtropical condition. Winter and autumn seasons are favorable season for semen characteristics when compared to summer and rainy season. Higher age of bull revealed good for all semen parameters except the semen motility.

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