Indian J Dairy Sci 76(6): 578-582
https://doi.org/10.33785/IJDS.2023.v76i06.009

RESEARCH ARTICLE

Effect of non-genetic factors on semen characteristics of Murrah bulls under tropical condition

Sunil Kumar Meena[1](✉), Prakash Chand Sharma[1], Samita Saini[1], Priyanka Swami[2,] Nirmal Kumar Jeph[3], Navav Singh[4] and Mahendra Kumar Meena[5]

Received: 18 October 2022 / Accepted: 10 April 2023 / Published online: 23 December 2023

Abstract: The objective of this study was to assess the effect of non-genetic factors on semen characteristics of 22479 ejaculates from 139 Murrah bulls during the period of 2017-2020 analyzed under tropical conditions, maintained and recorded at Frozen Semen Bank (FSB), Bassi, Rajasthan Cooperative Dairy Federation, Jaipur, Rajasthan, India as well as in Information Network for Animal Productivity & Health (INAPH) application maintained at National Dairy Development Board (NDDB). Results were recorded as 2.94±0.03 mL for volume, 1234.72±13.51 million/mL for sperm concentration and 68.44±0.25 percentage for motility. Statistical analysis presented a significant difference for all semen parameters with studied non-genetic factors except semen collector for motility traits. Also, seasonal dynamics presented winter as the most suitable season for semen collection under tropical conditions. Season of ejaculates was observed as most influencing factors for all semen characteristics in Murrah bulls. Age of bull showed significantly increased values for all semen traits with the increasing age of bull except motility traits. Data analysis in this regard may be utilized to enhance the fertility rate by increasing the semen quality.

Keywords: Age of Bull, Murrah Buffalo, Semen characteristics

Introduction

Ruminant production systems are very much dependent on the environment than non-ruminant system in tropical region (Bertoni et al. 2021). Buffalo showed adaptability to wider range of climate and excellent feed conversion efficiency making them ideal livestock for tropical countries. Additionally, nutritional make up and therapeutic components of buffalo milk may also serve in enhancing the nutritional security hence, the demand for buffalo milk-based products is increasing in developed and developing countries. Establishment of commercial dairy farms with high productive buffaloes has created a higher demand of quality frozen semen. Semen characteristics are important factors which determine fertility among buffalo population in tropical countries. The quantity and quality characteristics of semen straws produced per ejaculate depend on the volume of the ejaculate, the concentration of spermatozoa in the ejaculate, and the percentage of spermatozoa that are motile. Also, these ejaculate characteristics are known to be affected by environment and genetics (Biniova, 2017). In dairy animals, male fertility has received much less attention compared to female fertility (Butler et al. 2019). Hence, evaluation of semen in context of season, period and age of bull from well managed and genetically superior bulls is importantfor better conception and faster genetic progress in buffalo population. In the present study, effect of non-genetic factors on semen characteristics was assessed to improve the quality of frozen semen of Murrah in semi-arid region.

Materials and methods

A total of 22479 ejaculates related to 139bulls of Murrah buffalo maintained at Frozen Semen Bank (FSB), Bassi, Jaipur, Rajasthan, India were collected over a period of 4 years (2017-2020). The FSB is located in the hot semi-arid region (Koopen climate classification BSh) of India with dry and subtropical in characteristics with long, extremely hot summers and short, mild to warm winters. Bulls used in AI programme satisfy quality norms, semen was collected and processed in accordance with the standard protocols. Bulls were prepared for collection by giving two/three false mounts followed by restraint with the gap of half an hour to one hour depending on the bull. After examination of sperm concentration and initial motility, semen samples were primarily diluted with dilutor maintained at 34°C. Sperm concentration was checked preferably by a digital photometer with auto dilutor. To get a desired 20 million sperm per doses, initial semen volume was extended considering the sperm concentration per mL for estimation of total extended volume (TEV) and sperm concentration per dose (SCPD). After freezing, the semen straws were stored in a liquid nitrogen

[1] Department of Animal Genetics and Breeding, CVAS, Navania, Udaipur
[2]Department of Animal Genetics and Breeding, CVAS, Navania, Udaipur
[3]Department of Veterinary Medicine, PGIVER, Jaipur
[4]Department of Livestock Production Management, PGIVER, Jaipur
[5]Department of Veterinary Medicine, ACVM, Jaipur

Sunil Kumar Meena (✉)
Department of Animal Genetics and Breeding, CVAS, Navania, Udaipur, India
Email: drsunilkumarmeena@gmail.com

container. Post-thaw motility of semen was examined at 24 hours (after freezing). Preferably, the person involved in evaluation of freshly collected ejaculates did not check the post thaw motility. 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 were analyzed at Department of Animal Genetics and Breeding, PGIVER, Jaipur by using SPSS software. Parameters *viz*. ejaculation volume, total extended Volume (TEV), motility, sperm concentration and post thaw motility (PTM) were analyzed. Effect of non-genetic factors *viz*. season, period, age of bull, collectors, number of ejaculations for various genotypes were analyzed in the present study. Statistical analysis was carried out using least squares and maximum likelihood analysis method for non-orthogonal data as described by Harvey (Harvey, 1987) using following model.

Yijkl = μ + Pi +Sj+ ck+ eijkl

Yijkl = Observation on the lth individual in ith season, jth period and kth age group

μ = Overall population mean

Pi = Effect of ith season of semen collection

Sj = Effect of jth period of semen collection

ck = Effect of kth age group during semen collection

eijkl = 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

$$(Xi \text{"} Xj) = \frac{\sqrt{2}}{Cii+Cjj+Cij} > \sigma_e Zpn_2$$

Where,

Xi and Xj 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,

Zpn2 is ranged value in Duncan's table (0.05) at n2 degrees of freedom,

P is number of means in the range chosen,

σeis standard deviation of error,

n2 is degree of freedom for error.

Results and discussion

The effects of non-genetic factors were analyzed for semen characteristics and results of ANOVA have been presented in Table 1.The overall least square means along with standard error of physical characteristics of Murrah buffalo semen have been shown in Table 2. The overall mean of semen characteristics were estimated as 2.94 ±0.03 mL for ejaculates volume, 68.44±0.25 percentage for initial motility, 1234.27±13.51million per mL for sperm concentration, 39.32±0.61mLfor TEV, 20.71±0.07 for SCPD and 49.71 percentage for PTM.

Table 1 ANOVA mean sum of square for factors affecting semen of Murrah bulls

Source of variation	df	Volume (mL)	Concentration (Million/ml)	Motility (%)	TEV (mL)	SCPD (Million/ml)	PTM (%)
Season of collection	3	251.78***	15728299.15***	8661.98***	84299.68***	180.51***	47.61***
Period of collection	3	404.22***	47195249.64***	2054.16***	161886.28***	173.32***	163.95***
Collection by individual	5	44.07***	2442015.58***	308.83	12564.57***	18.27*	30.73***
Order of ejaculation	2	97.79***	21150340.72***	1885.80***	58205.93***	58.32***	70.85***
Age in Months		53.48*** (3)	24762107.38** (3)	3737.35** (3)	30252.71*** (3)	35.45*** (3)	23.69* (3)
Error		1.76 (22462)	275468.47 (22462)	88.43 (21495)	571.52 (22459)	6.97 (19345)	8.53 (19273)
R^2		0.064	0.062	0.025	0.090	0.009	0.007

*p>0.05; ** p >0.01;*** p>0.001

Table 2 Season, period, collection by individual, order of ejaculation and age of breeding bull, wise Least squares means and standard errors (Mean ±SE) of Semen of Murrah bulls

Effect	Volume (mL)	Concentration (Million/ml)	Motility (%)	TEV (mL)	SCPD (Million/ml)	PTM (%)
Overall	2.94±0.03 (22479)	1234.72±13.51 (22479)	68.44±0.25 (21511)	39.52±0.61 (22476)	20.71±0.07 (19362)	49.71±0.08 (19290) (19290)
Season						
Winter (Jan. - March)	3.03±0.03^{a} (5258)	1313.57±14.84^{a} (5258)	68.35±0.27^{a} (5069)	43.18±0.67^{a} (5259)	20.96±0.08^{a}(4589)	49.62±0.08^{a} (4569)
Summer (April-June)	2.71±0.03^{b} (6180)	1185.97±14.82^{b} (6180)	66.68±0.27^{b} (5800)	35.11±0.67 ^{b}(6177)	20.72±0.08^{b} (4993)	49.62±0.09^{a} (4993)
Rainy(July-September)	2.79±0.03^{c} (7194)	1215.33±14.37^{c} (7194)	69.28±0.26 ^{c}(6879)	37.16±0.65 ^{c}(7193)	20.49±0.07 ^{c}(6256)	49.76±0.08^{ab} (2954)
Autumn (Oct-Dec)	3.22±0.04^{d} (3847)	1223.99±15.90^{c} (3847)	69.47±0.29^{c} (3764)	42.65±0.72^{a} (3847)	20.69±0.08 ^{b}(3524)	49.85±0.09^{b} (3500)
Period						
2017	3.20±0.04^{d} (2838)	1450.27±16.48^{a} (2838)	68.21±0.30 ^{ac}(2771)	50.21±0.75^{a} (2836)	20.71±0.08^{a} (2574)	49.74±0.09^{a} (2574)
2018	2.58±0.03^{b} (7880)	1206.31±14.31^{b} (7880)	67.81±0.26^{a} (7315)	34.01±0.65 ^{b}(7889)	20.84±0.07^{a} (6589)	49.81±0.08^{a} (6568)
2019	2.75±0.03^{c} (6935)	1148.38±15.05^{c} (6935)	69.25±0. 27^{b}(6713)	34.15±0.68 ^{c}(6935)	20.46±0.08 ^{b}(6152)	49.89±0.09^{a} (6146)
2020	3.21±0.04^{a} (4826)	1133.91±16.35^{c} (4826)	68.50±0.30 ^{c}(4713)	39.73±0.74 ^{c}(4826)	20.85±0.08^{a} (4047)	49.41±0.09^{b} (4002)
Collection by Individual						
I	2.770±0.03^{a} (6500)	1210.69±14.18^{ac} (6500)	68.26±0.26 (6216)	36.54±0.64^{a} (6500)	20.82±0.07^{a} (5479)	49.53±0.08^{a} (5433)
II	3.1±0.07^{b} (334)	1332.67±31.20^{b}(3 34)	69.19±0.57 (325)	44.90±1.42 ^{b}(333)	20.58±0.16^{a} (303)	49.85±0.18^{a} (299)
III	3.05±0.04^{b} (1935)	1219.29±16.84^{ab} (1935)	67.81±0.31 (1875)	40.32±0.76 ^{c}(1936)	20.69±0.09^{a} (1671)	49.59±0.10^{a} (1666)
IV	3.00±0.04^{b} (1319)	1265.33±18.92^{b} (1319)	68.34±0.34 (1283)	41.33±0.86 ^{dc}(1319)	20.78±0.10^{a} (1176)	49.81±0.11^{b} (1175)
V	2.92±0.04^{b} (2222)	1170.68±16.64^{c}(2 222)	68.89±0.30 (2114)	37.33±0.75^{a} (2222)	20.61±0.08^{a} (1954)	49.81±0.09^{b} (1954)
VI	2.77±0.03^{a} (10169)	1209.63±13.37^{a}(1 0169)	68.16±0.25 (9699)	36.73±0.61^{a} (10166)	20.81±0.07^{b} (8779)	49.69±0.08^{a} (8763)
Ejaculation order						
1	3.07±0.01^{a} (13471)	1322.14±7.35^{a} (13471)	67.65±0.13^{a}(1307)	42.91±0.33^{a} (13471)	20.79±0.03^{ab} (11601)	49.64±0.04^{a} (11556)
2	2.88±0.02^{b} (8802)	1236.92±7.95 ^{b} (8802)	68.49±0.14 ^{b}(8297)	38.25±0.36 ^{b}(8800)	20.62±0.04 ^{b}(7583)	49.82±0.04^{b} (7559)
3	2.85±0.09^{b} (206)	1145.09±36.91^{c}(2 06)	69.18±0.69 ^{b}(188)	37.42±1.68 ^{b}(205)	20.73±0.20 ^{b}(178)	49.69±0.22^{b} (175)
Age of bull at ejaculation						
<42 Months	2.82±0.04^{a} (5621)	1119.68±15.70^{a} (5621)	69.41±0.29^{a} (5225)	35.46±0.71^{a} (5620)	20.63±0.08 ^{ac}(4820)	49.76±0.09^{ab} (4814)
43-78 months	2.89±0.03^{ab} (10298)	1243.68±14.02^{b}(1 0298)	69.03±0.26^{a} (9974)	39.19±0.64 ^{b}(10297)	20.81±0.07^{bc} (9010)	49.79±0.08^{b} (8962)
79-114 months	2.92±0.04^{b} (3435)	1324.39±15.91^{c}(3 435)	68.37±0.29 ^{b}(3298)	41.50±0.72 ^{c}(3434)	20.62±0.08 ^{c}(2964)	49.70±0.09^{a} (2954)
115 –and more	3.12±0.04^{d} (3125)	1251.11±16.34^{b}(3 125)	66.96±0.30 ^{c}(3015)	41.95±0.74 ^{c}(3125)	20.79±0.08 ^{c}(2568)	49.60±0.09^{a} (2560)

Season of ejaculation

Season of ejaculates was observed as most influencing factors for all semen characteristics in Murrah bulls. Least squares ANOVA showed significant effect of season of ejaculates on volume, motility, sperm concentration, TEV, SCPD and PTMin Murrah buffalo. Winter and autumn season were reported to be comparatively favorable season for all semen parameters in Murrah buffalo under tropical conditions. However, summer being the most unfavorable season for all semen parameters except PTM which may be attributed to the fact that post thaw motility is influenced by thawing rate instead of season of ejaculation. Due to standard practice of thawing and minimum variability for motility effect of season of ejaculation may diminish after thawing. The difference in semen characteristics between seasons might be attributed to variation in ambient temperature and relative humidity. In the present finding lower semen volume, motility and concentration of sperm cell of ejaculates during summer season may be due to climatically stressful environment. Being seasonal breeders, the maximum breeding activity occurs during the winter and autumn season. Similar to our findings, seasonal variation was observed and summer was considered as most unfavorable season for semen volume and sperm cell concentration in Karan Fries bull (Bhakat et al. 2015). Hot humid condition affects the body's normal physiological mechanism. Regulation of body temperature cannot cope with extreme environmental condition (Morrell2020). Summer stress under tropical environments 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 and other parameters. Thermal stresses causes testicular degeneration and hence lower the semen output (D'Andre et al. 2017).Similar to the present findings, summer season was unfavorable season and it might be due to seasonal alteration of fatty acid composition and cholesterol concentration (Orgal et al. 2012).

Period of ejaculation

Period of ejaculates had significant effect on semen parameters and it showed erratic trend over the period from 2017-19 and increased in the year of 2020. The significant difference in semen parameters between years may be observed due to changes in feed, climatic condition, management practices and techniques.

Age of bull at semen ejaculation

A significant effect of age of bull was observed on all semen parameters with an increasing trend for volume and TEV traits. Similar to other parameters of present findings, sperm concentration showed reducing trend over the age but reduction in sperm concentration was estimated in oldest age group (>115 Months). High ejaculated semen volume and concentration was observed in older age of Murrah buffalo. The total ejaculation volume was observed to be significantly enhanced with the increasing age in buffalo. Contrary to the volume, sperm concentration and TEV in reference to the age of bull, initial and post thaw motility was higher in younger bull as compared to old age of bull (<78 months of age).Murrah buffalo showed highest semen motility (69.41) in youngest age group bull (<42 months of age). These results are in agreement with those of most studies, which reported that an increase in semen production with age of bull (Boujenane and Boussaq 2013).The similar pattern was observed by Prastowo et al. (2014) in Bali cattle. It seems that ejaculate volume is increased with increasing testicular development since the size of testes increase for at least five years after puberty. In general, scrotal circumference, scrotal shape and testicular size increase with age (Ahirwar et al. 2018).Similar to this finding, low semen concentration associated with young bulls compared with older bulls corroborates with the findings of Murphy et al. (2018). Sonar et al. (2016) also reported the variation in semen motility due to age in Gir bull.

Semen collection by individual

A highly significant (P<0.001) variability in semen characteristics was observed for the factor of semen collectors except semen motility may be hypothesized due to the systematic training of the individual engaged in semen collection, and may be considered as an important factor in Murrah buffalo for higher quality semen parameters. Collector expertise along with the ability to judge the sexual behavior may enhance the semen quality and needs further exploration.

Order of ejaculates

Order of ejaculates had significant effect on all semen traits with highest semen parameters were observed in first ejaculation. A significant reduction in the semen quality over the orders of ejaculates and it may be a useful strategy for grading the quality of Frozen Semen Doses FSDs on the basis of ejaculates order. Similar to this finding, significant variation in sperm cell was also reported by Bhave et al. (2020) in buffalo. This may be primarily due to lower semen production with the collection of multiple ejaculates on the same day.

Conclusion

Non genetic factors need attention for quality semen production especially in buffalo bulls of developing tropical countries like India. Seasonal dynamics presented winter and autumn as comparatively better to summer season in tropical environment. A significant effect of age of the bull son better semen quality parameters as well as for initial and post thaw motility traits was observed. However, semen traits for volume, concentration, TEV and SCPD was better in old age bulls than young bulls. Individual semen collector effect showed highly significant effect on semen quality and needs proper attention.

Acknowledgement

The authors thank the General Manager, RCDF and team of FSB Bassi for permission to utilize the data and constant support during the study. The authors acknowledge the department of Animal Genetics and Breeding, PGIVER(RAJUVAS) for facilities provided.

Reference

Ahirwar MK, Kataktalware MA, Prasad K, Pal RP, Barman D, Thul M, Rawat N (2018) Effect of non-genetic factors on semen quality in bulls: A review. J Entomol Zool Stud 6(4): 38-45

Bertoni A, Álvarez-Macías A, Mota-Rojas D, Dávalos JL, Minervino AHH (2021) Dual-Purpose Water Buffalo Production Systems in Tropical Latin America: Bases for a Sustainable Model. Animals (Basel) 11(10):2910

Bhakat M, Mohanty, TK, Singh S, Gupta A K, Chakravarty AK,Singh P (2015) Influence of semen collector on semen characteristics of Murrah buffalo and Crossbred bulls. AdvAnim Vet Sci 3(4): 253-258

Bhave K, Koilpillai TPJ, Ragothaman V, Sontakke S, Joshi G, Ducrocq V (2020) Semen production and semen quality of indigenous buffalo breeds under hot semiarid climatic conditions in India. Trop Anim Health Prod 52:2529-2539

Biniova Z, Duchacek J, Stadnik L, Dolezalova M, Hajek R (2017) Effects of Climatic Conditions on Bovine Semen Characteristics. *Acta Univ Agric Silvic Mendel* 65(1): 17–23

Boujenane I, Boussaq K (2013) Environmental effects and repeatability estimates for sperm production and semen quality of Holstein bulls. Arch Tierz 56: 971-979

Butler ML, Bormann JM, Weaber RL, Grieger DM,Rolf MM (2019) Selection for bull fertility: a review. Transl Anim Sci 4(1): 423-441

D'Andre HC, Rugira KD, Elyse A, Claire I, Vincent N, Celestin M,Christine K (2017) Influence of breed, season and age on quality bovine semen used for artificial insemination. Int J Livest Prod 8(6): 72-78

Harvey WR. 1987 Least squares analysis of data with unequal sub-class numbers. Washington, DC: ARS H-4, USDA

Kramer CY (1957) Extension of Multiple Range Tests to Group Correlated Adjusted Means. Int Bio Soci 13(1): 13-18

Morrell JM (2020) Heat stress and bull fertility. Theriogenology153: 62-67

Murphy EM, Kelly AK, O'Meara C, Eivers B, Lonergan P, Fair S (2018) Influence of bull age, ejaculate number, and season of collection on semen production and sperm motility parameters in Holstein Friesian bulls in a commercial artificial insemination centre. J Anim Sci 96(6): 2408-2418

Orgal, S, Zeron Y, Elior N, Biran D, Friedman E, Druker S, Roth Z (2012) Season-induced changes in bovine sperm motility following a freeze-thaw procedure. J Reprod Develop 58(2): 212-218

Prastowo S, Kusuma M S T, Widyas N, Ratriyanto A, Pramono A, Setyawan A (2018) Effect of season on semen production and quality parameter in Indonesian Bali Cattle (*Bosjavanicus*) International Conf. Sci. Appl. Sci. (ICSAS) AIP Conf. Proc. 2014 020005-1–020005-3

Sonar BP, Tiwari RP, Poyam MR, Mishra GK, Pandey AK, Nair A K, Sahasrabudhe SA (2016) Characteristics and freezability of Gir bull semen. Indian J Anim Sci 86 (3): 264-272