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Effects of Various Non-genetic Factors on Fertility Traits in Murrah Bulls in an Organized Herd

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ABSTRACT

Background: In a herd going through improvement by selective breeding, bulls contribute more than half of the genetic gain due to higher selection intensity and wider coverage through AI. On the other side, meritorious sires might have a lower fertility due to various reasons which would render them less fit in their contribution to genetic improvement. Thus dairy buffalo breeders must find a delicate balance between production and reproduction superiority for selection of sires, for which an accurate assessment of genetic and non-genetic component of conception is essential.

Methods: Present study was conducted on thirty five years data comprising of 5349 AI records on 1302 Murrah buffaloes using 86 bulls maintained and semen analysis of cryopreserved semen samples of 130 Murrah maintained at ICAR-National Dairy Research Institute Karnal were analyzed to study bull conception rate and sperm quality traits. Data were classified into four periods; four seasons of AI *viz*., Summer (April-June), rainy (July-September), autumn (October-November), winter (December-March); five parity (first, second, third, fourth, fifth and above) and five female AI number (1,2,3,4 and ≥ 5 AI) to study the conception rate. Age at freezing (Years), period of freezing and season of freezing were analysed to observe their effects on semen quality traits. Mixed model analysis (SAS 9.3) and the analysis of variance were used to see the effects of various factors on conception rate as well as on semen quality parameters.

Result: The analysis of variance revealed that period of AI and season of AI had significant (P<0.05) effect on conception rate while parity and female AI number were not found to affect the conception rate of bulls. For seminal parameters, acrosome integrity and HOST were significantly affected by age at freezing. Season affected all the semen quality traits while the effect of period of freezing was found to be non-significant for all the traits. Over all autumn season was found to be most suitable with respect to better seminal parameters. Considering season of AI, rainy season (July-September) had the lowest conception rate. The overall conception rate (%) in the study was 38.43±0.99; where Sires showed a wide variation for conception rate ranging from 20.00% to 60.46%. Concluding with the fact that there exists variation among bull fertility that can be utilized for not only herd conception rates but also be used as a criteria for bull selection and evaluation.

Key words: Age, Bull conception rate, Murrah bulls, Period, Season, Semen quality traits.

INTRODUCTION

The Indian livestock biodiversity is endowed with the finest buffalo breeds and houses the largest buffalo population in the world. Despite nearly half of the cattle population, buffaloes contribute more than cows to the country's milk (Anonymous, 2019) which reflects the gravity of buffalo in the Indian dairy industry. Indian breeds of buffaloes have the remarkable genetic potential for production and adaptability to cater for the huge demand for milk in our country. Sire and dam, contribute equally to the inheritance of milk production; but sires contribute more genetic gain than a dam; since the higher intensity of selection can be realized for bulls, owing to frozen semen technique. Hence a faster genetic improvement in herd performance can be achieved through sire selection (Norman *et al*., 2003).

Keeping a record of service perconception, conception rate, non-return rate and calving, is necessary in order to properly evaluate the reproductive efficiency. Fertility of females, records of semen parameters as well as bull wise conception rates are also required to make a reproductive management decision. Ideally, with an 80% pregnancy rate on the first insemination, a maximum of 1.3 services per conception and an average service period of 85 days, optimum economic fertility could be achieved (Morrow, 1980). Conception is the first prerequisite of a buffalo entering into a productive life and greatly influences the lifetime productivity of the individual buffaloes. The

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conception rate directly determines the total profitability of a dairy farm enterprise. Specifically, among buffaloes, a large number of females remain barren or unproductive around the year and become a burden for the farmers (Cicek *et al*., 2017). On the other hand, many factors, both genetic and non-genetic, *viz*. genotypes, age, parity and reproductive health of females; genotype, libido and semen quality of bulls; heat detection, season and time of AI *etc*., have a direct influence onincreasing conception rate.

Meager buffalo population is contributing much in milk production as compare to cattle demands adequate supply of excellent germplasm or frozen semen doses for their further genetic improvement. Selection of bulls for semen production considering the various factors (Age of bull, season and period at collection/ preservation) affecting post-thaw semen quality traits plays a great role as frozen semen samples have been used widely in the field resulting good conception rate (Nordin *et al*., 1990; Parmar *et al*., 2021). The information regarding the trends of these influences semen attibutes would greatly help to know the requirement of bulls to meet the demand of frozen semen and to provide any suitable additional managerial requirements time to time. Considering these, the present study was planned with the aim to evaluate the effects of concomitantfactors affecting conception rate and to recommend an optimum approach for achieving maximum conception rate among buffaloes.

MATERIALS AND METHODS

The present investigation was conducted at Artificial Breeding Research Centre (ABRC) and Livestock Research Centre of ICAR-National Dairy Research Institute, Karnal; which is situated at an altitude of 250 meters above the mean sea level in the Indo-Gangetic alluvial plains on 29° 42′ N latitude and 72°02′ E longitude. The farm's climate is subtropical and the temperature shows broad variation froma near freezing point in winter months to a maximum around 46°C in summer. The average relative humidity ranges from 41% to 85%. The locality of the farmgets an annual rainfall of about 760 to 960 mm, mostly during the monsoon (July to August). Thus it is obvious that the Murrah herd at the institute has been exposed to a wide range of meteorological factors.

Records on 5349 AI of Murrah bulls and their mates during the period 1980 to 2014 for estimation of conception rate were collected from Dairy Cattle Breeding Division, Livestock Research Centre and Artificial Breeding Research Centre at National Dairy Research Institute, Karnal, Haryana (India). Semen analysis was done by collecting frozen semen samples of 130 Murrah bulls. The data were analyzed to study the effects of various non-genetic factors on conception rate as well as sperm quality traits in Murrah bulls, classifying the data into four periods of AI/ semen freezing *viz*., Period-1 (1980-1996), Period-2 (1997-2002), Period-3 (2003-2008), Period-4 (2009-2014); four seasonsof AI and collection/freezing of semen *viz*. Summer (April-June), Rainy (July-September), Autumn (October-November) and Winter (December-March); five parity groups (Heifer, first, second, third and fourth and more parity) and five female AI number groups (first, second, third, fourth and five or more AI after calving), respectively. The age of the bull was divided into four age groups *i.e*. the first group with less than three years, next as 3-4 years, 4-5 years and last group as 5 years or more at the time of freezing of semen has been considered to see the effect on sperm quality parameters and shown significant effect on post-thaw semen parameters. A minimum of 3 semen straws from each bull per available season (total 3 replication) were used for estimation of seminal parameters and the average of three replications of seminal parameters was used for further analysis. Sperm quality traits like Post-thaw motility, sperm abnormality, sperm viability, acrosomal integrity and hypo osmotic swelling test (HOST) test were performed to assess the quality parameters in Murrah bulls semen using standard protocol performed in most of the semen processing laboratory. Further, the estimation of conception rate was done using the following formula:

Conception rate of i^{th} bull (%) =

$$\frac{\text{No of pregnancies confirmed from } i^{th} \text{ bull}}{\text{Total number of inseminations from } i^{th} \text{ bull}} \times 100\%$$

Least-squares analysis was performedin SAS version 9.3 (SAS Institute, Cary, NC, USA) and the analysis of variance for the season of AI, period of AI, parity and female AI number affecting bull's conception rate under model was computed. The difference of means between subclasses was tested for significance using Duncan's multiple range test (DMRT) as modified by Kramer (1957). The following model was used for the present investigation;

$$Y_{ijkn} = \mu + S_i + P_j + Pa_k + FAI_l + e_{ijkln}$$

Where,

Y_{ijkn}= Observation of n^{th} bull in l^{th} female AI no.
k^{th}= Parity of cow.
j^{th}= Period of AI and i^{th} season of AI.
μ= Overall mean.
S_i= Fixed effect of i^{th} season of AI (1-4).
P_j= Fixed effect of j^{th} period of AI (1-4).
Pa_k= Fixed effect of k^{th} parity of cow (1-5).
FAI_l= l^{th} female AI number and.
e_{ijkn}= Random error ~ NID (0, σ^2_e).

Similarly, Least-squares analysis was performed to see the effects of various non-genetic factors on semen quality traits of Murrah bulls using the following model:

$$Y_{ijkn} = \mu + AG_i + S_j + P_k + e_{ijkl}$$

Where,

Y_{ijkn}= Observation of n^{th} bull in k^{th} period of freezing.
j^{th}= Season of freezing.
i^{th}= Bulls age group.
μ= Overall mean.
AG_i= Fixed effect of i^{th} age of bulls at freezing (1-4),
S_j= Fixed effect of j^{th} season of freezing (1-4).

P_k= Fixed effect of k^{th} period of freezing (1-4).
e_{ijkl}= Random error ~ NID (0, σ^2_e).

RESULTS AND DISCUSSION

Factors affecting conception rate

Overall conception rate was estimated by the total number of pregnancies in each female out of total numbers of AI and it was expressed in percentage. The overall conception rate (%) in the present study was 38.43±0.99% with individual bull's conception rate ranging from 20.00% to 60.46%. The range of conception rate for Murrah bulls was reported as 20.51 to 52.77% by Jadhav (1998) and 22.66 and 54.54% by Taraphdar (1999), which is very close to the present observations. Taraphdar (1999) reported the overall CR as 40.75% and Mir *et al*. (2015) estimated the average overall conception rate (%) as 39.19±1.55 for Murrah bulls. Segura-Correa *et al*. (2017) concluded the overall conception rate to 44.6% at 120 days postpartum. It is noteworthy that, overall conception rate (OCR) varies depending on not only genetic factors but predominantly on the environmental and managemental factors. The effect of various factors on the conception rate of bulls were studied by taking the period of AI, the season of AI, parity of female and female AI number with a remark that all the animals belonged to the same breed and herd and thus were provided similar feeding and housing management. The analysis of variance revealed that the period of AI and season of AI had a significant (*P*<0.05) effect on conception rate while parity and female AI number was not found to affect the conception rate of bulls (Table 1). Segura-Correa *et al*. (2017) found breed, parity number, season and year of calving to have significant effects on conception rate. In the study of Mir *et al*. (2015), parity and stage of lactation were found to be non-significant while the same for period and season was significant.The conception rate was found to be maximum *i.e*. 39.63±0.22 percentage in the period 1997 to 2002 and the lowest CR *i.e*. 36.37±0.23 percentage was found in the period between 2003 and 2008. Mir *et al*. (2015) found a significant effect of the period on conception rate (higher 47.88±1.13 in July 2011-December 2012 and lower 27.13±0.96 in the January 2010- June 2011). Considering the season of AI, the rainy season (July-September) had the lowest conception rate *i.e*. 37.12±0.23%, while autumn, summer and winter had a conception rate between 38.25±0.23% and 38.75±0.20% (Table 2). Segura-Correa *et al*. (2017) similarly found that the least conception rates in cattle belong to the rainy season. Bhave *et al*. (2016) found the different distribution of inseminations in lean (Jan- June) and favourable (July-December); however, at the same time, they could not find a significant effect of season of insemination on conception rate. Ron and Bar-Anan (1984) suggested that insemination month accounted for most variation of conception rate of cows; with the lowest conception rate of heifers during summer. Liu *et al*. (2018) reported that to achieve a high CR, lactating cows should be bred in winter and spring (from

Table 1: Least square means±S.E. of factors affecting conception rate in Murrah bulls.

SN and classes	Factors	No of AI	Means ± S.E.
	Period of AI*		
P1	1980-1996	1349	37.72^{b}±0.23
P2	1997-2002	1533	39.63^{d}±0.22
P3	2003-2008	1291	36.37^{a}±0.23
P4	2009-2014	1266	38.73^{c}±0.24
	Season of AI*		
S1	Summer (Apr-June)	1380	38.25^{b}±0.23
S2	Rainy (July-Sep)	1285	37.12^{a}±0.23
S3	Autumn(Oct-Nov)	940	38.33^{b}±0.27
S4	Winter (Dec-Mar)	1834	38.75^{b}±0.20
	Parity of female		
Pa1	Heifer	1226	38.44±0.24
Pa2	First	1241	38.12±0.24
Pa3	Second	930	37.83±0.27
Pa4	Third	748	37.79±0.30
Pa5	≥ Fourth	1294	38.38±0.24
	Female AI No		
FAI1	First	2305	38.28±0.17
FAI2	Second	1349	38.52±0.22
FAI3	Third	797	37.63±0.28
FAI4	Fourth	447	38.11±0.38
FAI5	^{3} Fifth	541	38.03±0.34

Dissimilar superscript indicates significant (*P*<0.05) difference of means.

Table 2: Least squares means of post-thaw semen attributes in Murrah bulls.

Effects	Post-thaw motility (%)	Viability (%)	Abnormality (%)	Acrosome integrity (%)	HOST (%)
Overall (μ)	55.83±0.53	65.87±0.63	8.07±0.18	73.20±0.50	52.40±0.36
Age at freezing					
Significance				*	*
<3 year	53.89±1.31	65.39±1.55	8.69±0.45	70.39^{b}±1.27	50.23^{b}±0.88
3-4 year	55.34±0.92	66.44±1.09	8.27±0.32	72.75^{ab}±0.9	52.95^{a}±0.62
4-5 year	57.38±0.95	66.37±1.13	7.77±0.33	74.67^{a}±0.93	53.38^{a}±0.64
>5 year	55.99±1.08	64.82±1.28	7.7±0.37	73.75^{ab}±1.05	51.58^{ab}±0.72
Period of freezing					
1980-1996	55.05±1.24	64.99±1.47	8.09±0.43	72.89±1.21	50.72±0.83
1997-2002	55.63±1.06	67.2±1.26	8.12±0.37	71.48±1.03	53.1±0.71
2002-2008	56.59±1.11	65.68±1.32	7.98±0.38	73.75±1.08	52.21±0.75
2009-2014	55.34±0.84	65.15±0.99	8.23±0.29	73.45±0.82	52.12±0.56
Season of freezing					
Significance	*	*	*	*	*
Summer (Apr-Jun)	54.66^{bc}±1.06	64.63^{bc}±1.26	7.96^{b}±0.37	72.20^{ab}±1.04	51.70^{bc}±0.71
Rainy (Jul-Aug)	50.88^{c}±1.02	60.40^{c}±1.21	7.53^{b}±0.35	70.32^{a}±1.00	49.30^{c}±0.69
Autumn (Sept-Nov)	59.74^{a}±1.05	70.66^{a}±1.25	9.30^{a}±0.36	74.67^{b}±1.03	54.88^{a}±0.70
Winter (Dec-Mar)	57.32^{ab}±1.00	67.33^{ab}±1.18	7.63^{b}±0.34	74.37^{b}±0.97	52.26^{b}±0.67

Dissimilar superscript indicates significant (P<0.05) difference of means; LSM: Least square means.

December to May) from the start of the seasonal breeding program, whereas the heifer should be allowed to breed in summer under subtropical climatic conditions. The present study and Mir *et al*. (2015) found non-significant effects of parity, contradicting the study of Bhave *et al*. (2016) which revealed the significant effects of lactation order on conception rate in buffaloes reared under field conditions. Similarly, Correa *et al*. (2017) found to have a significantly lower conception in the first parity of the cows.

Factors affecting semen quality parameters

In the present study, the age of bull at freezing had a significant effect on acrosome integrity whereas seasons of freezing were found to significantly affect almost all the post-thaw seminal parameters. No significant changes over the period of semen collection/freezing on semen attributes were found; which is expected since no genetic selection for seminal parameters were performed. The overall least squares mean for different semen quality parameters *viz*. Post-thaw motility, sperm abnormality, sperm viability, acrosome integrity and HOST were found as 55.83±0.53%, 65.87±0.63%, 8.07±0.18%, 73.20±0.50% and 52.40±0.36%, respectively. Bulls with age at freezing between 4-5 years showed higher acrosome integrity whereas less than 3 years of age had a lower percentage of acrosome integrity. The average HOST percentage of frozen semen was found significantly higher *i.e*. 53.38±0.64 and 52.95±0.62 for the age of bulls at freezing 4-5 years and 3-4 years, respectively, while less than 3 years of age of bulls at freezing had the lowest percentage of HOST *i.e*. 50.23±0.88. More or less similar, slightly higher or lower estimates of average post-thaw motility (%) were reported between 55.0 and 76.3% by Shivahre (2013), as 64.14% by Bhosrekar *et al*. (1994), from 60.8±1.5 to 69±4.5% by Kumar *et al*. (1993) and as 46.44±1.74 by Kumar *et al*. (2014). Acrosome integrity and HOST were significantly affected by age of bull at freezing (P<0.05). Ramajayan *et al*. (2021) observed higher estimates for acrosome integrity for bulls with an age group between 4-5 years while lower estimates were found for less than 3 years of age. Bulls aged from 3.5 years to ≥11 years showed higher acrosome integrity between 68.74 and 71.89% and a lower estimate was found in bulls with 18 to 42 months of age. However, no significant impact of age on HOST was observed. Similar findings have been reported by Bhosrekar *et al*. (1991), Chowdhury *et al*. (2017), Bhakat *et al*. (2015) as 92.00%, 73.74% and 70.10%, respectively in Murrah and Surti buffalo bulls. Season of the semen collection/freezing had a significant effect (P<0.05) on all the post-thaw motility parameters of Murrah bulls. The average PTM was found to be maximum *i.e*. 59.74±1.05 percentage for the semen frozen in the autumn, while frozen in the rainy season had the lowest PTM as 50.88±1.02 percentage. The average sperm abnormality was found to be maximum *i.e*. 9.3±0.36 per cent for the semen frozen in the autumn and rainy season had lower sperm abnormality *i.e*. 7.53±0.35 per cent. The average live spermatozoa percent in frozen semen was found maximum *i.e*. 70.66±1.25 percentage in the autumn season, while the rainy season had a lower percentage of live spermatozoa *i.e*. 60.40±1.21 percent. The average acrosome integrity percentage from frozen semen was found higher *i.e*. 74.67±1.03 and 74.37±0.97 percent in autumn season and winter, while lower *i.e*. 70.32±1.00 in the rainy season. Maximum (54.88±0.70) percent of HOST reacted spermatozoa were in the autumn season whereas minimum (49.30±0.69) in the rainy season.

Higher estimates of acrosome integrity had been reported by Bhosrekar *et al*. (1991) in the autumn season followed by Summer; whereas Bhakat *et al*. (2015) found a higher estimate in the Winter. Shahzad *et al*. (2021) reported the non-significant effect of two seasons *viz*. hot humid summer season (August-September) and the spring season (February-March) on semen kinematics including DNA integrity in Nili-Ravi buffalo bulls. Parmar *et al*. (2021) also found significant seasonal variations in extreme hot and cold weathers for the average percent of cryopreserved motility (58.85±0.56 and 56.35±0.43), sperm deformity (8.62±0.43 and 9.08±0.51), live and dead count (67.06±0.77 and 65.62±0.76), acrosomal integrity (75.22±0.27 and 74.29±0.22), HOST reactive sperm (58.52±0.67 and 55.43±0.60) and first AI conception rate (45.75±0.21 and 43.50±0.11) with better estimates for semen quality and first conception rate during cold as compare to hot season in buffalo bulls.

CONCLUSION

The study was carried out in Murrah bulls with the objectives to find out the effects of various factors on bulls' conception rate. The results of the study indicated that period and season were having a significant effect on conception rate whereas no effect of parity and female AI number on CR. The selection of bulls in a breeding program may improve male fertility that may further result in a higher success rate of Artificial Inseminations leading to better reproductive efficiency of the herd. This study highlights that the bulls of age group between 3 and 5 years have shown good semen quality and thus this age must be preferred for good quality semen cryopreservation. Semen collection and freezing in summer and rainy season had low to poor semen quality which indicates seasonality in the semen quality and warrants that the frequency of semen collection should be reduced during adverse seasons. Semen quality had no significant fluctuations over the period, possibly indicating that seminal traits did not face sufficient selection pressure. The information may be used as an aid for the selection of animals with high fertility.The genetic evaluation of bulls considering the conception rate along with productivity and correction of data for significantly affecting factors would be highly useful for the economic success of the dairy sector.

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