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REGULAR ARTICLES

Genetic analysis for semen quality traits in buffalo bulls

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Abstract

This study was attempted to estimate the genetic parameters of semen quality traits in buffalo bulls. The study data consisted of 10975 ejaculates from 45 Murrah buffalo bulls (aged 24–72 months) used for breeding program during year 2010 to 2020. Semen quality traits (ejaculate volume, concentration of sperm, mass activity, initial and post-thaw motility, number of sperms per ejaculate, motile sperm number and discard rates) were studied. It was observed that average ejaculate volume was 2.82 ± 1.45 mL with mean concentration of 1040.12 ± 523.26 million/mL. Higher heritability was observed for number of sperms per ejaculate, number of motile sperm and sperm concentration. Significant phenotypic correlation was obtained between volume and number of sperms per ejaculate as well as volume and number of motile sperms. Likewise, significant phenotypic correlation was evident between sperm concentration with sperm number per ejaculate. Highest phenotypic correlation was obtained between sperm count per ejaculate and motile sperm count. Estimated genetic trends showed significant change in volume and motile sperm number. In conclusion, this study ascertains that genetic parameters of semen traits can be considered during the selection of buffalo bulls in breeding program.

Keywords Buffalo · Bulls · Genetic correlation · Heritability · Repeatability · Semen

Introduction

In several countries, breeding programs have given more attention to female production traits (milk yield) or reproduction traits (first calving, service period and calving interval), with less focus on male fertility traits (Berry et al. 2011; Gebreyesus et al. 2021) including buffalo species (Shao et al. 2021). Male fertility includes behavior and libido of the bull, as well as semen quality and these traits can be measured directly from males which act as indicators of male fertility (Butler et al. 2021). Improvement of semen quality augments the success of artificial insemination and maximize the semen production process' efficiency as it affects the profitability of animal rearing system (Berry et al. 2011).

Male reproductive traits are moderately heritable, but variation exists in these parameters across different populations (Berry et al. 2019). The published heritability estimates of bull semen traits were low to medium (range: 0.02 to 0.31). Considerable efforts to quantify the genetic parameters of male reproduction of different bull breeds, show vast differences in their estimates (Corbet et al. 2012; Carvalho Filho et al. 2020). Moreover, semen traits are complex as their heritability estimates vary between and within traits due to several factors viz. sample size, population, breed, age and study methodology (Berry et al. 2019).

Though, genetic parameters regarding semen traits are reported in bulls (Olsen et al. 2020; Rostellato et al. 2021), but very sparse reports in buffalo species related to semen quality traits have been reported (Bhave et al. 2020). In this context, this study was carried out to estimate the genetic parameters for buffalo bull fertility traits as it shall aid in the identification of key male reproductive traits essential for planning buffalo breeding programme.

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Materials and methods

Location of study

The present study was carried out on the records of Murrah buffalo bulls present at ICAR-Central Institute for Research on Buffaloes, Hisar, Haryana. ICAR-Central Institute for Research on Buffaloes, Hisar located at 29.18N latitude and 75.7E longitude. All experimental procedures were carried out as per guidelines of Institutional Animal Ethics Committee (IAEC) of ICAR-Central Institute for Research on Buffaloes, Hisar, India.

Animals and study traits

All the male buffalo bulls taken for study aged between 24–72 months. The present data were collected during the year 2010 to 2020 for eight semen traits viz. ejaculate volume (VOL, mL) was read directly from a graduated collection tube (graduation: 1/15 mL), sperm concentration (SPC, millions/ml) was measured using a spectrophotometer, mass activity (MA, defined as motility score measured on a scale of 0 to 5 with the undiluted semen post collection), initial (BFM, % motile sperms post dilution), and post-thaw motility (PTM, % of motile sperms after dilution and after thawing) which was evaluated under microscope by experienced technicians. Number of sperms per ejaculate (NSP) and the number of motile sperm (NMSP) was calculated according to Yin et al. (2019) and details of ejaculates rejected (either prior or post freezing) were considered as discard rates (DR, %). Considering this, the data consisted of 10975 ejaculates from 45 breeding bulls used for breeding program.

Statistical analysis

Kolmogorov–Smirnov test was used for normality of the data which was normally distributed and F-test (*Levene's* test) was used to check the homogeneous of variances and found equal. Data was analysed by least squares model using SPSS software (Version 21), to identify significant non-genetic factors to be included in the repeatability model i.e. period, season and age with interaction.

Animal model and estimation of (Co) variance component

Covariance components were estimated by restricted maximum likelihood (REML) method using average information (AI) algorithm of WOMBAT. Linear model used for describing animal model for single records was $y = Xb + Za + e$. This animal model for single records in mixed model equation form is expressed as

$$\begin{bmatrix} X'X & X'Z \\ ZX & Z'Z + A^{-1}\alpha \end{bmatrix} \begin{bmatrix} \hat{b} \\ \hat{a} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \end{bmatrix}$$

where, $\alpha = \sigma^2 e / \sigma^2 a$ and A is the numerator relationship matrix of all the animals, which has non-zero off-diagonals only for the animal's parents, progeny, and mates. The elements of A can contain additive genetic effects, non-additive genetic effects, maternal effects, and permanent environmental effects (Henderson 1998). Estimates of (co)variance components and heritability for various traits was estimated by fitting a univariate animal models using average information restricted maximum likelihood (AIREML) algorithm (Meyer 2007). For genetic and phenotypic correlation bivariate animal model were used.

Repeatability animal model

The univariate animal repeatability model was run using WOMBAT for genetic evaluation of seminal parameters. When there are more than one records for an animal for a single trait, then the genetic evaluation and breeding value prediction was done by repeatability model. The repeatability model used in this study is shown as below

$$y = Xb + Za + Wpe + e$$

where, pe = vector of permanent environmental effects and non-genetic effects; W = incidence matrix relating records to permanent environmental effects. The permanent environmental effects and residual effects were assumed to be normally independently distributed with means zero and variance $\boldsymbol{\sigma}^{\mathbf{2}} pe$ and $\boldsymbol{\sigma}^{\mathbf{2}} e$, respectively.

$$var \begin{bmatrix} pe \\ e \\ a \end{bmatrix} = \begin{bmatrix} I\sigma^2_{pe} & 0 & 0 \\ 0 & I\sigma^2_e & 0 \\ 0 & 0 & A\sigma^2_a \end{bmatrix}$$

where, $\mathrm{var(e)} = \mathrm{I}\sigma^2\mathrm{e} = \mathrm{R}$ and $\mathrm{var(y)} = \mathrm{ZAZ'}\boldsymbol{\sigma}^{\mathbf{2}}\mathrm{a} + \mathrm{WI}\,\mathbf{I}\boldsymbol{\sigma}^{\mathbf{2}}\mathrm{peW'} + \mathrm{R}$. The mixed model equation for repeatability mode is shown as below

$$\begin{bmatrix} \hat{b} \\ \hat{a} \\ \hat{pe} \end{bmatrix} = \begin{bmatrix} X'R^{-1}X & X'R^{-1}Z & X'R^{-1}W \\ Z'R^{-1}X & Z'R^{-1}Z + A^{-1}1/\sigma^2_a & Z'R^{-1}W \\ W'R^{-1}X & W'R^{-1}Z & W'R^{-1}W + I\left(1/\sigma^2_{pe}\right) \end{bmatrix}^{-1} \begin{bmatrix} X'R^{-1}y \\ Z'R^{-1}y \\ W'R^{-1}y \end{bmatrix}$$

Dropping R-1 from both sides of the equation, the MME considered is shown below

$$\begin{bmatrix} \hat{b} \\ \hat{a} \\ \hat{pe} \end{bmatrix} = \begin{bmatrix} \grave{X}X & \grave{X}Z & \grave{X}W \\ \grave{Z}X & \grave{Z}Z + A^{-1}\alpha_1 & \grave{Z}W \\ \grave{W}X & \grave{W}Z & \grave{W}W + I\alpha_2 \end{bmatrix}^{-1} \begin{bmatrix} \grave{X}y \\ \grave{Z}y \\ \grave{W}y \end{bmatrix}$$

where, $\boldsymbol{\alpha}_1 = \sigma^2 e/\sigma^2 a$ and $\boldsymbol{\alpha}_2 = \sigma^2 e/\sigma^2 pe$ [24].

These estimates were fitted into univariate and bivariate repeatability model (considering effect of permanent environment on the production and fertility traits). The following repeatability model was used for uni- and bivariate analysis,

$$y = Xb + Za + Wpe + e$$

where, y, observation vector of records; X, incidence matrix for fixed effects; Z, incidence matrix for random effect; W, incidence matrix relating records to permanent environmental effects; a, vector of random effect; b, vector for fixed effects; pe, vector of permanent environmental effects; e, vector of random residual effects. Assumption: V (u) = G, V (e) = R and Cov with the likelihood maximized was derived as below:

$$\text{Log L} = -\,{}^{1}\!/_{2}\left[\text{constant} + \log|v| + \log|\text{X}^{*}\text{V} - \text{IX}^{*}| + \left(\text{y} - \text{x}\hat{\text{b}}\right)\text{v} - 1\prime\left(y - \text{x}\hat{\text{b}}\right)\right]$$

Genetic trends

Breeding values were regressed on test-year (simple linear regression) to assess genetic time trends. The slopes were considered significant and highly significant, when P was ≤ 0.05 and ≤ 0.01, respectively. To compare the rate of genetic trends across traits, a measure of relative change per trait was calculated as per Olsen et al. (2020).

Results

Descriptive statistics of semen traits

The descriptive statistics of semen traits under study is shown in Table 1. The average semen VOL was 2.82 ± 1.45 (range 0.5—15 mL). The average SPC of semen from buffalo bulls was 1040.12 ± 523.26 (range 17–3544 millions/mL). BFM and PTM was observed to be 72.83 ± 10.47 and 49.79 ± 10.73%, respectively. The DR (%) was observed to be 15.74 (range: 10–21%). The mean NSP and NMSP were found to be 2877.55 ± 2081.18 (range:22–33063) and 2086.75 ± 1511.97 (17–23144), respectively (Table 1).

Heritability, phenotypic and genetic correlation of semen quality traits

From this study, higher heritability was observed for semen quality traits viz., NSP, NMSP as well as SPC i.e. 0.41 ± 0.02, 0.34 ± 0.00 and 0.34 ± 0.00 as compared to MA (0.10 ± 0.07), VOL (0.10 ± 0.01), BFM (0.17 ± 0.02), PTM (0.04 ± 0.01) and DR (0.04 ± 0.01) (Table 2).

Significant phenotypic correlation ($P < 0.01$) was obtained between VOL and NSP (0.63 ± 0.03), between VOL and NMSP (0.58 ± 0.01). Likewise, significant phenotypic ($P < 0.05$) correlation was obtained between SPC with NSP (0.59 ± 0.02), NMSP (0.60 ± 0.01) and DR (0.61 ± 0.03). Moreover, highest phenotypic correlation ($P < 0.01$) was obtained between NSP and NMSP (0.96 ± 0.02), among the seminal traits under study (Table 2). Higher genetic correlation was obtained between NMSP and NSP (0.98 ± 0.01) as well as between SPC and DR (0.91 ± 0.03). Likewise, MA had higher genetic correlation with DR (0.85 ± 0.04) and SPC (0.81 ± 0.05) and

Table 1 Descriptive statistics of semen quality traits (N = 45)

Trait	n	Mean	Standard deviation	Minimum	Maximum	CV (%)
VOL (ml)	10975	2.82	1.45	0.5	15	51.63
SPC (millions/ml)	10975	1040.12	523.26	17	3544	50.30
MA	10975	3.04	1.05	0	5	34.83
BFM (%)	10975	72.83	10.47	5	95	14.38
PTM (%)	10975	49.79	10.73	5	80	72.32
NSP	10975	2877.55	2081.18	22	33063	72.45
NMSP	10975	2086.75	1511.97	17	23144	21.56
DR	10975	15.74	0.07	10	21	0.44

N number of animals; *n* number of observations; *VOL* volume; *MA* mass activity; *SPC* concentration; *BFM* Pre-freeze motility; *PTM* Post-thaw motility; *NSP* Number of sperm per ejaculate; *NMSP* Number of motile sperm; *DR* Discard rates

Table 2 Heritability ± SE (diagonal), phenotypic correlation ± SE (above diagonal) and genetic correlation ± SE (below diagonal) of semen quality traits

Traits	VOL	MA	SPC	BFM	PTM	NSP	NMSP	DR
VOL	0.10 ± 0.01	-0.13 ± 0.02	-0.13 ± 0.01	-0.11 ± 0.06	-0.09 ± 0.05	0.63** ± 0.03	0.58** ± 0.01	-0.12 ± 0.01
MA	-0.41 ± 0.13	0.10 ± 0.07	0.5 ± 0.01	0.30 ± 0.02	0.20 ± 0.03	0.24 ± 0.02	0.29 ± 0.03	0.53 ± 0.04
SPC	-0.5 ± 0.11	0.81 ± 0.05	0.34 ± 0.003	0.09 ± 0.01	0.06 ± 0.06	0.59* ± 0.02	0.60* ± 0.04	0.61* ± 0.03
BFM	-0.52 ± 0.12	0.498 ± 0.12	0.266 ± 0.14	0.17 ± 0.02	0.48 ± 0.03	-0.03 ± 0.04	0.19 ± 0.01	0.13 ± 0.07
PTM	-0.53 ± 0.11	0.35 ± 0.14	0.24 ± 0.15	0.78 ± 0.07	0.04 ± 0.01	-0.03 ± 0.01	0.07 ± 0.03	0.08 ± 0.19
NSP	0.56 ± 0.11	0.34 ± 0.14	0.41 ± 0.13	-0.32 ± 0.14	-0.32 ± 0.14	0.41 ± 0.02	0.96** ± 0.02	0.33 ± 0.14
NMSP	0.49 ± 0.12	0.44 ± 0.12	0.47 ± 0.12	-0.14 ± 0.15	-0.19 ± 0.15	0.98 ± 0.01	0.34 ± 0.001	0.34 ± 0.13
DR	-0.53 ± 0.11	0.85 ± 0.04	0.91 ± 0.03	0.34 ± 0.14	0.28 ± 0.14	0.33 ± 0.14	0.39 ± 0.13	0.04 ± 0.01

VOL volume; *MA* mass activity; *SPC* concentration; *BFM* Pre-freeze motility; *PTM* Post-thaw motility; *NSP* Number of sperms per ejaculate; *NMSP* Number of motile sperms; *DR* Discard rates; *$P < 0.05$; **$P < 0.01$

PTM had higher genetic correlation with BF (0.78 ± 0.07) (Table 2).

Repeatability, breeding value and genetic trends for semen quality traits

It was observed that highest repeatability was observed for NMSP (0.77 ± 0.001) followed by BFM (0.68 ± 0.002), NSP (0.66 ± 0.006), SPC (0.38 ± 0.002), VOL (0.37 ± 0.06) and MA (0.36 ± 0.02) (Table 3). The breeding value of the traits under study is shown in Table 4 and genetic trends showed significant change ($P = 0.005$) for two traits i.e. VOL and NMSP with years (Table 4).

Discussion

This is the first study to report the estimation of genetic parameters (heritability, repeatability, breeding value, genetic trend, genetic as well as phenotypic correlation) for eight semen traits in buffalo bulls. Physical as well as morphological semen traits present, in general, showed lower heritabilities in comparison with testicle biometric traits, since they are highly influenced by the environment. Our lower heritability for VOL agreed with earlier report (Panda et al. 2020; 0.218). From this study, the heritability of VOL agreed with Olsen et al. (2020) (0.14), but lower than Gebreyesus et al. (2021) (0.39). In Nellore bulls, Carvalho Filho et al. (2020) found out sperm progressive motility,

Table 3 Repeatability and variance components of semen quality traits

Trait	Repeatability	Standard error	Variance components			
			σ^2a	σ^2c	σ^2p	σ^2e
VOL	0.37	0.06	0.2464	0.6161	2.3432	1.48
SPC	0.38	0.002	99,353.1	9857.07	285,154	180,485.01
MA	0.36	0.02	0.1040	0.2601	1.0249	0.66
BFM	0.68	0.002	92.5693	113.1550	524.62	98.27
PTM	0.09	0.001	4.8672	5.6705	113.09	103.55
NSP	0.66	0.006	62,359.7	31,239.5	151,615.0	48,015.4
NMSP	0.77	0.001	108,027.0	129,616.0	309,451.0	71,807.5
DR	0.05	0.000	0.5127E-02	0.1215E-02	0.126512	0.12

VOL volume; *MA* mass activity; *SPC* concentration; *BFM* Pre-freeze motility; *PTM* Post-thaw motility; *NSP* Number of sperm per ejaculate; *NMSP* Number of motile sperm; *DR* Discard rates; σ^2a Additive genetic variance, σ^2c Permanent environment variance, σ^2p Phenotypic variance, σ^2e error variance; ^{a}Estimates of variance components σ^2a, σ^2c and σ^2e; *h2*: σ^2a/σ^2p, c^2: σ^2c/σ^2p and *r* (Repeatability): $(\sigma^2a+\sigma^2c)/(\sigma^2a+\sigma^2c+\sigma^2e)$

Table 4 Breeding value and genetic trends of different semen quality traits

Trait	Estimated Breeding value (Range)	Genetic trends			
		Intercept	Slope	Slope/SD	P value
VOL	2.71 (2.19 to 3.32)	-218.18	0.11	0.076	0.005
SPC	1137.35 (798.77 to 1959.46)	6317.07	-2.57	-0.005	0.750
MA	2.99 (2.69 to 3.34)	-9.20	0.006	0.006	0.776
BFM	71.55 (67.43 to 74.66)	-1337.66	0.699	0.067	0.082
PTM	48.43 (45.53 to 51.45)	-721.85	0.382	0.000	0.193
NSP	3132.95 (1414.82 to 5259.17)	-176152.41	88.95	0.059	0.058
NMSP	2227.22 (1588.64 to 3049.33)	-147846.34	74.46	6.934	0.005
DR	12.56 (10 to 21)	5.55	-0.001	-0.014	0.962

VOL volume; *MA* mass activity; *SPC* concentration; *BFM* Pre-freeze motility; *PTM* Post-thaw motility; *NSP* Number of sperms per ejaculate; *NMSP* Number of motile sperms; *DR* Discard rates

presented low heritability (0.07 ± 0.08) concordant with our study which indicates that these traits are under great influence of environmental factors.

The reason for lower heritability obtained may be the limited number of bulls, which exhibited the highest average age at collection. The moderate heritability for semen quality traits in this study corroborates with the earlier estimates (Berry et al. 2011; Gebreyesus et al. 2021). The lower heritability of PTM agreed with Gebreyesus et al. (2021) (0.17). Lower heritability in our study might be attributed to the lower age of the studied animals. Fewer heritability estimates have been published for NSP and NMSP. For NSP, heritability was lower (0.08–0.24) as deduced earlier (Rostellato et al. 2021) and heritability estimates of NSP and NMSP are 0.411 and 0.349, respectively and the difference, as compared to earlier reports, may be attributed to a different breed, genetic and different models used for parameter estimation. Lower heritability of semen quality traits (0.12 to 0.22) indicates a significant influence of environmental factors that affect semen quality which can be controlled by optimum managemental practices. Moreover, large variation in heritability may be attributed to breed difference, study animals' age and models used for estimation.

Regarding genetic correlations**,** antagonistic relationship between VOL and SPC and agreed with Gebreyesus et al. (2021) (-0.004) and Panda et al. (2020) (-0.007) in buffalo bulls. Our findings of SPC and PTM correlation was similar to the previous findings [Panda et al. 2020 (-0.0017); Butler et al. 2021 (-0.15)]. Negative correlation between VOL and SPC agrees with Butler et al. (2021); (-0.10) in bulls, but Gredler et al. (2007) calculated a value of 0.06, representing a significantly lower genetic correlation between these two traits. In our study, SPC and BFM were genetically positively correlated as reported earlier (Butler et al. 2021; 0.09). Nonetheless, the negative genetic correlation between VOL and SPC shows that genetic selection for single trait i.e. semen VOL shall result in poorer genetic gain for semen SPC and the genetic correlations between SPC and PTM were positive.

Likewise, PTM with VOL (0.17) and SPC (-0.15) showed positive and negative correlation, respectively, as reported by Butler et al. (2021); but Olsen et al. (2020) identified positive genetic correlation (0.83) between PTM and SPC. A strong genetic correlation (0.78) between BFM and PTM (0.92) as well as DR with NSP (0.69) deduced agrees with Berry et al. (2011; 2019). Positive genetic correlation of PTM with MA hints the similar group of genes controlling these traits (Filho et al. 2020). In all cases, NSP was positively correlated with other seminal characters. Highest genetic correlation (0.98) of NMSP and NSP agreed with Yin et al. (2019) (0.99) and Rostellato et al. (2021) (-0.15) reported lesser genetic correlation. The estimates for the genetic correlation between SPC and PTM slightly agreed with Rostellato et al. (2021) (0.13), but higher than earlier report (Butler et al. 2021; -0.15). The genetic correlations observed suggest that selection for one semen quality trait will not render unfavourable effects on other semen traits.

With respect to phenotypic correlation, positive phenotypic correlation between VOL with NSP and NMSP agreed with earlier report (Butler et al. 2021; 0.66). The phenotypic correlation between the semen quality traits were both positive and negative as compared to previous report in bulls (Gebreyesus et al. 2021). In this study, VOL and PTM which were phenotypically and genetically negatively related was supported by earlier finding (Rostellato et al. 2021; -0.01), but was found to be positive as reported by Butler et al. 2021 (0.16). Statistically significant correlation was between NSP and NMSP (0.96) agreed with Yin et al. (2019) in bulls. Lower phenotypic correlation between VOL and SPC agreed with other report (Rostellato et al. 2021; -0.23) which was evident from this study. It was noteworthy that all phenotypic correlations showed similar trend as the corresponding genetic correlations (Yin et al. 2019) and differences could be attributed to breed, age and study sample size.

Concerning repeatability, comparable results with Bhave et al. (2020) were obtained for VOL (0.41 ± 0.029), SPC (0.40 ± 0.029); but higher values for MA (0.09 ± 0.01) and BFM (0.16 ± 0.016). Repeatability with respect to PTM was lower similar to Bhave et al. (2020) (0.12 ± 0.013) in Murrah buffalo bulls. NSP and NMSP had higher repeatability than Bhave et al. (2020) (0.24 ± 0.021). In bulls (Olsen et al. 2020; Rostellato et al. 2021) higher repeatability for VOL, SPC, BFM, MA and NSP traits were reported. Difference in repeatability results may be due to dissimilar sample size as well as genetic and non-genetic factors as lower repeatability indicates that semen attributes differed with study bulls. Our findings of positive breeding value for the traits under study have corroborated that all study animals were of from selection (Olsen et al. 2020) and genetic gain with respect to two traits (VOL and NMSP) was in contrast with earlier studies (Olsen et al. 2020). The relative largest genetic changes for traits recorded were found for VOL and NMSP agreed with Butler et al. (2021) and Olsen et al. (2020) arising due to indirect selection, indicating that the fertility remained same throughout the study period.

In conclusion, this study showed that semen quality traits display significant genetic variation. The estimated heritability values suggest the feasibility of selective breeding to improve semen quality traits of buffalo bulls. The results obtained in this investigation may be used to develop genomic predictions, and increase the accuracy of breeding values for semen quality traits for buffalo species which in turn facilitate the selection of high genetic merit breeding bulls for genetic improvement.

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Data availability The data used in this study is the property of Indian Council of Agricultural Research, New Delhi. The data can be obtained from the corresponding author on request to the Director, ICAR-CIRB.

Code availability Could be shared on request.

Declarations

Conflict of interest None.

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