Genetic evaluation of sperm abnormality traits in Holstein Friesian crossbred bulls using random regression models

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ABSTRACT

Genetic parameters of sperm abnormality traits were evaluated in HF crossbred bulls using Bayesian univariate, bivariate and random regression models. Data from 161 bulls on 1,527 ejaculates collected from 2010 to 2020 at BAIF (Bharatiya Agro Industries Foundation) was utilized for this study. Four sperm abnormality traits viz. total abnormalities (A), head abnormality (HA), mid-piece abnormality (MA) and tail abnormality (TA) were included in the analysis. Gibbs sampling was done to implement a Bayesian framework. Means for A, HA, MA and TA were 10.77%, 2.99%, 2.61% and 5.10% respectively. Heritability and repeatability estimates obtained from univariate analysis were below 0.1. Genetic correlations obtained for the total abnormalities with semen production traits like ejaculate volume, sperm concentration, initial sperm motility, post-thaw motility and the total number of spermatozoa were negative. Heritability and repeatability estimates were higher in the case of random regression models where the values tend to increase with the age of the bulls. Heritability ranged from 0.025 (3 years) to 0.139 (10 years) for total abnormality estimates. In general, the estimates of genetic parameters for abnormality were low and found to increase with age.

Keywords: Bayesian framework, Genetic correlation, Legendre polynomials

India has the world’s largest cattle population (192.5 million with 145.1 million cows, according to the 20th Livestock Census, 2019), where the exotic population increased by 26.9% as compared to the previous census. During the fourth five-year plan (1969-1974), crossbreeding with exotic animals was widely practiced in India, contributing to India’s status as the world’s leading milk producer.

Most dairy development programs only incorporate milk production traits, therefore bulls are often chosen solely on their dams’ milk-producing potential. Hagiya et al. (2017) found negative genetic correlations between milk production and sperm production traits. When selecting purely for milk production qualities, there is a risk of lowering fertility, which will reduce genetic gain and profitability in the long term. As a result, analysis and the incorporation of semen traits into a breeding program are critical to the success of the breeding programme.

Sperm abnormality traits are important semen traits that are influenced by both genetic and environmental factors. There are very few studies done on sperm abnormality traits in HF crossbreds (Engidawork 2018, Seyoum et al. 2021). Kumaresan et al. (2021) also reviewed the causes of male sub-fertility in crossbred bulls where they demonstrated the need to study the quality of semen in crossbreds. Random regression models (RRM) have proven to be beneficial in modelling such repeatable traits over the age of the bulls at semen collection with less computational abilities required than conducting a multivariate analysis considering each age as a separate trait (Meyer and Hill 1997). This emphasizes the importance of this study on semen abnormality traits prior to insemination with the objective of modelling the sperm abnormality traits in Holstein Friesian crossbred bulls using random regression models in a Bayesian framework.

MATERIALS AND METHODS

Location and climate: Information on sperm abnormality traits was collected from frozen semen stations of BAIF, located at Uruli Kanchan on the outskirts on Pune in Maharashtra. It is situated at 18.5°N and 73.8°E at an altitude of 559 m above sea level. The climate can be roughly divided into three different seasons namely summer (March to June), monsoon (July to October), and winter (November to February) according to www.pmc.gov.in. Average temperature ranges from 19 to 33°C with an annual rainfall of 722 mm.

Traits and number of records: Sperm abnormality traits...
give the percentage of defective spermatozoa present in the fresh semen. Records (1527) collected from 2010 to 2020 on sperm abnormality traits in 161 Holstein Friesian crossbred bulls were included. These traits included head abnormality (HA), mid-piece abnormality (MA), tail abnormality (TA) and total abnormalities (A) which were expressed as percentages. Records beyond mean±4SD were excluded before analysis as outliers to reduce the effect of sampling error.

Factors included: Different factors that could affect the above semen traits were identified from the data available and these factors were included as fixed effects, whereas the effect of the semen collector and bull were included as random effects for all the traits. Collectors with less than 20 records and bulls with less than 30 records were eliminated from the analysis. Fixed factors included were breed (50%, 62.5% and 75% crossbreds), order of ejaculate (first or second which is collected 30 min apart), season of semen collection (Summer- March to June, monsoon- July to October, and winter- November to February), year of semen collection (2010 to 2020), age of the bull taken as a class for understanding the effect of the non-genetic factors (≤ 2, 3, 4, 5, 6, 7, 8, 9, 10 and >10 years) and as a covariate for obtaining the genetic parameter estimates, collection interval (≤ 2, 3, 4 and ≥5days) and collection time (hourly intervals between 5 AM and 12 pm).

Statistical analysis: Exploratory and descriptive analysis of the traits was done using the “psych” package in R software. Bayesian approach was used for obtaining the posterior distributions of parameter estimates using MCMC (Markov Chain Monte Carlo) and Gibbs sampling algorithm (Magnabosco et al. 2000) to generate Gibbs samples. The average of the samples (posterior mean) as a point estimate of variance components was calculated. The standard deviation of samples (posterior SD), which is corresponding to the standard error in a frequentist approach (e.g., REML) was also obtained.

For each trait, 8 models were studied by including different combinations of fixed effects as interaction effects or by taking age as a class or covariate. Animal effect was added as a random effect. The most parsimonious model was deduced based on the lowest DIC (Deviance Information Criterion).

The selected model which was used for obtaining the genetic parameter estimates can be represented as follows:

\[ Y_{ijkmnopq} = \mu + A_i + B_j + S_k + R_l + T_m + (S \times R)_l + U_o + SC_p + e_{ijkmnopq} \]

where \( Y_{ijkmnopq} \) Semen abnormality trait record; \( \mu \), Overall mean; \( A_i \), Age taken as a linear covariate; \( B_j \), Fixed effect of \( j^{th} \) breed of the bull; \( S_k \), Fixed effect of \( k^{th} \) season of semen collection; \( R_l \), Fixed effect of \( l^{th} \) year of semen collection; \( C_m \), Fixed effect of \( m^{th} \) collection interval; \( T_n \), Fixed effect of \( n^{th} \) collection time; \( U_o \), Fixed effect of \( o^{th} \) bull; \( SC_p \), Random effect of \( p^{th} \) semen collector; \( e_{ijkmnopq} \), Random error associated with each record, NID (0, \( \sigma_e^2 \)).

Univariate and random regression analyses were done to obtain variance components of direct genetic and permanent environmental effects where the significant factors were included as fixed effects. Gibbs samples (2,00,000) were generated with a burn-in of 20,000 samples and a thinning interval of 50 samples. Post-Gibbs samples (3,600) were used for the final analysis for the genetic parameter estimation for each trait. Trace plots were used to ascertain the criteria decided for Gibbs sampling.

A representative of the trace plots (total abnormalities) obtained to ascertain the stability of the MCMC chains for the parameters of the model like the number of iterations and burn-in required are given in Fig. 2.

In the case of random regression models, age of the bull was taken as the control variable and the range for age in days was wide from 16 to 194 months. The best model with the most appropriate order of fit for the Legendre polynomials for each trait was selected based on the DIC.
value. The various combination of orders for animal and perennial environment were modelled up to the order of fit of 3. The most parsimonious model was selected based on the lowest DIC (Deviance Information Criterion). Error variances were modelled as a homogenous class of random effect. The RRM could be represented as

\[ Y_{ij} = X_{ij}b + Z_{ij}a_k + \sum_{p=0}^{k-1} Z_{ij}p + C_{ij} + \epsilon \]

where \( Y_{ij} \) is the semen trait of \( i \)th animal at \( j \)th age; \( b \) is a vector of significant fixed effects, is the known incidence matrix relating fixed effects with \( Y \); \( a_k \) are the set of \( n \) values (\( n \) = number of animals (bulls)) of \( k \) random regression coefficients corresponding to animal effects, with the order of \( \text{fit} \ k \); \( p \) are the set of \( n \) values (\( n \) = number of animals (bulls)), of \( k \) random regression coefficients, corresponding to individual permanent environment effects, with the order of \( \text{fit} \ k \); \( Z_{ij} \) and \( Z_{ij}p \) are incidence matrices with dimensions \( n \times k \) and \( n \times k_p \) respectively; \( C_{ij} \) is the random effect of semen collector with mean 0 and variance 0 and ‘\( \epsilon \)’ is the random residual heteroscedastic error variance. The model also included fixed regression of age at collection with the order of that of the animal effect.

The genetic (co)variance between ages was estimated from the matrix of random regression coefficients using the general expression

\[ G_{ij} = \Phi_{xj} \Phi'_{xj} \]

Where \( G_{ij} \) is the (co)variance matrix for \( x = \text{animal or individual permanent environment and} \Phi_{xj} \) is the vector of Legendre polynomials for the random effect of \( x \) and \( j \)th age group.

In the case of bivariate analyses, which were done to obtain the (co)variance among the semen production traits, 5,000,000 Gibbs samples were generated with a burn-in of 1,000,000 and a thinning interval of 50. Total 8,000 post-Gibbs samples were generated for each pair of traits which were used for obtaining genetic correlations. Again, the number of iterations and longer burn-in for bivariate analyses were increased based on the examination of trace plots. The stability of the chains was ascertained using a trace plot which helped decide the number of iterations, as well as the burn-in period.

MCMCglmm package from the R software was used to understand the effect of genetic and non-genetic factors whereas the variance components were obtained using the BLUPF90 family of software (Misztal et al. 2018). THRGIBBS1F90 was used for generating Gibbs samples. Post-Gibbs analyses were done with POSTGIBBSF90. BayesFactor package in R was used for obtaining phenotypic correlations using Gibbs sampling.

### RESULTS AND DISCUSSION

The descriptive analysis of the sperm abnormality traits in HF crossbred bulls are given in Table 1.

The estimates of sperm abnormality traits were on the lower side of the estimates reported by other studies in HF cross in India and Ethiopia (Mandal et al. 2009, Bhakat et al. 2014, Engidawork 2018) while Bhakat (1996) in Jersey while Bhakat et al. (2018) while Seyoum (2014) in HF purebreds (Druet et al. 2009, Engidawork 2018) whereas the variance components were obtained using the BLUPF90 family of software (Misztal et al. 2018).

<table>
<thead>
<tr>
<th>Trait</th>
<th>No. of bulls</th>
<th>No. of sires</th>
<th>No. of dams</th>
<th>No. of records</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (%)</td>
<td>161</td>
<td>77</td>
<td>145</td>
<td>1522</td>
<td>10.77</td>
<td>3.92</td>
<td>36.40</td>
</tr>
<tr>
<td>HA (%)</td>
<td>161</td>
<td>77</td>
<td>145</td>
<td>1524</td>
<td>2.99</td>
<td>1.71</td>
<td>57.19</td>
</tr>
<tr>
<td>MA (%)</td>
<td>161</td>
<td>77</td>
<td>145</td>
<td>1523</td>
<td>2.61</td>
<td>1.61</td>
<td>61.69</td>
</tr>
<tr>
<td>TA (%)</td>
<td>161</td>
<td>77</td>
<td>145</td>
<td>1519</td>
<td>5.10</td>
<td>2.97</td>
<td>58.24</td>
</tr>
</tbody>
</table>

A: Total abnormalities (%); HA, Head abnormality (%); MA, Mid-piece abnormality (%); TA, Tail abnormality (%); SD, Standard deviation; CV, Coefficient of variation.
Table 2. Least-square means along with 95% lower and upper posterior densities for sperm abnormality traits in HF-crossbred cattle

<table>
<thead>
<tr>
<th>Trait</th>
<th>A</th>
<th>HA</th>
<th>MA</th>
<th>TA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breed</td>
<td>11.80</td>
<td>11.00</td>
<td>11.20</td>
<td>11.70</td>
</tr>
<tr>
<td>Season</td>
<td>12.06</td>
<td>13.02</td>
<td>9.61</td>
<td>11.96</td>
</tr>
<tr>
<td>Year</td>
<td>10.00</td>
<td>11.71</td>
<td>7.67</td>
<td>5.95</td>
</tr>
<tr>
<td>Age</td>
<td>12.80</td>
<td>14.50</td>
<td>11.10</td>
<td>13.50</td>
</tr>
<tr>
<td>Collection time</td>
<td>12.00</td>
<td>12.90</td>
<td>12.10</td>
<td>13.40</td>
</tr>
<tr>
<td>≤2 years</td>
<td>11.00</td>
<td>11.60</td>
<td>11.70</td>
<td>11.70</td>
</tr>
<tr>
<td>3 years</td>
<td>11.60</td>
<td>11.70</td>
<td>11.60</td>
<td>11.80</td>
</tr>
<tr>
<td>4 years</td>
<td>11.70</td>
<td>11.60</td>
<td>11.70</td>
<td>11.60</td>
</tr>
<tr>
<td>5 years</td>
<td>11.70</td>
<td>11.60</td>
<td>11.70</td>
<td>11.80</td>
</tr>
<tr>
<td>6 years</td>
<td>11.70</td>
<td>11.60</td>
<td>11.70</td>
<td>11.60</td>
</tr>
<tr>
<td>7 years</td>
<td>11.70</td>
<td>11.60</td>
<td>11.70</td>
<td>11.60</td>
</tr>
<tr>
<td>8 years</td>
<td>11.70</td>
<td>11.60</td>
<td>11.70</td>
<td>11.60</td>
</tr>
<tr>
<td>9 years</td>
<td>11.70</td>
<td>11.60</td>
<td>11.70</td>
<td>11.60</td>
</tr>
<tr>
<td>10 years</td>
<td>11.70</td>
<td>11.60</td>
<td>11.70</td>
<td>11.60</td>
</tr>
<tr>
<td>≥11 years</td>
<td>11.70</td>
<td>11.60</td>
<td>11.70</td>
<td>11.60</td>
</tr>
</tbody>
</table>

All the abnormality traits had very low heritability and repeatability estimates which were in accordance with the ones reported by Olsen et al. (2020) in Norwegian Red and Bhave (2021) in HF crossbred bulls. Though the heritability for bull fertility is low, it depends largely on the occurrence of the number of abnormal spermatozoa which have underlying genetic control (Chenoweth 2005). Lower heritability estimates for abnormality traits observed indicate that it would not be beneficial to include these traits in a breeding program where selection is done for improvement in the semen production traits. However, the difference between production and abnormality traits is that repeatability is also low in abnormality traits, indicating that all the variance is due to the temporary environment. This trait is dependent on the handling of semen in the laboratory, which could cause the variability unaccounted
of the number of abnormal spermatozoa present in the semen. These results also corroborate with Druet et al. (2009), Carvalho Filho et al. (2019) and Olsen et al. (2020) in HF, Nellore and Norwegian Red bulls respectively. These negative correlations are beneficial as the improvement in any of the production traits would decrease the occurrence of sperm abnormalities.

The trajectories obtained from RRM for the heritability and repeatability of the abnormality traits in HF-crossbreds are plotted in Fig. 3. The trend for the additive genetic and permanent environmental variance for total abnormalities in HF-crossbreds was similar and gradually increased till 6 years of age after which there was almost no variation. The heritability and repeatability also followed a similar trend for by genotype and permanent environment.

Genetic correlations were obtained between the total sperm abnormality and semen production traits. The genetic correlations of total abnormalities with ejaculate volume, sperm concentration, initial sperm motility, post-thaw motility and the total number of spermatozoa were -0.363±0.592, -0.214±0.687, -0.779±0.263, -0.821±0.224 and -0.305±0.572 respectively. Ejaculate volume, sperm concentration and total number or spermatozoa showed moderate negative correlation whereas the motility traits like initial sperm motility and post-thaw motility showed higher negative correlation. This high negative correlation between the motility and abnormality traits could result in a lower bull fertility as studied by Karabinus et al. (1990) and Söderquist et al. (1991) who demonstrated a negative correlation between sire conception rates and the presence of abnormal spermatozoa.

The trajectories obtained from RRM for the heritability and repeatability of the abnormality traits in HF-crossbreds are plotted in Fig. 3. The trend for the additive genetic and permanent environmental variance for total abnormalities in HF-crossbreds was similar and gradually increased till 6 years of age after which there was almost no variation. The heritability and repeatability also followed a similar trend.

<table>
<thead>
<tr>
<th>Trait</th>
<th>$\sigma^2_a$</th>
<th>$\sigma^2_{pe}$</th>
<th>$\sigma^2_s$</th>
<th>$\sigma^2_e$</th>
<th>$h^2$</th>
<th>$PSD$</th>
<th>$r$</th>
<th>$PSD$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (%)</td>
<td>0.37</td>
<td>0.64</td>
<td>0.51</td>
<td>12.86</td>
<td>14.38</td>
<td>0.026</td>
<td>0.023</td>
<td>0.071</td>
</tr>
<tr>
<td>HA (%)</td>
<td>0.04</td>
<td>0.06</td>
<td>0.04</td>
<td>2.53</td>
<td>2.68</td>
<td>0.017</td>
<td>0.016</td>
<td>0.038</td>
</tr>
<tr>
<td>MA (%)</td>
<td>0.03</td>
<td>0.03</td>
<td>0.05</td>
<td>1.87</td>
<td>1.97</td>
<td>0.013</td>
<td>0.011</td>
<td>0.029</td>
</tr>
<tr>
<td>TA (%)</td>
<td>0.07</td>
<td>0.08</td>
<td>0.31</td>
<td>6.40</td>
<td>6.85</td>
<td>0.010</td>
<td>0.010</td>
<td>0.022</td>
</tr>
</tbody>
</table>

A, Total abnormalities; HA, Head abnormality; TA, Tail abnormality; $\sigma^2_a$, additive genetic variance; $\sigma^2_{pe}$, permanent environmental variance; $\sigma^2_s$, variance of the semen collector; $\sigma^2_e$, error variance; $\sigma^2_p$, total phenotypic variance; $h^2$, heritability; $r$, repeatability; PSD, Posterior standard deviation equivalent to the standard error.

Table 3. Variance components, heritability and repeatability estimates HF-crossbred bulls for sperm abnormality traits from univariate analysis

Fig. 3. Heritability and repeatability over the age for sperm abnormality traits (Horizontal green line is the heritability for respective traits obtained through univariate analysis).
where the heritability was lowest (0.025) at 3 years and highest (0.139) at 10 years of age for total abnormality. There was more variability in the case of head abnormality where the values for additive and permanent environmental effects increased followed by a dip and then increased again. The repeatability showed higher variation than the heritability as the range was narrower for heritability and lied between 0.026 and 0.095.

In the case of mid-piece abnormality, the estimates were lower initially after which there was a drastic increase at 8 years of age for the additive effects while the permanent environmental effects gradually increased throughout. The heritability and the repeatability also showed an increasing trend where heritability had a very high range of 0.014 to 0.383. The values of heritability indicated a steep rise after 8 years, which could be due to the end effect of polynomials caused by lower number of observations. Thus, the range of values of heritability for the uniform stable part of the curve till 8 years was below 0.1.

Trajectories for the tail abnormality also resembled the total abnormalities with a slightly higher inclination. The heritability estimates ranged from 0.014 to 0.115.

Table 4 summarizes the estimates for additive genetic effect, permanent environmental effect, heritability and repeatability for different ages for sperm abnormality traits in HF-crossbred bulls. In general, the estimates of genetic parameters for abnormality were low and found to increase with age.

In general, the means of all the abnormality traits were low. Estimates of heritability and repeatability for all the traits were very low and hence improvement through individual selection is not a possibility. The high negative genetic correlation between abnormality and motility traits indicates that an increase in sperm abnormalities could reduce bull fertility. Random regression models were able to provide the genetic parameter estimates for all ages of semen collection. Estimates obtained were higher compared to that obtained through univariate analysis. Thus, random regression models can be used for modelling the sperm abnormality traits and further refining the objectives of any breeding programme for semen production.

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REFERENCES


Bhave K G. 2021. ‘Genetic evaluation of semen production and fertility traits in exotic, crossbred and indigenous cattle.’ Ph.D. Thesis, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India.