Analysis of Genetic Parameters for Reproductive Traits in Crossbred Dairy Cattle Maintained at Holetta Agricultural Research Center

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ABSTRACT
This study was aimed to generate information on variance components and the resulting genetic parameters (heritability, repeatability, genetic and phenotypic correlations and genetic trends) of some economic traits of Borena and its crosses with Holstein Friesian dairy cattle maintained at Holetta agricultural research center dairy farm. Traits studied were age at first service (AFS), age at first calving (AFC), calving interval (CI), days open (DO) and number of service per conception (NSC). Overall, 11331 dairy cattle reproductive performance records were used for the study. WOMBAT, which is a software package for quantitative genetic analysis of continuous traits, fitting a linear, mixed model; estimates of covariance components and the resulting genetic parameters were employed and obtained. Heritability values of reproductive traits were from very low (0.071, 0.082 and 0.012) for CI, DO and NSC to moderate (0.3 and 0.22) for AFC and AFS traits. Repeatability estimate for CI, DO and NSC were low (0.17, 0.17 and 0.129). Strong and positive genetic correlation (0.98) was appeared between AFS and AFC traits. Negative genetic correlations were observed between AFS and DO (-0.001), AFC and DO (-0.05), AFS and NSC (-0.022), AFC and NSC (-0.29) and CI and NSC (-0.31). AFS were negative phenotypic correlation with CI, DO and NSC. Similarly, AFC was negative phenotypic correlation with CI and DO. Low phenotypic correlation was observed between AFC and NSC, CI and DO, CI and NSC and, DO and NSC. Strong and positive phenotypic correlation was appeared between AFS and AFC. The regression coefficient of mean breeding value for NSC, CI, DO, AFC and AFS on year of birth were -0.0066x+13.25 times/year, -1.19x+2387.4 days/year, -1.23x+2445.6 days/year, 0.2x-410 months/year and 0.48x-980 months/year, respectively.

Key words: Borena, Crossbred dairy cattle, Genetic parameter, Holeta, Holstein friesian, Reproductive trait.

INTRODUCTION
Reproductive performance of modern dairy cow is declining and is wide spread in United States of America, Ireland, United Kingdom and Australia (Lucy, 2001). In the tropics, decreased reproductive performance of exotic dairy cows has also been reported and became evidence in the mid-1980’s (Million et al. 2010; Fekadu et al. 2011). It is not yet clear which factors cause or predispose the modern dairy cow to reduced fertility. However, reproductive traits are highly influenced by genetic, nutrition and other environmental factors. The reproductive efficiency of a cow is measured by parameters such as AFS, AFC, CI, DO and NSC and genetic parameter evaluations of animals are often based on these traits recorded on the dairy cattle herd.

Most population of indigenous tropical livestock have been subjected to less selection pressure for reproduction and the expectation of heritability estimates is high. However, the few available estimates of heritability for reproduction traits in indigenous tropical breeds have invariably been based on insufficient amount of data. Additionally, most studies in these region have suffered from poor experimental design, poor animal management and have obviously resulted in large environmental variations. Thus, the lower heritability estimates have often resulted from large environmental variation rather than from small genetic variation and the low reproductive performance of tropical cattle may largely be due to environmental, mainly nutritional stresses and poor heat detection.

Reproductive traits have been neglected in the past in most dairy cattle genetic improvement programs worldwide, mainly because these traits are known to exhibit a low heritability. Their improvements are carried out by adjustment of management practices (Wasike, 2006). The low heritability is caused not only by a low genetic variance but also by a higher phenotypic variance due to small size of the herd and by random or unidentified environmental factors (Khalid et al. 2001).

Some reproductive traits (CI, DO and NSC) of dairy cows are repeated over time. With repeat measurements...
of the same trait, it can be possible to calculate the repeatability estimate, which in fact is the fraction of variance that is due to permanent difference between individuals. In dairy cattle, the measure of repeatability estimate refers to strength of the relationship or correlation between repeated records for a trait in a population and this may be utilized to assess the real producing ability of individual cows in a population (Olubami and Salako, 2010). Heritability, repeatability and genetic correlation estimates can be increased by providing uniform environment, use of multiple measurements, adjustment of records and accurate measurement of data. Different estimates of genetic parameters may be found for the same trait in different populations or in one population at different times.

Study on genetic parameter estimation in Ethiopia for reproductive traits was scanty. Demeke et al. (2004) and Kefena et al. (2011) have been estimated genetic parameters for some reproductive traits of Holstein Friesian and jersey crosses with Boran at Holetta research center by using parts of the center data. Data obtained from small herd size (small number of records) might distort the estimation and there needs to be estimate by including large and updated data. Therefore, the objective of this study was thus to estimate variance components and genetic parameters for AFS, AFC, CI, DO and NSC traits using a multivariate repeatability animal model.

MATERIALS AND METHODS
Description of the study area
The current research was conducted at Holetta Agricultural Research Center (HARC) some 29 km away from the capital city, Addis Ababa, Ethiopia. The geographical location of HARC is 9° 00’ N latitude and 38° 30’ E longitude with an altitude of 2400 meter above sea level. The climate of the farm is cool tropical in nature. The area receives an average annual rainfall of 1100mm. The average temperature is 15°C with minimum of 6°C and maximum of 24°C. The average monthly relative humidity is 60%.

Data Source and data collection
Data for the study was obtained from long term (1974 - 2017) crossbred research of dairy cattle herds of Ethiopian Borena and their cross of Holstein Friesian dairy cattle maintained at the research station and therefore, Borena and different crossbred ranging from 50% to 75% of HF (Holstein Friesian) blood level have used in this study. The following data were obtained from individual animal card history and from the center database for the subsequent trait analysis.

- Identification number of each cow, sire of cow and dam of cow
- Date of birth
- AFS, AFC, DO and NSC
- Parity and genetic group (breed)

Animal management
The cattle were herded based on breed, pregnancy, lactation stage, sex and age. Regular feeding and management practices were adopted for all animals. Natural grazing, hay and concentrate supplement constitute the major feed supply. Animals were left to grazing from early morning 8.00 AM to 4.00 PM in the afternoon and allowed to graze around 8 hours. Concentrate mixture composed of wheat middling (32%), wheat bran (32%), noug (Guizocia abyssinica) cake (34%) and salt (2%) were supplemented based on their body weight, productivity and physiological stages. Milking cows, heifers and calves were supplemented with concentrate mixture at a rate of 4, 1-1.5 and 0.25-1kg per day, respectively depending on the availability of concentrate feed. The cows had free access to clean tap water all the time.

After birth, calves were received colostrum from its dam for about four days. Within 24 hours after birth, Weight data were collected and calves identification were given. After 4 days, calves were taken in to calf rearing pen and continued to feed recommended amount of whole milk around three months through artificial rearing system (bucket feeding) except the F1 calves, which have been suckled their dams until winning since 2002. Vaccination and treatment of the herd against any incidence of diseases was a routine practice.

Breeding program
Pure Borena dams were mated with pure Friesian semen to produce 50% F1 crosses while the 50% F1 was back crossed with pure Friesian semen to produce the 75% first generation. The later generations F2 (50% F1 dam x 50% F1 sire), F3 (F2 dam x F2 sire) and 75% second generations were produced by inter se mating using 50% and 75% genotypes. Semen for AI to produce the required generations was brought from NAIC (national artificial insemination center) and WWS (worldwide sire). Sometimes natural service was used when animals became repeat breeder with AI.

Early 2000, seasonal breeding was undertaken. Thenceforth the mating practice in the farm was changed and undertaking throughout the year. Bulls born in the farm were selected for breeding based on dam milk performance and physical conformation for semen collection at NAIC and used for on station breeding activities and care was taken during bull selection for NAIC to avoid genetic relationship. Selection of bulls based on their breeding value is a recent work in the research station. The use of WWS semen as genetic improvement of the herd was started since 2010.

In addition to herdsmen, teaser bulls were reared with cows for heat detection every day. Cows detected in heat were mated using AI by qualified technicians. Cows not seen in heat after service for longer were diagnosed for pregnancy after 60 days of service. The on-station selection program was implemented by using 50% F1 and 75% crossbred cows and bulls but not on Borena breed, which has only been used as dam line for F1 generations.

Data management
Data screening and censoring was made to avoid manmade errors during data entrance on individual animal card or on
the computer writing. AFS below 10 month and above 80 months and, AFC below 20 month and above 90 months were truncated. Parities more than eighth were pooled together in to eight because of few in number.

Cows have on average 285 days gestation length and 45 days voluntary waiting period after calving (330 days CI). Based on this assumption, cows that recorded below 330 days calving interval (CI) were removed from the analysis. A cow after calving needs some rest period for the uterus to involutes and normal cycle to take place. For this reason, a voluntary waiting period ranging from 45 to 60 days were allowed before the cow was inseminated or bred (Gebregziabher et al. 2005). As a result, animals, which showed estrus and bred earlier of 45 days, were removed from the analysis. Repeat breeder cows more than 10 times were very few compared from the rest of the population and were removed. The animals that have abnormal calving, i.e., abortions and stillbirths were not included in the analysis of breeding data. Records of animals without pedigree information and dates of birth and date of calving were excluded. Animals ID (name) that appeared before their parents were edited and recoded for the pedigree analysis.

Method of data analysis

Multivariate analysis for five traits (AFS, AFC, CI, DO and NSC) were undertaken for estimation. WOMBAT, a genetic analysis software package version 23-07-2019, which is developed by Karin Meyer, was used to determine variance components and genetic parameters (heritability, repeatability and genetic and phenotypic correlations). Year, season, parity and breed (genetic group) were fitted as a fixed effect in the model.

Breeds included in the analysis were pure Borena and cross of Borena and HF (50% F1, F2, F3 and 75% first generation, second generation) (Table 1). The Borena breed genetic contribution for crossbred generation was immense (25% and 50%) in the present study and the breed was fitted in to the genetic parameter analysis. Months of the year were classified into 3 seasons based on rainfall distribution. Dry season from October to February, short rain season from March to May and main rain season from June to September.

Repeatability animal model was fitted, where direct additive effects plus permanent environmental effect due to repeated records per cow were fitted as random effects. For traits (AFS and AFC), however, direct additive genetic effect was the only random effect fitted. The model was presented as follows:

\[ Y = Xb + Z_a + W_d + e \]

where;
- \( Y \) is a vector of observations for the traits of interest
- \( b \) is a vector of fixed effects (breed/genetic group, year, season and parity).
- \( a \) is a vector of random individual additive effects
- \( d \) is a vector of permanent environmental effects
- \( X \) matrices relating records to fixed effects
- \( Z \) matrices relating records to incidence random individual additive effect
- \( W \) matrices of permanent environmental effects.
- \( e \) vector of random residual effect

The model was assumed as expected value of \( Y \) to be \( Xb \). The vector random individual additive effects, permanent environmental effects and residual effects are assumed to be uncorrelated and have expected mean of zero and variances \( \sigma^2_a, \sigma^2_d \) and \( \sigma^2_e \), respectively. From these expectations, WOMBAT is estimated the direct (co) variance, genetic and phenotypic correlations and breeding values of each animal for each trait from multigenerational pedigree data as the software already recognized the formula. Heritability and repeatability were calculated by using user-defined formulas as follows:

\[
\begin{align*}
\sigma^2_a &= \sigma^2_p + \sigma^2_e \\
\hat{h}^2 &= \frac{\sigma^2_a}{\sigma^2_p} \\
\hat{r}^2 &= \frac{\sigma^2_a}{\sigma^2_p + \sigma^2_e}
\end{align*}
\]

\( \hat{h}^2 \): Heritability value
\( \hat{r}^2 \): Repeatability value
\( \sigma^2_p \): is phenotypic variance (total variance)
\( \sigma^2_a \): additive genetic variance
\( \sigma^2_d \): permanent environmental variance
\( \sigma^2_e \): residual variance

Regression analysis was used to estimate genetic trends for the five reproductive traits using SAS 2004 version 9.0. The mean additive genotype in a particular year of birth was defined as the mean predicted breeding values of cows born in that year. Consequently, changes of mean additive

| Table 1: Number of records used for genetic analysis. |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Traits            | Number of observations in genetic groups (breeds) |                  |                  |                  |                  |                  |
|                   |                  |                  |                  |                  |                  |                  |
|                   | 50%              |                  |                  |                  |                  |                  |
|                   |                  |                  |                  |                  |                  |                  |
| Age at first service | 54              | 461              | 89               | 60               | 143              | 26               | 833              |
| Age at first calving       | 59              | 470              | 89               | 60               | 143              | 26               | 847              |
| Calving interval            | 1147             | 1295             | 162              | 89               | 167              | 22               | 2882             |
| Days open                   | 1124             | 1271             | 161              | 87               | 163              | 22               | 2828             |
| Service per conception      | 1410             | 1762             | 258              | 150              | 313              | 48               | 3941             |
| Total                        | 3794             | 5259             | 759              | 446              | 929              | 144              | 11331            |
genotype between the years reflected additive genotypic differences. The overall additive genetic trend in a trait was estimated by regressing the mean predicted breeding values on the respective year of birth in that trait \cite{Rege_1989}.

**RESULTS AND DISCUSSION**

**Heritability estimation**

Variance components, heritability ($h^2$) and repeatability ($r$) estimates for AFS, AFC, CI, DO and NSC are presented in Table 3.

**Age at First Service**

The heritability estimate for AFS was $0.22 \pm 0.08$. The present result was very lower than Haile \textit{et al.} \cite{Haile_2009} 0.6 ± 0.145 estimated for Ethiopian crossbred dairy cattle and slightly lower than the estimate of Belay \cite{Belay_2014} who found 0.26 for Fogera x HF crosses at Metekel ranch, Ethiopia. The difference might be due to breed, geographical situation and analysis type.

**Age at First Calving**

The present result 0.30 ± 0.08 for AFC was higher than the report of Ashutosh \textit{et al.} \cite{Ashutosh_2013} who found that 0.26 and 0.23 for two and three breed crosses and, 0.149 by Belay \cite{Belay_2014} for Fogera x HF. From the literature, lower estimate of heritability (0.235 and 0.215) for this trait was also reported \cite{Gutierrez_2002, Oyama_2002}. Higher values of heritability were reported across Ethiopia 0.44 ± 0.05 by Demeke \textit{et al.} \cite{Demeke_2004}, 0.7 ± 0.16 by Haile \textit{et al.} \cite{Haile_2009} and 0.4 by Kefena \textit{et al.} \cite{Kefena_2011}. The moderate heritability value of AFC in this study would be gave a chance for further improvement by selection of this trait or other correlated traits.

**Calving Interval**

Heritability estimate for CI in this study was 0.071 ± 0.03 and at the lower edge (0.08 ± 0.03) of Demeke \textit{et al.} \cite{Demeke_2004} and the upper edge of Ashutosh \textit{et al.} \cite{Ashutosh_2013} who reported 0.05, 0.05 and 0.05 for HF x Local, HF x Sahiwal and HF x Sahiwal x Local. This result was higher than the report of Oyama \textit{et al.} \cite{Oyama_2002} who found 0.047 for Japanese black cattle and Belay \cite{Belay_2014} 0.049 for Fogera x HF. However, higher values from the present study were reported by Gutierrez \textit{et al.} \cite{Gutierrez_2002} 0.125 for beef crossbred, Sarder \textit{et al.} \cite{Sarder_2004} 0.38 ± 0.05 for Bangladesh crosses, Mahmudur \textit{et al.} \cite{Mahmudur_2007} 0.499 for HF and Jersey crossed with local, Haile \textit{et al.} \cite{Haile_2009} 0.1 ± 0.046 and Kefena \textit{et al.} \cite{Kefena_2011} 0.17 for Ethiopian crossbred dairy cattle. Apart from actual breed differences and environmental variation, methods of analysis and models contribute to the differences.

**Days Open**

The heritability estimate for DO (0.082 ± 0.03) was in the true range (low) of reproductive traits but high when compared with the literature average of 0.047 ± 0.009 for wagyu cattle reported by Oyama \textit{et al.} \cite{Oyama_2002}, 0.04 ± 0.03 by Demeke \textit{et al.} \cite{Demeke_2004} for Borena crosses with HF and Jersey and 0.01 by Belay \cite{Belay_2014} for Fogera x HF. However, higher values compared with the present result were reported 0.1 ± 0.047 by \cite{Haile_2009}.

**Table 2:** Number of records on pedigree characteristics for genetic parameter estimation.

| Number | Pedigree characteristics                          | Number of records |
|--------|--------------------------------------------------|-------------------|
| 1      | Number of animal IDs in data file                 | 1095              |
| 2      | Number of animal IDs in total                     | 1299              |
| 3      | Number of animals without offspring               | 667               |
| 4      | Number of animals with offspring                  | 535               |
| 5      | Number of animals with unknown sire               | 376               |
| 6      | Number of animals with unknown dam                | 413               |
| 7      | Number of animals with both parents unknown      | 355               |
| 8      | Number of sires with progeny in the data          | 96                |
| 9      | Number of dams with progeny in the data           | 439               |
| 10     | Number of animals with maternal grandsire        | 342               |
| 11     | Number of animals with maternal grand dam         | 313               |

**Table 3:** Variance components, heritability ($h^2 \pm se$) and repeatability ($r \pm se$) of reproductive traits from multivariate analysis.

|        | AFS       | AFC       | CI         | DO         | NSC       |
|--------|-----------|-----------|------------|------------|-----------|
| Va     | 9759.44   | 15187.6   | 1510.23    | 1721.95    | 0.198     |
| Vc     | -         | -         | 2163.37    | 1804.90    | 0.213     |
| Ve     | 33890.6   | 35344.62  | 17482      | 17396      | 1.56      |
| Vp     | 43650     | 50532.3   | 21156      | 20922.8    | 1.79      |
| $h^2$  | 0.22 ± 0.08| 0.30 ± 0.06| 0.071 ± 0.03| 0.082 ± 0.03| 0.012 ± 0.003|
| r      | -         | -         | 0.17 ± 0.02| 0.17 ± 0.02| 0.129 ± 0.03|

$Va$ = additive variance, $Vc$ = permanent environmental variance, $Ve$ = error variance, $Vp$ = phenotypic variance, $h^2$ = heritability and $r$ = repeatability, $se$ = standard error
Number of Service per Conception

The estimated heritability value for NSC was 0.012 ± 0.003. The estimate of this study is lower than the finding of Demeke et al. (2004) 0.07 ± 0.02, Sarder et al. (2004) 0.08 ± 0.05 and Haile et al. (2009) 0.1 ± 0.071. Heritability value of Number of service per conception was generally very low and expected which indicating that this trait is highly influenced by environmental factors such as inaccurate insemination technique, neglected heat detection, use of poor quality of semen for insemination and poor fertility of cows resulted for high residual variance.

Repeatability Estimation

Repeatability estimate for CI, DO and NSC were low (0.17, 0.17 and 0.129) and this value were within the limits often reported by different literatures (Demeke et al. 2004; Haile et al. 2009). This indicated that an animal evaluation and genetic improvement using heritability value for these reproductive traits were not reliable. This shows that improvement of these traits should be done by improving management. Inconsistent and lower repeatability estimate was reported by Oyama et al. (2002) for DO and CI (0.087 and 0.092) but Sarder et al. (2004) found higher (0.46) repeatability value for NSC trait. The lower repeatability estimate for these traits on the present study could be due to higher influences of specific environmental effects on a given record that may inflate the variability of repeated animal records.

Genetic and phenotypic correlations

The direct genetic and phenotypic correlations between five reproductive traits (AFS, AFC, CI, DO and NSC) are summarized in Table 4. The present study showed that negative, weak positive and strong positive genetic correlations were observed among reproductive traits. An inverse correlation between AFS and DO (-0.001), AFC and DO (-0.05), AFS and NSC (-0.022), AFC and NSC (-0.29) and CI and NSC (-0.31) were found. The negative genetic correlation of these traits showed that there might be the result of separate gene action. Strong and positive genetic correlation (0.98) was appeared between AFS and AFC traits. However, moderate (0.36) genetic correlation was observed between CI and DO. The correlation between AFS and AFC with DO and NSC in this study was different from the finding of Haile et al. (2009) who reported positive, and 0.51 for AFS and DO, 0.19 for AFC and DO, 0.38 for AFS and NSC and 0.65 for AFC and NSC, respectively. Other study carried out by Gutierrez et al. (2002) and found comparatively higher correlation (0.23) between AFC and CI for beef crossbred. However, a perfect positive genetic correlation (1) between AFS and AFC and CI and DO for Fogera crosses were reported by (Belay, 2014). These difference might be due to variation in breed, size of the data set, estimation procedure and analysis type.

The positive genetic correlations among traits (eg. AFS with AFC) in the present study might be arises due to the pleiotropic effect of gene and some linkage among genes.

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**Table 4:** Genetic and phenotypic correlations of reproductive traits.

|       | AFS | AFC | CI  | DO  | NSC |
|-------|-----|-----|-----|-----|-----|
| AFS   | 0.98|     | 0.001| -0.001| -0.022|
| AFC   |     | 0.05| -0.05| -0.29|
| CI    | -0.03| -0.02| 0.36| -0.31|
| DO    | -0.03| -0.02| 0.25| 0.08|
| NSC   | -0.006| 0.09| 0.19| 0.07|

Genetic correlations (above diagonal) and phenotypic correlations (below diagonal).

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**Fig 1:** genetic trend for NSC, CI, DO, AFC and AFS traits.
However, traits, which showed negative or zero correlations (AFS with DO, AFC with NSC and CI with NSC) is considered as the result of independent gene action and uncorrelated of each other and selection to improve single trait might disfavor other traits.

Phenotypic correlation between reproductive traits were negative (AFS with CI, DO and NSC and AFC with CI and DO), low (AFC with NSC, CI with DO, CI with NSC and DO with NSC) and strong positive correlation between (AFS and AFC). Belay (2014) found very strong phenotypic correlation between AFS and AFC (0.86) and CI and DO (0.99). The variation of the present study from others literature might be due to breed, number of observation studied and software procedure used for analysis. The present study showed that both random environmental and genetic effects could be influence phenotypic correlation of the studied traits.

**Genetic trend**

The overall genetic trend for AFC and AFS were non-significant (p > 0.05) and not in the right direction (positive) which indicate that an increasing numbers of months across the observed years. However, NSC, CI and DO were negative but insignificant which mean that a decreasing trend over the observed years (Fig 1).

The regression coefficient of mean breeding value for NSC, CI, DO, AFC and AFS on year of birth were -0.0066x+13.25 times/year, -1.19x+2387.4 days/year, -1.23x+2445.6 days/year, 0.2x+410 months/year and 0.48x-980 months/year, respectively. Thus, this means that breeding value for NSC, CI and DO were decreased by 0.0066 times, 1.19 days and 1.23 days while breeding value for AFC and AFS were increased by 0.2 months/year and 0.48 months/year, respectively across the study period. This might be the within year difference between estimated breeding value of cows in each trait.

**CONCLUSION**

The present result showed that heritability values of reproductive traits were very low for NSC and moderate for AFC traits. The lower proportion of additive genetic to phenotypic variance in the present study had resulted for lower heritability estimate for reproductive traits. Even though the heritability estimate was low, genetic variations among individual dairy cows in the farm were certainly existed. The higher proportion of phenotypic variance indicated that reproductive traits were highly influenced by unfavorable environmental condition and should be improved through improving management and feeding system of the herd. The lower repeatability estimate for reproductive traits on the present study could be due to higher influences of specific environmental effects on a given record that may inflate the variability of repeated animal records. The only positive genetic correlations between all reproductive traits in this study implies that they all are being more or less controlled by similar gene and indicating that selection of one trait for improvement will improve other correlated traits in a desired direction which will helping the breeding process as a whole.

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