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Genetic Evaluation of Gir Cattle for Success Rate to Conception using GLIMMIX Model

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ABSTRACT

Background: The objective of this study was to assess the genetic and phenotypic parameters associated with the success rate to conception using 90-days non-return rate (NRR90F), conception rate (CR) and number of insemination to conception (INS) traits in service bull.

Methods: A total of 2590 insemination including 1776 first time insemination was carried out during the period of 2018-2020 in the Gir cattle distributed in the semi-arid region of RajasthanNRR90F and CR were analysed as binary variable and INS as multi-nominal trait. Generalized linear models were used to for the evaluation of non-genetic factors and generalized linear mixed models for genetic effects.

Result: The average success rate of artificial insemination was estimated as 0.35 for NRR90F and 0.41 for CR. Further, cumulative logit link function was used to analyse the number of successful insemination to conception (INS) and cumulative estimates was observed as 0.95 for two number of insemination to conception. Period and season were the non-genetic factors that influenced the traits studied. Decreasing trend was observed over the period for success of insemination for all studied traits. Summer season showed highest odd ratio and estimated as 1.213 for NRR90F, 1.388 for CR and 1.440 for INS with reference to autumn season. Heritability was estimated as 0.015 for NRR90F, 0.028 for CR and 0.086 for INS.

Key words: Conception rate, Gir, Heritability, INS, NRR90F.

INTRODUCTION

Economic loss due to failure of insemination to conception is of major concern to dairy sector around the world. Reducing the number of insemination to conception and increasing the rate of success at first insemination to conception will lead to improve the reproductive performance of dairy cattle. Better herd management can improve the reproductive performance but this increases the cost andtherefore a combination of better managementand proper breeding strategyare required. The need for inclusion of fertility traits in selection programme has been strengthen to achieve the optimum reproductive parameters. A very broad range of indicators has been standardised under international genetic evaluation system for male and female fertility traits (ICAR, 2019). In contrast to conventional indicator traits *i.e* open days and days to first service that do not account for elapsed time from first to last insemination and veterinary cost, the variable non-return rate to insemination (NRR), conception rate (CR) and number of insemination to conception (INS) takes partly these issues into consideration. Therefore, the identification and evaluation of reproductive performance of service bull that can be easily measured and have meaningful impacts on selection are required. Diagnosis of early pregnancy is regular field activity and consider as easily measured trait for reproductive performance since it comprises the categories of conception (pregnant/ non pregnant). Recording of pregnancy does not require additional costs for recording, since the categories of conception is the best indicator trait for reproductive performance. However, one of the major challenges of using categorical traits in breeding programme is the development of adequate algorithm for estimation of parameters. One of the methods frequently used for this purpose is generalised linear mixed model (GLIMMIX) (Garcia *et al.*2012).

Bull fertility has reportedly been genetically and phenotypically correlated with traits of economic gain in the dairy sector because it affects overall efficiency of dairy herds. Keeping in view of the above economic importance, there is an imperative requirement to estimate the genetic and phenotypic parameter associated with the success rate

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of insemination using non-return rate at 90days (NRR90F), conception rate (CR) and number of insemination to conception (INS) in Gir bull under the assumption of threshold model.

MATERIALS AND METHODS

Data related to the project of High Genetic Merit (HGM) Bull production were utilized for the study on insemination records during the period of 2018 to 2020 comprising 2950 insemination records of 1776 registered Gir cattle from the breeding tract of Rajasthan. Breeding tract of Gir cattle of Rajasthan is located in the semi-arid eastern plain region at 26°25′ to 27°51′N latitude and 74°55′ to 76°10′E longitude. Information related to artificial insemination of the registered cattle was recorded in Information Network for Animal Productivity and Health (INAPH) maintained by National Dairy Development Board (NDDB) in association with Rajasthan Livestock Development Board (RLDB). All registered cows were regularly monitored by AI technician and reported in INAPH. INAPH data file consisted of insemination, pregnancy and calving records of registered cattle were used to analyse the trait of interest.

The fertility traits selected for this study were investigated for success in first insemination and not returned to insemination within a 90 days (NRR90F); success of insemination (CR) and number of insemination to conception (INS). NRR90F and CR were considered as binary traits and insemination outcome was defined as 1for positive pregnancy diagnosis after 90 days of insemination otherwise 0 for failure and again verified from calving record. INS trait was analysed as categorical trait considering the category1 for first insemination for conception, 2 for second insemination for conception, 3 for third insemination for conception and category 4 for more than 3 inseminations to conception. Conception from 3 or more insemination (under absence of detectable abnormality) was considered as repeat breeding (category 3 and 4). The data were classified into non-genetic factors such as period and seasons. The model included the fixed effect of period (2018; 2019; 2020) and season (December to February; March to June; July and August; September to October).

Preliminary least squares analyses were conducted for all traits using Generalised Linear Model (GLM) procedure of SAS (OnDemandforAcademic) to assess the effect of non-genetic factors *i.e.* period and season on fertility traits. Fixed effect were analysed using PROC GLM syntax as:

```
proc logistic data = Work. import;
Class Period season/param = glm;
model n/t = Period season/link = logit ;
lsmeans period season/cl ilink;
run;
```

Odd ratios were calculated for interest traits. The odd ratios compare two opposite probabilities which one has higher chance of occurrence. The significance of odd ratio is given by the confidence interval, usually the 95% confidence interval (Schmidek, 2013). Reference classes were established to evaluate the risk of occurrence compared with the reference class.

The following linear predictor was common to NRR90F and CR traits:

$$\eta = \beta_o + \beta_{xi} + \beta_{yi}$$

Where

η = A function of expected value of NRR90F and CR.

β_o, $_x$ and β_y represented the estimated parameter for intercept, period(x_i=1 to 3) and season (y_i=1 to 4) the probability of success (p) for interest trait was estimated as:

$$\mu_{xi} = \frac{_e\beta_o + \beta_{xi}}{1 + {_e\beta_o} + \beta_{xi}}$$

$$\mu_{yi} = \frac{_e\beta_o + \beta_{yi}}{1 + {_e\beta_o} + \beta_{yi}}$$

The overall mean (μ) of the distribution of depends on the independent variables (x) and (y) through the inverse link function (g^{-1})

$$\mu = g^{-1}(\eta)$$

Estimation of variance componentand prediction of breeding value of Sire using SAS (On demad for academics)

A sire model was fitted to estimate the genetic parameters of the studied traits. The data were arranged in univariate form to estimate the sire component for binary and ordered categorical data. PROC GLIMMIX was used to estimate the variance component for NRR90F, CR and INS traits. The following GlIMMIX statements fit a model with logit link for the probabilities by maximum likelihood where the marginal log likelihood was approximated by adaptive quadrature (Schabenberger, 2013).

```
procglimmix data = Work. import method = quad;
class sire period season;
modelnumber_of_ conception/total_AI = period season/s
link = logitdist = binomial;
randomint/sub = sire s cl;
lsmeans period season/cl ilink;
ods output solutionr = solr;
freq n;
run;
```

Number of insemination to conception trait (INS) was scored in four ordered categories. In order to analyse the data as multinomial with PROC GLIMMIX, the data need to be arranged in univariate form. The sire were selected at random, a model for the four categories with fixed regression effect for period and season with random sire effects was considered. Because the response categories were ordered, a proportional odds model was chosen (McCullagh, 1980). The following GLIMMIX statement fit a model with logit link for the cumulative probabilities by maximum likelihood where the marginal log likelihood was approximated by adaptive quadrature (SAS,2013):

```
procglimmix data = import method = quad;
class sire;
model trait = period season/s link = cumlogitdist =
multinomial;
randomint/sub = sire s cl;
```

```
ods output Solutionr = solr;
freq count;
run;
```

The variance of sire effect was estimated with estimated asymptomatic standard error. Heritability for binary (NRR90F and CR) and ordered trait (INS) were calculated using the variance of the logit link function. This implies a correction of the residual variance by factor $\pi^2/3$(Southey *et al* 2003). For the logit function, the residual variance is an approximation based on the variance of the logistic regression and is described as:

$$\sigma^2_{el} = \frac{3.1416^2 * b^2}{3} = 3.2899$$

Where,

b = Standard deviation of the logistic distribution which is generally assumed to be 1 (Southey *et al*., 2003; Garcia *et al*., 2012) as in the present study. Thus following expression can be used to calculate the heritability (h^2):

$$h^2 = \frac{4\sigma^2_s}{\sigma^2_s + \sigma^2_{el}}$$

Where,

σ^2_s = Sire variance.

Comparision of sire evaluation methods for stability of NRR90F,CR and INS traits

Thespearman'srank correlation between the rank of breeding values of sires for NRR90 F, CR and INSwas used to find thestabilityof various indicator traitsThe rank correlation was estimated as:

$$r_s = 1 - \frac{6\Sigma d_i^2}{n\,(n^2 - 1)}$$

The significance of rank correlation was tested by t-testas given below

$$t = r\sqrt{\frac{(n - 2)}{n\,(1 - r^2)}}$$

Where,

r_s = Rank correlation coeffeicient.

n = Number of sires under evaluation.

d_i = Difference of rank between paired items under two traits.

t = Calculated value was compared with (n-2)degree of freedom.

RESULTS AND DISCUSSION

The results of model effect with phenotypic means and standard errors were presented in Table 1. Fishers scoring

Table 1: Season and period wise least squares means and standard errors of success to insemination traits (fertility) of Gir cattle.

Effect	NRR90F	CR	INS (cumulative)		
			First AI	Second AI	Third AI
Number of successful AI	759	1074	759	218	69
Number of total AI	1776	2590		1074	
Wald chi-square/DF	0.71	1.21		1.68	
conception rate intercept	0.42**	0.41**	0.71**	0.91**	0.97**
(Reference 2020/Autumn)	(-1.14±0.20)*	(-1.288±0.16)*	(0.31±0.29)*	(1.76±0.28)*	(3.08±0.33)*
Period F/waldchisquare	28.99	44.49		17.2439	
(Pr>F)	(0.001)	(0.0001)		(P<0.008)	
2018	0.50±0.03	0.49±0.03	0.85±0.03	0.96±0.01	0.99±0.003
	(101/206)	(120/251)	(101/120)	(117/120)	(118/120)
2019	0.44±0.02	0.43±0.01	0.68±0.02	0.90±0.01	0.97±0.01
	(585/1299)	(841/1916)	(585/841)	(760/841)	(819/841)
2020	0.26±0.04	0.25±0.02	0.60±0.05	0.87±0.02	0.96±0.01
	(73/271)	(113/423)	(73/113)	(100/113)	(109/113)
Season F	1.904	7.3757		16.6582	
(Pr>F)	(P>0.59)	(P>0.06)		(P<0.05)	
Winter	0.39±0.02	0.39±0.02	0.75±0.03	0.93±0.01	0.98±0.004
	(160/423)	(231/621)	(160/231)	(51/231)	(16/231)
Summer	0.41±0.02	0.41±0.02	0.77±0.03	0.93±0.01	0.98±0.004
	(355/809)	(489/1132)	(355/489)	(98/489)	(29/489)
Rainy	0.40±0.03	0.39±0.03	0.67±0.04	0.90±0.02	0.97±0.007
	(123/263)	(185/409)	(123/185)	(40/185)	(12/185)
Autumn	0.37±0.03	0.33±0.02	0.70±0.04	0.91±0.02	0.97±0.006
	(121/281)	(169/428)	(121/169)	(29/169)	(12/169)

*logit scale **Data scale.

optimisation technique and wald chi-square statistics criteria were used to assess the goodness of fit. The ratio between Chi-square statistics and degree of freedom was estimated as close to one for all fertility indicator traits and it indicates that the variability in the data has been properly modelled.

Non Return Rate at 90 days (NRR90F)

The number of individual for NRR90F was estimated as0.42 on data scale. The intercept of NRR90F along with standard error were estimated as (-) 1.14±0.20 on log it scale. Negative intercept value indicated that proportion of non-return rate at 90 days was lower than the alternative outcome *i.e* heat return rate after insemination (0.58).The present findings showed lower success rate of insemination than the failure rate to conception. It may be due to that success of insemination is reflected by large number of environmental factors compared to alternative outcome.

Subjecting the data to statistical analysis, NRR90 was significantly influenced (P<0.001) by period of AI on indicating that the NRR 90 is not equally successful for conception during the period. The cattle inseminated in period of 2020 had low estimates for non-return rate at 90 days compare to the year of 2018 and 2019. The odds ratio NRR90 was estimated as 2.751 for in the year of 2019 compare to the year of 2020. The LS estimates have been converted back to the data scale by the inverse link function and results have been presented in Table 1. NRR90 during the period showed that success rate of non-return at 90 days was reduced by 22 per cent in the year of 2020. The effect of insemination period on NRR90 may be expected due to no uniform AI practices during the period. It led to the conclusion that effect of period on non-return rate may be mediated through the management practices.

Success rate of AI was assessed among the seasons using odds ratio and results of odds ratio and 95% confidence intervals (CIs) were presented Table 2. In relation to season of AI, it was found to be non-significant for NRR90F. In general, insemination during the summer season showed higher success rate for NRR90 and it was estimated as 1.101 times higher to the autumn season. Lowest success rate of NRR90 was observed in the rainy season. In agreement with our findings,

It is possible that decreasing day light during autumn season negatively influences the hormonal secretion responsible for the reproductive function.

Conception rate (CR)

The overall means of CR was estimated as 0.43 on data scale. Intercept on logit scale was recorded as -0.85±0.19 for conception rate in Gir cattle (Table 1). Statistical analysis for fixed effects showed significant difference among CR during the period. The conception rate was lower by 22 percent in the year of 2020 as compared to the year of 2019.In order to predict predict the success rate of AI, odds ratio was estimated among the period and seasons. It was estimated as 1.547 for the year of 2019 compared to the

Table 2: Estimates of oddsratio and confidence limitfor conception in Gir cattle with frozen thawed semen.

Variable	NRR90F			CR			INS								
							First AI			Second AI			Third AI		
	Point estimate	Confidence limits 95% Wald		Point estimate	Confidence limits 95% Wald		Point estimate	Confidence limits 95% Wald		Point estimate	Confidence limits 95% Wald		Point estimate	Confidence limits 95% Wald	
Period	P<0.001			P<0.001						P<0.05					
2018	2.751**	1.815	4.169	2.855**	2.003	4.071	12.022**	1.585	91.206	4.939	0.600	40.647	0.731	0.040	13.501
2019	2.219**	1.630	3.022	2.214**	1.724	2.842	3.675	0.889	15.187	2.991	0.691	12.963	2.813	0.584	14.035
2020	Reference														
Season	P>0.05	P>0.05	P<0.05												
Winter	1.120	0.804	1.559	1.277	0.972	1.677	5.537	1.151	26.628	5.907	1.164	29.972	3.711	0.644	21.389
Summer	1.213	0.908	1.618	1.388	1.094	1.761	4.414	1.437	13.557	4.211	1.295	13.692	2.487	0.687	9.051
Rainy	1.190	0.846	1.672	1.301	0.987	1.716	0.793	0.290	2.169	1.001	0.339	2.957	0.667	0.189	2.347
Autumn								Reference							

year of 2020. Proportion of CR observed in present study was lower than the reports of NDRI, (2015) who found that the overall conception rate (CR) as 0.43 in herd maintained at NDRI, Karnal. Highest estimates for conception rate was observed in summer season (0.42) followed by (0.40). It can be inferred that summer season of AI is appropriate for higher CR and effect of season may be variable due to the inter-annual random change of climatic factors. CR may be decreased under stress of rainy. It necessitated the need to take effective measures for standardised AI practices for achieving optimum fertility.

Similarly, the present study was similar to that have reported non-significant effect of season of artificial insemination on conception rate was observed in field animals by Bhagat and Gokhale (2016). The period of AI had exerted a significant effect (P<0.001) on NRR90 and it showed decreasing trend over the period. The present findings may be observed due to the inter-annual random change of climatic factors. Incidence of NRR90 may be decreased under stress of heat and cold. It necessitated the need to take effective measures for standardised AI practices for achieving optimum fertility.

Number of insemination to conception (INS)

The wald chi- square estimates and least squares means along with standard error for period and season have presented in Table1. The data from 1074 conception was analysed and average cumulative proportion to conception was estimated as 0.71, 0.91 and 0.97 from first, second and third insemination cycle, respectively. Present studies was estimated as 91 percent conception from 2 insemination and rest 9 percent conception was from3 or more insemination *i.e.* incidence of repeat breeding in Gir population. Intercept was estimated as positive 0.55±0.27, 2.05±0.28 and 3.28±0.32 on cumulative log it scale for one, second and third insemination cycle indicating that maximum conception was achieved from first AI cycle compared to other categories. However, variation to conception is marked in ordered insemination and it might be due to environmental and genetic factors of inseminations. Subjecting to the statistical analysis, ordered insemination to conception were significantly influenced (P<0.002) by season and (P<0.04) period of AI. Higher proportion of conception was estimated in winter season and lowest was found in rainy season (Table.1).

The 95% confidence interval (CI) was used to estimate the precision of the OR and results are presented in Table 2. High estimates of OR for INS traits indicates a low level of precision of the OR, whereas a small CI indicates a higher precision of the OR.

In the absence of reproductive disorders such as cystic ovaries, anoestrus and chronic endometritis, overall success to conception from two AI was estimated as 0.90. The present study was in agreement with the findings of Gustafsson *et al*., (2002). It led to the conclusion that in female cattle with normal fertility the incidence of fertilisation failure is approximately 10% and early embryonic death within 3 weeks following fertilisation accounts for approximately 30% leading to a total early pregnancy loss of close to 40% during the first 21 days post AI. This means that on average 40% females will return to oestrus after each AI or mating. Several environmental factors e.g. nutrition, climate, as well as intrinsic animal factors have been suggested to be the cause behind this early embryonic loss in cattle. It has also been proposed that early embryonic loss should be regarded as “normal” due to an early elimination of unfit genotypes.

Estimation of heritability for NRR90, CR and number of insemination to conception (INS)

Estimated variance component on the underlying scales based on sire model were presented in Table 3. Variance component of service sire for all indicator traits showed very low estimate. Heritability using logit sire model were estimated as 0.02, 0.03 and 0.09 for NRR90F, CR and INS, respectively. The heritability estimates for INS washigher than others. However, multinomial trait INS showed higher additive genetic variance related to NRR90 F and CR as binary traits. Additive genetic variance for NRR90F was the least among all indicator traits. The present finding was more or less in agreement with those of workers who have estimated heritability values in the Holstein breed for fertility traits (Averill *et al*. 2004; Rahbarr, 2016). In the case of fertility traits, the range of heritability was reported as 0.02 to 0.07(Rahbar, 2016). As shown in Table 3, estimates of additive genetic correlation varied from 0.26 for CR and INS to 0.92 for NRR90F and CR. Sun *et al*. (2009) estimated very low heritability for non-return rate and number of insemination to conception in Holstein cows. Wall *et al*. (2003) also reported very low estimates for non-return rate

Table 3: Estimation of Variance components for non-return rate at 90 days, conception rate and Number of insemination to conception.

Parameters	NRR90F (Non-return rate 90 days)	CR (Conception rate)	INS (Number of insemination to conception)
Sire variance	0.01274±0.00859	0.02364±0.01499	0.07758±0.06989
Additive genetic variance	0.05096	0.09456	0.31032
Phenotypic variance	3.34086	3.38446	3.60022
Heritability	0.015254	0.027939	0.086195
Genetic correlation			
NRR90F	1	0.92	0.48
CR	0.92	1	0.26

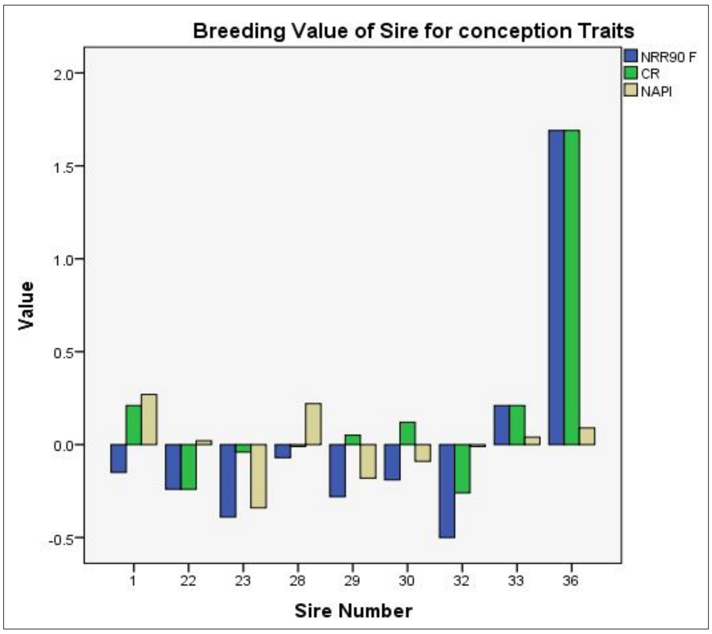

Fig 1: BreedingValue of siresforNRR90F, CR and INS.

after 56 days and number of inseminations to conception in dairy cows using maternal grandsire model. Heritability estimates of heifer conception rate on first service were 0.005 from the linear model and 0.01 from the threshold model in Holstein cattle (Kuhn *et al.,* 2006). The low heritability in this study suggested that improvement in success rate to conception could be achieved by improving the reproductive management. The high and positive correlation among NRR90F, CR and INS in this study suggests that these traits are genetically equivalent and influenced by the same genes. Integration of information related to these traits may be more effective for efficient selection strategies.

Multi-trait sire evaluation

The breeding values using best linear unbiased predictor method (BLUP) formulti-traits (NRR90F, CR and INS) were obtained duriring GLIMMIX analysis. Breeding valuesofeach traitand corresponding ranking are presentedin Fig 1. Its howed wider range of variation in breeding value for all indicator traits. The percent bullabove mean BV were found as 22.22% for NRR90F, 55.55% for CR and INS in Gir cattle population. Theranking of BV among Girbulls for NRR 90F, CR and INS traits was very high and aproaching to unity. It can be inferred from the present study that earlier observed trait NRR90 F could be used for evaluation of sire for successful insemination traits and this could in turn saves the cost of production.

CONCLUSION

The present study was conducted for evaluation of conception ratein GirCattle in semi arid region andit was estimated as 0.35fornon return rate at 90 days. Conception traits were affected by non genetic factors and summer season was considered as favorable season for successful artificial in semination and it showed highest odd ratio and estimated as 1.213 for NRR90F, 1.388 for CR and 1.440 for INS with reference to autumn season. Heritability estimates for the conception traits were in the range of low to very low and improvement of conception could be achieved through standardizing the herd for optimum reproductive performance. Theselection of bulls based on the breeding value of early expressed conception trait (NRR90 F) showed the positive and high correlation with other conception trait in the studyand selection of sire on the basis of early conception trait could in turn saves the cost of production.

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Competing interest

The authors declare no competing interests.

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