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In-depth Pedigree Analysis and Inbreeding Effect on Reproduction Traits in Sahiwal Nucleus Herd

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ABSTRACT

Background: Loss of genetic diversity is allied with adverse incidents such as inbreeding depression in fitness related traits, addiction of favorable alleles and enhances fluctuation on selection response. Therefore, the study aims to monitor population structure and avoid unfavorable effects of inbreeding for sustainable improvement in breeding programme.

Methods: Pedigree information on 3449 animals born from 1955-2018 was analyzed. The effect on inbreeding on Age at first calving (AFC), Service period (SP), Daughter pregnancy rate (DPR), Calving interval (CI) and pregnancy rate was seen by regression analysis.

Result: The pedigree completeness index (PCI) were found to be (%) 92.32, 80.26, 65.22, 49.43% and 32.54 respectively from first to fifth generations. The estimated average inbreeding rate was 2.30% and average genetic diversity loss was 2.64%. Study also revealed significant effect of inbreeding on (AFC, CI and SP) with slightly increasing trends. Hence, it is suggested that more precise pedigree recording and planned mating strategies should be adopted to avoid negative effect of inbreeding in future generation.

Key words: Genetic diversity, Inbreeding, Pedigree analysis, Population structure, Sahiwal cattle.

INTRODUCTION

Today the whole world animal breeder worldwide we are talking about towards the genomic selection to start it the basic requirement, needs a very strong pedigree information and population structure for development of reference population to full filled this the following study were undertaken to open that burnt door towards under population structure of Sahiwal cattle taking ICAR-NDRI, Karnal herd as a model. Sahiwal is one of the best milch breed of India known for their higher milk production. These breeds are maintained since more than four decades in NDRI, with systematic recordings of all the performance traits. Lack of pedigree knowledge of ancestors and introduction of purchased animals especially from field resulted into in accurate estimation of in breeding coefficient and other important parameters (Goyache *et al*., 2003), leading to misinterpretation of genetic structure of herd.

Modern animal breeding practice is mainly focused on the precise evaluation of breeding values as well as the adoption of advanced reproduction technologies; lead to rapid genetic improvement but chances of inbreeding also increases simultaneously by selecting only few superior individuals or families inbreeding programme (Weigel, 2001). However, it is also to keep in mind that inbreeding if not affecting the performance traits, then also monitoring is needed with cautious planning so as not to cross the threshold level. The selection of Sahiwal germplasm or bull based on the different parameter of population structure and genetic diversity will lead to more response to selection followed by more improved and increase genetic trend and ultimately more genetic gain per year in the ongoing breeding goal.

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MATERIALS AND METHODS

Pedigree data

The compete pedigree include 3449 animals born between (1955-2018) of Sahiwal cattle collected from record room of Animal Genetics and Breeding Division. The software programs ENDOG version 4.6. (Gutiérrez and Goyache, 2005) was utilized in generating these parameter.

Pedigree completeness index (PCI)

It was computed for each individual according to (MacCluer *et al*., 1983) by using following formulas:

$$I_d = \frac{4\, I_{dpat}\, I_{dmat}}{I_{dpat} + I_{dmat}} \qquad I_{dk} = \left(\frac{1}{d}\right) \sum_{i=1}^{d} a_i$$

Where,

k = Maternal or paternal line.

a_i = Known ancestors proportion in i^{th} generation.

d = Generation considered for pedigree completeness.

Inbreeding coefficient (F_x)

It was calculated using the formula as modified by (González-Recio *et al*., 2007 and Gutiérrez *et al*., 2009).

$$\Delta F_i = 1 - \sqrt[t_i - 1]{1 - F_i}$$

Where,
F_i= Inbreeding coefficient for the individual
i and t_i= Equivalent complete generations for this individual.

Average relatedness coefficient (AR)

It is defined as the probability that an allele randomly chosen from the whole population in the pedigree belongs to a given animal. It interpreted as the representation of the animal in the whole pedigree regardless of knowledge of its own pedigree, calculated by c' = (1/n) 1 'A, with A being the numerator relationship matrix (Henderson, 1976) 1 is a vector of one's (1 x n) matrix.

Effective population size (N_e)

Calculated by two methods first based on individual inbreeding rate (N_eF_i) (Henderson, 1976).

$$N_eF_i = \frac{1}{2\Delta F}$$

and second based on the effective population size (N_eC_i) from increase in coancestry (ΔC_{ij}) for all pairs of individuals i and j (Cervantes *et al*., 2011)

$$\Delta C_{ij} = \sqrt[(t_i - t_j)/2]{1 - C_{ij}}$$

Where
C_{ij} is the coancestry coefficient between the individuals i and j and t_i and t_j are their equivalent complete generations.

Generation interval (GI)

The generation interval was defined as average age of parents at the birth of their progeny that were subsequently kept for reproduction. It was calculated by four selection pathways; sire-son (SS), sire-daughter (SD), dam-son (DS) and dam-daughter (DD).

Effective number of founders

The effective number of founder was obtained to evaluate the concentration of the origin of both animals and genes. It is defined as the number of equally contributing founders that would be expected to produce the same genetic diversity as observed in the population under study (Lacy, 1989) computed as:

$$f_e = \frac{1}{\left[\sum_{k=1}^{f} q_k^2\right]}$$

Where,
qk is the expected proportional genetic contribution of founder k, calculated by the average relationship of the founder to each animal in the current population and f is the total number of founders.

Effective number of ancestors

This parameter complements the information offered by the f_e by accounting for the losses of genetic variability caused by the unbalanced use of reproductive individuals producing bottlenecks, calculated as minimum number of animals (founders or non-founders) those are necessary to explain the complete genetic diversity of the study population.

Founder genome equivalents

It can be defined as the number of founders that would be expected to produce the same genetic diversity as observed in the population under study if the founders were equally represented and no loss of alleles occurred (Ballou and Lacy, 1995).

The f_{ge} was calculated using the method proposed by (Caballero and Toro 2000) and calculated as:

$$f_{ge} = \frac{1}{2 f_g}$$

Where,
f_g = Average coancestry of the individuals included in the reference population.

Genetic diversity (GD)

The GD in the reference population accounting for loss of diversity due to genetic drift and unequal founder contribution was calculated by given formula (Lacy, 1995).

$$GD = 1 - \frac{1}{2 f_{ge}}$$

Effects of inbreeding on performance traits

The first lactation reproduction performance data spread over a period of 53 years (1966-2018) of Sahiwal cattle collected. The following traits were studied AFC, SP, DP, CI and pregnancy rate. The data were classified into five inbreeding groups (Table 1).

The following regression model was used to study the effect of inbreeding on first lactation reproductive traits as follows:

$$Y_{ij} = a + bX_i + e_{ij}$$

Where,
Y_i is reproduction trait of j^{th} cow with intercept as 'a' and regression coefficient as 'b' having i^{th} inbreeding coefficient value as 'x_i' and Random error as e_{ij} (NID 0, σ2).

RESULTS AND DISCUSSION

Pedigree completeness index

Current study shown that the pedigree data for the Sahiwal cattle was well maintained to acquire significant information regarding population structure of the breed. The proportionate contribution of parents, grandparents (GP) and great grandparents (GGP) (Fig 1) indicating low estimate of PCI in the earlier generation due to unavailable of pedigree records at the earlier generation. It was higher in the dam pathway (94.67%) as compared to sire pathway (89.97%) while considering the recent generation (Table 2). The PCI exhibited an increasing trend over the generations (Fig 2) similar to earlier estimates reported by Muasya *et al*. (2011) in Sahiwal and Santana *et al*. (2014)

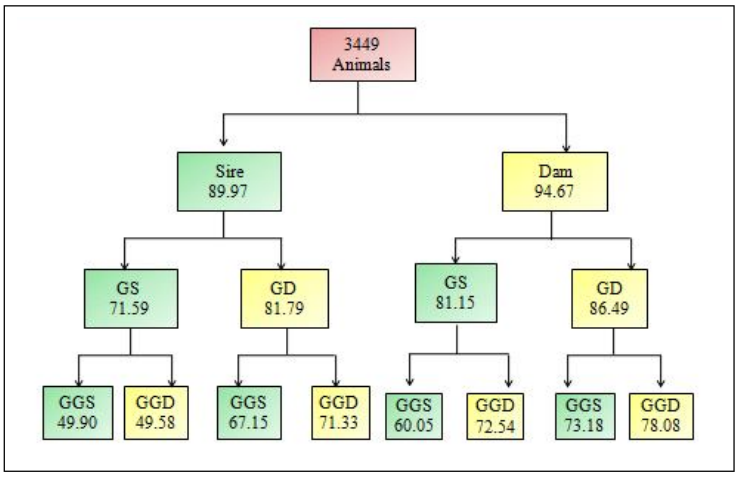


Fig 1: Proportionate of parent, grandparents and great grandparents in Sahiwal cattle.

Table 1: Classification of inbreeding coefficient.

Inbreeding group	Code given
0	1
>0 - <1.25	2
>1.25 - <5	3
>5 - <10	4
>10	5

Table 2: Description of pedigree since inception in Sahiwal cattle breed of India.

Parameter	Values
Data	1955-2018
Number of animals	3449
Male	1737
Female	1712
Individuals had known parent (%)	89.10
1st Generation	92.32
2nd Generation	80.26
3rd Generation	65.22
4th Generation	49.43
5th Generation	32.54
Mean maximum generations	7.79
Mean complete generations	1.87
Mean equivalent generations	3.51

in Brazilian Gir. Importantly, the pedigree information of dam line in Sahiwal is more inclusive than sire line due to more retention of females in the herd to produce milk. Low pedigree information of paternal ancestors due to frequent use of few sires considerably hinders the knowledge of descendent. It was observed that up to sixth generations more than 7% ancestors were known. However, after the eighth generations, very small number of individuals had known pedigree, creating the intricacy in obtaining information beyond that. The knowledge of pedigree to best identified generations is very crucial to have precise estimates of inbreeding, gene flow, *etc*.

Inbreeding coefficient and average relatedness

The F_x and AR values of whole pedigree population (Fig 3). The highest inbred mating was calculated and it was found that two (0.06%) mating were between full sibs; seventy seven (2.23%) were between half sibs; and fourteen (0.41%) mating were between parent-offspring. There were total of 1299 (37.66%) inbred animals (Fig 4) with wide range of inbreeding level (0.1-32.57%) was reported in the breed with 23.16% of the inbred animals having inbreeding levels less than 1% (Table 3). There was general increase in inbreeding at an annual rate of 0.38% per generation, much lower than the recommended level of 1% by (FAO, 1998) however is higher than report of (Muasya *et al*., 2011) in Sahiwal cattle of Kenya. The average F_x are similar to earlier reports of Rege and Wakhungu, (1992) in Sahiwal cattle in Kenya. However, lower estimates than the present value was reported by Muasya *et al*. (2011) and Kamiti *et al*. (2016). The AR in herd attains 1.1% (Fig 5) three years before the inbreeding accessible between individuals indicating AR could be used to predict future inbreeding. In our study, the mean percentage of inbred sires and dams was not more than 10%, a fact that may not rapidly lead to an increase of the average level of F_x in the population if constant mating structure is intended in future. Immediate changes were observed in percentage of inbred sires and dams between 2009 and 2015. This could be due to the changes in the number of equivalent generations.

Effective population size

The population effective size is characterized by the number of individuals that would give rise to calculated rate of inbreeding if they breed in a manner of an idealize population. The obtain result of estimate of realized effective population size was 50.01±1.10 (Table 4), a value considered to be low due to intra herd breeding policy. This estimate was little lower than the estimate of 94 by Santana *et al*. (2014) for Gir cattle of Brazillian. It is important to note that although the current estimated N_e reported in Sahiwal is within the limits as recommended, however estimates of N_e

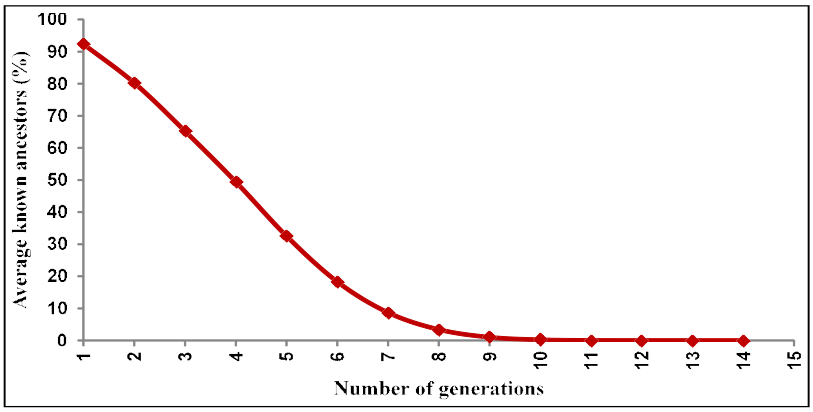


Fig 2: Average known ancestors (%) per generation of Sahiwal cattle.

Table 3: Distribution of inbreeding level in Sahiwal cattle.

Inbreeding levels	Individuals	Inter class (%)	Intra inbred classes (%)
0	1299	37.66	-
0 > F≤1.0	498	14.44	23.16
2.0 > F ≤ 3.0	286	8.29	13.30
3.0 > F ≤ 4.0	323	9.37	15.02
4.0 > F ≤ 5	180	5.22	8.37
5.0 > F ≤ 10.0	389	11.28	18.09
F > 10.0	130	3.77	6.05

Table 4: Effective population size whole population of Sahiwal cattle.

	Average
N_e[1]	
N_eC_i[2]	50.01 ± 1.10
N_eF_i[3]	51.23 ± 7.26
N_eC_i/N_eF_i ratio	0.97

[1] Effective population size; [2] Individual increase in co ancestry; [3] Individual increase in inbreeding.

Table 5: Generation interval (years) for four selection pathways.

Path	Number of animals	Generation interval
Sire to bull	100	9.64 ± 1.09
Sire to bow	886	9.0 ± 0.30
Dam to bull	106	7.01 ± 0.30
Dam to bow	936	5.55 ± 0.08
Total	2028	7.79 ± 0.16

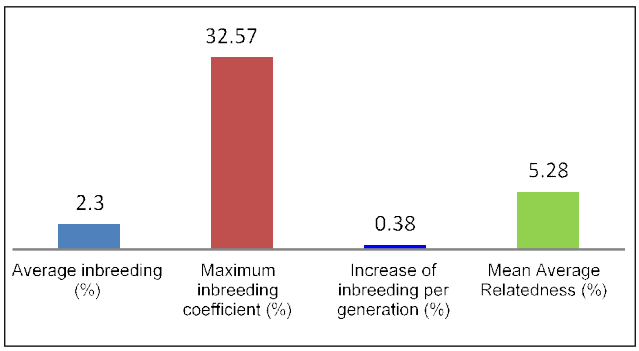


Fig 3: Average inbreeding coefficient and average relatedness of Sahiwal cattle.

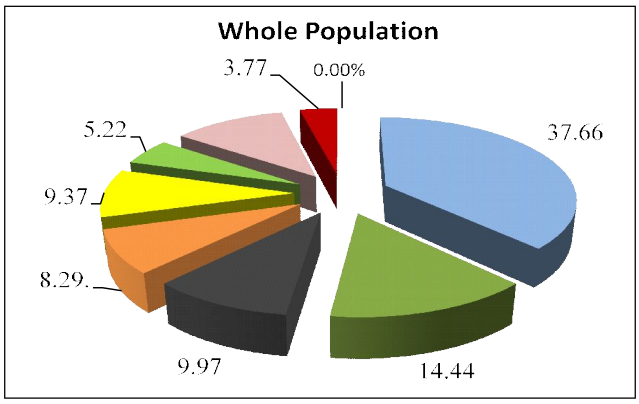


Fig 4: Distribution of inbreeding level (%) in Sahiwal cattle breed of India.

are not constant and do change with time according to change in inbreeding level of the population. Based on the effective population sizes and its ratio (N_eC_i/N_eF_i) indicated, population is still highly structured. According to the FAO (1992), the loss of genetic diversity over 10 generations is around 18, 10, 4, 1.6 and 0.8% when the N_e is 25, 50, 125, 250 and 500, respectively. Therefore, depth of the pedigree and the monitoring of the effective size are suggested.

Generation interval

The paternal interval being larger than maternal interval (Table 5) due to the time taken in progeny tested bulls. Similar estimate of GI as 7.2 years have been reported by Parveen, (2016) for Sahiwal breed. Muasya *et al.* (2011) reported generation interval of 6.9 years and longer for the sire-son pathway reported by of Sahiwal breed in Kenya. Kamiti *et al*. (2016) and Faria *et al*. (2009) reported the mean GI for the total Sahiwal cattle population was 6.3 and about 8 years respectively. The shorter generation intervals especially of females are explained by the use of young female and early replacement of older ones in breeding programme. It is an important parameter for sustainable genetic improvement because it directly influences the annual genetic

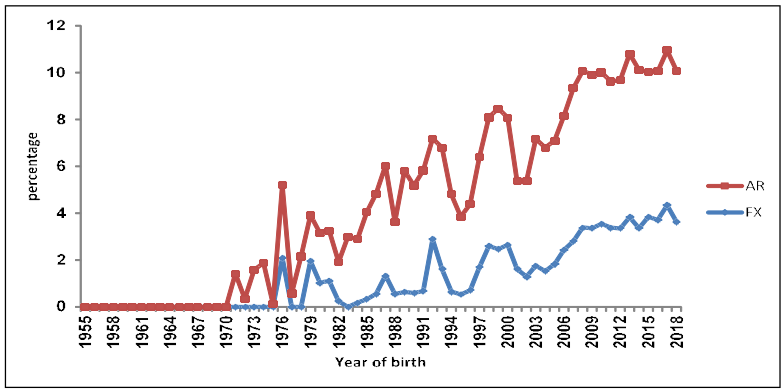


Fig 5: Trends of inbreeding and Average relatedness coefficient in Sahiwal cattle.

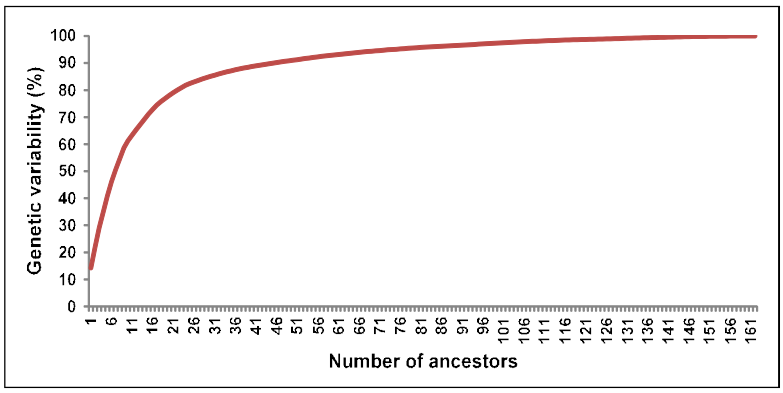


Fig 6: Genetic variability (%) in the population as per number of ancestors.

gain in selected traits. The higher GI the lower genetic gain which may represent an increase in the genetic lag.

Genetic diversity

The evolution of the number of founders accounting for different percent of populations (Fig 6). However, f_a/f_e (Table 6) deviation in genetically improving herd it is common since selective breeding is practiced and utmost care have been given to maintain the optimum level of inbreeding and diversity, thus even the ratio is quite low does not lead to bottle neck condition. Silva *et al*. (2016) reported higher (f_e= 473 and f_a= 471) in the Holstein breed in Brazil. The difference between f_a and f_e was due to unbalance utilize of ancestors and founders in the reference population. This condition occurs due to a disproportionate use of few breedable animals, especially superior sires (as regular practice in breeding programme).

Loss of genetic diversity occurred to a lesser extent in our population was 2.64. The results are in agreement with Santana *et al*. (2014) observed loss 2.3% in the Gir breed.

Effects of inbreeding on reproduction traits in Sahiwal cattle

Table 7 indicating significant influence of inbreeding of economically important traits, except first lactation dry period and pregnancy rate. The regression analysis of the fertility traits indicated positive effects of inbreeding that is undesirable, since our breeding goal is to have high yielders

Table 6: Parameters characterizing the probability of gene origin in the Sahiwal cattle.

Parameters	Sahiwal
Number of animals in the reference population	1173
Number of founders (f)	188
Number of ancestors contributing to the reference population	162
Effective number of founders (f_e)	37
Effective number of ancestors (f_a)	20
f_e/f	0.20
F_a/f_e	0.54
Founder genome equivalent (f_{ge})	18.94
Number of ancestors explaining more than 50% Genetic variability	7
Genetic diversity (%)	97.36

Table 7: Effects of inbreeding on first lactation reproduction traits of Sahiwal cattle.

Traits (days)	Number of observations	Mean ± S.E.	Regression
AFC	694	1128.44 ± 5.56	1.48*
FSP	494	113.32 ± 5.34	1.54*
FDP	479	124.12 ± 3.60	1.31
FCI	494	443.34 ± 4.47	1.55*
PR (%)	456	79.24 ± 0.22	0.08

* Significant at p≤0.05.

with high reproductive efficiency. In the present study, reduction in performance due to inbreeding was not very high in most of the traits since the level of inbreeding was quite low in Sahiwal population. Although, a few inbred animal had inbreeding of more than 12.5 per cent, but majority of the population was not inbred. Every percent increase in inbreeding there was significant increase in AFC, SP and CI by 6.53, 0.52 and 2.51 days respectively.

CONCLUSION

This is the first-time study of population structure and genetic variability by pedigree analysis in *Bos indicus* of Indian origin. The study indicated that the current population is well balanced, low inbreeding and AR than the recommended level. Moreover, focus should also be given to improve population structure with emphasis to increase effective population size with shorter generation interval. In studied population founder effects were minimal and there was no indication of bottleneck effect. However, very few ancestors was explaining the genetic variation in the Sahiwal population, but not affecting the population structure. The effect of inbreeding on most of the traits is significant except for DP and pregnancy rate although, the difference between inbred and non-inbred animals was very small. Hence, it can be overcome by adopting proper breeding strategy in the herd and population should be managed with an affinity towards evasion of inbreeding in the offspring. This information assists to create reference population using genetic tools and a small step towards giant leap for genomic selection in indigenous cattle.

Conflict of interest

All authors declare that they have no conflict of interest.

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