Full Length Article

FACTORS AFFECTING SEMEN PRODUCTION TRAITS IN HOLSTEIN FREISIAN PUREBRED, HOLSTEIN FREISIAN CROSSBRED AND INDIGENOUS BULLS

Vrinda B. Ambike[1], R. Venkataramanan [2*], S.M.K. Karthickeyan[3], K.G. Tirumurugaan[4], Kaustubh G. Bhave[5] and Jayant Khadse[6]

Department of Animal Genetics and Breeding
Madras Veterinary College
Tamil Nadu Veterinary and Animal Sciences University
Chennai – 600 007, Tamil Nadu

ABSTRACT

Data on 1,64,962 ejaculates of 486 bulls maintained at BAIF (Bharatiya Agro Industries Foundation) Research Foundation was utilized for this study. Six semen production traits viz. ejaculate volume (EV- ml), sperm concentration (SC- 10^9/ml), initial sperm motility (ISM- %), post-thaw motility (PTM- %), total number of spermatozoa per ejaculate (TNS- 10^9/ejaculate) and the theoretical number of semen doses (TNSD) were included in the analysis. Effect of different factors like location, season, year, collection time, collection interval, ejaculation order, breed and age of the bulls was studied on the semen production traits and least-squares means were obtained for these fixed factors while adjusting for the random factors like bull and semen collector. Bayesian method using Gibbs sampling was used to obtain precise least-squares estimates along with 95 per cent posterior standard densities. Better yield was obtained in the early hours of the day before 7 AM, when the ambient temperatures were lower and the first ejaculate was better than the second ejaculate for all the traits. EV was highest when the bulls were six years old, whereas, ISM gradually declined as the age advanced.

Keywords: Bayesian framework, Gibbs sampling, Repeatability model

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* Corresponding author: venkyvet@gmail.com
1 Postgraduate scholar
2 Associate Professor, Office of Vice-Chancellor, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai-600 051, Tamil Nadu, India
3 Professor and Head, Department of Animal Genetics and Breeding, MVC, Chennai - 600 007.
4 Director,Translational Research Platform for Veterinary Biologicals, TANUVAS, Chennai-600 051, Tamil Nadu, India
5 Thematic Program Executive, BAIF, Development Research Foundation, Uruli Kanchan, Pune- 412 202, India
6 Research Director, BAIF, Development Research Foundation, Uruli Kanchan, Pune- 412 202, India

INTRODUCTION

India has the largest cattle population in the world (https://dahd.nic.in/invest-india) but the average milk yield per cow is lower compared to other developed countries. Milk production traits are only included in most of the dairy development programmes where the bulls are chosen solely on their dams' milk-producing ability. A negative correlation between milk production and semen traits was reported by Hagiya *et al.* (2018), as a result of this, the semen traits deteriorate which could result in a decline in fertility.

Generally, the motility traits like initial sperm motility and post-thaw motility which are closely related to fertility have very low heritability estimates which suggest that there is a higher environmental influence on them. Semen production data is repeatable with thousands of records available on each bull which makes it voluminous. Bayesian framework using Gibbs sampling can aid in the analysis of such data. (Al-Kanaan *et al*., 2015). With this background, the current study was carried out to study the effect of different genetic and non-genetic factors on the semen production traits in Holstein Friesian (HF) purebred, Holstein Friesian(HF) crossbred and indigenous bulls reared under hot and semi-arid conditions of India.

MATERIALS AND METHODS

Location and climate

Information on semen production and abnormality was collected from Uruli Kanchan and Dharouli frozen semen stations of BAIF (Bharatiya Agro Industries Foundation), Pune, Maharashtra. The frozen semen station at Uruli Kanchan is situated at 18.5° N and 73.8° E at an altitude of 559 m above sea level at the outskirts of Pune and the frozen semen station at Dharouli is located in Haryana on the outskirts of Jind and is situated at 29.2° N and 76.2°E at an altitude of 227 m above sea level.

Data structure

Semen production data were available for 11 years (2010 to 2020) with records of 120, 177 and 189 HF purebred, HF crossbred and indigenous bulls respectively.The founder population was imported during 1967 to 1974 from Canada, USA, Denmark and Netherland, where these bulls were proven through progeny testing. Information on the location of semen station, bull's name, date of birth, ejaculate number, collector's name, date of collection, semen colour, ejaculate volume, sperm concentration, motility and number of straws produced were available.

Semen collection

Semen collection was done using a teaser bull and an artificial vagina when the bulls were sufficiently stimulated after two to three false mounts. The time between the two mounts differed between the bulls. One or two ejaculates were collected in a glass tube and stored at 37° C. Ejaculate volume was recorded directly and sperm concentration was estimated using a digital photometer (IMV technologies). Initial motility was assessed subjectively after semen dilution and 0.25 ml straws with 20×10^6 spermatozoa per straw were prepared. Sealed and printed semen straws were then cooled to 4° C for 3 hours followed by stepwise cooling to reach -140° C over a span of 7 to 8 minutes and then immersion stored in liquid Nitrogen at -196°C using a programmable freezer (IMV technologies). Post-thaw motility was also assessed subjectively 24 hours post-freezing using a phase-contrast microscope. The ejaculates which did not fulfil the minimum standards (https://www.nddb.coop) were discarded though the records have been included in the analysis.

Traits studied

Semen production traits that were directly available from the data were ejaculate volume (EV- ml), sperm concentration (SC- 10^9/ml), initial sperm motility (ISM- %) and post-thaw motility (PTM- %). From these traits, composite traits like the total number of spermatozoa per ejaculate (TNS- 10^9/ ejaculate) and the theoretical number of semen doses (TNSD) were calculated.

$$TNS = EV \times SC$$

$$TNSD = \frac{EV \times SC \times PTM}{10 \text{ million}}$$

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, while the effect of the semen collector and bull were included as random effects for all the traits. The fixed effects were location (Pune or Jind), order of ejaculate (first or second), the season of collection (summer, monsoon, or winter), the year of collection (2010 to 2020), age of the bull (≤2 to >10 years and each year in between), collection interval (≤2, 3, 4, ≥5days) and a collection time (5 AM to 6 AM, 6 AM to 7AM, 7 AM to 8 AM, 8 AM to 9 AM, 9 AM to 10 AM, 10 AM to 11 AM and 11 AM to 12 PM).

Statistical analysis

Markov Chain Monte Carlo (MCMC) procedure was used to obtain random samples using Gibbs sampling (Magnabosco *et al.*, 2000) from the marginal posterior distribution which results in the formation of chains for the parameters of interest.

Records beyond mean ± 4 SD were excluded before analysis as outliers to reduce the effect of sampling error. Exploratory and descriptive analysis of the traits was done using the "psych" package in R software.The univariate repeatability animal model was used for studying the effect and understanding the significance of genetic and non-genetic factors. The model can be represented as given below.

$$Y_{ijklmnopqr} = \mu + L_i + O_j + C_k + T_l + A_m + S_n + R_o + (S \times R)_{no} + W_p + U_q + e_{ijklmnopqr}$$

$Y_{ijklmnopqr}$	=	Semen production trait record
μ	=	Overall mean
L_i	=	Fixed effect of i^{th}location
O_j	=	Fixed effect of j^{th}order of ejaculate
C_k	=	Fixed effect of k^{th} collection interval
T_l	=	Fixed effect of l^{th} collection time
A_m	=	Fixed effect of m^{th} age class
S_n	=	Fixed effect of n^{th} season of semen collection

R_o	=	Fixed effect of o[th]year of semen collection
$(S \times R)_{no}$	=	Interaction effect between season and year of semen collection
W_p	=	Random effect of p[th] semen collector, NID (0, σ_s^2)
U_q	=	Random effect of q[th] bull, NID (0, σ_a^2)
$e_{ijklmnopqr}$	=	Random error associated with each record, NID (0, σ_e^2)

"MCMCglmm" package from the R software was used with default values.

RESULTS AND DISCUSSION

The descriptive analysis of the semen production traits in HF purebred, HF crossbred and Indigenous bulls have been summarized in Table I and the least-squares means of factors affecting semen production traits along with their 95% posterior densities have been summarized in Tables II, III and IV.

The significant factors like age, season and year of collection, collection interval, breed, bull and semen collector were in accordance with other studies (Everett *et al.,* 1978; Everett and Bean, 1982; Sarder, 2003; Boujenane and Boussaq, 2013; Bhakat *et al.,* 2011 Argiris *et al.,* 2018; Gopinathan *et al.,* 2018; Sontakke *et al.,* 2020; Bhave *et al.,* 2021) reported in HF purebred, HF crossbred and indigenous bulls. Only EV and ISM were not affected by location and breed.

In HF, similar EV and SC were reported by Ha *et al*. (2012) and Bhave (2021) in the tropics and by Mathevon *et al*. (1998) in Canada. ISM and PTM means were in close accordance with those obtained by Bhave (2021) and Seyoum *et al*. (2021). In HF crossbred bulls, EV, ISM and PTM were within the range of the estimates reported by earlier studies whereas SC and TNS were higher in the present study. The estimates for other traits from this study were higher compared to those reported by Sarder (2003) and Gopinathan *et al*. (2018) whereas they were similar to Bhave (2021) and Seyoum *et al*. (2021). TNSD in crossbreds was also higher in the present study compared to Sarder (2003). The means in indigenous bulls were lower compared to HF purebreds and crossbreds except for the motility traits which could also indicate better fertility in the indigenous bulls. Mandal *et al*. (2014) and Bhave *et al*. (2020) reported higher estimates in Gir bulls which could be due to combining several indigenous breeds under the same genetic group in the present study.

Ejaculate Volume gradually increased till 6 years of age after which it again showed a declining trend. The trend of higher EV at 6 to 7 years of age was also observed by Amann *et al.* (1974). Sperm Concentration (SC) was significantly affected by all the factors included and was lowest at extreme ages. Initial Sperm Motility (ISM) was inversely related to the age of the bulls as motility decreased as the age advanced, whereas, PTM did not show any specific pattern across different age groups of bulls. Total Number of Spermatozoa (TNS) and TNSD were lowest

Table 1: Descriptive statistics for semen production traits in HF purebred, HF crossbred and indigenous bulls

Traits	HF purebred				HF crossbred				Indigenous			
	N	Mean	SD	CV	N	Mean	SD	CV	N	Mean	SD	CV
EV (ml)	42866	6.40	2.10	32.81	62334	6.87	2.33	33.92	59591	5.76	2.03	35.24
SC (10^9/ml)	42862	1.39	0.56	40.29	62396	1.30	0.54	41.54	59311	1.34	0.64	47.76
ISM (%)	42582	74.18	12.61	16.70	61785	74.24	13.12	17.67	59147	74.64	11.8	15.81
PTM (%)	37139	55.98	3.31	5.91	53692	56.38	3.48	6.17	52042	56.10	2.90	5.17
TNS (10^9/ ejaculate)	42768	8.80	4.31	48.98	62243	8.74	4.30	49.20	59465	7.69	4.41	57.35
TNSD	37984	505.37	237.59	47.01	55008	509.28	241.75	47.47	52916	446.79	243.07	54.40

N: Number of ejaculates, EV: Ejaculate volume (ml), SC: Sperm concentration (10^9/ml), ISM: Initial sperm motility (%), PTM: Post-thaw motility (%), TNS: Total number of spermatozoa (10^9/ejaculate), TNSD: Theoretical number of semen doses, SD: Standard deviation, CV: Coefficient of variation.

Table 2: Least-squares means for location, breed and age along with lower and upper 95 per cent posterior densities for semen production traits HF purebred, HF crossbred and indigenous bulls

Trait	EV			SC			ISM			PTM			TNS			TNSD		
Fixed effects	Mean	Lower HPD	Upper HPD	Mean	Lower HPD	Upper HPD	Mean	Lower HPD	Upper HPD	Mean	Lower HPD	Upper HPD	Mean	Lower HPD	Upper HPD	Mean	Lower HPD	Upper HPD
Location		NS			**			**			**			**			**	
Pune (n=109977-128419)	5.74	5.52	5.98	1.25	1.20	1.30	71.40	70.20	72.40	56.07	55.87	56.29	7.08	6.65	7.46	409.25	386.23	430.92
Jind (n= 32896-36413)	6.03	5.67	6.40	0.88	0.81	0.96	75.40	73.80	76.80	56.76	56.41	57.06	5.26	4.73	5.87	313.42	278.53	345.03
Breed		**			**			NS			**			*			**	
HF (n=37139-42866)	6.19	5.88	6.47	1.07	0.99	1.14	74.00	72.60	75.50	56.44	56.14	56.74	6.57	6.09	7.10	384.12	356.22	412.83
HF- cross (n=53692-62396)	6.50	6.24	6.76	0.95	0.88	1.00	73.10	71.90	74.40	56.72	56.47	56.99	6.10	5.68	6.63	362.09	336.35	392.02
Indigenous (n=52042-59591)	4.98	4.72	5.23	1.20	1.13	1.25	73.10	71.80	74.30	56.09	55.84	56.34	5.83	5.35	6.26	337.95	313.87	365.47
Age		**			**			**			**			**			**	
≤2 years (n=12046-13707)	5.08	4.84	5.28	1.04	0.99	1.09	75.60	74.60	76.60	56.48	56.30	56.70	5.00	4.59	5.36	292.63	271.49	313.07
3 years (n=25669-29535)	5.61	5.38	5.83	1.07	1.02	1.12	75.00	74.00	75.90	56.49	56.29	56.69	5.91	5.51	6.26	346.68	325.54	366.46
4 years (n=26975-31091)	5.95	5.73	6.18	1.06	1.10	1.10	74.40	73.40	75.40	56.6	56.40	56.78	6.24	5.86	6.61	364.76	342.28	384.24
5 years (n=20937-24307)	6.23	5.99	6.45	1.05	1.00	1.10	73.80	72.80	74.80	56.61	56.42	56.82	6.53	6.12	6.86	381.13	358.30	400.56
6 years (n=17563-20369)	6.42	6.19	6.66	1.06	1.01	1.11	73.60	72.60	74.60	56.53	56.32	56.74	6.75	6.30	7.09	396.29	373.91	417.23
7 years (n=13912-16202)	6.26	6.02	6.49	1.06	1.01	1.11	73.20	72.20	74.30	56.42	56.20	56.62	6.60	6.23	7.03	382.62	360.35	405.04
8 years (n=9379-10783)	6.11	5.86	6.33	1.07	1.02	1.12	72.80	71.60	73.80	56.35	56.14	56.58	6.50	6.11	6.93	380.79	357.66	403.89
9 years (n=5708-6639)	5.90	5.65	6.13	1.12	1.06	1.17	71.80	70.70	73.00	56.25	56.02	56.51	6.42	5.98	6.83	376.69	350.33	399.93
10 years (n=3913-4402)	5.84	5.59	6.10	1.10	1.04	1.15	72.00	70.80	73.20	56.36	56.08	56.62	6.29	5.84	6.73	371.76	346.87	397.11
≥11 years (n=6771-7766)	5.54	5.26	5.80	1.04	0.98	1.10	71.80	70.60	73.20	56.05	55.80	56.38	5.45	4.97	5.88	319.31	293.57	348.10

n: Number of records, EV: Ejaculate volume (ml), SC: Sperm concentration (10^9/ml), ISM: Initial sperm motility (%), PTM: Post-thaw motility (%), TNS: Total number of spermatozoa (10^9/ejaculate), TNSD: Theoretical number of semen doses, HPD: 95 per cent highest posterior density, NS: Not significant, * :significant at $P<0.05$, ** :significant at $P<0.01$

Table 3: Least-squares means for season and year along with lower and upper 95 per cent posterior densities for semen production traitsHF purebred, HF crossbred and indigenous bulls

Trait	EV			SC			ISM			PTM			TNS			TNSD		
Fixed effects	Mean	Lower HPD	Upper HPD	Mean	Lower HPD	Upper HPD	Mean	Lower HPD	Upper HPD	Mean	Lower HPD	Upper HPD	Mean	Lower HPD	Upper HPD	Mean	Lower HPD	Upper HPD
Season		**			**			**			**			**			**	
Summer (n=42234-48983)	5.98	5.74	6.20	1.07	1.02	1.12	73.30	72.30	74.40	56.35	56.17	56.58	6.30	5.88	6.66	368.41	343.88	388.69
Monsoon (n=51804-60150)	5.84	5.60	6.06	1.05	1.00	1.10	73.30	72.30	74.30	56.51	56.32	56.74	6.01	5.61	6.39	353.55	332.23	376.24
Winter (n=48835-55658)	5.87	5.63	6.08	1.08	1.03	1.13	73.60	72.70	74.70	56.38	56.15	56.58	6.20	5.81	6.58	361.91	338.91	383.39
Year		**			**			**			**			**			**	
2010 (n=3077-3645)	6.22	5.97	6.50	0.94	0.88	1.00	78.80	77.70	80.20	56.72	56.41	57.00	5.80	5.34	6.25	343.35	315.30	369.27
2011 (n=8137-9093)	6.26	6.00	6.50	0.94	0.88	1.00	77.30	76.20	78.60	56.68	56.41	56.95	5.81	5.37	6.26	347.12	322.24	373.87
2012 (n=7906-9220)	5.99	5.71	6.21	0.92	0.86	0.97	73.90	72.80	75.10	57.49	57.25	57.74	5.24	4.85	5.69	324.71	298.35	347.19
2013(n=8777-10109)	5.61	5.35	5.84	1.11	1.06	1.17	71.00	69.80	72.10	57.22	56.98	57.45	6.02	5.64	6.48	361.72	338.12	386.81
2014 (n=11116-13059)	5.55	5.29	5.76	1.13	1.08	1.18	71.30	70.30	72.40	55.99	55.76	56.21	6.10	5.71	6.51	350.28	329.37	375.15
2015 (n=16412-18298)	5.69	5.46	5.91	1.13	1.08	1.17	73.90	72.90	75.00	55.99	55.77	56.20	6.32	5.91	6.70	363.55	339.65	384.55
2016 (n=22457-25895)	5.61	5.37	5.82	1.08	1.03	1.13	72.70	71.70	73.70	55.85	55.64	56.05	5.94	5.53	6.29	345.13	322.94	365.96
2017 (n=17789-20571)	5.94	5.71	6.15	1.10	1.06	1.16	72.20	71.20	73.20	55.43	55.22	55.62	6.49	6.11	6.86	368.74	347.12	389.30
2018 (n=17300-20780)	6.04	5.77	6.23	1.09	1.04	1.14	71.10	70.00	72.10	56.07	55.87	56.26	6.52	6.09	6.86	374.57	353.39	395.28
2019 (n=16702-19219)	5.88	5.64	6.09	1.22	1.17	1.26	72.00	71.10	73.10	56.80	56.63	57.02	7.04	6.63	7.39	415.91	395.08	436.31
2020 (n=13200-14971)	6.04	5.81	6.26	1.10	1.05	1.14	73.10	71.90	74.00	56.33	56.13	56.54	6.58	6.14	6.94	380.21	357.38	400.27

n: Number of records, EV: Ejaculate volume (ml), SC: Sperm concentration (10^9/ml), ISM: Initial sperm motility (%), PTM: Post-thaw motility (%), TNS: Total number of spermatozoa (10^9/ejaculate), TNSD: Theoretical number of semen doses, HPD: 95 per cent highest posterior density, NS: Not significant, * :significant at P<0.05, ** :significant at P<0.01

at extreme ages similar to SC and maximum at six years of age like that of EV. But, the differences in the production levels according to the age of the bulls were not very high, which could be due to the routine culling practices followed at BAIF which ensured that optimum production levels were maintained. The traits showed higher production before 7 AM, which declined gradually as the ambient temperature increased, which shows the effect of environmental temperature on semen production.

Al-Kanaan *et al.* (2015) have also demonstrated higher environmental temperatures had detrimental effects on semen production traits. This could also be the reason why means of SC, ISM and PTM were higher during the winter season. Boujenane and Boussaq (2013) also reported higher estimates during the winter months in Morocco, whereas, higher estimates were reported by Murphy*et al.* (2018) in the USA during the summer months which provided a more comfortable climate compared to the winter months. The results of lower EV and SC in the second ejaculate concur with Everett *et al.* (1978) though they reported no significant difference between the motility traits. The difference in the motility estimates due to the order of the ejaculates was less than one per cent. This demonstrates how semen production can be increased by collecting semen twice without deteriorating the semen quality.

Theoretical Number of Semen doses was almost twice in the first ejaculate compared to the second but the overall number of straws generated was higher and so the collection of second ejaculate in bulls is justified (Murphy *et al.,* 2018). Collection interval of more than 5 days was better in all the traits though increasing the collection frequency maximizes the weekly output while having no detrimental effect on the productive and reproductive performance (Almquist, 1982) whereas Al-Kanaan *et al.* (2015) also reported impaired male fertility when the collection interval was less than 3 days.

Semen production traits were affected by non-genetic factors like location, age of the bulls at semen collection, season and year of collection, collection interval, time of semen collection, order of the ejaculate and breed of the bulls. Maintaining lower ambient temperature will enhance semen productivity and the collection of 2 ejaculates per day will be beneficial for increasing the semen output without deteriorating the semen quality.

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