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Effect of age, season and sire on semen quality traits in Frieswal breeding bulls

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ABSTRACT

The present study was undertaken to assess the effect of season of semen collection, age and sire on semen quality of Frieswal breeding bulls. The data on 8113 semen ejaculates of 155 bulls born to 34 Frieswal sires were utilized for the study. The effect of age of bulls on semen volume, sperm concentration (million) per ml and sperm motility (%) in fresh semen (directly after collection) was highly significant. Additionally, it was discovered that the sire effect of bulls on different semen quality parameters and the influence of season on sperm concentration and motility both were statistically significant. Heritability estimates of different semen quality traits studied were moderately high and exceptionally significant. For semen volume, the estimate was found to be 0.336±0.078 while for concentration and pre freezing sperm motility values were 0.296±0.068 and 0.407±0.089, respectively. From the results, it may be concluded that the age and sire of bulls significantly affected the semen volume, sperm concentration and sperm motility in Frieswal bulls. The season of semen collection caused significant variations in sperm concentration and motility but not the semen volume. The semen volume and concentration were superior in ejaculates collected during winter season while the summer season was conducive for increased sperm initial motility. The Frieswal bulls up to 4-5 years are able to produce semen of better quality. The moderately high heritability estimates of semen quality traits provide indication for genetic improvement of the traits through selection.

Keywords: Age, Fertility, Frieswal. Season, Semen quality

Selection of bulls for artificial insemination is one of the important aspects of dairy farming in order to achieve higher conception rate and milk yield in future generation. In our country, the indigenous cattle breeds are poor milk producers and hence the crossbreeding was initiated using the temperate dairy breeds like Holstein-Friesian and Jersey predominantly to produce the crossbred cattle with higher milk production. Frieswal is one of the important crossbred cattle developed by All India Coordinated Research Project on Cattle. With planned selection and mating strategies the lower grades were upgraded with pure HF and the higher grades were downgraded by using the Frieswal bulls to have a stabilized blood level of 62.5% Holstein-Friesian and 37.5% Sahiwal in Frieswal.

The fertility status of young bulls has significant impact on milk production and economy of cattle production. DeJarnette *et al.* (2004) reported that bull subfertility due to low semen quantity and poor semen quality causes reproductive failures. The significant impact of bull fertility on cattle production is also reported by Braundmeier and Miller (2001). The recent advancements in genomic selection facilitate the early and accurate selection of young bulls at an early age for achieving faster genetic progress by reducing the generation interval (Goddard and Hayes 2007). Barth *et al.* (2008) reported within and between breed variations in reproductive performance of young bulls.

The semen quality traits of bulls can be affected by a wide range of factors of which the age of bulls, season of semen collection and sire of bulls are proven to be the major factors (Fuerst-Waltl *et al.* 2006, Snoj *et al.,* 2013, Sirohi, *et al.* 2017). The bull fertility traits are low to moderately high heritable and hence bulls can be selected for the genetic improvement of these traits. Murphy *et al.* (2018) reported that the age of bulls in semen collection significantly affects semen volume, sperm concentration and pre freeze gross and total motility score. As reported by Nichi *et al.* (2006) the micro-environmental variations can also have impact on bull fertility.

Reports on the effect of season and age of bulls on semen quality parameters are available in different breeds of cattle (Mandal *et al.* 2000, Mathur *et al.* 2002, Mandal *et al.* 2010). But scanty information is available on Frieswal bulls as this breed is newly evolved. With the above facts in mind the present study was undertaken to assess the effect of season on semen collection, age and sire on some important semen quality parameters of Frieswal bulls.

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MATERIALS AND METHODS

Study location, animals and their management: The study was conducted at Male Germplasm Unit of ICAR-Central Institute for Research on Cattle, Meerut, India. For this study crossbred bulls (Frieswal: 5/8 Holstein-Friesian and 3/8 Sahiwal) of 1.5 to 7 years of age were kept in individual pens having covered and open area with equal floor space/bull in loose house sheds. All the bulls were managed and fed under standard farm conditions. They had free access to water, fed on 22-25 kg green fodder, 6-7 kg wheat straw and 3.5 kg of concentrate mixture containing 18% crude protein and 70% total digestible nutrients per head per day.

Semen collection and assessment: The semen samples were collected by artificial vagina technique twice a week from each bull during stay of these bulls for semen collection at semen station. The fresh semen ejaculates were subjected to evaluation for volume (ml), initial progressive sperm motility (%), sperm concentration ($\times 10^6$/ml). Initial progressive sperm motility was scored at 200× magnification with phase contrast microscope equipped with a warm stage. It was observed at four to five areas of the slide before recording of average values. The concentration of spermatozoa was measured with Accucell photometer (IMV Technologies, France). The fresh semen samples passed with minimum criteria for freezing were extended with egg yolk citrate diluter and further processed for freezing. The frozen semen samples were examined for post thaw sperm motility (PTM) after 24 h period of freezing.

Data collection and analysis: The data for present study were collected for the period from 2019 to 2021 from the semen collection records of the Semen Freezing Laboratory attached with the Male Germplasm Unit of the Institute. Data on 8113 semen ejaculates of 155 bulls born to 34 sires were utilized for the study. The whole year was divided into three seasons namely winter (November to February), summer (March to June) and rainy (July to October). According to the age, the bulls were divided into five groups as 1-2 years, 2-3 years, 3-4 years, 4-5 years and above 5 years. The data were initially tested using standard statistical tests in the univariate procedure of SAS (version 9.1.3; SAS Institute, Cary, NC, USA) to ensure the homogeneity of variance and normality. MIXED model analysis of SAS was performed by including the season of semen collection and the age of bulls as fixed factors and the effect of sire of bulls as random factor. Mean differences were estimated by F-test using the type III sums of squares. The pair wise comparisons of means were done by using Duncan's Multiple Range Test (DMRT) as proposed by Kramer (1957). The heritability estimates for different traits were also estimated using the method described by (Becker 1975). The model used for the analysis is as follows:

$$Y_{ijkl} = \mu + S_i + T_j + A_k + e_{ijkl}$$

Where, Y_{ijkl}, semen quality parameter; μ is the population mean; S_i, random effect of i^{th} sire; T_j, effect of season of semen collection (j=1,2 and 3); A_k, age of bull under semen collection (k = 1, 2, 3, 4 and 5) and e_{ijkl}, random error which is normally and independently distributed with a mean of zero and variance of $\sigma^2 e$.

RESULTS AND DISCUSSION

Effect of age of bulls on semen quality parameters: The results of the effect of various factors (ANOVA) are given in Table 1. The effect of age of bulls on various semen quality traits studied, viz. semen volume (ml), sperm concentration (million) per ml and pre-freezing initial sperm motility (%) were highly significant (P<0.001). The semen volume was lowest for the younger bulls of 1-2 years age. The overall average semen volume obtained in the present study was higher than the volume reported by Shanmugavel and Singh (2002) and Mandal *et al.* (2005). Contrary to this, Kumar *et al.* (2015) reported higher estimates of semen volume in pure and crossbred Jersey bulls, respectively. The semen volume increased with advancement in the age of bulls as it was the maximum for bulls at 4-5 years of age (Fig. 1a). For bulls of 5 years and above age group, the volume reduced slightly. The findings that the semen volume consistently increased with the advancement in the age are in accordance with the findings of earlier studies (Argiris *et al.* 2018, Brito *et al.* 2002, Bhakat *et al.* 2011, Murphy *et al.* 2018). The rate of increase was 0.204 ml from 1-2 to 2-3 years, 0.228 ml from 2-3 to 3-4 years and 0.123 ml from 3-4 to 4-5 years. As reported by earlier workers (Mathevon *et al.* 1998, Perumal 2014, Murphy *et al.* 2018), the peripubertal bulls had the least

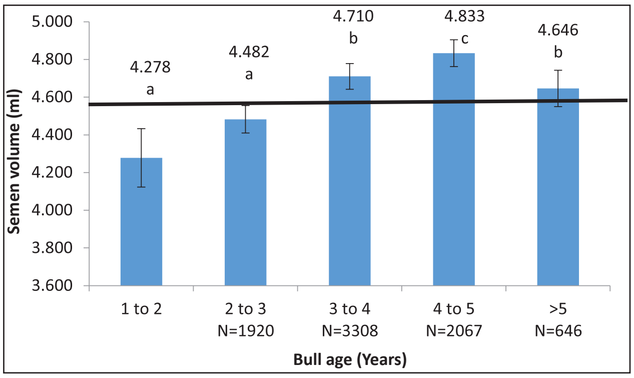

Fig. 1a. Effect of age of bull on semen volume (P<0.001).

Table 1. Effect of age of bull, season of collection and sire of bull on semen quality parameters in Frieswal cattle

Effect	Semen volume			Sperm concentration			Sperm initial motility		
	d.f.	MSS	Sig.	d.f.	MSS	Sig.	d.f.	MSS	Sig.
Age of bull	4	28.987	0.000	4	1343571.497	0.000	4	18056.059	0.000
Season of collection	2	6.854	0.125	2	5938296.578	0.000	2	4867.515	0.000
Sire of bull	31	71.848	0.000	33	4148641.575	0.000	33	8032.861	0.000

volume. The volume increase in the semen ejaculates with age advancement might be attributed to the increased hypothalamic-pituitary-testicular axis and the parallel development of testis and other accessory glands.

The results of sperm concentration revealed that the ejaculates from younger bulls has higher estimates. Thereafter, consistent reduction in sperm concentration was noticed in bulls as the age advanced and the lowest concentration was noticed in bulls of 4-5 years of age while the concentration increased in the bulls of above 5 years (Fig. 1b). The effect of age of bull on sperm concentration per ml of semen was highly significant (P<0.001) which is in accordance with the results reported by Murphy *et al.* (2018). Contrary to present findings, Ghosh (2004) and Bhakat *et al.* (2011) reported that age of bulls showed no effect on sperm concentration. The average sperm concentration obtained in the present study was lower than the values of 1171.01±56.09 and 1093.488±48.25 million/ml for pure and crossbred Jersey bulls reported by Kumar *et al.* (2015), respectively. However, the concentration estimate obtained in the present study is higher than the estimate reported by Bhakat *et al.* (2011) in Sahiwal bulls. The rate of decrease was 2.2% from 1-2 years to 2-3 years, 5.47% from 2-3 years to 3-4 years and 2.31% from 3-4 years to 4-5 year. The rate of increase from 4-5 years to 5-6 years was 2.06%.

The initial motility of sperm in pre freezing ejaculates showed increasing trend as the age advanced (Fig. 1c). In young bulls of 1-2 years of age, the initial motility was the least which increased in bulls of above 5 years of age. The average estimate for initial sperm motility in Frieswal bulls was 47.64% which was lower than the values reported by Singh *et al.* (2000), Mandal *et al.* (2005) and Bhakat *et al.* (2011) in Sahiwal cattle. The results of present study also revealed that the differences between different age groups of bulls were statistically significant (P<0.001). Similar to the present finding, Murphy *et al.* (2018) also reported significant effect of age of bulls on pre freezing gross motility in Holstein Friesian bulls. Further analysis revealed that the rate of increase in the initial motility was also increasing consistently as it was 3.75% between the age groups of 1-2 and 2-3 years, 5.95% between 2-3 to 3-4 years, 11.94% between 3-4 and 4-5 years and 10.07% between 4-5 and above 5 years of age. On the contrary, Birgit *et al.* (2006) reported inconsistent motility estimates having slight decreasing tendency with increase in age of the bulls.

Effect of season on semen quality parameters: The seasonal effect on semen volume was non-significant (P<0.125). However, the effect of season on sperm concentration (P<0.001) and initial motility (P<0.001) were highly significant (Fig. 2 a, b and c). Similar to the present finding, Mandal *et al.* (2005), Koivisto *et al.* (2009) and Murphy *et al.* (2018) also reported non-significant effect of season of collection on semen volume in Holstein Friesian cattle. Contrary to the present findings Bhakat *et al.* (2011) reported significant variations in the volume of semen collected during different seasons in Sahiwal bulls.

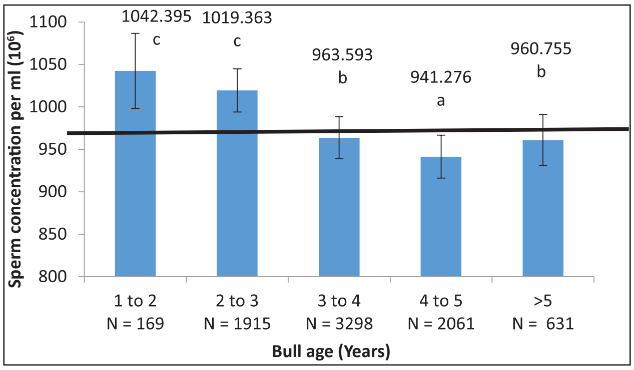

Fig. 1b. Effect of Age of bull on sperm concentration (P<0.001).

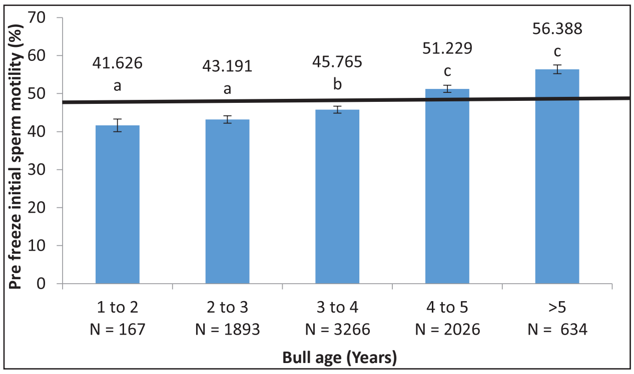

Fig. 1c. Effect of age of bull on pre-freezing initial sperm motility (P<0.001).

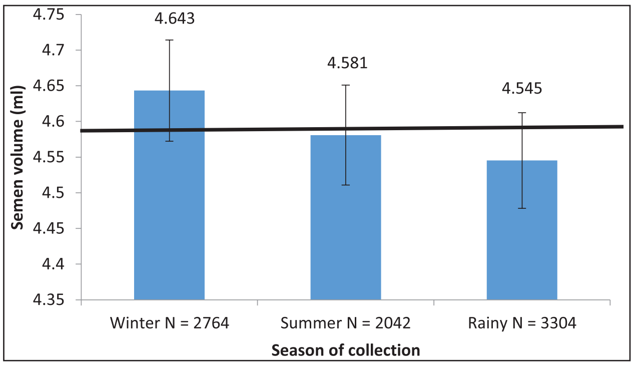

Fig. 2a. Effect of season of collection on semen volume (P<0.125 non-significant).

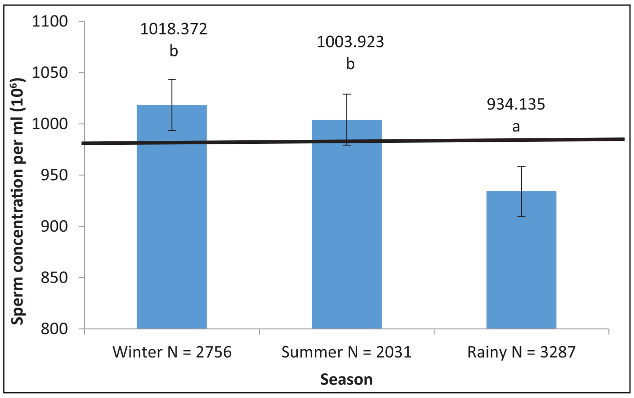

Fig. 2b. Effect of season of collection on sperm concentration (P<0.001).

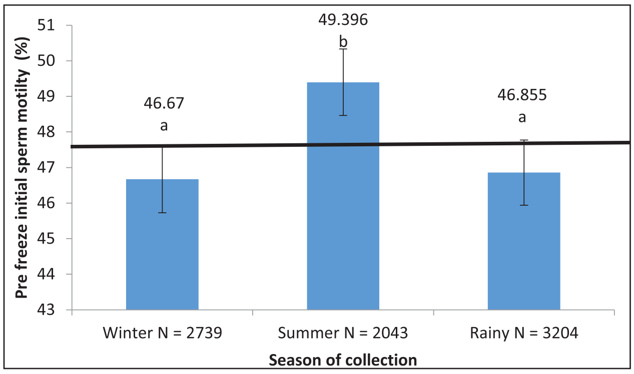

Fig. 2c. Effect of season of collection on initial sperm motility (P<0.001).

The semen volume was highest during the winter season and was least in the rainy season, however, the difference was not statistically significant. Bhakat (2011) reported highest semen volume during the rainy season and lowest volume during the winter season.

The seasonal variation in sperm concentration was found to be highly significant (P<0.001) as the estimates were higher in winter followed by summer and rainy seasons. The results obtained in the present study are in accordance with the results of Mandal *et al.* (2005) and Murphy *et al.* (2018) who reported significant effect of season of collection on sperm concentration. However, Bhakat *et al.* (2011) reported non-significant effect of season of semen collection on sperm concentration in Sahiwal cattle. The ejaculates collected during winter season had the highest sperm concentration while during the rainy season, the sperm concentration was the least. The sperm concentration of semen ejaculates collected during summer season was statistically similar to the concentration of ejaculates collected during winter season. However, Bhakat *et al.*

Table 2. Mean ± SE for semen volume, sperm concentration (10^6) and initial motility for sires of bulls

Sire No.	Semen volume (P<0.001)		Sperm concentration (10^6) / ml (P<0.001)		Pre-freeze initial sperm motility (%) (P<0.001)	
	N	Mean±SE	N	Mean±SE	N	Mean ± SE
Overall	8110	4.590±0.062	8074	985.477±23.462	7986	47.640±0.881
101	109	3.806±0.178	106	1363.49±47.899	107	59.063±1.787
102	210	4.219±0.133	209	1021.625±35.241	207	53.146±1.327
103	17	2.39±0.443	17	851.302±117.473	17	63.541±4.401
104	42	3.87±0.293	42	929.214±77.677	42	47.355±2.913
105	32	2.857±0.324	31	947.874±87.231	32	66.532±3.217
106	66	4.117±0.229	65	1190.252±61.108	66	69.055±2.273
107	48	4.663±0.275	48	1017.784±72.859	48	14.224±2.733
108	634	4.239 0.08	633	1047.345±21.084	625	56.511±0.795
109	196	4.97±0.139	196	858.165±36.815	194	48.844±1.387
110	1642	5.236±0.053	1639	872.995±14.056	1612	53.996±0.531
111	48	4.038±0.278	48	901.059±73.579	48	38.052±2.76
112	23	3.988±0.383	23	674.799±101.47	22	41.253±3.885
113	161	4.367±0.148	161	826.588±39.299	161	53.871±1.473
114	1135	4.893±0.06	1129	1090.245±16.046	1111	54.919±0.606
115	638	4.336 ± 0.079	636	1076.368±20.987	620	51.252±0.796
116	10	4.185±0.576	10	478.879±152.653	9	31.455±6.028
117	36	5.488±0.306	36	1611.382±81.132	36	22.676±3.039
118	146	6.307±0.155	146	782.886±41.145	146	48.017±1.542
119	7	4.048±0.689	7	1206.46±182.454	7	36.47±6.834
120	8	4.859±0.648	8	991.33±171.705	8	12.086±6.433
121	106	3.734±0.181	106	1145.852±48.043	102	47.427±1.833
122	296	5.891±0.112	296	1037.511±29.607	294	47.447±1.113
123	74	5.157±0.217	74	687.374±57.468	74	49.1±2.153
124	1290	5.303±0.063	1288	858.929±16.674	1276	53.932±0.628
125	-	-	1	826.275±481.833	1	59.497±18.048
126	37	5.127±0.312	37	882.442±82.653	37	59.111±3.100
127	6	7.331±0.747	6	966.538±197.927	6	20.343±7.415
128	-	-	2	984.217±340.645	2	55.419±12.759
129	140	4.881±0.16	139	1156.706±42.567	140	35.127±1.591
130	108	4.679±0.182	108	1066.903±48.13	104	62.338±1.835
131	606	5.024±0.084	605	952.421±22.192	595	60.375±0.839
132	182	4.928±0.15	166	1205.022±41.23	182	49.735±1.496
133	15	3.82±0.472	15	1281.821±124.902	15	61.005±4.679
134	42	4.122±0.286	41	714.15176.661	40	36.591±2.907

(2011) reported that the semen collected during the rainy season had the highest sperm concentration and the lowest was observed during the winter season. Murphy *et al.* (2018) also reported that the semen collected during winter season had the least sperm concentration. The variation in results of the effect of seasons on semen quality traits may be attributed to many factors like climatic conditions, humidity, ambient temperature, housing, feeding, etc. which may lead to variations in the findings of various studies reported.

The effect of season of collection on initial motility was highly significant (P<0.01) as the estimate was higher in semen collected during summer season (49.396%) while it was statistically similar during the winter (46.670%) and rainy (46.855%) seasons. The average initial sperm motility reported in the present study was lower than the values reported by Mandal *et al.* (2005) in Sahiwal cattle. Similar to the present findings, Bhakat *et al.* (2011) also reported the lowest initial motility in ejaculates collected during winter season.

Sire effect and heritability estimates of semen quality parameters: The sire effect of bulls on various semen quality parameters was highly significant (P<0.001). The semen volume for different sires ranged from 2.39±0.443 to 7.331±0.747 ml. Similarly, the sperm concentration ranged from 478.879±152.653 to 1611.382±81.132 (million) per ml of semen. The per cent sperm motility before freezing ranged from 12.086±6.433 to 69.055±2.273 (Table 2). The variations due to the sire effects on the semen quality parameters may be attributed to the variations in the genetic makeup of the sires and the variations in the average number of progenies per sire as it varied very widely.

The heritability estimates of different semen quality traits studied are presented in Fig. 3. The values depicted are moderately high and statistically highly significant (P<0.01). For semen volume the estimate was found to be 0.336±0.078 while for concentration and pre freezing sperm motility the values were 0.296±0.068 and 0.407±0.089, respectively. The heritability for semen volume obtained in the present study was higher than the values reported by Druet *et al.* (2009) and Suchocki and Syzda (2015) in Holstein breed of cattle. However, higher estimate of 0.65 was also reported by Ducrocq and Humblot (1995) in Normande cattle. The heritability estimate of sperm concentration obtained in the present study was lower than the values reported by Ducrocq and Humblot (1995) in Normande cattle, Mathevon *et al.* (1998) and Suchocki and Syzda (2015) in Holstein cattle which was higher than the values reported by Kaps *et al.* (2000) in Simmental cattle. The heritability estimate obtained for initial motility in the present study was higher than the values reported by Mathevon *et al.* (1998) and Suchocki and Syzda (2015) in Holstein breed of cattle. On the contrary, Druet *et al.* (2009) reported heritability estimate of 0.43 for the same trait in Holstein cattle. The variations in heritability estimates for different semen quality traits reported by different workers may be attributed to the genetic constitutions of different breeds studied, number of observations and the method of estimation of heritability. The moderately high heritability estimates obtained in the present study provide scope for their improvement through genetic selection of Frieswal bulls for these traits.

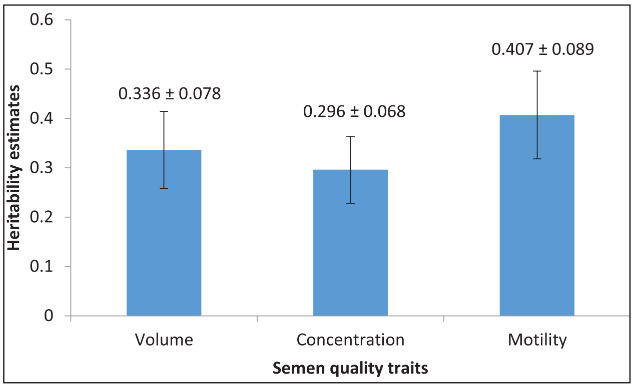

Fig. 3. Heritability estimates for different semen quality traits.

From the results of the present study, it may be concluded that the age and sire of bulls significantly affected the semen volume, sperm concentration and initial sperm motility in Frieswal bulls. The season of semen collection caused significant variations in sperm concentration and initial sperm motility but not the semen volume. The semen volume and initial motility of sperms increased with the increase in age and correspondingly the sperm concentration decreased. The semen volume and concentration were superior in ejaculates collected during winter season while the summer season was conducive for increased sperm initial motility. The Frieswal bulls up to 4-5 years are able to produce semen of better quality. The moderately high heritability estimates of semen quality traits provide indication for genetic improvement of the traits through selection.

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