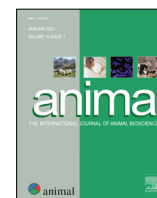




Animal

The international journal of animal biosciences



Estimates of non-genetic effects and genetic parameters for semen traits in Piemontese bulls



R. Rostellato^a, V. Bonfatti^{a,*}, V.A.D. Dias^b, S. Savoia^c, V. Spalenza^c, A. Albera^c, P. Carnier^a

^a Department of Comparative Biomedicine and Food Science, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy

^b Faculdade de Ciências Agrárias e Veterinárias (FCAV), Universidade Estadual Paulista, Via de Acesso Prof. Paulo Donato Castellane s/n, CEP 14884-900 Jaboticabal, SP, Brazil

^c Associazione Nazionale Allevatori Bovini di Razza Piemontese (Anaborapi), strada Trinità 32a, 12061 Carrù (CN), Italy

ARTICLE INFO

Article history:

Received 21 December 2020

Revised 21 May 2021

Accepted 27 May 2021

Available online 7 July 2021

Keywords:

Genetic parameters

Male fertility

Motility

Semen volume

Sperm concentration

ABSTRACT

Male reproductive performances are often ignored in cattle breeding programmes, although semen traits might be used to improve bull breeding soundness. Effects of genetic and environmental factors on semen production and quality traits were estimated in 693 Piemontese bulls with the aim of providing the first estimates of genetic parameters for semen traits for this breed. Volume and concentrations of individual ejaculates (up to three per each test-day), and volume, concentration, total number of spermatozoa and post-thawing progressive motility of within test-day pooled semen were available for 19 060 ejaculates. Bulls reached the maximum amount of daily semen production after their third year of age, with concentration rapidly increasing until 23 months of age, and then slowly decreasing. Semen volume was at its highest when collection days were at least 15 days apart, whereas the maximum concentration was reached when the interval was 6 days. Heritability estimates were generally moderate (0.14–0.26), and low for progressive motility (0.08). Estimates of genetic correlation among the volumes of the individual ejaculates were high and positive (≥ 0.79), as were the genetic correlations among their concentrations (≥ 0.46). Genetic correlations among volume and concentration traits varied from -0.47 (with a 95% high posterior density interval ranging from -0.65 to -0.23) to -0.32 (with a 95% high posterior density interval ranging from -0.55 to -0.09). Progressive motility was unrelated with the other traits, but moderately positively correlated with volumes of the second and third ejaculates. The magnitude of heritabilities showed that selection for semen traits is possible. However, the unfavourable relationship between volume and concentration must be taken into account if a future selection programme is to be established.

© 2021 The Author(s). Published by Elsevier B.V. on behalf of The Animal Consortium. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Implications

We investigated the influence of non-genetic factors and we estimated genetic parameters for ten traits related to volume and quality of semen sampled from Piemontese bulls. Age of bulls and the interval between two consecutive test-days affected semen characteristics. An antagonistic correlation was observed between semen volume and concentration, whereas progressive motility was unrelated with concentration, but moderately and positively correlated with semen volume. Moderate heritabilities were found for the investigated traits, suggesting that selective breeding for these traits is feasible in the Piemontese population.

Introduction

Improving reproductive performances is one of the major challenges in both dairy and beef cattle. Improvement of semen quality may allow breeding organizations to increase the success of artificial insemination (AI) (Christensen et al., 2005) and maximize the efficiency of the semen production process. However, while female fertility is included in most cattle breeding programmes, male reproductive traits are often ignored (Berry et al., 2011). Several environmental factors are known to influence semen production and quality, such as age of bulls, season, month and year of collection, interval between collections, number of ejaculates, bull handler and semen collector (Mathevon et al., 1998; Fuerst-Waltl et al., 2006). Besides these factors, differences across breeds were also reported (Boujenane and Boussaïq, 2014) and scientific evidence suggests that male reproductive traits are generally

* Corresponding author.

E-mail address: valentina.bonfatti@unipd.it (V. Bonfatti).

moderately heritable, but wide variation in the genetic parameter estimates across populations exists (Berry et al., 2019).

In Italy, the current focus of the breeding goal for the Piemontese cattle population is the improvement of beef traits (i.e., daily gain and live fleshiness) and calving ease (Albera et al., 2004a). Purebred Piemontese bulls enrolled in AI are selected after a two-stage testing programme. In the first stage, young bulls (pres-selected according to their pedigree index) are selected on the basis of their breeding value for beef traits recorded in a central station testing programme which lasts on average 11 months (Albera et al., 2001). These bulls are then enrolled in a progeny testing programme for calving performance (second stage) where genetic evaluations are based on birth records of their progeny (Albera et al., 2004b). After the first stage selection, the bulls enter the AI station of the Italian Association of Piemontese Cattle Breeders (Anaborapi, Carrù, Italy), which is responsible for the collection, processing and distribution of semen for progeny testing and for all AI service sires. On average, the first and second stages take approximately 33 months.

Male fertility traits are not yet considered in the breeding programme, although nearly 50% of the inseminations are performed artificially and a large fraction of natural service sires is progeny or grand-progeny of AI sires (Bonfatti et al., 2013). Furthermore, data on semen production and quality are routinely collected for all bulls selected for AI, ensuring the availability of phenotypes for genetic evaluations. The inclusion of male fertility among the selected traits requires first the estimation of the extent of genetic variation in semen traits, but genetic parameters of semen traits have never been investigated in Piemontese cattle.

Aims of this study were to investigate non-genetic effects influencing variation in semen characteristics and to provide the first estimate of genetic parameters for semen production and quality traits in Piemontese bulls. The present study also investigated for the first time the genetic relationships between volumes and concentrations of individual ejaculates sampled on the same test-day.

Material and methods

Animals and data records

Data used in this study were measures of sperm volume and quality recorded at the AI station of the Italian Association of Piemontese Cattle Breeders (Anaborapi, Carrù, Italy) from April 1995 to March 2015 for bulls born between December 1988 and December 2013 ($n = 693$). Animals entered the AI station at 17 ± 11 months of age, with a minimum age of 12 months. Semen was sampled on 2 176 different test-days from nine bulls in each test-day on average. Up to three ejaculates were collected from each bull in a single test-day using an artificial vagina. The number of test-days per bull was on average of 27.5 ± 27.7 and ranged from 3 to 272. Age at semen collection was on average 30 ± 22 months, ranging from 12 to 139 months. The average interval between two consecutive test-days was 12.7 ± 28.7 days and ranged from 1 to 688 days. After arrival on the collection floor, bulls were generally allowed up to two false mounts (although it differed by bull preferences), with an approximate 2–3 min between consecutive mounts. The volume of each ejaculate (three ejaculates as a maximum) produced on the same test-day (ml) was quantified using a graduated tube, whereas sperm concentration (SC1, SC2 and SC3, 10^9 cells/ml) was measured using a Ciba-Corning 257 colorimeter (Ciba Corning Analytical, Halstead, England) set at a wavelength of 546 nm. Each ejaculate was diluted 1:1 (v:v) in Bullxcell medium (IMV Technologies, France) after collection. Subsequently, ejaculates produced by the same bull on a given test-day were pooled together. The total sperm number (PSN, 10^9 cells) of the

pooled semen was the sum of total sperm number of single ejaculates, which was obtained as the product of the volume times the concentration. The sperm concentration of the pooled semen (PSC, 10^9 cells/ml) was calculated as the ratio of PSN to pooled semen volume (PSV, ml). Within 24-h of freezing, two straws per pooled semen sample were randomly sampled and thawed in a water bath at 36°C for 1 min. Progressive motility after thawing (%) was assessed by an expert technician using a light microscope and was the average progressive motility of the two straws.

To obtain more accurate estimates of test-day effects, only test-days with at least five bulls in production were considered in the analysis. For each trait, records with values lower than -4 SD and higher than 4 SD from the mean (eight records in total) were considered outliers and eliminated. After data editing, records for 19 060 ejaculates of 693 bulls were available.

Bulls were progeny of 261 AI purebred sires and 655 dams, all registered in the Italian Piemontese Herd Book. Pedigree information included the bulls with phenotypic information and all their known ancestors (6 855 animals).

Statistical analysis

Before statistical analysis, frequency distributions of individual ejaculate volumes were normalized by applying a natural logarithmic transformation (LnSV1, LnSV2 and LnSV3). Frequency distributions of LnSV1, LnSV2 and LnSV3 are shown in Fig. 1.

Estimation of (co)variance components for the investigated traits was performed through univariate and bivariate Bayesian analyses using repeatability animal models implemented in the software TM (Legarra et al., 2011). Each trait was first analysed with the following univariate model:

$$y = Xb + Zd + Wa + Vpe + e$$

where: y is a vector of observed phenotypes; b is a vector of non-genetic effects, including age class of bulls (six classes of 2-month intervals until 24 months, four classes of 6-month intervals until 48 months and the last class including records with age >48 months), the number of days between two consecutive test-days (eight classes of 4-day intervals, two classes of 30-day intervals, one class of 60-day interval and the last class including records with interval between test-days greater than 150 days), and, for PSV, PSN and PSC, the number of ejaculates produced in the test-day (3 classes; class 1: one ejaculate, class 2: two ejaculates, class 3: three ejaculates); d is the vector of test-day effects; a is a vector of additive genetic effects of animals (6 855 levels); pe is an unknown vector of permanent environmental effects; e is a vector of residual effects; X , Z , W and V are incidence matrices relating y to b , d , a and pe , respectively. Effects in b were a priori assumed to follow a bounded uniform probability density, whereas prior densities for d , a , pe and e were normal probability densities.

Adjusted posterior means for effects in b were calculated as the sum of the estimated model intercept, the solution for the considered effect and the average of the solutions of the other effects in b .

Relationships between the investigated traits were evaluated using a set of bivariate analyses. The general model, in matrix notation, can be written as:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \cdot \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \cdot \begin{bmatrix} d_1 \\ d_2 \end{bmatrix} + \begin{bmatrix} W_1 & 0 \\ 0 & W_2 \end{bmatrix} \cdot \begin{bmatrix} a_1 \\ a_2 \end{bmatrix} + \begin{bmatrix} V_1 & 0 \\ 0 & V_2 \end{bmatrix} \cdot \begin{bmatrix} pe_1 \\ pe_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

where y_1 and y_2 are vectors of phenotypes for traits 1 and 2, respectively; b_1 and b_2 are vectors of non-genetic effects as specified for the univariate model; X_1 and X_2 are known incidence matrices relating effects in b_1 and b_2 to y_1 and y_2 , respectively; d_1 and d_2

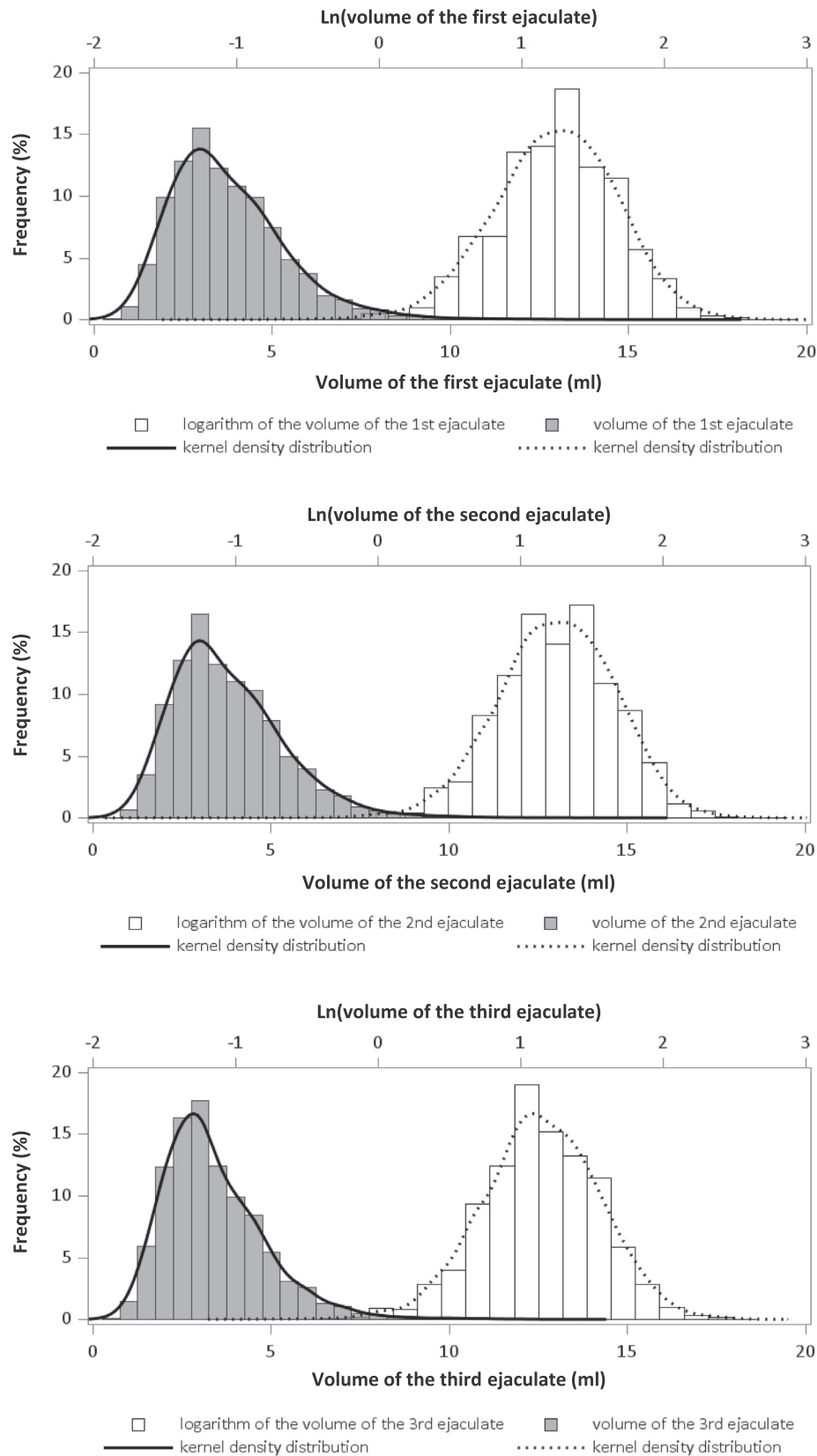


Fig. 1. Frequency distributions of raw data and natural logarithm (Ln) transformation for the volume of the first, second and third ejaculates in a test-day in Piemontese bulls.

are vectors of effects of test-day assumed to be normally distributed. Pairs of records from different individuals are assumed to be conditionally independent given the parameters, but a correlation between effects acting on the same individual is allowed.

Hence, sorting the data by test-day, the test-day (co)variance matrix can be written as $Q \otimes I$ with Q being the (co)variance matrix between test-day effects on both traits and I an identity matrix of appropriate order; Z_1 and Z_2 are known incidence matrices relating

d_1 and d_2 to y_1 and y_2 , respectively; a_1 and a_2 are vectors of additive genetic effects assumed a priori to follow a multivariate normal probability density. Sorting the data by individual, the animal (co)variance matrix can be written as $G \otimes A$ where G is the (co)variance matrix for animal genetic effects on both traits and A is the numerator of Wright's relationship matrix. W_1 and W_2 are known incidence matrices relating a_1 and a_2 to y_1 and y_2 , respectively. pe_1 and pe_2 are the vectors of permanent environmental effects, assumed to follow a multivariate normal distribution. Sorting the data by individual, the permanent environmental (co)variance matrix can be written as $P \otimes I$ with P being the (co)variance matrix between permanent environmental effects on both traits. V_1 and V_2 are known incidence matrices relating pe_1 and pe_2 to y_1 and y_2 , respectively. e_1 and e_2 are vectors of residual effects, assumed to follow a multivariate normal distribution. The residual (co)variance matrix can be written as $R \otimes I$, where R is the (co)variance between residual effects for both traits. Inverse Wishart prior distributions, which are very vague priors (Blasco, 2001), were assumed for all (co)variances in matrices Q , G , P and R .

Numerical integration through the Gibbs sampler, as implemented in the TM software (Legarra et al., 2011), was used to estimate marginal posterior densities of (co)variance components and genetic parameters. A unique Gibbs chain of 500 000 iterations was run for each analysis, one Gibbs sample was saved every 200 iterations and the length of burn-in was 50 000 iterations. The posterior median was used as a point estimate of (co)variance components and parameters. The lower and upper bounds of the highest 95% posterior probability density interval for (co)variance components and genetic parameters were obtained from the estimated marginal densities. Probabilities for the estimated parameter of being >0.2 were calculated for heritability and repeatability. The probability for the estimated parameter of being >0.1 for positive estimates or, alternatively, of being <-0.1 for negative estimates was calculated for genetic and phenotypic correlations. Correlations were considered relevant if this probability was >0.80 .

Heritability of a trait was defined as:

$$h^2 = \sigma_a^2 / (\sigma_d^2 + \sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2)$$

where σ_a^2 is the additive genetic variance, σ_d^2 is the variance due to test-day, σ_{pe}^2 is the variance due to permanent environmental effects, and σ_e^2 is the residual variance. The proportion of phenotypic variance due to test-day effects was computed as:

$$d^2 = \sigma_d^2 / (\sigma_d^2 + \sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2)$$

Repeatability was defined as:

$$c^2 = (\sigma_a^2 + \sigma_{pe}^2) / (\sigma_d^2 + \sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2)$$

Results

Non-genetic effects

Bulls were able to produce three ejaculates in more than 75% of the test-days. Only in 1.6% of the test-days, the bull semen sample derived from a single ejaculate. Average volumes of the first and second ejaculates were similar and greater than the volume of the third ejaculate (Table 1). Compared to the first ejaculate, the sperm concentration of the second and third ejaculates were 12 and 32% lower, respectively.

The adjusted means for the age class effects on the investigated traits are shown in Fig. 2. Volumes of the three individual ejaculates, PSV and PSN increased with increasing age at sampling, with bulls producing the largest amount of semen after their third year

of age. Sperm number increased up to 75 months of age, but the rate of increase dropped after 24 months of age.

Different patterns for age effects were detected for SC1, SC2, SC3 and PSC. Concentration of the first ejaculate increased until 16 months of age and then remained constant. In contrast, SC2 increased until 20 months of age, it was stable from 20 months onwards and decreased for bulls older than 4 years. The third ejaculate exhibited a decrease in concentration, albeit at a slow rate, for bulls older than 22 months. The concentration of the pooled semen showed a pattern similar to SC2. Motility after thawing was low in young bulls, increased until 19 months of age and then remained relatively constant. All patterns for concentrations and motility must, however, be interpreted as tendencies as differences between adjusted means for age classes were, to a large extent, not statistically significant.

The interval between two consecutive test-days affected semen characteristics as shown in Fig. 3. Semen volumes increased with increasing interval length ($P < 0.05$), up to 15 days, and it remained relatively constant for longer intervals. The maximum semen concentration was observed when the interval between consecutive test-days was equal to 6 days. With longer intervals and up to 30-day intervals, concentrations decreased with the increase in the interval length. For sampling intervals greater than 30 days, semen concentration remained constantly low. Being dependent upon variation in PSV and PSC, PSN was low when intervals between test-days were shorter than 10 days and it remained relatively high and constant for longer intervals. Post-thawing progressive motility was not affected by the interval between test-days.

Estimates of (co)variance components and genetic parameters

Considerable genetic variability was observed for PSV, PSC and PSN, with coefficient of genetic variation estimates of 14–17%. Among the investigated traits, only progressive motility after thawing exhibited a low coefficient of genetic variation (2.9%). Point estimates and highest 95% posterior probability density interval of variance components, the proportion of phenotypic variance due to test-day effects, heritability, and repeatability obtained from univariate analyses are reported in Table 2. In general, the permanent environmental variance estimates were as large as those of the additive genetic variance. The only exceptions were PSN, for which the permanent environmental variance was 62% of the additive genetic variance, and progressive motility after thawing, for which the permanent environmental variance was 4.7 times higher than the additive genetic variance. The proportion of the phenotypic variance due to test-day effects was small and ranged from 2.1 (for LnSV1 and SC1) to 8.4% (for progressive motility after thawing), indicating that test-day effects contributed weakly to the observed variation in the investigated traits.

The estimated heritability for LnSV1, LnSV2 and LnSV3 was 0.14, 0.16 and 0.17, respectively. Similar heritability estimates were obtained also for concentrations of the individual ejaculates. The probabilities for such estimates of being >0.2 were low, ranging from 0.01 (for LnSV1) to 0.48 (for SC3). The estimated heritability was higher when traits were measured on the pooled semen (heritability was 0.22 for PSV, 0.23 for PSN and 0.26 for PSC). Such estimates had a probability of being >0.2 of 70%, 82% and 92%, respectively. A low heritability estimate (0.08) was observed for progressive motility after thawing. Estimated values of repeatability were low to moderate, ranging from 0.26 for LnSV1 to 0.51 for PSC.

Estimated genetic and phenotypic correlations between traits are reported in Table 3. The volumes of the individual ejaculates were genetically highly correlated, with values ranging from 0.79 (between LnSV1 and LnSV3) to 0.95 (between LnSV2 and LnSV3).

Table 1Number of records and descriptive statistics for semen production and quality traits in Piemontese bulls.¹

Trait	N	Mean	SD	P1	P99
Volume of the first ejaculate (ml)	19 060	3.8	1.6	1.0	9.0
Volume of the second ejaculate (ml)	18 770	3.8	1.5	1.5	8.5
Volume of the third ejaculate (ml)	14 760	3.4	1.4	1.0	8.0
Concentration of the first ejaculate (10 ⁹ cells/ml)	19 060	1.20	0.49	0.30	2.43
Concentration of the second ejaculate (10 ⁹ cells/ml)	18 770	1.06	0.40	0.30	2.02
Concentration of the third ejaculate (10 ⁹ cells/ml)	14 760	0.82	0.35	0.27	1.78
Volume of the pooled semen (ml)	19 060	10.14	3.39	4.00	20.00
Number of sperm in the pooled semen (10 ⁹ cells)	19 060	10.33	3.83	3.43	21.56
Concentration of the pooled semen (10 ⁹ cells/ml)	19 060	1.06	0.33	0.42	1.87
Post-thawing progressive motility (%)	18 213	45.57	4.84	34.00	58.00

¹ P1: 1st percentile; P99: 99th percentile.

Such estimates had a probability of being >0.8 greater than 90% (data not reported in table). The genetic correlation between SC1 and SC2 and between SC2 and SC3 (0.77 and 0.91, respectively) was much higher compared to the correlation of SC1 with SC3 (0.46). Given that PSV originated from the sum of the volume of the three individual ejaculates, the additive genetic correlations between the volume of the three ejaculates and PSV were spurious and, as expected, were positive and high. Likewise, concentrations of the three individual ejaculates were positively and highly correlated with PSC.

Moderate negative additive genetic correlations were detected between volume and concentration of individual ejaculates, ranging from −0.43 (between LnSV1 and SC1) to −0.47 (between LnSV3 and SC3). The probability that these correlations were negative was 100%. Sperm number in the pooled semen was positively correlated with all the investigated traits, with the exception of progressive motility after thawing. In general, the latter exhibited trivial genetic relationships with the other traits. The magnitude of these estimates was small, while the highest 95% posterior probability density intervals were wide and included zero for all correlations. Only the genetic correlations between progressive motility after thawing and LnSV2 (0.29) or LnSV3 (0.35) were considered statistically relevant (i.e., the probability of these correlations of being >0.1 was greater than 0.8). Phenotypic correlations were consistent in sign with genetic correlations, but weaker. Estimates were all statistically relevant, with the exception of those between progressive motility after thawing and the other traits.

Discussion

Non-genetic effects

In the literature, semen traits are usually measured on the first ejaculate. The volume of the first ejaculate observed in our study (3.8 ml) was in the lower range of the values (3.5–5.5 ml) reported for other breeds (Murphy et al., 2018; Berry et al., 2019; Burren et al., 2019). Concentration of the first ejaculate (1.20 · 10⁹ cells/ml) was also in the lower range of other reports (1.22–1.55 · 10⁹ cells/ml) performed on bulls of the same age range (Murphy et al., 2018; Berry et al., 2019; Burren et al., 2019). Average progressive motility after thawing (46%) was consistent with findings on other breeds (on average 49%) reported by Karoui et al. (2011). A decrease in the ejaculate volume and concentration with increasing number of ejaculates per day was previously reported (Brockett et al., 1994). However, in our study, the differences in volume and concentration between the first and second ejaculates were small in comparison with those reported by Murphy et al. (2018) in Holstein bulls. They observed a greater volume and sperm concentration in the first than in the second ejaculate and reported that first ejaculates had a number of total sperm twice as high as the one of second ejaculates.

The large variability in the age of bulls at semen sampling and in the intervals between two consecutive test-days is due to changes in the management of animals that occurred over time. The AI station was set up in 1995 and some bulls, already in use in the population as service sires from other AI stations, entered the station before 1996 at advanced ages (about 30 months). From 1996 to 2003, semen sampling was suspended for bulls awaiting proofs from the progeny testing programme and restarted only for selected bulls, resulting in possible long intervals between two consecutive test-days. Since then, semen sampling is continuously performed until a predefined number of straws are obtained for each bull.

The estimated effects of non-genetic factors on semen quality traits are in agreement with the literature (Murphy et al., 2018; Berry et al., 2019). Peak ejaculate volumes and total sperm number are achieved at different ages in different breeds (Snoj et al., 2013), but similar effect of bull's age on semen volumes and number of spermatozoa was detected in previous studies. Taylor et al. (1985), Mathevon et al. (1998) and Karoui et al. (2011) reported that semen volume increased with age in different Holstein populations. Consistent with the results of these studies, an increase in semen volume with age was also described by Berry et al. (2019) on 787 bulls of 16 different breeds. The increase in the volume of semen is primarily believed to be due to physiological changes such as an increase in body mass (Balić et al., 2012) and the simultaneous development of the testis and accessory glands during sexual maturation, which continues for up to 5 years postpuberty (Almqvist, 1978).

The decrease in sperm concentrations observed in Piemontese old bulls is in agreement with Fuerst-Waltl et al. (2006) and Berry et al. (2019) findings, who reported that the highest semen concentrations were reached between 19 and 33 months of age. This may be explained by a dilution of bull semen resulting from the considerable increase in sperm volume not accompanied by an equal increase in the number of spermatozoa. As PSC decreased at a very low rate after 22 months of age, the increase in PSN with age is to be ascribed to the increase in ejaculate volume, consistent with the findings of Berry et al. (2019).

In agreement with our results, several authors reported a marked effect of the interval between test-days on both semen volume and total sperm per ejaculation, with an interval between 3 and 7 days maximizing sperm concentration (Mathevon et al., 1998; Fuerst-Waltl et al., 2006; Karoui et al., 2011). Given that both semen volumes and concentrations are fundamental to produce the highest number of doses, intervals of 10–20 days between collections are recommended in order to maximize semen production.

Genetic parameters

The considerable genetic variability observed in the Piemontese breed for most of the investigated traits is consistent with results

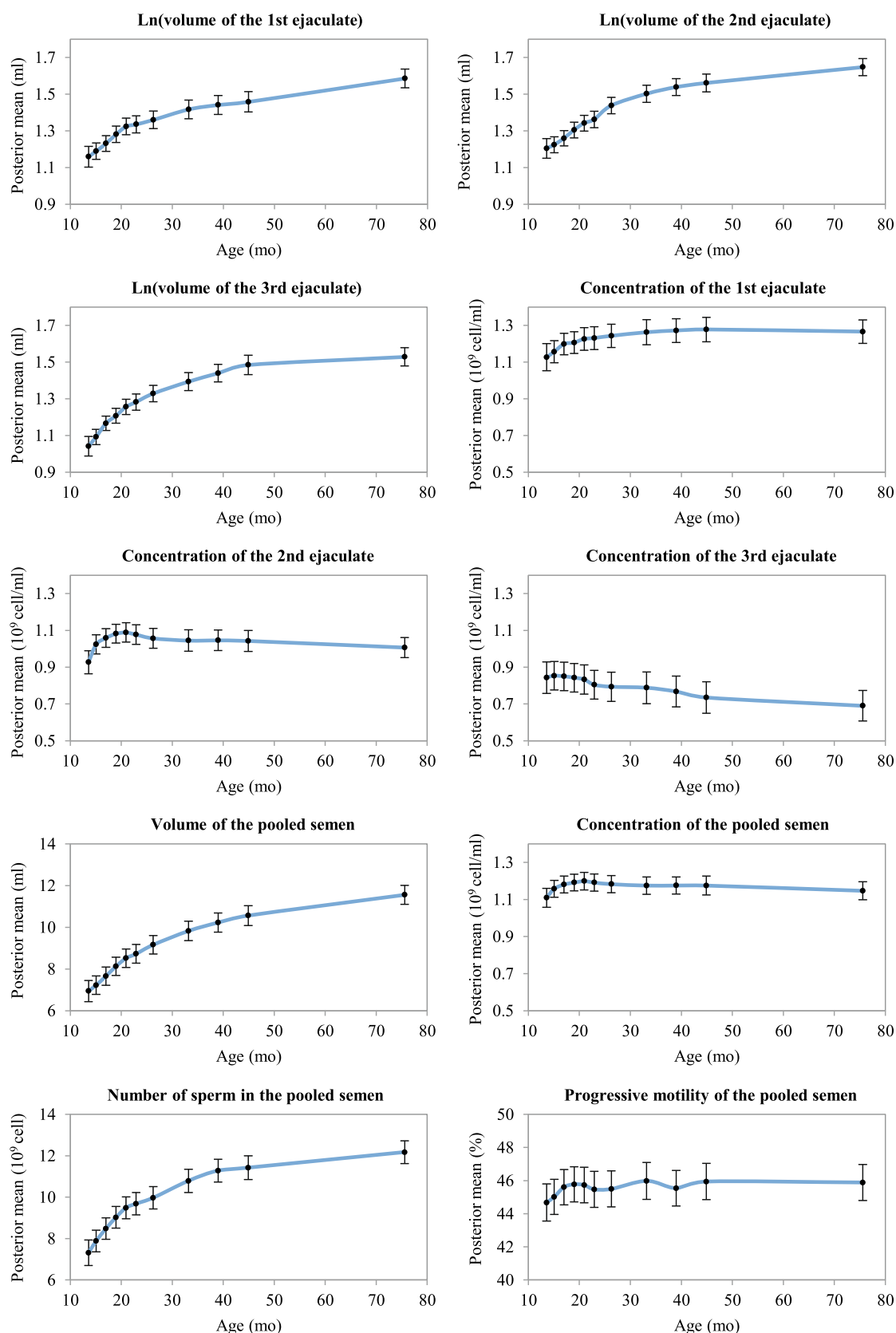


Fig. 2. Adjusted means of semen production and quality traits for age class effects in Piemontese bulls.

obtained by [Berry et al. \(2019\)](#). The variability of male fertility traits is greater than the one commonly documented for many performance and female fertility traits ([Berry et al., 2019](#)).

Following the approach used by [Berry et al. \(2019\)](#) and assuming approximately 15 million sperm per AI straw on average, the estimated genetic standard deviation of 1 713 million sperms per

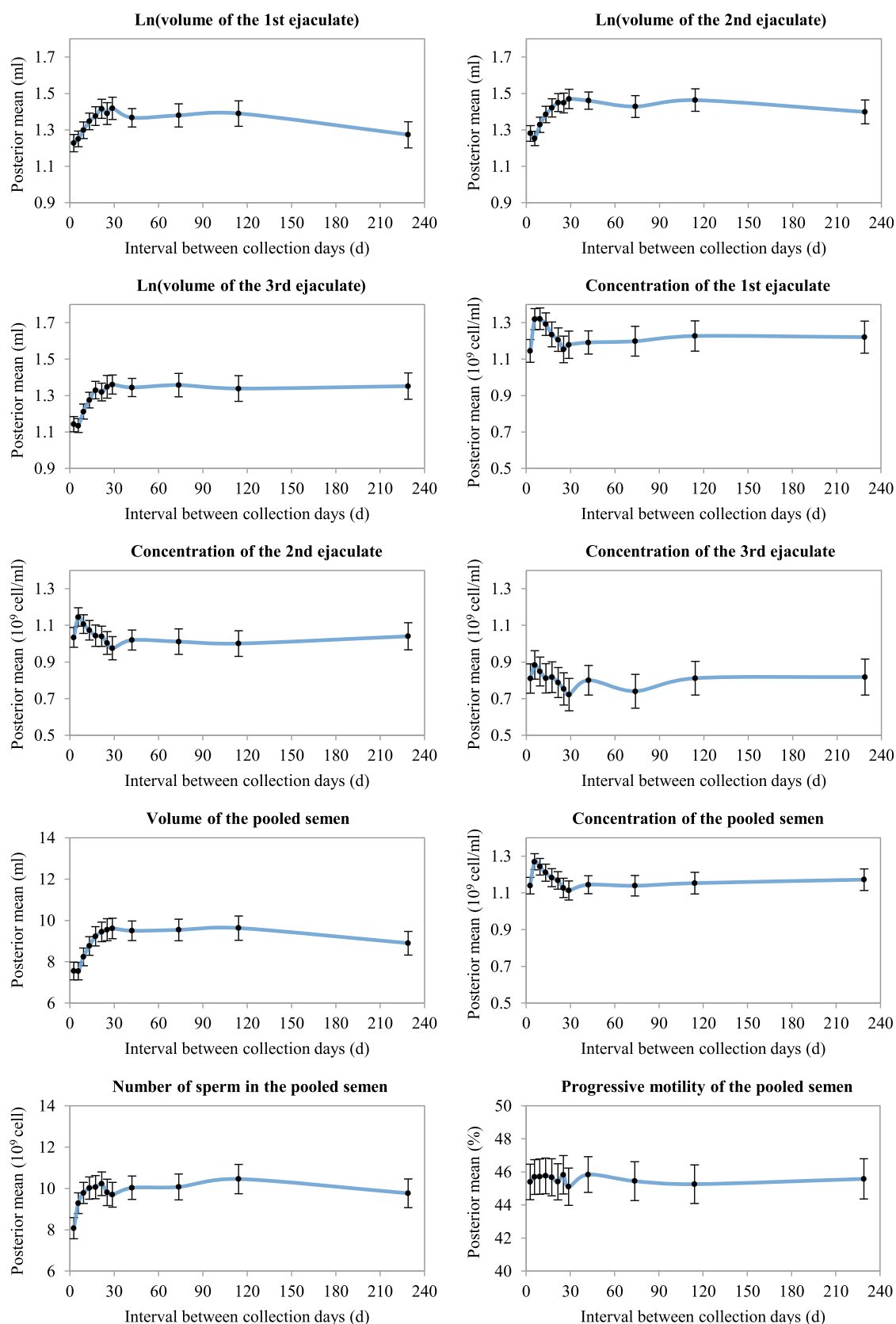


Fig. 3. Adjusted means of semen production and quality traits for the interval between two consecutive semen test-days in Piemontese bulls.

test-day implies an expected difference of 2 398 million sperm between the top 20% bulls and the population mean. This translates into a difference of approximately 160 straws per test-day.

A wide range of heritability estimates has been reported in the literature for male fertility traits (Butler et al., 2020). Differences in methods of measurement of traits, characteristics of the

Table 2

Estimates (median of the posterior density), 95% highest posterior density interval (HPD95%), and posterior probability of the estimate being greater than 0.2 ($P_{0.2}$) for variance components and parameters for semen production and quality traits in Piemontese bulls.^{1,2}

Trait	σ_d^2	σ_a^2	σ_{pe}^2	d^2	h^2			c^2		
					Estimate	HPD95%	$P_{0.2}$	Estimate	HPD95%	$P_{0.2}$
LnSV1	0.003	0.021	0.018	0.021	0.137	0.092, 0.184	0.01	0.258	0.232, 0.282	1.00
LnSV2	0.004	0.021	0.018	0.032	0.155	0.099, 0.210	0.06	0.290	0.262, 0.316	1.00
LnSV3	0.004	0.024	0.017	0.028	0.169	0.117, 0.225	0.14	0.288	0.260, 0.318	1.00
SC1	0.005	0.038	0.050	0.021	0.158	0.089, 0.125	0.22	0.367	0.336, 0.395	1.00
SC2	0.004	0.032	0.027	0.026	0.199	0.138, 0.261	0.48	0.369	0.338, 0.398	1.00
SC3	0.004	0.024	0.020	0.028	0.186	0.127, 0.241	0.31	0.341	0.157, 0.304	0.78
PSV	0.402	1.946	1.753	0.045	0.219	0.150, 0.290	0.70	0.419	0.389, 0.451	1.00
PSN	0.590	2.934	1.830	0.046	0.231	0.166, 0.302	0.82	0.377	0.348, 0.412	1.00
PSC	0.004	0.027	0.026	0.038	0.259	0.180, 0.348	0.92	0.510	0.477, 0.541	1.00
PM	1.916	1.759	8.199	0.084	0.077	0.013, 0.143	0.00	0.441	0.431, 0.470	1.00

¹ LnSV1: logarithmic transformation of the volume of the first ejaculate; LnSV2: logarithmic transformation of the volume of the second ejaculate; LnSV3: logarithmic transformation of the volume of the third ejaculate; SC1: concentration of the first ejaculate (10^9 cells/ml); SC2: concentration of the second ejaculate (10^9 cells/ml); SC3: concentration of the third ejaculate (10^9 cells/ml); PSV: volume of the pooled semen (ml); PSN (10^9 cells): number of spermatozoa in the pooled semen; PSC (10^9 cells/ml): concentration of the pooled semen; PM: post-thawing progressive motility of the pooled semen (%).

² σ_d^2 : variance due to test-day; σ_a^2 : additive genetic variance; σ_{pe}^2 : permanent environmental variance; d^2 : proportion of variance due to test-day; h^2 : heritability; c^2 : repeatability.

Table 3

Estimates and 95% highest posterior density interval of additive genetic (above diagonal) and phenotypic (below diagonal) correlations between semen production and quality traits in Piemontese bulls.¹

Trait	LnSV1	LnSV2	LnSV3	SC1	SC2	SC3	PSV	PSN	PSC	PM
LnSV1	–	0.93 (0.86, 0.98)	0.79 (0.68, 0.89)	–0.43 (–0.66, –0.15)	–0.47 (–0.65, –0.23)	–0.47 (–0.66, –0.26)	0.95 (0.90, 0.97)	0.44 (0.24, 0.63)	–0.46 (–0.66, –0.25)	0.05 (–0.34, 0.44)
LnSV2	0.39 (0.33, 0.37)	–	0.95 (0.89, 0.99)	–0.37 (–0.65, –0.09)	–0.33 (–0.56, 0.07)	–0.32 (–0.55, –0.09)	0.99 (0.98, 0.99)	0.56 (0.37, 0.73)	–0.39 (–0.61, –0.15)	0.29 (–0.10, 0.67)
LnSV3	0.28 (0.25, 0.30)	0.37 (0.35, 0.40)	–	–0.40 (–0.63, –0.12)	–0.41 (–0.62, –0.18)	–0.39 (–0.60, –0.16)	0.93 (0.89, 0.97)	0.44 (0.24, 0.63)	–0.47 (–0.67, –0.26)	0.35 (–0.02, 0.75)
SC1	–0.11 (–0.14, –0.08)	–0.13 (–0.16, –0.10)	–0.13 (–0.16, –0.10)	–	0.77 (0.62, 0.89)	0.46 (0.13, 0.67)	–0.43 (–0.64, –0.14)	0.47 (0.24, 0.69)	0.88 (0.81, 0.95)	–0.27 (–0.74, 0.18)
SC2	–0.30 (–0.33, –0.27)	–0.23 (–0.26, –0.20)	–0.19 (–0.23, –0.16)	0.35 (0.320, 0.377)	–	0.91 (0.83, 0.99)	–0.42 (–0.62, –0.21)	0.56 (0.37, 0.72)	0.97 (0.94, 0.99)	–0.15 (–0.63, 0.26)
SC3	–0.23 (–0.26, –0.21)	–0.31 (–0.33, –0.28)	–0.24 (–0.27, –0.21)	0.21 (0.18, 0.24)	0.35 (0.33, 0.38)	–	–0.40 (–0.36, –0.30)	0.43 (0.22, 0.61)	0.85 (0.76, 0.92)	–0.06 (–0.42, 0.32)
PSV	0.73 (0.72, 0.74)	0.75 (0.74, 0.76)	0.71 (0.69, 0.72)	–0.17 (–0.20, –0.13)	–0.32 (–0.35, –0.29)	–0.33 (–0.36, –0.30)	–	0.51 (0.36, 0.70)	–0.44 (–0.63, –0.22)	0.17 (–0.24, 0.52)
PSN	0.43 (0.40, 0.45)	0.40 (0.37, 0.43)	0.34 (0.31, 0.37)	0.54 (0.51, 0.56)	0.37 (0.33, 0.40)	0.26 (0.23, 0.29)	0.53 (0.50, 0.68)	–	0.56 (0.31, 0.68)	–0.08 (–0.48, 0.30)
PSC	–0.23 (–0.26, –0.20)	–0.28 (–0.31, –0.25)	–0.31 (–0.34, –0.28)	0.78 (0.77, 0.79)	0.75 (0.74, 0.76)	0.63 (0.61, 0.65)	–0.35 (–0.38, 0.31)	0.56 (0.53, 0.59)	–	–0.20 (–0.62, 0.18)
PM	–0.07 (–0.10, –0.04)	–0.01 (–0.04, 0.03)	–0.01 (–0.04, 0.03)	0.02 (–0.01, 0.06)	0.13 (0.09, 0.16)	0.13 (0.09, 0.16)	–0.04 (–0.08, –0.01)	0.05 (0.01, 0.09)	0.11 (0.07, 0.15)	–

¹ LnSV1: logarithmic transformation of the volume of the first ejaculate; LnSV2: logarithmic transformation of the volume of the second ejaculate; LnSV3: logarithmic transformation of the volume of the third ejaculate; SC1: concentration of the first ejaculate (10^9 cells/ml); SC2: concentration of the second ejaculate (10^9 cells/ml); SC3: concentration of the third ejaculate (10^9 cells/ml); PSV: volume of the pooled semen (ml); PSN (10^9 cells): number of spermatozoa in the pooled semen; PSC (10^9 cells/ml): concentration of the pooled semen; PM: post-thawing progressive motility of the pooled semen (%).

investigated populations and statistical models used to estimate (co)variance components may affect the magnitude of the estimates (Gredler et al., 2007). The moderate heritability estimates obtained in the present study are in agreement with findings reported in other breeds. Butler et al. (2020) reported that the average heritability estimated in the literature is 0.26 (from 0.13 to 0.52) for sperm concentration, 0.23 (from 0.04 to 0.65) for semen volume and 0.24 (from 0.03 to 0.38) for number of spermatozoa, in line with our findings.

In general, larger heritability estimates were observed for volume of ejaculate (Karoui et al., 2011), whereas motility score or mass motility, subjectively measured on a discrete scale, showed the lowest estimates, lower than 0.05 in Gredler et al. (2007) for Simmental bulls, although large values (0.50 for motility score and 0.43 for progressive motility) have also been reported (Druet et al., 2009). Heritability estimates for motility and progressive motility differ greatly across studies, perhaps due to the different scales and methods used (visual inspection or computer-assisted

semen analysis). The heritability for progressive motility after thawing detected in our study was in the lower range of the estimates previously reported using the same assessment method (Burren et al., 2019; Yin et al., 2019; Butler et al., 2020) and in agreement with values obtained by Olsen et al. (2019) on refrigerated semen.

The heritability estimates reported in the present study for semen volumes and concentrations suggest that, in the Piemontese breed, selective breeding to improve male fertility might be feasible, especially considering that the assessment of Piemontese bull semen quality is performed routinely at the AI station, ensuring the availability of phenotypes.

Genetic and non-genetic permanent effects affect the ability of each bull to produce semen throughout its productive life. Estimates of repeatability, which represents the correlation between repeated measures of phenotype of the same bull, indicate that semen characteristics are moderately repeatable in the Piemontese population. Our estimates are consistent or slightly lower than those previously reported (Druet et al., 2009; Karoui et al., 2011; Olsen et al., 2019). Differences in repeatability estimates for semen traits across studies may be due to variation in age of bulls at sampling. As discussed by Mathevon et al. (1998), semen production is expected to be more stable in adult bulls compared to young animals. In our study, 28% of ejaculates came from bulls younger than 30 months and it may be partly responsible for the moderate repeatability estimates obtained. In general, also the standardization of semen sampling procedures might increase the repeatability, improving accuracy of breeding value estimates and the response to selection.

Genetic correlations between traits

Our estimates of the genetic correlations between semen concentration and semen volume or total number of sperm per ejaculate were consistent with those reported in the literature review of Butler et al. (2020). The negative genetic correlation estimated between semen volume and semen concentration implies that a selection aimed at improving the former would lead to a worsening of the latter. The improvement of semen volume should not be at the expense of genetic merit for concentration and thus both traits should be considered in the definition of the breeding goal.

Gredler et al. (2007) and Druet et al. (2009) reported that genetic correlations between semen volume and concentration were greater than the correspondent phenotypic correlation, with the latter being weak or close to zero. In our study, the same pattern was observed, although phenotypic correlations between sperm volumes and concentrations were slightly greater than previous estimates (Gredler et al., 2007; Druet et al., 2009).

In the literature, inconsistent results have been obtained on the genetic correlation between motility and ejaculate volume or concentration. In contrast with our results, in most of the studies, greater sperm motility was associated with greater semen concentration, but the association of motility with volume was weak or negative (Butler et al., 2020). However, positive correlations between these traits were also found (Yin et al., 2019; Olsen et al., 2019). Heterogeneous estimates have been reported also for the genetic relationship between the number of sperm and motility (Butler et al., 2020). Given its favourable genetic correlation with semen concentration and its weak negative correlation with volume, progressive motility after thawing is not expected to be negatively affected from the genetic improvement of semen concentration or volume.

Druet et al. (2009) reported an unfavourable relationship between sperm concentration and abnormal sperm percentage. However, sperm abnormalities are not currently recorded during the routine sampling of semen on Piemontese bulls. In the light

of these results, genetic relationships between semen production and quality should be examined in depth also in the Piemontese population.

In the present study, we investigated for the first time in cattle the genetic relationships between volumes and concentrations of individual ejaculates sampled on the same test-day. The marked and positive genetic correlations between the characteristics of individual ejaculates and pooled semen indicate that such observations can be considered as repeated measures of the same trait. This evidence is supported by the high correlations between the breeding values estimated using the phenotypes of the pooled and individual ejaculates (data not reported in tables). Spearman's correlations between rankings of bulls based on EBV for PSV and LnSV1, LnSV2, LnSV3 were 0.90, 0.93 and 0.87, respectively. Furthermore, Spearman's correlation coefficients between PSC and SC1, SC2 and SC3 were 0.87, 0.90 and 0.77, respectively. This provided evidence that the ranking of breeding candidates differed only slightly when volumes and concentrations of the pooled semen were used as an alternative to those of individual ejaculates to estimate genetic merit of bulls. In order to simplify the operational routine, genetic evaluations of bulls might rely on phenotypes for PSV and PSC, if these traits are to be included in the breeding goal of the breed.

This study showed that most of the investigated male fertility traits display considerable genetic variation. The estimated heritability values suggest the feasibility of selective breeding to improve semen quality traits of Piemontese bulls. Many traits related to semen quality undergo routine recording and inclusion of such traits in the genetic evaluation programme of the Piemontese population is tempting. The results provided by this study may be used in the near future to develop genomic predictions, which would be particularly useful to increase the reliability of breeding values for young bulls and may facilitate the selection of traits with antagonistic relationships, such as sperm concentration and volume. In addition, the market that still exists for natural mating sires could benefit from estimates of the genetic merit of individual bulls for male reproductive traits. In the near future, genetic relationships between semen traits and traits included in the breeding goal of the Piemontese population need to be estimated to elucidate possible correlated effects of current selection on male reproductive performances.

Ethics approval

Animal Care and Use Committee approval was not needed because observations used in this study were from the routine data recording which is conducted during semen sampling performed at the AI station of the Italian Association of Piemontese Cattle Breeders, (Anaborapi, Carrù, Italy). Semen collections were carried out at the AI station by trained staff. The AI centre operates in compliance with Italian and European regulations concerning animal protection and welfare.

Data and model availability statement

No data were deposited in an official repository. The data that support the findings of this study are available from Anaborapi (Carrù, Italy). Restrictions apply to the availability of these data, which were used under license for this study. Data are available from the authors with the permission of Anaborapi.

Author ORCIDs

R. Rostellato: <https://orcid.org/0000-0001-5538-3377>
V. Bonfatti: <https://orcid.org/0000-0003-3970-5764>

S. Savoia: <https://orcid.org/0000-0003-3537-0768>

P. Carnier: <https://orcid.org/0000-0002-6009-6601>

Author contributions

R. Rostellato: Formal analysis, Writing - Original Draft; **V. Bonfatti:** Writing - original draft, writing - review & editing. **V.A.D. Dias:** Formal analysis; **S. Savoia:** Data curation, writing - review & editing; **V. Spalenza:** Writing - review & editing; **A. Albera:** Conceptualization, writing - review & editing; **P. Carnier:** Conceptualization, supervision, writing - review & editing.

Declaration of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors thank the Italian Association of Piemontese Cattle Breeders (Anaborapi, Carrù, Italy) for providing the data.

Financial support statement

V.A. D. Dias benefited from financial support of CAPES Foundation, Ministry of Education of Brazil (PDSE/CAPES Scholarship - Process n° 99999.010090/2014-02).

References

- Albera, A., Mantovani, R., Bittante, G., Groen, A.F., Carnier, P., 2001. Genetic parameters for daily live-weight gain, live fleshiness and bone thinness in station-tested Piemontese young bulls. *Animal Science* 72, 449–456.
- Albera, A., Carnier, P., Groen, A.F., 2004a. Definition of a breeding goal for the Piemontese breed: economic and biological values and their sensitivity to production circumstances. *Livestock Production Science* 89, 66–77.
- Albera, A., Groen, A.F., Carnier, P., 2004b. Genetic relationships between calving performance and beef production traits in Piemontese cattle. *Journal of Animal Science* 82, 3440–3446.
- Almquist, J.O., 1978. Bull semen collection procedures to maximize output of sperm. In: *Proceedings of the 7th Technical Conference on Artificial Insemination and Reproduction of the National Association of Animal Breeders*, 14–15 April 1978, Madison, WI, USA, pp. 33–36.
- Balić, I.M., Milinković-Tur, S., Samardžija, M., Vince, S., 2012. Effect of age and environmental factors on semen quality, glutathione peroxidase activity and oxidative parameters in simmental bulls. *Theriogenology* 78, 423–431.
- Berry, D.P., Eivers, B., Dunne, G., McParland, S., 2019. Genetics of bull semen characteristics in a multi-breed cattle population. *Theriogenology* 123, 202–208.
- Berry, D.P., Evans, R.D., McParland, S., 2011. Evaluation of bull fertility in dairy and beef cattle using cow field data. *Theriogenology* 75, 172–181.
- Blasco, A., 2001. The Bayesian controversy in animal breeding. *Journal Animal Science* 79, 2023–2046.
- Bonfatti, V., Albera, A., Carnier, P., 2013. Genetic associations between daily BW gain and live fleshiness of station-tested young bulls and carcass and meat quality traits of commercial intact males in Piemontese cattle. *Journal Animal Science* 91, 2057–2066.
- Boujenane, I., Boussaïd, K., 2014. Environmental effects and repeatability estimates for sperm production and semen quality of Holstein bulls. *Archiv für Tierzucht* 56, 1–6.
- Brockett, C.C., Pressice, G.A., Foote, R.H., Kaproth, M.T., Rycroft, H.E., 1994. Semen quality and behaviour of Holstein-Friesian bulls exposed to estradiol-treated bulls for mounts. *Journal of Dairy Science* 77, 124–131.
- Burren, A., Joerg, H., Erbe, M., Gilmour, A.R., Witschi, U., Schmitz-Hsu, F., 2019. Genetic parameters for semen production traits in Swiss dairy bulls. *Reproduction in Domestic Animals* 54, 1177–1181.
- Butler, M.L., Bormann, J.M., Weaver, R.L., Grieger, D.M., Rolf, M.M., 2020. Selection for bull fertility: a review. *Translational Animal Science* 4, 423–441.
- Christensen, P., Boelling, D., Pedersen, K.M., Korsgaard, I.R., Jensen, J., 2005. Relationship between sperm viability as determined by flow cytometry and nonreturn rate of dairy bulls. *Journal of Andrology* 26, 98–106.
- Druet, T., Fritz, S., Sellem, E., Basso, B., Gerard, O., Salas-Cortes, L., Humblot, P., Druet, X., Eggen, A., 2009. Estimation of genetic parameters and genome scan for 15 semen characteristics traits of Holstein bulls. *Journal of Animal Breeding and Genetics* 126, 269–277.
- Fuerst-Walzl, B., Schwarzenbacher, H., Perner, C., Sölkner, J., 2006. Effects of age and environmental factors on semen production and semen quality of Austrian Simmental bulls. *Animal Reproduction Science* 95, 27–37.
- Gredler, B., Fuerst, C., Fuerst-Walzl, B., Schwarzenbacher, H., Sölkner, J., 2007. Short Communication: Genetic parameters for semen production traits in Austrian dual-purpose Simmental bulls. *Reproduction in Domestic Animals* 42, 326–328.
- Karoui, S., Diaz, C., Serrano, M., Cue, R., Cellorio, I., Carabano, M.J., 2011. Time trends, environmental factors and genetic basis of semen traits collected in Holstein bulls under commercial conditions. *Animal Reproduction Science* 124, 28–38.
- Legarra, A., Varona, L., Lopez de Maturana, E., 2011. Threshold model. Retrieved on 15 January 2020 from http://genoweb.toulouse.inra.fr/~alegarra/tm_folder/manualtm.pdf.
- Mathevon, M., Buhr, M.M., Dekkers, J.C., 1998. Environmental, management, and genetic factors affecting semen production in Holstein bulls. *Journal of Dairy Science* 81, 3321–3330.
- Murphy, E.M., Kelly, A.K., O'Meara, C., Eivers, B., Lonergan, P., Fair, S., 2018. Influence of bull age, ejaculate number, and season of collection on semen production and sperm motility parameters in Holstein Friesian bulls in a commercial artificial insemination centre. *Journal of Animal Science* 96, 2408–2418.
- Olsen, H.B., Heringstad, B., Klemetsdal, G., 2019. Genetic analysis of semen characteristic traits in young Norwegian Red bulls. *Journal of Dairy Science* 103, 545–555.
- Snoj, T., Kobal, S., Majdic, G., 2013. Effects of season, age, and breed on semen characteristics in different bos Taurus breeds in a 31-year retrospective study. *Theriogenology* 79, 847–852.
- Taylor, J.F., Bean, B., Marshall, C.E., Sullivan, J.J., 1985. Genetic and environmental components of semen production traits of artificial insemination Holstein bulls. *Journal of Dairy Science* 68, 2702–2722.
- Yin, H., Fang, L., Qin, C., Zhang, S., 2019. Estimation of the genetic parameters for semen traits in Chinese Holstein bulls. *BMC Genetics* 20, 51–55.