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Genetic and environmental effects on semen quality of Austrian Simmental bulls

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Abstract

Semen production data from an Austrian AI centre collected between 2000 and 2004 were analysed. In total, 12,746 ejaculates from 301 AI bulls were examined considering different effects on ejaculate volume, sperm concentration, percentage of viable spermatozoa in the ejaculate, total spermatozoa per ejaculate and motility. The model included the fixed effects of age of bull, collection interval, number of collections on collection day, bull handler, semen collector, year and month of collection, and a random additive genetic component. Age of bull, collection interval, number of collection on collection day, and year and month significantly affected most semen quality traits. The collection team (bull handler and semen collector) had relevant effects on semen traits. All semen production traits were moderately heritable and correlated. Heritabilities for volume, concentration, percentage of viable spermatozoa, total spermatozoa and motility were 0.18, 0.14, 0.10, 0.22 and 0.04, respectively. Correlations between estimated breeding values of sperm quality traits and routinely estimated breeding values for male fertility were low and ranged from 0.10 to 0.20.

1. Introduction

The ability of bulls to produce sufficient quantities and qualities of semen is of great importance for the successful implementation of modern cattle breeding programs as well as for obvious economic reasons for artificial insemination organizations. AI centres have to guarantee providing enough semen doses for high demanded bulls. Superior semen quality may result in more produced semen doses per ejaculate and also a higher male fertility. However, various genetic, management and environmental factors have been reported to affect semen production traits. Many studies dealt with selection strategies for semen quality, but estimates of genetic parameters considerably differed (Pirchner, 1968; Taylor and Everett, 1985; Distl and Averdunk, 1988; Stålhammer et al., 1989; Makulska et al., 1993; Ducrocq and Humblot, 1998). Several studies found evidence that age of bulls significantly influences semen production characteristics. Generally, ejaculate volume or total number of sperms was found to increase with age of bull (Everett and Bean, 1982; Mathevon et al., 1998a; Brito et al., 2002a). Management effects like the interval between collections and the number of collections on collection day were observed to have an impact on semen traits (Everett and Bean 1982; Mathevon et al. 1998a, 1998b).

Many authors indicate a significant effect of season on semen traits (Stålhammer et al., 1989; Mathevon et al., 1998a). The effect of season may consist of different factors such as temperature, day length, humidity, feed composition and quality. Optimal ambient temperatures for semen production were found to be in the range of 15°C to 20°C (Taylor et al., 1985; Parkinson, 1987). Not only temperature on collection day turned out to influence semen traits, but also about 65 to 70 days before collection during epididymal maturation and spermatogenesis (Stephan et al., 1971; Meyerhoeffer et al., 1985; Dorst, 1991). Feed quality was also found to affect semen quality (Peter, 1991).

The objectives of this study were (1) to evaluate the effect of several management and environmental factors on semen production of Austrian Simmental bulls, (2) to estimate genetic parameters for ejaculate volume, sperm concentration, percentage of viable spermatozoa, total number of spermatozoa and motility, and (3) to estimate the relationship between semen quality and male fertility using correlations between breeding values for semen production traits and male fertility.

2. Material and methods

Data on semen production characteristics obtained from the AI centre Oberösterreichische Besamungsstation GmbH, Hohenzell in Upper Austria, collected between April 2000 and November 2004 were evaluated. The average daily ambient temperature during the investigation period ranged from -13.4°C to +24.8°C with an

average of 10.1°C. The average relative humidity was 76% (range 42-99%) and average length of day 12:28 h (range 8:20-16:06 h).
In total 12,746 ejaculates from 301 Simmental bulls were analysed. All bulls were housed in tie-stalls. On average bulls were 3.4 years old. Semen is routinely collected on three days a week by artificial vagina. Most bulls mounted a dummy, but a teaser animal was also used. For sexual stimulation and preparation bulls were also allowed false mounts. Additional stimulation was provided by the collection team, consisting of bull handler and semen collector. Bulls were handled by 9 and semen was collected by 4 different persons. Bulls were collected up to 3 times a day. 53% of ejaculates came from first, 44% from the second, and 3% from the third collection per day. 26% of ejaculates were collected between intervals of 1 - 3 days, also 26% were between 4 - 6 days, 35% between 7 - 9 days and 13% showed an interval of >10 days. The 301 bulls were sons of 114 sires. The whole pedigree set consisted of 2470 bulls.

Traits recorded in the routine evaluation of bull semen are ejaculate volume, sperm concentration, percentage of viable spermatozoa in the ejaculate, total number of spermatozoa per ejaculate and motility.
Ejaculate volume was weighed by an electronic scale in mg and converted to ml afterwards. Sperm concentration was measured with an appropriate calibrated spectrophotometer. Total number of spermatozoa per ejaculate was computed as the product of ejaculate volume and sperm concentration. Percentage viable spermatozoa and motility were subjectively assessed under the microscope before dilution. Determining motility mass and forward movement was regarded scaled from 1 (worst) to 5 (best motility). For the analysis it was assumed that results of subjective evaluation were not influenced by technicians. Table 1 shows means and standard deviations for all traits and first, second and third collections.

Table 1. Arithmetic means and standard deviations for ejaculate volume, sperm concentration, percentage of viable spermatozoa, total number of spermatozoa and motility and for first (1), second (2), and third (3) collection.

Trait	**1**	**2**	**3**
	N=6778	**N=5638**	**N=330**
Volume (ml)	5.55±2.45	5.36±2.25	4.64±2.09
Concentration (10^9/ml)	1.24±0.42	1.02±0.38	0.88±0.38
Percentage viable (%)	63.4±11.5	66.3±8.9	66.9±7.9
Total (10^9)	6.72±3.48	5.25±2.60	3.90±2.20
Motility	2.87±0.42	2.93±0.39	2.94±0.34

All statistical analyses were performed using SAS Version 8.0 (SAS Institute Inc., 1999). To analyse the impact of various environmental effects on ejaculate volume, sperm concentration, percentage of viable spermatozoa and total number of spermatozoa per ejaculate the procedure MIXED was used. Motility classes 1 and 2 and 4 and 5 were combined. Since sperm motility was not normally distributed, the procedure GENMODE for analyses of multinomially distributed data was applied. Data of AI bulls with less than 10 collections and also collections with missing records for semen production traits and environmental effects were excluded from the analysis. The very first collection per bull was not regarded because there was no information about interval between collections. All two-way interactions between fixed effects were tested but removed when found to be not significant.

The following statistical model was used:

$Y_{ijklmnop} = \mu + a_i + age_j + handler_k + collector_l + interval_m + number_n + year_o + month_p + (age*interval)_{jm} + (age*month)_{jp} + \varepsilon_{ijklmnop}$
where
$Y_{ijklmnop}$ is the individual observation,
μ is the overall mean,
a_i is the random effect of animal i (i = 1 to 301),
age_j is the fixed effect of age class j of the bull at collection (j = 1 to 8; 1 = 16-18 months, 2 = >18-20 months, 3 = >20-22 months, 4 = >22-24 months, 5 = >24-36 months, 6 = >36-48 months, 7 = >48-72 months, 8 = >72 months),
$handler_k$ is the fixed effect of bull handler k (k = 1 to 9),
$collector_l$ is the fixed effect of semen collector l (l = 1 to 4),
$interval_m$ is the fixed effect of interval in days since last collection (m = 1 to 4; 1 = 1-3d, 2 = 4-6d, 3 = 7-9d, 4 = >10d),
$number_n$ is the fixed effect of number of collection within day of collection (n = 1 to 3),
$year_o$ is the fixed effect of year o at collection (o = 2000 to 2004),
$month_p$ is the fixed effect of month p at collection (p = 1 to 12),
$(age*interval)_{jm}$ is the fixed effect of interaction between age class j and interval m,
$(age*month)_{jp}$ is the fixed effect of the interaction between age class j and month p, and
$\varepsilon_{ijklmnop}$ is a random residual effect.

Pearson correlation coefficients for phenotypic values were calculated using procedure CORR (SAS Institute Inc., 1999). Heritabilities and genetic correlations of semen production traits were estimated by REML using the computer program VCE4 (Groeneveld, 1998). The same model, as described previously, was used except that the random bull effect was divided into a random additive genetic effect (a) and a

permanent environmental effect (pe) to account for repeated measurements of a bull. Genetic relationships among bulls were included which gave the following variance-covariance structure for random effects:

$$Var\begin{bmatrix} a \\ pe \\ e \end{bmatrix} = \begin{bmatrix} A\sigma_a^2 & 0 & 0 \\ 0 & I\sigma_{pe}^2 & 0 \\ 0 & 0 & I\sigma_e^2 \end{bmatrix}$$

where A is the genetic relationship matrix among bulls, I is the identity matrix, σ_a^2 is the additive genetic variance, σ_{pe}^2 is the permanent environment variance, and σ_e^2 is the residual variance.
Heritabilities and repeatabilities for each semen production trait were calculated as follows:

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

$$R = \frac{(\sigma_a^2 + \sigma_{pe}^2)}{(\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2)}$$

3. Results and discussion

3.1 Environmental effects

In Table 2 levels of significance for all fixed effects on semen production traits are shown.

Age class of bull at collection
All semen production traits were highly affected by age class of bull. Age class significantly interacted with collection interval for sperm concentration. Percentage of viable spermatozoa and motility were slightly affected and for total number of spermatozoa only a trend could be observed. Interaction between age class and month of collection was highly significant for all semen traits (Table 2). Ejaculate volume showed a continuous increase with age class except for bulls older than 72 months a small decline was observed (Table 3). Sperm concentration was highest for bulls aged between 18 and 20 months. Results for bulls in higher age classes indicated a general decrease of sperm concentration with a minimum of 0.96×10^9/ml.

Except for the youngest bulls percentage of viable spermatozoa reduced with increasing age. The optimum was found for bulls between 20 and 22 months old.

Table 2. Significance level for fixed effects on ejaculate volume (Vol), sperm concentration (Conc), percentage of viable spermatozoa (%viable), total number of spermatozoa (Total), and motility (Mot).

Effect	**Vol**	**Con**	**%viable**	**Total**	**Mot**
Age class	***	***	***	***	***
Bull handler	***	***	***	***	**
Semen collector	***	+	ns	ns	ns
Collection interval	***	***	***	***	**
Number of collection	***	***	***	***	***
Year	***	***	***	***	***
Month	***	***	***	**	***
Age class * Collection interval	ns	***	*	+	*
Age class * Month	***	***	***	***	***

+ P<0.10; * P<0.05; ** P<0.01; *** P<0.001

Total number of spermatozoa per ejaculate generally increased with age class with a maximum value of 6.56 ml in age class >72 month. Motility values were inconsistent with a slight tendency to decrease with higher age. The increase of ejaculate volume and total number of spermatozoa with age of bull was also found by Everett and Bean (1982), Taylor et al. (1985), Schwab et al. (1987), Mathevon et al. (1998a and 1998b) and Brito et al. (2002a). An enhancement of ejaculate volume until 7 years of age was reported by Taylor et al. (1985). After this period ejaculate volume did not vary until 9 to 10 years of age which is also confirmed by Brito et al. (2002a). Mathevon et al. (1998a) observed an increase of sperm concentration up to 20-22 months of age, which is in agreement of findings in the present study. Similar results were also reported by Schwab et al. (1987) which refer to an increase up to 34-36 months of age. Sperm concentration was not influenced by age of bull in the studies of Garner et al. (1996) and Brito et al. (2002a). Fuerst-Waltl et al. (2004), who analysed partly the same data, obtained similar results for percentage of viable spermatozoa and motility, whereas contrary results were found in other studies (Stålhammer et al., 1989; Makulska et al., 1993) indicating an increased motility with age of bull.

Table 3. Least Squares Means for different age classes of bulls for ejaculate volume (Vol), sperm concentration (Con), percentage of viable spermatozoa (%viable), total number of spermatozoa per ejaculate (Total) and arithmetic means for motility (Mot).

Age class in months	**N**	**Vol (ml)**	**Con (10^9)**	**%viable**	**Total (10^9)**	**Mot[1]**
16-18	3556	4.30	1.16	67.5	4.93	2.96
>18-20	1968	4.36	1.21	68.6	5.18	2.98
>20-22	693	4.53	1.18	68.8	5.31	2.97
>22-24	331	4.77	1.16	66.5	5.60	2.95
>24-36	459	5.08	1.12	65.9	5.69	2.89
>36-48	476	6.19	1.06	62.9	6.40	2.80
>48-72	3315	6.64	0.96	61.3	6.40	2.80
>72	1948	6.33	1.01	63.7	6.56	2.86

[1] The LSMEANS statement is not available for multinomial distributed models for ordinal response data (SAS Institute Inc., 1999). Therefore arithmetic means for the subclasses are presented.

Bull handler and semen collector

Bull handler was highly significant for all semen production traits whereas semen collector was found to have a relevant effect only on ejaculate volume and a very slight effect on sperm concentration (Table 2). The collection team is responsible for appropriate sexual preparation of bulls. This may explain the significant influence of the bull handler and partly the semen collector on semen output. Ejaculate volume, total number of spermatozoa and number of motile cells per ejaculate were highly affected by the collection team reported by Mathevon et al. (1998a), while sperm concentration and motility were not influenced by the collection team. Duration of sexual preparation was described to have significant effects on volume, number of doses per ejaculate and post-thaw motility (Kommisrud and Andersen Berg, 1996). Almquist (1973) concluded that sexual preparation including false mounts resulted in increasing sperm output.

Collection interval

The collection interval strongly influenced all semen characteristics (Table 2). Ejaculate volume and total number of spermatozoa per ejaculate continuously increased with longer intervals between collections. For sperm concentration optimum values were found with an interval of 4 to 9 days between collections. Percentage of viable spermatozoa was highest with an interval of 1 to 3 days and decreased with an increasing interval between collections (Table 4). Motility was also affected by the collection interval and little higher with an interval of 4 to 9 days (results not shown). Results observed in the present study for volume and total numbers of spermatozoa per ejaculate are in accordance with previous studies (Everett et al., 1978; Everett and Bean, 1982; Mathevon et al., 1998a and 1998b).

Present findings for sperm concentration and percentage of viable spermatozoa agree with Fuerst-Waltl et al. (2004).

Table 4. Least Squares Means for different collection interval, number of collection, year and month of collection for ejaculate volume (Vol), sperm concentration (Conc), percentage of viable spermatozoa (%viable) and total number of spermatozoa per ejaculate (Total)

Collection interval	**N**	**Vol (ml)**	**Conc (10^9)**	**%viable**	**Total (10^9)**
1-3	3391	4.73	1.08	66.6	4.95
4-6	3305	5.08	1.13	66.5	5.62
7-9	4413	5.50	1.13	65.4	6.15
>=10	1637	5.80	1.08	64.0	6.31
Number of collection					
1	6778	5.49	1.30	63.6	7.04
2	5638	5.33	1.08	66.3	5.60
3	330	5.00	0.94	67.0	4.64
Year					
2000	2009	4.80	1.14	66.6	5.53
2001	2897	5.16	1.08	67.3	5.45
2002	2997	5.40	1.11	62.9	5.88
2003	2458	5.63	1.09	64.3	6.03
2004	2385	5.38	1.11	67.1	5.92
Month					
January	939	5.13	1.17	67.0	5.91
February	985	5.28	1.17	66.2	6.20
March	980	5.10	1.11	65.5	5.62
April	1157	5.16	1.10	66.2	5.67
May	1377	5.02	1.08	65.2	5.42
June	1551	5.18	1.06	65.2	5.51
July	1091	5.42	1.08	65.0	5.74
August	304	5.67	1.03	62.2	5.55
September	1194	5.41	1.08	65.5	5.68
October	1386	5.54	1.10	66.4	5.96
November	1012	5.21	1.17	66.8	5.96
December	770	5.20	1.15	66.5	5.90

Number of collections on collection day

Number of collections was highly significant (P<0.001) for all semen quality traits (Table 2). First collection resulted in significantly higher volumes, concentration and total number of spermatozoa (Table 4). For percentage of viable spermatozoa and motility best results could be observed for second and third collection. Increased

ejaculate volumes, sperm concentration and total number of spermatozoa of first collections are in agreement with several other studies (Everett et al., 1978; Everett, 1982; Everett and Bean, 1982; Taylor et al., 1985). Slightly enhanced values for percentage of motile sperms of second and third ejaculates were obtained by Everett (1982) and Fuerst-Waltl et al. (2004), while in contrast to present results a significant higher percentage of motile sperms of first ejaculates were found by Everett et al. (1978) and Everett and Bean (1982).

Year and month of collection

The fixed effect of year had a significant impact on all semen quality traits (Table 2). Volume continuously increased until the year 2003 up to 5.63 ml and afterwards decreased in the year 2004 (Table 4). Nearly the same development for the total number of spermatozoa was obtained, except that the year 2001 yielded in higher total number of sperms than found in 2000. For concentration, percentage of viable spermatozoa and motility no clear pattern could be observed.

The effect of month was included to account for an effect of season with different climatic conditions. All semen production traits appeared to be highly affected by month (Table 2). Best values for ejaculate volume were found between July and October, whereas the lowest values were obtained in January and the months of spring. However, for all other traits smallest sperm output was obtained during the hot summer months. January, February, November and December were superior for sperm concentration. Optimum values for percentage viable spermatozoa and total number of spermatozoa were also observed in January, February, October, November and December (Table 4). Motility was highest in April and showed lowest values in August and September. The very low estimates for all semen traits in August, with the exception of volume, may be due to the small number of observations (Table 4). Schwab et al. (1987) reported the highest ejaculate volume, sperm concentration and total number of sperms between April and June, while lowest values for all semen traits, except concentration, were observed between October and December. According to Everett et al. (1978) lowest semen output appeared in January, February, and March. Better semen characteristics in summer were also obtained by Stålhammer et al. (1989) and Makulska et al. (1993). Contrary results were found by Everett and Bean (1982) reporting the poorest semen output in July and August. Mathevon et al. (1998a) observed optimum sperm concentration and total number of sperms during winter and spring. The highest percentage of motile spermatozoa for mature bulls was usually gained in summer and fall. Ejaculate volume and motility was not affected by season. Brito et al. (2002b) also found no seasonal influence on semen traits. Results presented in literature considerably vary. As season can include several environmental effects such as temperature, day length, humidity, feed composition and quality results should be carefully interpreted and compared.

3.2 Genetic parameters

Estimated heritabilities, genetic and phenotypic correlations, and repeatabilities for all semen production traits are shown in Table 5. In general estimated values for heritabilities were low to medium, ranging from 0.04 to 0.22. The highest values were found for total number of spermatozoa and ejaculate volume. The results obtained for ejaculate volume and sperm concentration were similar to those by Taylor et al. (1985) for Holstein bulls with heritabilities of 0.18 and 0.10 for volume and concentration. However, for total number of spermatozoa they estimated a very low heritability of 0.03. Makulska et al. (1993) also reported comparable heritabilities for Simmental bulls for volume and total number of spermatozoa of 0.19 and 0.20, respectively. However, they obtained values of 0.18, 0.26, 0.23 for mass movement, individual motility and concentration, respectively, which were higher than results found in this study. Taylor and Everett (1985), Gipson et al. (1987), Lang et al. (1988) as well as Stålhammer et al. (1989) refer to low to medium heritabilities for semen traits ranging from 0.02 to 0.28. Whereas high heritabilities were observed by Ducrocq and Humblot (1995, 1998), Diarra et al. (1997) and Mathevon et al. (1998a) varying between 0.24 to 0.65 for volume, 0.36 to 0.52 for concentration, 0.23 to 0.51 for motility, and 0.38 to 0.54 for total number of spermatozoa. The large variation among heritability estimates may be likely due to different breeds and ages of animals regarded, and also the different models used for parameter estimation. In this study the random additive effect and permanent environmental effect may be confounded because the two effects only differ in the genetic relationship matrix. The 301 bulls examined were sons of 114 sires indicating that the bulls were not highly related. Therefore heritability estimates ought to be carefully interpreted.

Table 5. Heritabilities with standard errors (diagonal), genetic correlations with standard errors (above diagonal), phenotypic correlations (below diagonal), and repeatability for ejaculate volume (Vol), sperm concentration (Conc), percentage of viable spermatozoa (%viable), total number of spermatozoa per ejaculate (Total) and motility (Mot).

	1	2	3	4	5
1. Vol	**0.18**±0.02	0.06±0.13	0.31±0.15	0.83±0.13	0.21±0.17
2. Conc	-0.17	**0.14**±0.04	0.41±0.17	0.60±0.07	0.48±0.17
3. %viable	-0.13	0.27	**0.10**±0.03	0.54±0.11	0.90±0.05
4. Total	0.70	0.52	0.07	**0.22**±0.02	0.50±0.13
5. Mot	-0.12	0.23	0.55	0.06	**0.04**±0.01
Repeatability	0.29	0.35	0.21	0.24	0.08

Repeatabilities for all semen production traits were very low to moderate (Table 5). Highest value was found for sperm concentration, the lowest value was estimated for motility. Results observed in the present study were quite lower than by Stålhammer et al. (1989) ranging from 0.52 to 0.59 and by Mathevon et al. (1998b) ranging from 0.41 to 0.64. Similar results for volume, concentration and total number of sperms were obtained by Taylor et al. (1985). Taylor and Everett (1985) estimated a repeatability coefficient for volume of 0.24 but higher values for concentration and total number of spermatozoa.

Genetic and phenotypic correlations for semen characteristics are presented in Table 5. Phenotypic values indicate a slight antagonistic relationship between volume and concentration, percentage of viable spermatozoa and motility. Between volume and total number of spermatozoa an obvious positive correlation of 0.70 was observed. Corresponding genetic correlations were generally positive and varied between 0.06 and 0.90. Genetic correlations obtained in the present study were contrary to literature findings. Diarra et al. (1997) found genetic correlations of -0.47 between volume and concentration. Negative relationship between volume and concentration was confirmed by Ducrocq and Humblot (1998) who estimated a correlation of -0.43. Genetic correlations of -0.30 and -0.39 between volume and concentration and mass movement were reported by Lang et al. (1988). Unexpected genetic correlations estimated in this study may be caused by the small dataset of only 301 bulls and the low relationship among bulls.
Breeding values of all bulls for the five semen production traits were estimated by Best Linear Unbiased Prediction applied to the animal model used for parameter estimation. Correlations between estimated breeding values of sperm quality traits and routinely estimated breeding values for male fertility (Fuerst and Egger-Danner, 2002) were low and ranged from 0.10 to 0.20. Highest correlations were obtained between estimated breeding values for total number of spermatozoa, percentage of viable spermatozoa, and motility and male fertility.

Concluding remarks

Number of collection on collection day, age of bull and collection interval showed the highest influence on semen production traits. First ejaculates resulted in increased ejaculate volume, sperm concentration and total number of spermatozoa per ejaculate. However, for economical reasons a second collection per day is recommended. The high impact of age of bull may be partly caused by preferential treatment of older high demanded bulls. To obtain a sufficient amount of semen with adequate quality a collection interval between 4 and 6 days is suggested. As month of collection was found to affect semen quality AI centres should take care of climatic conditions like temperature and humidity.

In general, considerable genetic variation was observed in the semen production traits studied. Heritabilities were low to medium and repeatabilities allow the prediction of semen quality to some extent. However, in order to obtain more reliable estimates for heritabilities, repeatabilities and genetic correlations, higher number of bulls should be considered. Further studies are planned to investigate the relationship between semen characteristics and fertility and the possible implementation of semen information in the genetic evaluation for fertility.

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