

# EFFECT OF SELECTED FACTORS ON QUALITATIVE AND QUANTITATIVE SEMEN PARAMETERS OF CZECH FLECKVIEH BULLS

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## Abstract

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The aim of the study was to estimate genetic characteristics for important qualitative and quantitative parameters of Czech Fleckvieh bulls' ejaculate and to define factors which influence these parameters. a total number of 2,929 entries about samples from 163 bulls of a selected artificial insemination centre were used for calculation. the analysed ejaculate characteristics were: volume of ejaculate, sperm concentration, total sperm count, native sperm activity and activity after thawing. In all evaluated semen characteristics, the age of individual, coefficient of inbreeding, interval between collection and year of collection were statistically significant ( $P < 0.05$ ). In the case of season of collection, statistical significance ( $P < 0.01$ ) was proved in all monitored semen parameters, except concentration of spermatozoa where statistically significant difference was not found ( $P > 0.05$ ). Estimated coefficients of heritability correspond to the values standardly specified for reproductive parameters ( $h^2 = 0.003 - 0.14$ ), except for the volume of ejaculate ( $h^2 = 0.31$ ). the highest value of genetic correlation was recorded between the volume of ejaculate and the total sperm count ( $r_G = 0.9$ ), on the contrary, the lowest value was between the volume of ejaculate and the sperm concentration ( $r_G = 0.32$ ).

Keywords: artificial insemination, bovine semen, coefficient of inbreeding, czech fleckvieh bulls, genetic variance, heritability, interval between collection, season of collection.

## INTRODUCTION

Artificial insemination (AI) is one of the most powerful and the most valuable biotechnological methods. It allows dairy cattle breeders to use quality proven AI sires and thus to improve genetic potential and increase profitability of their herds. Knowledge of factors affecting sperm production and semen quality is of a high importance with regard to reproductive efficiency and thus genetic improvement as well as for the productivity and profitability of AI centers (Fuerst-Waltl *et al.*, 2004). Evaluation of qualitative and quantitative parameters of bovine semen was done by many

authors (Vilakazi *et al.*, 2004; Sarder, 2007; Igna *et al.*, 2010) who consider external influences, namely the effect of season, stable microclimate and sampling techniques, the most important factors affecting semen quality. Internal factors are described too, mainly the genetic ones (Mathevon *et al.*, 1998a; Mathevon *et al.*, 1998b; Brito *et al.*, 2002; Gedler *et al.*, 2007; Karoui *et al.*, 2011), variation between individuals and age of the sire (Bezdicsek *et al.*, 2007; Stolc *et al.*, 2009; Strapak *et al.*, 2010). the increasing rate of AI application during the past few decades has resulted in widespread use of only a few top sires (Behmorad and Ghorbani, 2015).

the best animals accumulate in pedigrees, therefore it is nowadays practically impossible to find animals without multiple genetic ties to certain individuals in a given dairy cattle breed (Croquet *et al.*, 2006). the objective of this study was to estimate genetic and environmental factors affecting semen parameters of Czech Fleckvieh bulls. Better knowledge of internal and external factors influencing bovine semen parameters can improve management of AI centre (AIC).

## MATERIALS AND METHODS

The project was realized at AIC of "Breeding Cooperative Impuls" in the Czech Republic. In period from May 2008 to December 2014, the study was carried out on a biological material consisting of 2929 semen samples from 163 Czech Fleckvieh bulls. All ejaculates were collected by the sampling team of AIC, in a room specially adapted for this task, using the sampling method to artificial vagina on a dummy. For sexual stimulation and preparation, bulls were allowed on false mounts. Additional stimulation was provided by the collection team, consisting of a bull handler and a semen collector (Gredler *et al.*, 2007). a standard bovine artificial vagina at a temperature of 42 °C was used (Vilakazi *et al.*, 2004). Immediately after collecting, macroscopic and microscopic examination was performed for all samples, which included measurement of the volume of ejaculate, sperm concentration, native sperm activity and activity of sperm after thawing. the volume of ejaculate was detected directly by reading from the scale on calibrated collection containers (Mathevon *et al.*, 1998a). the native sperm activity was assessed by subjective method according to the percentage of motile sperms in the native ejaculate (Student Microscope Model SM 5 with phase contrast by INTRACTO MICRO, spol. s r. o., lens 10 × /0,25 PHD). We evaluated the percentage of sperms with progressive direct movement after the head (Sarder, 2007) and sperm concentration was evaluated by Minitube Photometer SDM6 calibrated for bull ejaculate. the total sperm count was determined by calculation of the sperm concentration per mm<sup>3</sup> and a total volume of ejaculate (Fuerst-Waltl *et al.*, 2006). For the purpose of genetic evaluation, a pedigree of 7 generations was created and total number of 3016 individuals were included in the calculation. the proper prediction of population parameters and subsequently also breeding values was performed using multi-trait animal model with repeatability, where restricted maximum likelihood method REML was used for the best estimate of variance components. For this prediction, program REMLF90 was used (Misztal *et al.*, 2002). For data preparation and selection of a requisite model, software SAS 9.4 (SAS Institute Inc., 2005) was used.

Coefficients of inbreeding ( $F_x$ ) used in the model were calculated from the matrix of relatedness using

program FSpeed v2.04a (Tenset Technologies Ltd., 2009) based on the formula (Mrode, 1996):

$$F_x = \sum (1/2)^n (1 + F_A)$$

where:  $n$  is a number of generations to a common ancestor and  $F_A$  is an inbreeding coefficient of common ancestor.

Fixed and random effects were selected based on literature and our own experiences from collection of samples at the AIC. Mainly commonly used fixed effects of the AIC, the collection team and order of collection were not taken into account. the reason for this is that the set of data was collected at one AIC by one collection team with unchanged staff and only the first ejaculates of each bull were taken to the laboratory. Following fixed effects were taken into consideration in calculation: age of bull, inbreeding coefficient, interval between individual collections, season of the year, year of collection and individual interactions between the fixed effects. the random effects included besides the additive genetic effect also the effect of permanent environment, because all the bulls were collected more than once (from 2 to maximum 95 collections in data set), therefore there was repetition of measurements. the selected fixed effects and their mutual interactions were tested using GLM method in SAS 9.4 (SAS Institute Inc., 2005) software. Significance level for exclusion of effects from the model was set to  $\alpha = 0.05$  (test statistics: F-value).

Equations of mixed multi-trait model were different for each dependent variable (volume of ejaculate, native sperm activity, sperm concentration and activity of sperm after thawing):

$$y_{ijklmno} = \mu + a_i + f_j + i_k + s_l + r_m + (af)_{ij} (ai)_{ik} + (as)_{il} + (ar)_{ji} + (fi)_{jk} + (fs)_{jl} + (fr)_{jm} + (is)_{kl} + (ir)_{km} + (sr)_{lm} + u_n + pe_n + e_{ijklmno}$$

where:  $y_{ijklmno}$  is dependent variable  $o$  (ejaculate volume in ml) of  $n^{th}$  bull of age  $i$ , inbreeding coefficient  $j$ , with interval between collections  $k$ , season  $l$  and year of collection  $m$ . the  $a_i$  is the fixed effect of the  $i^{th}$  age class at the time of collection ( $i = 1$  to 8). the age was divided into 8 classes: Class 1 for youngest bulls from 277 to 487 days of age; class 2 for 488 to 548 days; class 3 for 549 to 609 days; class 4 for 610 to 731 days; class 5 for 732 to 1097 days; class 6 for 1098 to 1829 days; class 7 for 1830 to 2195 days and class 8 for 2196 to 3981 days. the  $f_j$  is the fixed effect of the  $j^{th}$  class of inbreeding coefficient ( $j = 1$  to 8), where the class 1 contains non-inbred bulls (with  $F_x = 0.0$  %); class 2 with  $F_x = 0.1$ – $0.2$  %; class 3 with  $F_x = 0.3$ – $0.4$  %; class 4 with  $F_x = 0.5$ – $0.9$  %; class 5 with  $F_x = 1.0$ – $1.4$  %; class 6 with  $F_x = 1.5$ – $1.9$  %; class 7 with  $F_x = 2.0$ – $2.9$  % and class 8 with  $F_x = 3.0$ – $12.8$  %. the  $i_k$  is the fixed effect of the  $k^{th}$  interval between collections ( $k = 1$  to 10), where the interval is expressed as the number of days from the previous collection. Class 1 consists of the data from the first

collection in the certain bull's life. Other classes are following: class 2 = 1–3 days; class 3 = 4–5 days; class 4 = 6 days; class 5 = 7 days; class 6 = 8 days; class 7 = 9–11 days; class 8 = 12–14 days; class 9 = 15–19 days and class 10 = 20–48 days. the  $s_l$  is the fixed effect of the  $l^{\text{th}}$  season of the year ( $l = 1$  to 4), where the classes are: class 1 = December–February; class 2 = March–May; class 3 = June–August; class 4 = September–November. the  $r_m$  is the fixed effect of the  $m^{\text{th}}$  year of collection of the bull ( $m = 1$  to 10), where each year stood for an individual class. the  $(af)_{ij}$  is the fixed effect of the interaction between age  $i$  and inbreeding coefficient  $j$ . the  $(ai)_{ik}$  is the fixed effect of the interaction between age  $i$  and interval  $k$ . the  $(as)_{il}$  is the fixed effect of the interaction between age  $i$  and season  $l$ . the  $(ar)_{im}$  is the fixed effect of the interaction between age  $i$  and year  $m$ . the  $(fi)_{jk}$  is the fixed effect of the interaction between inbreeding coefficient  $j$  and interval  $k$ . the  $(fs)_{jl}$  is the fixed effect of the interaction between inbreeding coefficient  $j$  and season  $l$ . the  $(fr)_{jm}$  is the fixed effect of the interaction between inbreeding coefficient  $j$  and year  $m$ . the  $(is)_{kl}$  is the fixed effect of the interaction between interval  $k$  and season  $l$ . the  $(ir)_{km}$  is the fixed effect of interaction between interval  $k$  and year  $m$ . the  $(sr)_{lm}$  is the fixed effect of the interaction between season  $l$  and year  $m$ . the  $u_n$  is the random effect of the  $n^{\text{th}}$  individual ( $n = 1$  to 3016). the  $pe_n$  is the random effect of permanent environment of the  $n^{\text{th}}$  donor bull ( $n = 1$  to 163) and  $e_{ijklmno}$  is random residual error. Assumption is (model shown for only two characteristics):

$$\text{var} \begin{bmatrix} u_1 \\ u_2 \\ pe \\ e_1 \\ e_2 \end{bmatrix} = \begin{bmatrix} u_{11} & u_{12} & 0 & 0 & 0 & 0 \\ u_{12} & u_{22} & 0 & 0 & 0 & 0 \\ 0 & 0 & pe_{11} & pe_{12} & 0 & 0 \\ 0 & 0 & pe_{12} & pe_{22} & 0 & 0 \\ 0 & 0 & 0 & 0 & e_{11} & 0 \\ 0 & 0 & 0 & 0 & 0 & e_{22} \end{bmatrix}$$

where  $u_{11}$  is genetic variance for characteristic 1,  $u_{22}$  is genetic variance for the second characteristic and  $u_{12}$  is genetic covariance of the two characteristics. Variance-covariance matrix  $G$  ( $5 \times 5$ ) composed of these characteristics is multiplied by matrix of relatedness:  $G \otimes A$ .

Matrix  $PE$  ( $5 \times 5$ ) of the permanent environment of each donor bull is created similarly, defined in the model as:  $I \otimes PE$ .

Individual components of variance and covariance ( $G$ ,  $PE$  and  $E$ ) are outputs of the model.

Based on these values, coefficient of heritability ( $h^2$ ) and coefficient of repeatability ( $R_{pe}$ ) were calculated as follows:

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

$$R_{pe} = (\sigma_u^2 + \sigma_{pe}^2) / (\sigma_u^2 + \sigma_{pe}^2 + \sigma_e^2)$$

## RESULTS AND DISCUSSION

The mean value (Tab. I) of the volume of ejaculate was 7.95 ml, the native sperm activity 71.96 %, the sperm activity after thawing 35.54 %, the sperm concentration  $1.31 \times 10^9$  spz.ml<sup>-1</sup> and the total sperm count was  $10.38 \times 10^9$  spz/per ejaculate

### The influence of age

Age of individual at the time of collection was divided into 8 classes. In all evaluated characteristics, the age was highly statistically significant ( $P < 0.01$ ), which corresponds to other authors (Wilakazi *et al.*, 2004; Fuerst-Waltl *et al.*, 2006; Igna *et al.*, 2010). Increasing age was associated with growing activity of the native sperm activity (Fig. 1), the volume of ejaculate (Fig. 2) and the sperm concentration in the ejaculate (Fig. 3), with the most prominent increase in the volume of ejaculate (Brito *et al.*, 2002). Increase of the total sperm count (Fig. 4) corresponded to an increase in the volume of ejaculate (Boujenane and Boussaïq, 2013) and an increase, albeit slight, was recorded also in the sperm activity after thawing (Fig. 5).

### The influence of inbreeding coefficient

The effect of inbreeding depression on bovine semen production was expressed by inbreeding coefficients ( $F_x$ ). In order to investigate, bulls were divided into 8 classes to allow meaningful statistical analysis. In the analysed classes, the inbreeding coefficient ranged from 0.0 to 12.8 %. Although almost every bull was inbred to some extent, the 50.0 % of all tested bulls did not exceed the 0.5 % value of inbreeding level and only 10.0 % of them had inbreeding coefficients higher than 2.0 %. Possible preselection of inbred AI bulls with insufficient semen production may be another reason for the low inbreeding level in the observed group. Still a statistically significant

I: The base statistics of the evaluated traits

TRAIT	Unit	Number	Mean	SD	SE
Native sperm activity	%	2,897	71.96	6.37	0.12
Volume of ejaculate	ml	2,914	7.95	3.15	0.06
Sperm concentration	$\times 10^9$ spz.ml <sup>-1</sup>	2,838	1.31	0.48	0.01
Total sperm count	$\times 10^9$ spz	2,837	10.38	5.62	0.11
Activity after thawing	%	2,682	35.54	7.04	0.14

SD = standard deviation, SE = standard error, spz = spermatozoa.

## II: Significance of fixed effects on evaluated traits

FIXED EFFECT	Act	Vol	Con	TSC	Aat
Age of individual	**	**	**	**	**
Coefficient of inbreeding	**	**	**	**	**
Interval between collections	**	**	**	*	*
Season of collection	**	**	NS	*	**
Year of collection	**	**	**	**	**

Act = native sperm activity, Vol = volume of ejaculate, Con = sperm concentration, TSC = total sperm count, Aat = activity after thawing, \*\* = ( $P < 0.01$ ), \* = ( $P < 0.05$ ), NS = ( $P > 0.05$ ).

effect ( $P < 0.01$ ) of inbreeding coefficient on all monitored semen parameters was found (Tab. II). In similar publications, only a small number of authors studied the effect of inbreeding depression on bovine semen production (Maximini *et al.*, 2011; Behmorad and Ghorbani, 2015). Despite the quite low inbreeding level, the effect of inbreeding depression on semen quality traits was observed in earlier studies in cattle (Smith *et al.*, 1989; Flade *et al.*, 1992).

### The interval between collections

The interval between collections is considered to be a very important factor affecting ejaculate characteristics in bulls. In the observed population, it had again highly statistically significant effect ( $P < 0.01$ ) on all parameters, while in the case of total sperm count and sperm activity after thawing, the level of statistical significance was  $P < 0.05$  (Tab. II). a significant influences of interval between collection were also proved by Fuerst-Waltl *et al.* (2006), Karoui *et al.* (2011) and Boujenane and Boussaq (2013). In mean values of individual parameters, depending on the length of interval between collections, a slight decrease was recorded in the sperm concentration (Fig. 3) with lengthening interval, on the contrary, the volume of ejaculate (Fig. 2) increased as expected. Mathevon *et al.* (1998b) observed higher sperm concentration and a significant increase of the total sperm count per ejaculate with longer intervals between collections. No trend was recorded in the native sperm activity and the sperm activity after thawing, the total sperm count grew in relation to the increase in the volume of ejaculate (Fuerst-Waltl *et al.*, 2004).

### The season and the year of collection

Season and year of collection belong to significant environmental factors, mainly temporal. the year of collection can reflect many changes, regarding animal care, climatic conditions and many other hardly definable events, which disables the search for particular reasons of decrease or increase in individual parameters in dependence on the year of collection. the effect of the year of collection was significantly different ( $P < 0.01$ ) in all observed parameters. Also a considerable trend of significant increase can be observed in the volume of ejaculate (Fig. 2) and the native sperm activity (Fig. 1), while

a decrease was observed in the sperm concentration (Fig. 3). Taylor *et al.* (1985) reported that sperm production in Holstein bulls (volume of ejaculate, sperm concentration and total sperm count) was greater during summer. However, Mathevon *et al.* (1998a) and Boujenane and Boussaq (2013) documented that Holstein bulls produced more spermatozoa (higher concentration of spermatozoa and total sperm count) with greater motility during winter and spring which is in agreement with our results and other authors (Brito *et al.*, 2002). the season of collection, divided into 4 classes according to the season of the year, was confirmed to be statistically significant ( $P < 0.05$ ). In each parameter, the lowest and the highest values are reached in a different class of season and no evident trend can be searched in this effect. These results are in accordance with those of Karoui *et al.* (2011) and Boujenane and Boussaq (2013). This factor, similarly to the year of collection, covers many environmental factors, such as animal care, climatic changes, temperature, humidity, feed quality, eventually length of day (Mathevon *et al.*, 1998a).

### Estimating of population genetic parameters

The estimated values of genetic, permanent environmental and residual variance are presented in Table III. These values also served for calculation of coefficients of heritability, where the highest heritability ( $h^2 = 0.31$ ) was recorded in the volume of ejaculate, while the lowest ( $h^2 = 0.003$ ) in the concentration of spermatozoa. the remaining parameters showed rather lower heritability, in the levels around  $h^2 = 0.1$ . Recorded coefficients of heritability correspond to the generally estimated values in parameters associated with reproduction. Mathevon *et al.* (1998b), Gredler *et al.* (2007) and Karoui *et al.* (2011), documented heritability of the volume of ejaculate in a wide range of values ( $h^2 = 0.18–0.65$ ). In case of the concentration of spermatozoa, the heritability reaches much higher values ( $h^2 = 0.14–0.37$ ). In the native sperm activity and the sperm activity after thawing, our values correspond to the values reported by other authors. Coefficients of repeatability were also calculated. They describe the degree of similarity between consecutive measurements or collections, affected by random environmental influences. the highest coefficient of repeatability was found



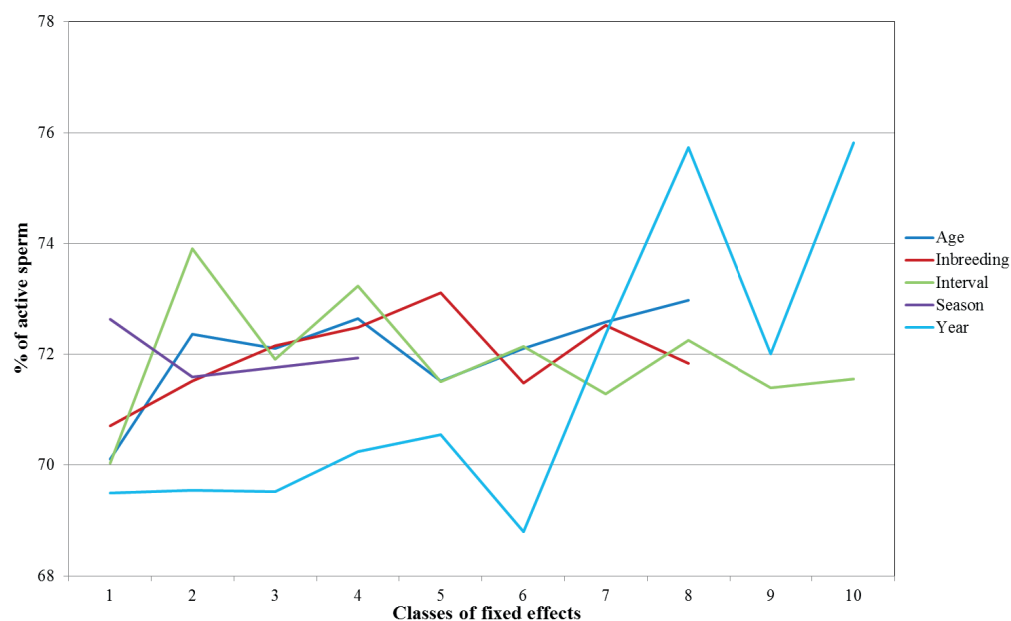
in the native sperm activity ( $R_{PE} = 0.59$ ), which shows the second lowest heritability ( $h^2 = 0.08$ ). Similar results were found in the concentration of spermatozoa ( $R_{PE} = 0.38$ ;  $h^2 = 0.003$ ), therefore in parameters, where a considerable part of variance is influenced by controlled but also uncontrolled environmental effects, while genetically determined individuality of a bull plays only a minimal role here. Different results were found in the volume of ejaculate ( $R_{PE} = 0.35$ ;  $h^2 = 0.31$ ), where the values of genetically determined variance are at the levels of milk performance. Mathevon *et al.* (1998b) and Karoui *et al.* (2011) achieved mostly similar results of the coefficients of heritability, with an exception of native sperm activity with much higher value of the coefficient ( $h^2 = 0.08$ ) recorded in our study. The values of genetic variance and heritability are very similar, but in the case of the sperm activity after thawing much higher variance was recorded

caused by uncontrolled environmental effects to the detriment of the permanent environment of individual, which is confirmed also by lower coefficient of repeatability in the sperm activity after thawing. Relatively high coefficients of repeatability in all parameters indicate a possibility to predict future development of the given characteristics of a bull on the basis of the first collections.

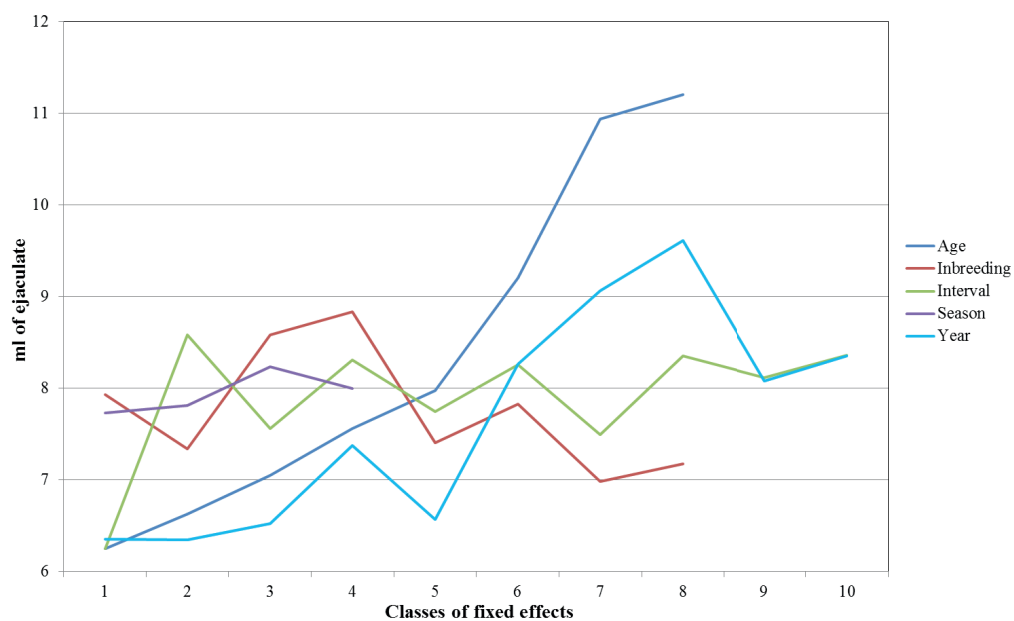
III: Genetic variance, permanent environmental variance, residual variance, coefficient of heritability and coefficient of repeatability for all monitored parameters

Population parameters	Act	Vol	Con	TSC	Aat
$\Sigma^2_u$	3.86	1.99	0.62	3.14	4.50
$\Sigma^2_{pe}$	25.73	0.23	83.78	4.17	12.22
$\Sigma^2_e$	20.81	4.11	139.00	15.72	30.40
$h^2$	0.08	0.31	0.003	0.14	0.10
$R_{PE}$	0.59	0.35	0.38	0.32	0.36

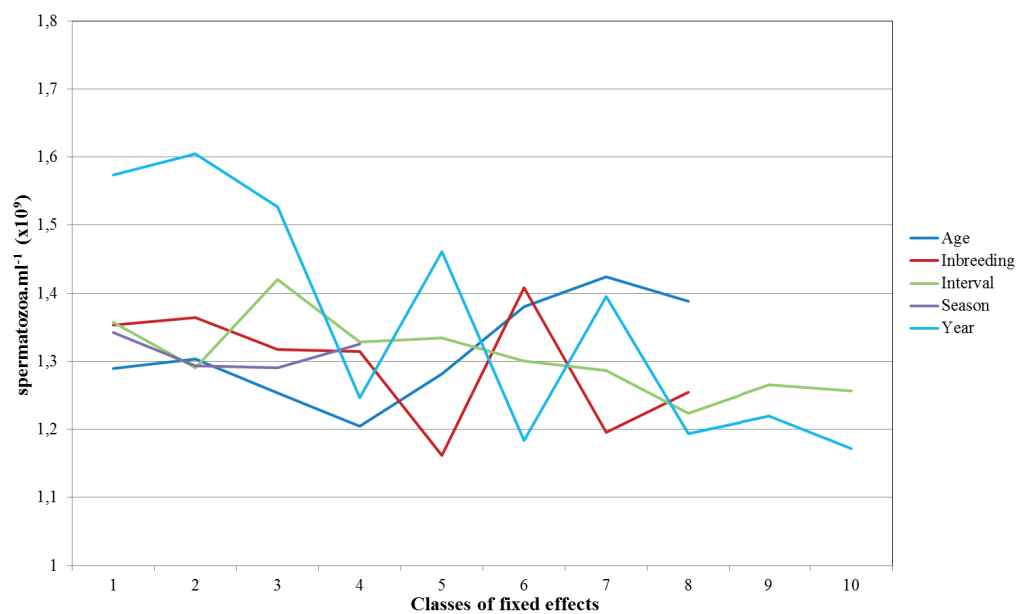
Act = native sperm activity, Vol = volume of ejaculate, Con = sperm concentration, TSC = total sperm count, Aat = activity after thawing,  $\sigma^2_u$  = genetic variance,  $\sigma^2_{pe}$  = permanent environmental variance,  $\sigma^2_e$  = residual variance,  $h^2$  = coefficient of heritability and  $R_{PE}$  = coefficient of repeatability.



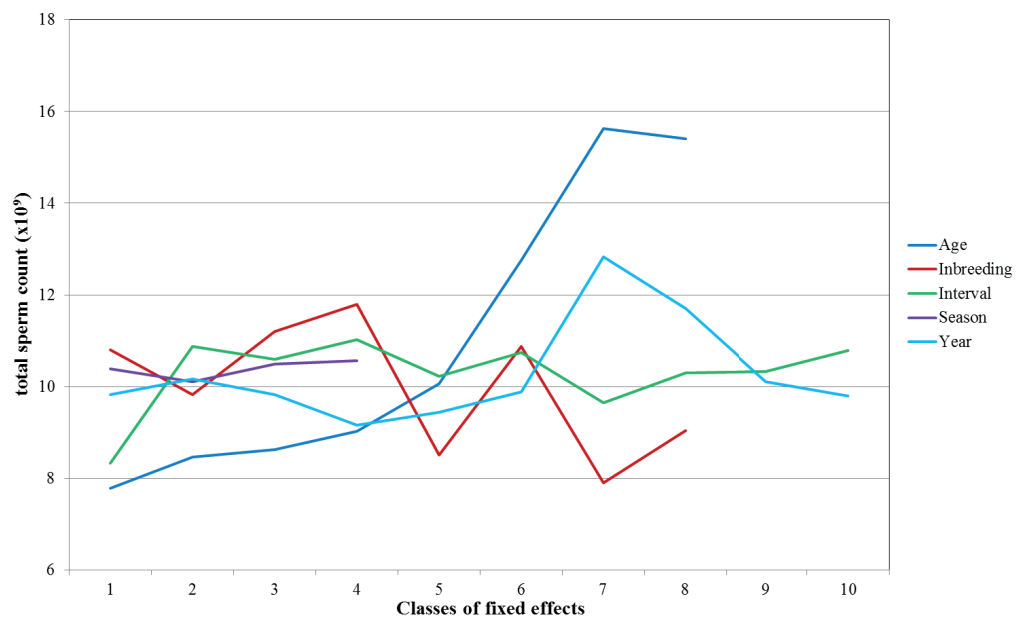
1: Mean values of the native sperm activity in various groups of the fixed effects.



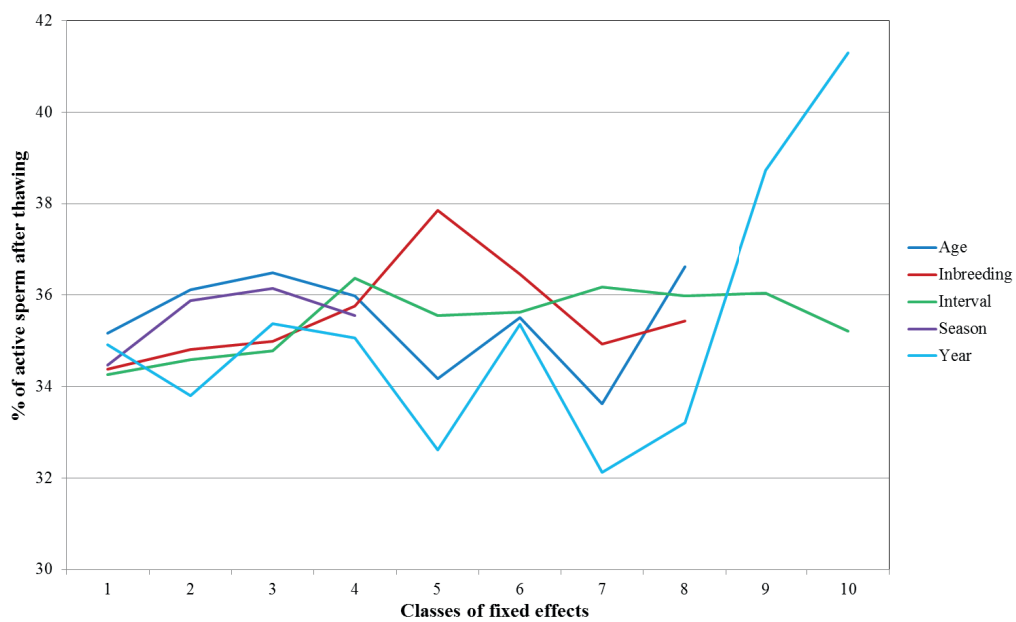
2: Mean values of ejaculate volume in various groups of the fixed effects.



3: Mean values of sperm concentration in various groups of the fixed effects.



4: Mean values of total sperm count in various groups of the fixed effects.



5: Mean values of sperm activity after thawing in various groups of the fixed effects.

## CONCLUSION

The presented results indicate that environmental factors influencing selected parameters of bull ejaculate of Czech Fleckvieh Cattle correspond to findings in other populations of Fleckvieh breed and other dual purpose breeds of cattle. Crucial factors were the season and the year of collection, the interval between collections, the age of bull at the time of collection and also the level of inbreeding. Also interactions between these effects have an important role. It is important to note that the evaluation included data from one AIC, collected by one collection team, therefore these major effects could not be analysed but at the same time, they did not have an effect on evaluated parameters. Estimated population genetic parameters mostly correspond to findings of other authors. Coefficients of heritability correspond to characteristics associated with fertility, thus they reach low levels. An important feature is repeatability between individual measurements, which determines good ability to predict future development of the parameters of ejaculate in young bulls and thus helps to decide about their stay at AIC. This is a more important fact for insemination companies than heritability of these parameters itself, since no selection pressure on bulls in ejaculate parameters can be expected. In conclusion, ejaculate parameters of bulls are affected by various number of environmental factors, but also genetic basis of an individual. All these aspects must be taken into account in analysis of bull ejaculate parameters for optimization of ejaculate production at AIC.

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