

## Effects in genetic evaluation for semen traits in Czech Large White and Czech Landrace boars

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**ABSTRACT:** Data on 75 567 ejaculates from 1 417 boars of the breeds Czech Large White and Czech Landrace collected in 23 AI centres between 2000 and 2007 were analyzed. Fixed effects were estimated from a four-trait animal model for semen volume, sperm concentration, motility and percentage of abnormal spermatozoa and from single-trait animal models for the total number of spermatozoa and the number of functional spermatozoa. Both the total number of spermatozoa and the number of functional spermatozoa were highest in winter and lowest in summer. Boar's age had a strong influence on semen volume, the total number and the functional number of spermatozoa; these traits increased especially in the first phase. The percentage of abnormal spermatozoa also increased with age. An interval between successive collections of 7 to 10 days yielded the best values for all semen traits. As semen traits are of direct economic importance for AI centres, it can be expected that the estimation of breeding value for semen traits will become important and that AI centres will choose among top boars for production and female reproduction traits the boars with better semen production.

**Keywords:** pig; boar; semen traits; breeding value estimation

Artificial insemination (AI) has become an important procedure in the global pig industry. Compared with natural service, artificial insemination allows the greater use of genetically superior sires (Oh et al., 2006). Selection practices for AI boars are universally based on the genetic evaluations for economically important traits (Robinson and Buhr, 2005). For example, boars of dam breeds kept in the Czech Republic are mainly selected for average daily gain from birth till the end of the performance test, lean meat content at the end of the test and number of piglets born alive (Wolf et al., 2005). However, no AI centre can restrict itself to the selection for production and female reproduction traits only, it must also consider factors that enhance the efficiency of the centre such as boar conformation and temperament and sperm quantity and quality. Semen volume, sperm con-

centration and gross sperm morphology are semen traits that affect the profitability of an AI centre (Robinson and Buhr, 2005).

It has been shown that semen traits are heritable traits with heritabilities in the same order of magnitude or higher than those for litter size traits (Rothschild, 1996; Grandjot et al., 1997; Smital et al., 2005; Oh et al., 2006; Wolf, 2009). Therefore, genetic evaluation of semen traits and selection for these traits are possible. On this basis, an animal model was developed and put into practical use for the genetic evaluation of semen traits of pig dam breeds kept in the Czech Republic. The genetic evaluation is based on a large data set and includes all AI boars of both dam breeds. The objective of the present investigation is to present and discuss the effects of the individual fixed factors in the animal model for semen traits.

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## MATERIAL AND METHODS

### Animals and traits

Data on semen collections from boars of the breeds Czech Large White and Czech Landrace collected between 2000 and 2007 were analyzed. The boars were located in 23 AI centres in the Czech Republic. The number of boars, total number of ejaculates and average number of ejaculates per boar for both breeds are summarized in Table 1. These numbers refer to the edited data set which was used for all calculations (for details see below).

On each ejaculate, the following semen traits were measured: semen volume (*Vol*) or ejaculate volume in ml (i.e. volume of the sperm rich fraction) measured with a graduated cylinder, sperm concentration (*Con*, in  $10^3$  sperm cells per  $\text{mm}^3$ ) measured by photocolometry, motility (*Mo*, progressive motion of spermatozoa in per cent, i.e. proportion of sperm cells actively moving straight-forward, evaluated microscopically) and percentage of abnormal spermatozoa (*Ab*, percentage of deformed or otherwise changed sperm cells, also evaluated microscopically). The total number of spermatozoa in the ejaculate ( $N_{total}$ , in  $10^9$  sperm cells) was calculated as follows:

$$N_{total} = Vol \times Con / 1\,000$$

and the number of functional spermatozoa ( $N_{funct}$ , in  $10^9$  sperm cells) was estimated in this way (Smital et al., 2004):

$$N_{funct} = N_{total} (Mo/100)(1 - Ab/100)$$

### Statistical analyses

The following model was used both for (co)variance and for breeding value estimation:

$$y_{ijklmno} = month_i + age_j + int_k + centre\_year_l + breed_m + p_n + a_n + e_{ijklmno}$$

where:

- $y_{ijklmno}$  = the semen trait measured on the  $o^{\text{th}}$  ejaculate of the  $n^{\text{th}}$  boar of the  $m^{\text{th}}$  breed
- $month_i$  = the effect of the season (month)
- $age_j$  = the effect of the age class of the boar
- $int_k$  = the effect of the interval between the present and the previous semen collection
- $centre\_year_l$  = the combined effect of the AI centre and year
- $breed_m$  = the effect of the breed of the boar
- $p_n$  = the permanent environmental effect of the boar
- $a_n$  = the additive genetic effect of the boar
- $e_{ijklmno}$  = the residual effect

The pedigree was traced back approximately to the year 1985.

To form age classes, first the boar's age in months at collection was calculated. Ejaculates from animals of less than eight months of age or older than 48 months were excluded from the data set. When forming the age classes, monthly intervals were used up to an age of 28 months. For animals aged between 29 to 38 months bimonthly intervals were

Table 1. Summary statistics for semen traits in Czech Large White and Czech Landrace boars

Variable	Czech Large White	Czech Landrace	Both breeds
Numbers			
Number of boars	672	745	1 417
Number of ejaculates	31 328	44 239	75 567
Average number of ejaculates per boar	47	59	53
Means			
Semen volume (ml)	276	273	274
Sperm concentration ( $10^3$ sperm cells/ $\text{mm}^3$ )	430	422	425
Motility (%)	76.0	75.6	75.8
Percentage of abnormal spermatozoa (%)	11.4	11.2	11.3
Total number of spermatozoa ( $10^9$ sperm cells)	112	107	109
Number of functional spermatozoa ( $10^9$ sperm cells)	75.5	72.6	73.8

Table 2. Estimates of genetic parameters with standard errors for semen volume, sperm concentration, motility and percentage of abnormal spermatozoa

Trait	<i>Vol</i>	<i>Con</i>	<i>Mo</i>	<i>Ab</i>
<b>Heritabilities (on diagonal) and genetic correlations</b>				
Semen volume ( <i>Vol</i> , ml)	0.24 ± 0.016	-0.73 ± 0.037	-0.09 ± 0.044	-0.02 ± 0.065
Sperm concentration ( <i>Con</i> , 10 <sup>3</sup> sperm cells/mm <sup>3</sup> )		0.18 ± 0.016	0.17 ± 0.067	0.06 ± 0.110
Motility ( <i>Mo</i> , %)			0.13 ± 0.023	-0.88 ± 0.109
Percentage of abnormal spermatozoa ( <i>Ab</i> , %)				0.07 ± 0.026
<b>Proportions of variance (on diagonal) and correlations caused by the permanent environmental effect of a boar</b>				
Semen volume ( <i>Vol</i> , ml)	0.18 ± 0.014	-0.52 ± 0.034	0.10 ± 0.035	0.03 ± 0.026
Sperm concentration ( <i>Con</i> , 10 <sup>3</sup> sperm cells/mm <sup>3</sup> )		0.19 ± 0.015	-0.04 ± 0.041	0.06 ± 0.039
Motility ( <i>Mo</i> , %)			0.21 ± 0.020	-0.27 ± 0.052
Percentage of abnormal spermatozoa ( <i>Ab</i> , %)				0.39 ± 0.026

formed. For animals over 38 months of age, the following three classes were formed: 39 to 41 months, 42 to 44 months and 45 to 48 months.

Preliminary analyses showed that all measured traits were most sensitive to changes in the interval between two semen collections from the same boar when that interval was short. Therefore, for intervals shorter than 11 days, classes with an interval of one day were formed. For intervals of 11 days and more, the following three classes were formed: 11 to 12 days, 13 to 15 days and 16 to 21 days. The first semen collection of each boar and semen collections with an interval of 1 day or more than 21 days were not included in the analyses.

Data were excluded from further analyses if one of the following conditions was not satisfied: the minimal number of ejaculates per AI centre and per AI centre and year must be 100 and 20, respectively, and the minimal number of semen collections per boar must be 5. Furthermore, the trait values must be within the following intervals: semen volume 50–600 ml, sperm concentration 50–900 thousand sperm cells per mm<sup>3</sup>, motility 50–100%, percentage of abnormal spermatozoa 0–30%, total number of spermatozoa  $5 \times 10^9 - 200 \times 10^9$  sperm. The means of all traits of the final data set are given in Table 1.

Restricted maximum likelihood (REML) and optimisation by a quasi-Newton algorithm with analytical gradients (Neumaier and Groeneveld, 1998) as implemented in VCE 5.0 program (Kovač et al., 2002) were used to estimate the variances and covariances. The PEST program (Groeneveld

et al., 1990) with the SMP solver was used for the prediction and estimation of random and fixed effects, respectively, in the model given above. Effects of fixed factors are usually presented as deviations from their average effect. A four-trait animal model was used for semen volume, sperm concentration, motility and percentage of abnormal spermatozoa. Single-trait animal models were calculated for the total number of spermatozoa and the number of functional spermatozoa.

To get an impression of the environmental trend, the effect of the year of collection was calculated from the combined effect for the AI centre and year using the GLM procedure of SAS<sup>®</sup> 9.1 software. Average breeding values of boars born in the same year were the basis for the estimation of the genetic trend.

## RESULTS

### Genetic parameters

The estimates of genetic parameters needed for breeding value estimation are summarized in Tables 2 and 3. Semen volume showed the highest heritability (approximately 0.25). With the exception of the percentage of abnormal spermatozoa, the heritabilities for the remaining traits were in the range from 0.10 to 0.20. The proportion of variance caused by the permanent effect was around 0.20 for nearly all traits; this value was considerably higher only in the percentage of abnormal spermatozoa.

Table 3. Estimates of genetic parameters with standard errors for the total number of spermatozoa and the number of functional spermatozoa

Genetic parameter	Total number of spermatozoa ( $10^9$ sperm cells)	Number of functional spermatozoa ( $10^9$ sperm cells)
Heritability	$0.10 \pm 0.020$	$0.11 \pm 0.020$
Proportion of variance caused by the permanent effect	$0.18 \pm 0.018$	$0.19 \pm 0.018$

High negative genetic correlations were observed between semen volume and sperm concentration and between motility and percentage of abnormal spermatozoa. The correlations caused by the permanent environmental effect of the boar behaved similarly like the genetic correlations.

### Seasonal effects

Table 4 shows a survey of seasonal effects on the six studied semen traits. The effect of season is expressed as the effect of the month of collection averaged across years. All effects are expressed as deviations from the mean. Semen volume had its highest values from September to November and was lowest from February to May. Sperm concentration was highest in late winter and spring (January to June) and lowest in late summer and

autumn (August to November). The seasonal effect on motility was relatively low, the maximal difference between two months being approximately 0.5%. In the percentage of abnormal spermatozoa, the absolute values of the effects were also low (less than 0.5%). Both the total number of spermatozoa and the number of functional spermatozoa were highest in winter and lowest in summer.

### Effect of boar's age at collection

Figures 1 to 3 show the impact of boar's age at the time of collection on semen traits. Semen volume increased until an age of about two years by approximately 100 ml and remained more or less constant thereafter. The dependence of sperm concentration on age started with a short increase until 12 months followed by a long-term moderate

Table 4. Effect of the month of collection (as a deviation from the overall annual average) on semen traits

Month	<i>Vol</i> <sup>a</sup>	<i>Con</i> <sup>b</sup>	<i>Mo</i> <sup>c</sup>	<i>Ab</i> <sup>d</sup>	<i>N<sub>total</sub></i> <sup>e</sup>	<i>N<sub>func</sub></i> <sup>f</sup>
January	4.5	8.4	0.10	-0.41	4.3	3.4
February	-4.7	13.5	0.20	-0.14	1.9	1.6
March	-13.6	19.6	0.28	-0.04	-0.1	0.2
April	-17.0	16.6	0.28	-0.08	-2.2	-1.0
May	-11.5	10.3	0.15	-0.18	-1.7	-0.9
June	-8.6	-0.3	0.06	0.09	-3.4	-2.3
July	-5.9	-3.6	-0.07	0.00	-2.9	-2.0
August	-3.2	-13.2	-0.13	-0.01	-4.2	-2.9
September	7.8	-20.7	-0.19	-0.06	-2.5	-1.9
October	16.2	-15.7	-0.23	0.16	1.7	0.8
November	18.6	-15.8	-0.19	0.37	2.5	1.2
December	17.4	0.9	-0.25	0.31	6.7	3.9

<sup>a</sup>semen volume (ml); <sup>b</sup>sperm concentration ( $10^3$  sperm cells/mm<sup>3</sup>); <sup>c</sup>motility (%); <sup>d</sup>percentage of abnormal spermatozoa (%); <sup>e</sup>total number of spermatozoa ( $10^9$  sperm cells); <sup>f</sup>number of functional spermatozoa ( $10^9$  sperm cells)

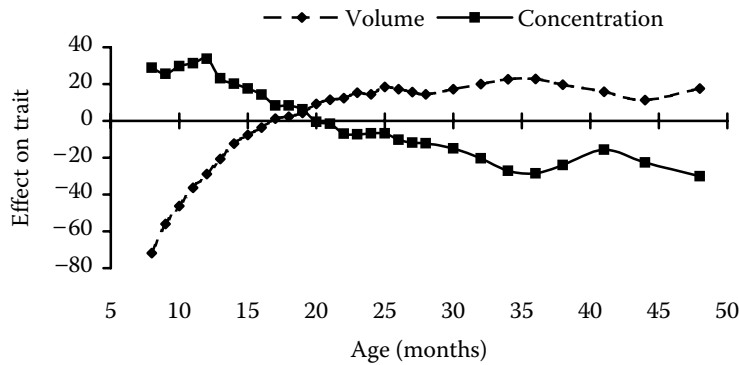


Figure 1. Effect of boar's age at collection on semen volume (ml) and sperm concentration ( $10^3$  sperm cells/ $\text{mm}^3$ ); the effect is defined as deviation from the average across all age classes

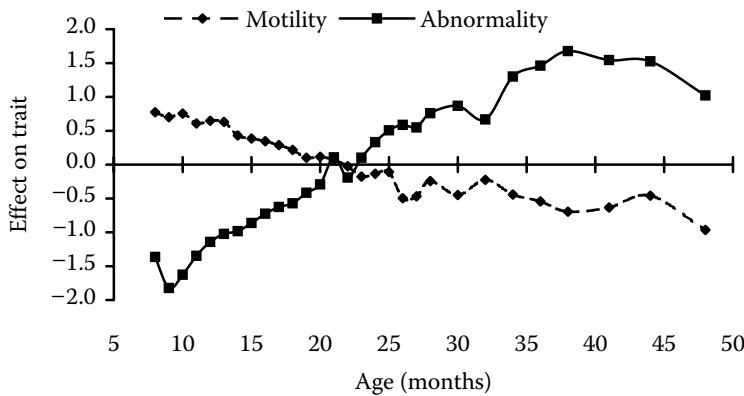


Figure 2. Effect of boar's age at collection on motility (%) and percentage of abnormal spermatozoa; the effect is defined as deviation from the average across all age classes

decrease until 3 years of age and a relative stabilization thereafter.

Motility decreased steadily with age, the overall decrease being approximately 1.7% (Figure 2), whereas the percentage of abnormal spermatozoa increased nearly over the whole productive lifetime of the boar amounting to a difference of more than 3% between the youngest and the oldest boars. Both the total number of spermatozoa and the number of functional spermatozoa started with a steep increase, reached the maximum at an age of 21 months and dropped slightly to the end of the investigated age range (Figure 3).

**Effect of the interval between successive collections**

The interval between successive collections had a large effect on sperm concentration (Figure 4). Prolonging the interval between two collections from 2 to 6 and 10 days raised the concentration by approximately  $100 \times 10^3$  and  $150 \times 10^3$  sperm cells per  $\text{mm}^3$ , respectively. The influence of the interval between two collections on semen volume was considerably lower than its effect on sperm concentration. A slight increase in semen volume was observed when the interval was prolonged

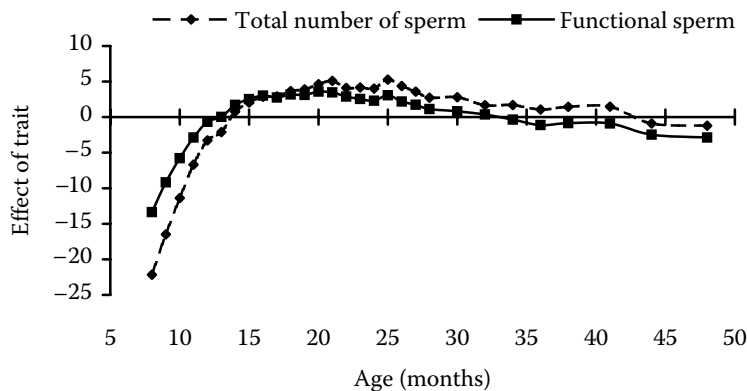


Figure 3. Effect of boar's age at collection on the total number of spermatozoa ( $10^9$  sperm cells) and the number of functional spermatozoa ( $10^9$  sperm cells); the effect is defined as deviation from the average across all age classes

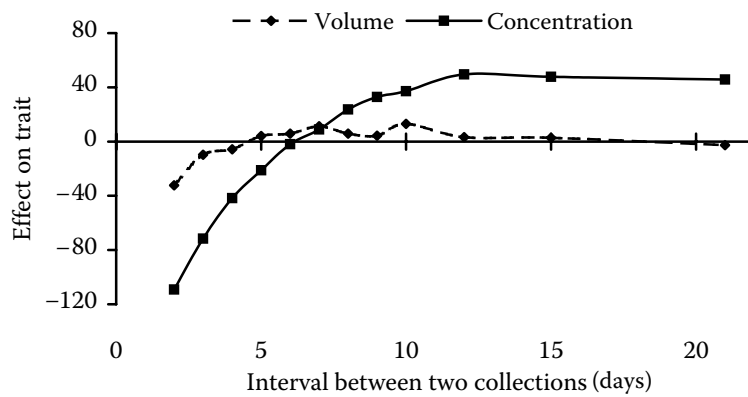


Figure 4. Effect of the interval between successive collections on semen volume (ml) and sperm concentration ( $10^3$  sperm cells/ $\text{mm}^3$ ); the effect is defined as deviation from the average across all interval classes

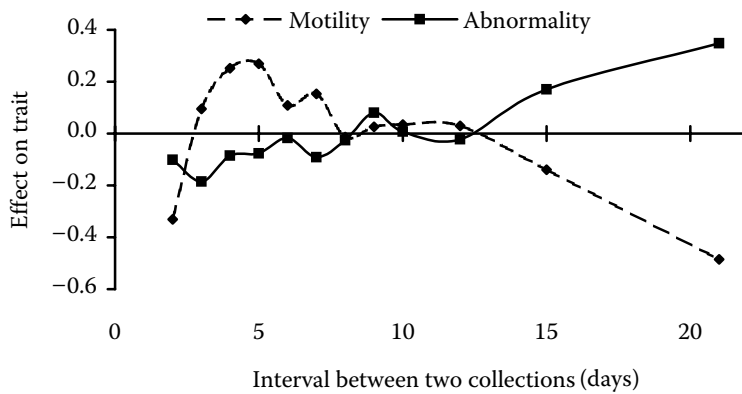


Figure 5. Effect of the interval between successive collections on motility (%) and percentage of abnormal spermatozoa; the effect is defined as deviation from the average across all interval classes

from 2 to 7 days; for longer intervals, the values changed only inconsiderably.

Motility and percentage of abnormal spermatozoa changed little with the interval between two collections; there was a certain tendency of increasing the percentage of abnormal spermatozoa and

decreasing the motility for intervals longer than 12 days (Figure 5). Both the total number of spermatozoa and the number of functional spermatozoa rose with the interval between two collections until 10 days (Figure 6). Later on these values slightly decreased.

Table 5. Effect of the year of collection on semen traits; the effect was defined as deviation from the effect of the year 2000

Year	Vol <sup>a</sup>	Con <sup>b</sup>	Mo <sup>c</sup>	Ab <sup>d</sup>	$N_{total}^e$	$N_{func}^f$
2000	0.0	0.0	0.00	0.00	0.0	0.0
2001	-9.7	28.0	0.36	-0.96	3.3	3.6
2002	2.4	11.3	0.40	-1.33	4.4	4.4
2003	-1.7	38.2	-0.08	-0.18	9.8	6.9
2004	2.9	23.0	-0.36	0.69	9.6	6.0
2005	3.8	31.6	-0.65	1.73	11.6	5.9
2006	-2.1	35.0	-1.09	2.05	9.5	3.9
2007	-1.4	35.0	-0.28	2.12	10.5	5.2

<sup>a</sup>semen volume (ml); <sup>b</sup>sperm concentration ( $10^3$  sperm cells/ $\text{mm}^3$ ); <sup>c</sup>motility (%); <sup>d</sup>percentage of abnormal spermatozoa (%); <sup>e</sup>total number of spermatozoa ( $10^9$  sperm cells); <sup>f</sup>number of functional spermatozoa ( $10^9$  sperm cells)

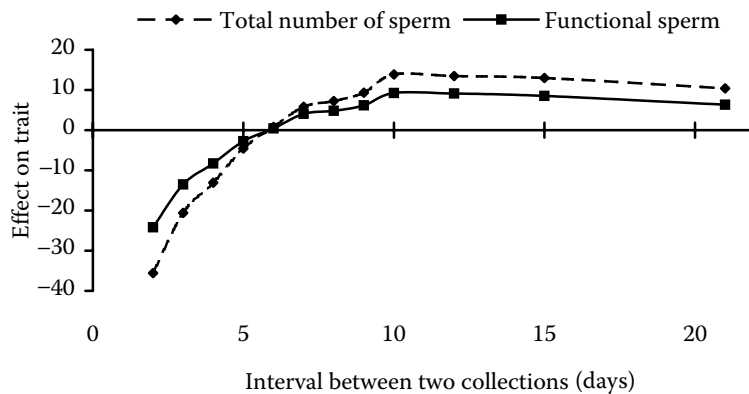


Figure 6. Effect of the interval between successive collections on the total number of spermatozoa ( $10^9$  sperm cells) and the number of functional spermatozoa ( $10^9$  sperm cells); the effect is defined as deviation from the average across all interval classes

### Breed effects

The differences between breeds in semen traits were small. Czech Large White showed slightly higher values in sperm concentration ( $+14 \times 10^3$  sperm/mm<sup>3</sup>), motility (+0.6%), total number of spermatozoa ( $+2.3 \times 10^9$  sperm) and number of functional spermatozoa ( $+1.8 \times 10^9$  sperm). Czech Landrace had a somewhat higher semen volume (+6 ml).

### Environmental and genetic trend

The environmental trend of the semen traits expressed as the effect of the year of collection is presented in Table 5. All values are given as deviations from the year 2000. Most traits did not exhibit any unique tendencies during the years. Only the percentage of abnormal spermatozoa clearly increased in the investigated time interval. In the total number of spermatozoa and the number of functional spermatozoa, an increase was observed until 2003 followed by a stagnation of the values thereafter. No genetic trend was manifest in the investigated traits (data not shown).

## DISCUSSION

In most literature sources, the estimates of effects are calculated only from phenotypic models whereby these effects may be fully unadjusted or adjusted for factors in a linear model. The estimates of effects presented in this paper were derived from an animal model taking into account all important factors influencing semen traits and all relationships between animals. Furthermore, in our

calculations the effects were estimated from data on all AI boars of the two breeds under consideration back to the year 2000. Therefore, contrary to experimental studies with a low number of boars, these effects will be representative of the whole population.

### General discussion on the model

In genetic evaluation preferably all traits should be analyzed in one multiple-trait model as these traits are measured on the same experimental unit (boar). But the total number of spermatozoa is a function of semen volume and sperm concentration and the number of functional spermatozoa is a function of all four measured traits so that both derived traits could not be included together with the four measured traits in a six-trait animal model. Functional relationships could cause numerical instabilities in the solutions and it does not make sense to calculate correlations between traits where a clear functional relationship is given. Therefore, a four-trait animal model for the four measured traits and single-trait animal models for the derived traits were used.

Random regression models as used by Oh et al. (2006) in pigs and Carabaño et al. (2007) in cattle may be an alternative to using classical animal models for the estimation of genetic parameters and breeding values. Random regression models allow for modelling both genetic and permanent effects as time functions. The disadvantage of random regression models is that care has to be taken when interpreting results at the extremes of the period (Carabaño et al., 2007). Furthermore, later performance may be harder to predict accurately from records at an early age (Oh et al., 2006).

Therefore classical animal models which need a substantially lower number of parameters than random regression models may be preferable because of their robustness.

### Genetic parameters

Our results have shown that semen traits are heritable traits with heritabilities between 0.06 and 0.24. These values are in a similar order of magnitude or higher than heritabilities for litter size traits. That means they are sufficiently high to allow for selection for these traits using an animal model. The functions of these traits such as the total number of spermatozoa in the ejaculate or the number of functional spermatozoa may also be used for selection purposes.

The negative genetic correlation between semen volume and sperm concentration is unfavourable for selection for the total number of spermatozoa. On the other hand, the negative correlation between motility and the percentage of abnormal sperm is favourable.

There is only a very limited number of literature sources presenting estimates of genetic parameters for boar's semen traits. They are summarized and discussed in Wolf (2009).

### Seasonal effects

In the investigation of Grandjot et al. (1997) the highest values in the total number of spermatozoa occurred, as in our investigation, in the last quarter of the year. Rutten et al. (2000), Smital et al. (2004) and Smital (2009) found that the number of usable doses per collection or the number of functional spermatozoa exhibited clear seasonality with the highest values from autumn to winter and the lowest values from spring to summer, which is in good agreement with our findings.

Seasonal effects on female and male reproductive traits occur in most farm animal species (Trudeau and Sanford, 1990; Chemineau et al., 2007). In the temperate climate, seasonal effects may be explained mainly by the influence of the photoperiod and temperature whereas in the tropics also humidity may be of importance (Murase et al., 2007). The separation of the influence of photoperiod and temperature is possible only in experimental data, but not in field data as used

in our investigation. Especially high temperatures have a negative effect on semen quality (Huang et al., 2000; Suriyasomboon et al., 2005). The negative effect of high temperatures may be diminished by management of the ambient control in the AI centre. According to Corcuera et al. (2002) boars were probably quite comfortable at 24°C, but if that temperature was coupled with a high stocking rate, high humidity and a high level of ammonia, they would not be comfortable.

### Effect of boar's age at collection

Huang and Johnson (1996) and Šerniene et al. (2002) reported an increase in the percentage of abnormal spermatozoa with age and Clark et al. (2003) found a dramatic increase in the average total number of spermatozoa between boars of 8–10 months and up to 14 months of age followed by constancy in this trait after 14 months of age. All these findings are in good agreement with our results. Smital (2009) also observed a rapid increase in sperm output with the boar's age, but the culmination was found at a later time (3.5 years of age). The results of Rutten et al. (2000) that the number of usable doses per collection increased only slowly with age is seemingly in contradiction with the results of the above cited papers and of our investigation. The slow increase may be explained by the fact that the interval between successive collections in the Rutten et al. (2000) analyses decreased with age, which shortened the influence of the age effect on the total number of spermatozoa.

### Effect of the interval between successive collections

Our investigations suggest that time interval of 7 to 10 days seems to be a good choice for getting the values of all semen traits near optimum. This was confirmed by Rutten et al. (2000), Frangež et al. (2005) and Smital (2009). Rutten et al. (2000) investigated collection intervals from 1 to 10 days and found that the highest number of doses per collection can be generated for intervals from 7 to 10 days. Frangež et al. (2005) reported that smaller ejaculate volumes, lower sperm concentrations and lower total sperm counts per ejaculate were obtained at collection frequencies of 7 and 3 times per



week than at twice and once per week. Significantly lower progressive sperm motilities at 7 than at 3, 2 and 1 times per week were observed.

Though longer intervals yield better results for the individual semen traits, an economic analysis showed that the highest profit could be achieved for the shortest interval between successive collections (Rutten et al., 2000). However, this analysis did not take into account that long-term high ejaculation frequency leads to the gradual deterioration of the biological value of spermatozoa and induces changes in the essential indices of semen quality (Strzezek et al., 1995). The authors concluded that high semen-collection frequencies stimulate an array of specific biochemical damaging changes in the spermatozoa which are similar to the apoptosis of somatic cells. Pruneda et al. (2005) reported that a high semen-collection frequency brings about an altered resorption and secretion pattern of the epididymal fluid, which results in defective sperm maturation and abnormal development of sperm motility.

### Breed effects

Breed differences between Czech Large White and Czech Landrace boars were relatively low. This is one argument for the joint genetic evaluation of both breeds. As the number of boars in both breeds is relatively low, the joint genetic evaluation is necessary to estimate the environmental effects (effect of AI centre and year and effect of the month of collection) with a minimal precision.

### CONCLUSION

In conclusion, the importance of semen traits in breeding programs will be shortly discussed. As until recently, also in future the greatest emphasis will be laid on production and female reproduction traits. Nevertheless, the knowledge of breeding values for semen traits is of economic importance for AI centres to ensure an efficient selection of boars for improved semen production. Therefore it can be expected that boars will mainly be selected for their breeding values in production and female reproduction traits, but AI centres will choose among top boars the boars with better semen production on the basis of breeding value estimation for these traits.

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