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Boar semen quality of the Přeštice black-pied breed
(Short Communication)

Soňa Frydrychová, Alena Lustyková, Jan Lipenský and Miroslav Rozkot

Department of Pig Breeding, Institute of Animal Science, Prague-Uhříněves, Workplace Kostelec nad Orlicí, Czech Republic

Abstract

The objective of this study was to examine semen quality parameters for the Přeštice black-pied breed over a test period of 8 years while considering the potential effects of collection month and boar age. Ninety-nine ejaculates were collected using the gloved-hand technique from healthy and fertile mature boars from selected farms. Ejaculate volumes were relatively low because the boars were accustomed to natural mating. Sperm motility, sperm concentration, percentage of morphologically abnormal spermatozoa (MAS), and sperm motility after 24 h of storage in Androhep extender (dilution rate 1:1.5) were assessed. Significant differences were found in sperm concentration and MAS rate in relation to collection months and boar age in the monitored years ($P<0.05$). A tendency for MAS to increase with monitored years was observed. Significant differences in sperm motility and motility after 24 h of storage were only observed in relation to collection months ($P<0.05$). Results of this study detected effects due to collection month and boar age on boar semen quality during the monitored years.

Keywords: boar, sperm quality parameters, Přeštice black-pied pig

Zusammmenfassung

Qualität des Eberspermas des Prešticer Schwarzbunten Schweins (Kurzmitteilung)

Ziel dieser Studie war es, die Spermaqualität von Ebern des Prešticer Schwarzbunten Schweins über einen Zeitraum von acht Jahren zu analysieren. Neunundneunzig Ejakulate wurden mittels Handschuhtechnik von gesunden und fruchtbaren Ebern gewonnen. Das Ejakulatvolumen war relativ gering, weil die Eber an natürliche Paarung gewohnt waren. Bewertet wurden die Beweglichkeit der Spermien, die Spermakonzentration und der Prozentsatz der morphologisch abnormalen Spermatozoen (MAS) sowie die Beweglichkeit der Spermien nach 24 Stunden im Spermaverdünner Androhep (Verdünnung 1:1.5). Im Beobachtungszeitraum wurden signifikante Unterschiede ($P<0.05$) in der Spermakonzentration und im MAS-Anteil in Beziehung zu den Entnahmemonaten und dem Eberalter festgestellt. In laufe der Jahre wurde eine steigende Zahl von MAS im Ebersperma des Prešticer Schwarzbunten Schweins beobachtet. Signifikante Unterschiede ($P<0.05$) bei der Beweglichkeit der Spermien und der Beweglichkeit der Spermien nach 24 Stunden wurden nur in Beziehung zu den Entnahmemonaten beobachtet.
Die Studie zeigt, dass im Beobachtungszeitraum die Spermaqualität vom Entnahmemonat und dem Eberalter beeinflusst wurde.

Schlüsselwörter: Eber, Qualität des Spermas, Prešticer Schwarzbuntes Schwein

Introduction

Reproduction plays a key role in the successful production of farm animals (Omelka *et al.* 2006). It is important to monitor the quality of fresh boar semen prior to its preservation because the quality of thawed semen depends upon the quality of the original, fresh semen. Tardif *et al.* (1999) pointed out that monitoring of semen quality is the first step towards improving pig fertility.

In the Czech Republic the Přeštice black-pied pig was included in 1992 into the programme for maintaining livestock genetic resources. Because their populations are less influenced by intensive selection in comparison to commercial hybrid lines, farm animals represent a very interesting area for research. The Přeštice black-pied pig is our national breed and probably originates from crossing of the Old-Bohemian Bristled pig and the Bavarian pig breed in Western Bohemia (in the area of Přeštice, Klatovy & Domažlice) during the second half of the 19th century. The importance of this breed lies in its genes that provide excellent reproduction performance, adaptability, rather easy care, good vitality, and resistance to diseases (Lustyková *et al.* 2008) This breed's disadvantages include relatively high back-fat thickness, lower percentage of lean muscle, and poorer feed conversion. It was recognized as a distinct breed in 1964, and since 1996 it has been bred in a closed population. Within that population, genetic markers in association with reproduction traits (Horák *et al.* 2004, Horák *et al.* 2005) and gene polymorphisms associated with meat performance traits (Zrůstová *et al.* 2009) have been studied, but until recently semen quality had not been evaluated. Therefore, the objective of this study, conducted over a test period from 2001 to 2009, was to examine the semen quality parameters of the Přeštice black-pied breed while taking into account the potential effects of collection month and boar age.

Material and methods

Ninety-nine ejaculates were obtained from boars of the Přeštice black-pied breed from 8 months to 5 years of age over a period of 8 years (2001-2009) on selected farms in the Czech Republic. Ejaculates were collected using the gloved-hand technique from healthy and fertile mature boars. Ejaculate volumes were relatively low because the boars were accustomed to natural mating. The gel portion was removed using double gauze. Semen volume and sperm motility were evaluated in the fresh boar semen. The volume of the sperm-rich fraction of the ejaculate was determined using a graduated cylinder. Sperm motility was assessed subjectively using phase contrast microscopy with a heating stage (38 °C) at 100 × magnification. A part of the fresh semen was taken to determine sperm concentration and spermatozoa morphology. The remaining semen was diluted using Androhep extender (Minitüb, Germany) at a semen:extender dilution ratio of 1:1.5 and samples were stored at the temperature of 17 °C until delivered to the laboratory for cryopreservation. The sperm concentration was determined by a cytometric method using a Bürker's chamber. Morphologically abnormal spermatozoa (MAS) were assessed according to the staining method of Čeřovský (1976). Sperm motility was evaluated 24 h after semen dilution. Boars

were divided by age into the following groups: less than 1 year, 1-2 years, 2-3 years, and more than 3 years.

Standard statistical parameters, including arithmetic mean, standard deviation, coefficient of variation, and significance (*P*) were calculated using the QC Expert program (TriloByte Statistical Software, s.r.o., Pardubice, Czech Republic). Statistical significance was determined using analysis of variance ANOVA at significance levels of $P<0.05$ and $P<0.01$.

Results and discussion

The overall quality of boars semen included into this study can be characterized as follows (mean±SD): semen volume 196.12±68.85 ml, sperm motility 78.79±9.90 %, sperm concentration 472.39±200.19×10^3/mm^3, MAS 23.20±20.53 %, and sperm motility after 24 h of storage 69.14±11.74 % (Table 1). The semen quality showed considerable variability. The highest coefficient of variation was detected in the proportion of MAS (88.49 %) and sperm concentration (42.38 %). Very low coefficients of variation were observed in sperm motility (12.57 %) and sperm motility after 24 h of storage (16.98 %). The values for semen volume were relatively lower in this study than would be normal in reality because most of the boars were accustomed to natural mating. Therefore, semen volume was not evaluated statistically. Kuciel *et al.* (1980) had studied phenotypic variability in the characteristics of ejaculates from boars of various breeds for 1 year from age 12 to 24 months and reported for the Přeštice black-pied breed values for sperm motility of 67.33 %, motility after 24 h of 60.67 %, and MAS of 7.01 %. Mašek *et al.* (1979) had pointed out that this breed had the highest volume compared to other breeds and reported sperm concentration was 338.53×10^3/mm^3. The semen quality measures for the Přeštice black-pied breed by years is presented in Table 1.

Table 1
Boar semen quality parameters according to years

Year	n	Semen volume, ml		Sperm motility, %		Sperm concentration, 10^3/mm^3		MAS, %		Sperm motility 24h, %	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2001	8	275.63	49.09	77.50	7.07	497.50	145.68	16.00^{a}	7.32	68.75	9.54
2002	19	170.84	81.25	76.05	19.33	479.84	121.58	12.29^{c,e}	15.00	68.16	19.52
2003	11	196.82	55.33	79.09	5.84	521.06	199.80	23.82	17.52	69.55	8.20
2004	10	180.00	44.72	81.00	4.59	557.20^{a}	232.76	20.95	16.42	69.50	4.97
2005	13	173.08	48.54	78.85	4.63	496.58	243.89	22.08	18.90	71.92	5.22
2006	7	175.71	16.18	82.14	3.93	342.50^{b}	65.44	25.21	30.04	66.43	20.76
2007	5	218.00	65.35	81.00	4.18	423.50	193.03	23.00	31.11	68.00	7.58
2008	7	203.57	106.29	76.43	6.90	427.14	294.50	32.93^{d}	18.98	64.29	9.76
2009	19	210.79	68.11	79.74	7.16	442.37	211.50	34.47^{b,f}	23.35	71.05	7.37
Total	99	196.12	68.85	78.79	9.90	472.39	200.19	23.20	20.53	69.14	11.74

MAS: morphologically abnormal spermatozoa, $^{a,b,c,d}P<0.05$, $^{e,f}P<0.01$

Significant differences in sperm concentration and MAS were found between years. Sperm concentration was different in 2004 and 2006 (557.20±232.76 vs. 342.50±65.44×10^3/mm^3; $P<0.05$). Differences in MAS were observed between years 2001 vs. 2009, 2002 vs. 2008 ($P<0.05$), and 2002 vs. 2009 ($P<0.01$). An increasing tendency in MAS was observed with the

passage of monitored years (from 16 % to 34.5 %). Sperm motility and sperm motility after 24 h of storage were relatively constant throughout the individual years and no significant differences were found.

The mean values of sperm motility, sperm concentration, MAS and sperm motility after 24 h of storage are presented by calendar month of collection in Table 2.

Table 2
Effect of month on quality of boar semen

Month	n	Semen volume, ml		Sperm motility, %		Sperm concentration, 10^3/mm^3		MAS, %		Sperm motility 24h, %	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
January	4	195.00	36.97	80.00	9.13	536.25	122.91	23.38^{c}	29.13	73.75^{b}	9.46
February	7	149.43	50.82	69.29^{a}	31.42	518.57	136.83	7.21^{a,e}	2.66	55.71^{a,e}	27.60
March	10	177.00	44.42	81.00^{b}	4.59	422.80^{a}	191.94	18.75^{e}	14.74	72.00^{f}	5.87
April	12	205.00	88.98	80.00^{b}	3.02	385.67^{e,c}	112.15	17.83^{e}	13.60	74.58^{f}	6.20
May	15	190.67	45.27	80.67^{b}	5.30	454.07^{a}	244.76	22.50^{c}	25.08	68.67^{b}	14.33
June	7	137.14	43.86	82.14^{b}	4.88	482.14	118.99	21.79^{c}	8.17	72.14^{f}	4.88
July	8	177.50	76.25	82.50^{b}	3.78	573.03^{d}	173.95	24.75^{c}	18.01	70.00^{b}	5.35
August	16	199.06	70.29	77.19	6.05	478.59^{a}	276.62	30.34^{b}	23.74	68.75^{b}	8.66
September	4	165.00	38.73	77.50	6.45	711.25^{b,f}	61.56	27.25	25.52	66.25	11.09
October	3	206.67	47.26	73.33	10.41	448.33	315.05	54.83^{d,f}	33.33	65.00	13.23
November	4	282.50	62.38	81.25	4.79	337.50^{e}	109.96	37.63^{b}	30.21	70.00	8.16
December	9	280.56	48.25	76.67	7.07	463.06^{a}	171.02	17.11^{e}	7.61	67.78^{b}	9.39

MAS: morphologically abnormal spermatozoa, $^{a, b, c, d}P<0.05$, $^{e, f}P<0.01$

The lowest motility value was in February, and motility values were highest in March to July ($P<0.05$). This is in contrast with the findings of Wolf & Smital (2009), who had observed sperm motility to be relatively constant throughout the year. The percentage of sperm motility after 24 h of storage was lowest in February (55.71±27.60 %) vs. January, May, July, October, December ($P<0.05$), March, April, and June ($P<0.01$). Statistically significant differences in sperm concentration were noted in March, May, August and December vs. September; in April vs. July ($P<0.05$); and in April and November vs. September ($P<0.01$). The highest value for sperm concentration was determined in September, at 711.25×10^3/mm^3. Wolf & Smital (2009) had noted that sperm concentration was highest in winter and early spring (December to April) and lowest in late summer and early autumn (August to October). The MAS percentage was higher from August to November and the value was lowest in February (7.21±2.66 %). This could have been caused by variation of temperature in the summer and in the autumn season. Several studies have shown that photoperiod (Anderson *et al.* 1998, Sancho *et al.* 2004), elevated ambient temperature, heat stress and/or hot weather have averse effects on semen production (Mauget *et al.* 1987, Colenbrander *et al.* 1993, Frydrychová *et al.* 2007, Wolf & Smital 2009) as well as semen quality (Larrson *et al.* 1984, Sonderman *et al.* 2008) in boars, and especially an increased proportion of MAS (Wettemann *et al.* 1976, Cameron *et al.* 1980). On the other hand, this could also have been an influence of the genetic background (Čeřovský *et al.* 2005). Falkenberg *et al.* (1994) had recorded a positive correlation between the percentage of normal spermatozoa in semen and number of piglets born in litters. Kawecka *et al.* (2008) also found a negative correlation between MAS rate (especially in relation to the

proximal droplet) and other ejaculate quality traits ($P<0.05$). We also observed a negative correlation between MAS rate and sperm motility (r=−0.40, $P<0.05$), sperm concentration (r=−0.35, $P<0.05$) and sperm motility after 24 h (r=−0.43, $P<0.05$). Colenbrander & Kemp (1990) had indicated that sperm production of boars may fluctuate by as much as 25 to 30 % throughout the year.

The dependence of semen quality parameters on boar age are summarized in Table 3.

Table 3
Boar semen quality parameters according to age

Year	n	Semen volume, ml		Sperm motility, %		Sperm concentration, $10^3/mm^3$		MAS, %		Sperm motility 24h, %	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<1	17	150.59	58.76	75.88	20.48	483.18	173.93	17.00^{e}	18.37	68.82	18.67
1-2	44	192.52	55.17	80.34	5.54	474.55	186.39	19.27^{e}	16.76	70.80	8.76
2-3	21	203.57	82.48	78.33	5.99	551.17^{a}	208.18	23.95^{a}	22.55	68.33	8.56
>3	11	274.27	67.21	78.82	6.43	401.82^{b}	225.93	38.64^{b,f}	19.47	68.64	6.36

MAS: morphologically abnormal spermatozoa, $^{a,b}P<0.05$, $^{e,f}P<0.01$

Significant differences were determined in sperm concentration and rate of MAS. Sperm concentration differed at age 2-3 years vs. >3 years (551.17±208.18 vs. 401.82±225.93×10^3/mm^3; $P<0.05$). According to the results of Wolf & Smital (2009), sperm concentration increased sharply up to 11 months of age, followed by a long-term moderate decrease until 3 years of age and stabilization thereafter. Differences in the MAS rate were found between ages <1 years and 1-2 years vs. >3 years ($P<0.01$), as well as for 2-3 years vs. >3 years ($P<0.05$). Wolf & Smital (2009) had reported that percentage of abnormal sperm increased over the entire productive lifetime of the boar. No significant differences were found in sperm motility and sperm motility after 24 h of storage in relation to boar age. Wolf & Smital (2009) had noted that motility was reduced with age. Jankeviciute & Zilinskas (2002) had observed significant impacts of age on volume, spermatozoa concentration and sperm morphology ($P<0.05$).

In conclusion, the present findings show effects in relation to collection month and boar age on semen quality parameters for the Přeštice black-pied breed during 2001-2009. This experiment suggests the possibility for utilizing boars as genetic resources for collection at age 2-3 years for the sake of highest sperm concentration and optimal rate of MAS. The findings show the best period for collection as measured by semen quality parameters to be from January to July, except for February. We also observed an overall increase of MAS in boar semen through the monitored years.

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Corresponding author:

Soňa Frydrychová
email: frydrychova.sona@vuzv.cz

Institute of Animal Science, Prague-Uhříněves, Department of Pig Breeding, Workplace Kostelec nad Orlicí, Komenského 1239, 517 41 Kostelec nad Orlicí, Czech Republic