

THE INFLUENCE OF SEASON ON SPERM MEMBRANE LIPID PEROXIDATION AND INSEMINATION DOSES QUALITY OF PŘEŠTICE BLACK-PIED BOARS

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Abstract

The aim of this study was to evaluate semen quality together with the detection of sperm membrane lipid peroxidation in insemination doses of Přeštice black-pied boars during the season. Přeštice black-pied pigs are included in the program of preservation of Animal Genetic Resources in the Czech Republic. The basic parameters of semen quality in fresh boar semen were evaluated. Insemination doses (ID) were evaluated using flow cytometry and Computer Assisted Sperm Analysis (CASA). The quality of ID was divided according to the season. Flow cytometry was used to detect membrane lipid peroxidation (LPO). ID evaluated in the autumn were characterized by a significantly higher ($P < 0.05$) proportion of live spermatozoa without LPO ($86.00 \pm 5.83\%$) compared with the winter ($78.22 \pm 10.80\%$) and the subsequent spring ($76.30 \pm 16.21\%$). Statistically significant differences ($P < 0.05$) in initial semen quality were recorded in sperm volume in summer ($291.67 \pm 137.10 \text{ ml}$) vs. autumn ($360.19 \pm 112.52 \text{ ml}$). The lowest values of sperm concentration were in autumn ($296.85 \pm 139.13 \text{ mm}^3 \times 10^3$) vs. winter ($373.06 \pm 129.93 \text{ mm}^3 \times 10^3$) and in morphologically abnormal spermatozoa were in summer ($18.52 \pm 6.33\%$) vs. autumn ($24.08 \pm 9.46\%$) and in winter ($24.79 \pm 9.90\%$). In conclusion, in this study statistically significant differences were found in the initial quality of native sperm and in the ID quality according to lipid peroxidation during the year. Season of the year had no significant effect on the total number of functional spermatozoa and on sperm motility evaluated by CASA.

Key Words: Boar, semen quality, season, flow cytometry, CASA, insemination dose, membrane lipid peroxidation

The season of the year affects boar semen quality (Smítal, 2009). Among the factors that influence seasonal changes are photoperiod (Knecht et al., 2013) and high temperatures (Kunavongkrit et al., 2005). Seasonal-related variations affect the reproductive tract functions in the boar, resulting in marked changes in the biochemical composition of the semen (Fraser et al., 2016). Seasonal infertility may be due, at least in part, to a combination of low motility, abnormal morphology including acrosomal abnormality, and early occurrence of the acrosome reaction in response to stimulus, possibly resulting from a decrease in acrosomal stabilizing proteins in the seminal plasma during

summer. These changes may be modulated by heat/humidity stress and/or photoperiod-regulated testosterone (Murase et al., 2007).

Lipid peroxidation in the sperm plasma membrane is a significant mechanism of sperm damage induced by reactive oxygen species (ROS). Lipid peroxidation causes sperm dysfunction associated with decreased membrane fluidity, loss of membrane integrity and sperm function (Sengupta et al., 2024, Wang et al., 2025). Sperm cells are particularly vulnerable to oxidative stress due to their unique characteristics, including high polyunsaturated fatty acid (PUFA) content in their membranes (Wang et al., 2025). Boar spermatozoa may be sensitive to membrane

lipid peroxidation due to the relatively high PUFA content in the sperm membrane (Johnson et al., 2000). Lipid peroxidation in boar sperm membranes have a major involvement in reductions in motility, vitality, membrane permeability and hence, storability of sperm (Amin et al., 2010).

Přeštice black-pied pigs are an original national breed in the Czech Republic and have been included in the program of preservation of Animal Genetic Resources. The aim of this study was to evaluate semen quality together with the detection of sperm membrane lipid peroxidation in insemination doses of Přeštice black-pied boars during the season.

Material and Methods

During two years, 125 ejaculates were collected from twelve fertile boars of the Přeštice black-pied breed. Ejaculates were collected during the years 2023 and 2024. All boars were included in the program of preservation of Animal Genetic Resources. Ejaculates were collected using the gloved-hand technique and the gel portion was removed by using double gauze. The boars were kept in the same housing, feeding and breeding conditions at the main insemination station for Přeštice black-pied boars in the Czech Republic.

The following sperm quantity and quality parameters were initially evaluated in the native semen: sperm volume, sperm motility, sperm concentration and morphologically abnormal spermatozoa (MAS). Sperm motility was subjectively assessed by microscopic estimation of the number of spermatozoa moving in the field of view of a phase-contrast microscope with a heated stage (38°C) at 200× magnification. Proportion of MAS were assessed according to the staining method of Čerovský (1976) and two hundred spermatozoa per slide were evaluated microscopically under oil immersion and 1500× magnification. The total number of functional spermatozoa (TNFS) in ID was calculated from the initially evaluated sperm quality parameters. The total number of functional sperms ($\times 10^9$) was calculated according to Wolf (2009) as

follows: $TNFS = (VO \times CO / 1000) \times (MO / 100) \times (1 - AB / 100)$ where VO is sperm volume (ml), CO sperm concentration (1000 mm^3), MO progressive sperm motility (%), AB percentage of MAS.

Semen was diluted in extender Androstar plus (Minitube, Germany) in dilution ratio 1+2 (semen + extender) and prepared insemination doses (ID) were stored at 17°C. The final volume of ID was 90 ml. After 24h storage time ID were evaluated using flow cytometry and CASA (Computer Assisted Sperm Analysis). The quality of insemination doses was divided according to the season (spring 3rd-5th, summer 6th-8th, autumn 9th-11th, winter 12th-2nd). CASA evaluation was performed using a 2 μl sample placed in a Leja 20 chamber and 500 sperm cells were evaluated by negative phase contrast microscopy with a heating stage (38°C) at 160× magnification. The following sperm motility parameters were determined using CASA (Sperm Class Analyzer, Microptic S.L., Spain): percentage of progressive sperm motility (VCL > 25 $\mu\text{m/s}$ and STR \geq 45%), rapid and medium (VCL 25 < medium < 45 < rapid $\mu\text{m/s}$), percentage of non-progressive sperm motility (VCL > 25 $\mu\text{m/s}$ and STR < 45%), kinetic characteristics – curvilinear velocity VCL ($\mu\text{m/s}$), average path velocity VAP ($\mu\text{m/s}$), straight line velocity VSL ($\mu\text{m/s}$), straightness index (STR, VSL/VAP, %), linearity index (LIN, VSL/VCL, %) and oscillation index (WOB, VAP/VCL, %). Sperm lipid peroxidation analysis was performed by flow cytometry as previously described by Partyka et al. (2011) followed by Frydrychova et al. (2024). Briefly, membrane lipid peroxidation (LPO) was assessed by flow cytometry (Guava easyCyte TM5) using C11-BODIPY581/591 (Lipid Peroxidation Sensor, BODIPY™ 581/591 C11, Invitrogen™, ThermoFisher, USA). A total of four sperm subpopulations were detected using flow cytometry – live spermatozoa without LPO, dead spermatozoa without LPO, dead spermatozoa with LPO and live spermatozoa with LPO.

Basic statistical characteristics of the results – arithmetic means, standard deviations and significance (P) were calculated by the QC Expert

program (TriloBite Statistical Software s.r.o., Pardubice, Czech Republic). All data were tested for normality before analysis. The data were analysed by statistical analysis of variance ANOVA followed by the Fisher test ($P < 0.05$). All data are expressed as mean \pm standard deviation (SD).

Results and Discussion

The autumn period was characterized by an increased volume of collected ejaculates associated with a lower sperm concentration (Table 1). While the highest sperm volume was recorded in autumn, the lowest sperm volume was observed in summer ($P < 0.05$). The lowest values of sperm concentration were in autumn vs. winter ($P < 0.05$). Zasiadczyk et al. (2015) reported significantly higher ejaculate volume, sperm concentration and the total number of spermatozoa during the autumn-winter period. The recorded variations in volume and sperm concentration of native semen were not limiting for the production of ID from boars of the Přeštice black-pied breed. This situation is due to the low pressure on the total number of ID produced. To maintain existing boar lines and genetic diversity within a small genetic resource population, a higher number of boars is kept in insemination than corresponds to the actual demand of breeders for ID. As a result of this situation, semen can be diluted at a very low dilution ratio of 1+2. Each ID therefore contains a large number of spermatozoa exceeding the standard of 1.5 billion spermatozoa per dose as reported by Feitsma (2009). In contrast to sperm volume and sperm concentration, season of the year had no significant effect on the total number of TNFS in the evaluated ID ($P > 0.05$). Due to sufficient sperm production, it is possible to increase the production of ID from boars of the Přeštice black-pied breed regardless of seasonal effects. The high potential for increasing production is well demonstrated by the recorded values of TNFS contained in ID. The highest number of TNFS per ID was recorded in summer, while the lowest was recorded in autumn ($P < 0.05$). These values clearly exceed the above-

mentioned standard of 1.5 billion spermatozoa per ID. Alm et al. (2006) recommends that under commercial circumstances the homospermic semen doses contain no less than 3 billion spermatozoa per dose. An association between seasonal effects and semen quality was further observed in the occurrence of MAS (Table 1). The autumn–winter period was characterized by an increased incidence of MAS. The lowest incidence of MAS was recorded during the summer months, compared with the highest incidence in autumn and winter ($P < 0.05$). This finding corresponds with previous results obtained at another commercially oriented station in the Czech Republic (Lipenský et al., 2010). The increased incidence of MAS observed in autumn and winter was almost at the recommended limit. The general recommendation is not to exceed a limit of 25% pathological spermatozoa in ejaculates of boars used in insemination. It is advisable to evaluate the incidence of MAS including the occurrence of proximal and distal cytoplasmic droplets (Čeřovský et al., 2005). The use of semen from boars with poor sperm morphology may negatively affect insemination outcomes. However, an increased number of spermatozoa per ID can partially compensate for the low quality of these spermatozoa (Alm et al., 2006). Given the limited number of Přeštice boars used in insemination, a high sperm concentration in produced doses may represent a significant advantage. To a certain extent, breeding and zootechnical considerations may therefore play a more prominent role in the decision to use a lower quality ID or keeping a boar at the insemination station. Season had no significant effect on sperm motility in ID evaluated by CASA and had only a limited effect on some of the monitored kinetic parameters of sperm movement (Table 2). This result can be considered as a positive finding. Many authors have observed the effect of season on sperm motility (Górski et al., 2017, Ibănescu et al., 2018, Szczeńiak-Fabiańczyk et al., 2019). In this study, significant differences ($P < 0.05$) were found in VCL in autumn vs. winter and in VSL in

summer vs. winter. Singh et al. (2025) showed a significant effect of season on the kinematics parameters VAP, VCL, ALH, BCF, LIN of boar sperm in a sub-tropical climate. Insemination doses evaluated in the autumn were characterized by a significantly higher proportion of live spermatozoa without LPO ($P<0.05$) compared with the winter and the subsequent spring (Table 3). Other statistically significant differences ($P<0.05$) were recorded in subpopulation dead spermatozoa without LPO in autumn vs. spring and winter. The highest proportion of subpopulation live spermatozoa with LPO ($P<0.05$) was noted in summer vs. winter, spring and autumn.

Although ID produced in autumn contained the lowest number of TNFS, the proportion of live spermatozoa without LPO was highest during the

same period. This may be due to the influence of the season on the antioxidant defence systems in the seminal plasma and fluids of the boar reproductive tract (Koziorowska-Gilun et al., 2011). Szczeńniak-Fabiańczyk et al. (2019) reported a higher value of mitochondrial activity and lower value of oxidative stress in boars collected in the autumn-winter period compared to the spring-summer period. In stallions, ROS concentrations and lipid peroxidation were higher and faster in winter than in summer, without a negative effect on sperm quality (Mislei et al., 2020). The recorded results suggest that a comprehensive approach to the evaluation of ID is appropriate to reveal the effects of seasonality on semen and ID quality. Based on the limited number of semen quality parameters makes it difficult to estimate the full impact of seasonal influences.

Table 1. The initial boar semen quality by season

Season	n	Sperm volume	Sperm concentration	MAS	TNFS in ID
		ml	$\text{mm}^3 \times 10^3$	%	$\times 10^9$
Spring	18	310.56±108.22	360.72±159.07	20.64±8.15	6.84±2.74
Summer	24	291.67±137.10 ^b	371.79±232.59	18.52±6.33 ^b	8.02±5.70
Autumn	52	360.19±112.52 ^a	296.85±139.13 ^b	24.08±9.46 ^a	5.87±2.95
Winter	31	311.61±96.47	373.06±129.93 ^a	24.79±9.90 ^a	7.19±2.62

^{ab} $P<0.05$ TNFS - Total number of functional spermatozoa MAS - Morphologically abnormal spermatozoa

Table 2. Sperm motility, kinetic and other movement characteristics in insemination doses by season

Season	N	Rapid PM	Medium PM	Non-PM	VCL	VAP	VSL	STR	WOB	LIN
		%	%	%	$\mu\text{m/s}$	$\mu\text{m/s}$	$\mu\text{m/s}$	%	%	%
Spring	18	55.56±4.17	39.54±3.36	4.71±3.68	83.43±12.26	46.78±4.88	25.41±2.48	53.14±3.04	57.26±3.48	31.56±3.73
Summer	24	55.06±4.85	39.52±3.15	5.20±3.94	83.12±12.07	47.61±5.38	26.11±3.33 ^a	53.48±3.54	58.71±5.01	32.68±5.09
Autumn	52	54.70±5.29	40.17±2.89	4.83±4.45	84.17±13.52 ^a	47.38±7.03	25.39±3.71	52.45±2.59	57.48±3.58	31.24±3.47
Winter	31	53.54±5.14	40.64±2.35	5.65±4.19	78.31±10.58 ^b	44.93±5.11	24.16±2.69 ^b	52.80±2.09	58.80±3.29	32.23±3.12

^{ab} $P<0.05$ PM - Progressive sperm motility VCL - Curvilinear velocity VSL - Straight-line velocity VAP - Average-path velocity

STR - Straightness index LIN - Linearity index WOB - Oscillation index

Table 3. Percentage of sperm subpopulations with membrane lipid peroxidation in insemination doses by season

Season	n	Live without LPO	Dead without LPO	Dead with LPO	Live with LPO
		%	%	%	%
Spring	18	78.22±10.80 ^b	20.07±10.80 ^b	1.28±4.22	0.42±0.87 ^b
Summer	24	79.22±16.90	18.27±9.52	0.48±0.62	2.02±2.30 ^a
Autumn	52	86.00±5.83 ^a	13.74±5.83 ^a	0.23±1.25	0.02±0.37 ^{b,c}
Winter	31	76.30±16.21 ^b	22.41±10.30 ^b	0.81±2.87	0.47±0.88 ^{b,d}

ab;cd P<0.05 LPO - Membrane lipid peroxidation

Conclusion

In the present study, only a limited effect of season on the semen quality and quantity, and consequently on insemination doses was observed. The recorded significant seasonal variations in the volume and sperm concentration of native semen did not have a limiting effect on the preparation of the insemination doses from boars of the Přestice black-pied breed. The incidence of morphologically abnormal spermatozoa in collected ejaculates increased during the autumn–winter period. The highest proportion of live spermatozoa without lipid peroxidation in their membranes was recorded in the autumn period. Furthermore, season did not affect sperm motility in the evaluated insemination doses. Another positive outcome for Přestice black-pied pig breeders is that no significant effect of season on the total number of functional spermatozoa in insemination doses was noted.

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