EFFECTS OF AGE, AND SEASON ON SPERM QUALITATIVE PARAMETERS IN LITHUANIAN WHITE AND PETREN BOARS

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Abstract. In the present work, we examined the effects of age, breed, and season on qualitative parameters of sperm from 45 boars bred by artificial insemination. Boars were assigned to the groups according to their age (young, 10 to 18; 18 to 24; 24 to 30; 30 to 36 month of the age and older, over 36 months of the age) and breed (Lithuanian White, [LtW] and Petren, PI). The following sperm parameters were analysed: motility, morphology of spermatozoa, and viability (eosin/nigrosin staining).

Analysis of the obtained results revealed that sperm motility was the only sperm parameter analysed that did not significantly differ between the different age groups of boars. Other qualitative sperm parameters were significantly ($P \leq 0.05$) influenced by the age of the boar. Within young (10 to 18 months) boars, from the LtW breed had the lowest ($P \leq 0.05$) sperm motility, when compared to the PI breed. They also had higher abnormal sperm counts and their sperm motility values were significantly correlated (r=-0.57) with abnormal sperm morphology results. The incidence of pathological spermatozoa and sperm viability were significantly ($P \leq 0.001$) influenced by the age of the boar. With increasing age, the total number of pathological spermatozoa increased, and the number of viable spermatozoa decreased ($P \leq 0.05$). Young (10 to 18 months) LtW breed boars had 34,0–47,0 % greater pathological sperm counts and 5,0 to 7,0 % lower ($P \leq 0.05$) sperm viability that PI breed boars.

Season had a significant ($P \leq 0.05$) effect on the intensity of spermatogenesis and qualitative sperm parameters. During the summer–autumn period, the incidence of pathological spermatozoa increased (54.2 %), but sperm motility and viability decreased (4.1 % and 9.2 %; $P \leq 0.05$, $P \leq 0.01$, respectively). Boars from the LtW breed during the summer–autumn period had a 30,0–34,0 % lower ($P \leq 0.01$) incidence of pathological spermatozoa than the PI breed boars, respectively.

Keywords: boar, age, season, breed, sperm quality.

AMŽIAUS IR SEZONO ĮTAKA LIETUVOS BALTŲJŲ IR PETRĖNŲ VEISLIŲ KUILIŲ SPERMOS KOKYBEI

Santrauka. Tyrimų metu buvo panaudoti 45 veisliniai kuiliai ir išanalizuota virš 400 spermos kokybinių rodiklių, atsižvelgiant į kuilio veislės, amžiaus ir sezono įtaką. Kuiliai buvo sugrupuoti pagal amžių į 5 grupes (jauni, 10-18 mėn., 18-24; 24-30; 30-36 ir vyresni nei 36 mėn.) ir pagal veislę į dvi (Lietuvos baltieji, LB; Petrėnai, PI). Darbo metu buvo vertinamas spermatozoidų judrumas, patologinių spermatozoidų skaičius ir gyvybingumas, dažant spermatozoidus eozino-nigrozino dažais.

Tyrimais buvo nustatyta reikšminga amžiaus įtaka patologiškai pakitusių bei gyvų spermatozoidų skaičiui kuilių ejakuliatuose ($p \leq 0,01$), spermatozoidų judrumui amžiaus veiksnys patikimos įtakos neturėjo. Kuiliams senstant bendras patologiškai pakitusių spermatozoidų ir spermatozoidų su patologinėmis uodegėlėmis skaičius patikimai didėjo, gyvų spermatozoidų skaičius ejakuliate - mažėjo ($p \leq 0,05$). Patologinių spermatozoidų skaičius buvo didelis tiek jaunų (10-18 mėn.) tiek senstančių (36 mėn. ir vyresnių) kuilių ejakuliatuose. Nustatyta reikšminga amžiaus įtaka ir patologinių spermatozoidų skaičiaus pasiskirstymui tarp veislių - jaunų LB veislės kuilių patologinių spermatozoidų skaičius yra 34,0-47,0% didesnis, gyvų spermatozoidų skaičius – 5,0-7,0% mažesnis, nei PI veislės kuilių ($p \leq 0,05$).

Sezono įtaka pasireiškia patologiškai pakitusių spermatozoidų skaičiaus padidėjimu (54,2%), spermatozoidų judrumo ir gyvų spermatozoidų skaičiaus ejakuliate sumažėjimu (4,1% ir 9,2% atitinkamai) vasarą ir rudenį ($p \leq 0,05$; $p \leq 0,01$). Analizuojant sezono įtaką skirtingoms veislėms mūsų tyrimais nustatytas 30,0-34,0% mažesnis LB veislės kuilių patologinių spermatozoidų skaičius vasaros ir rudens periodu ($p \leq 0,01$), nei PI veislių.

Raktažodžiai: kuiliai, amžius, sezonas, veislė, spermos kokybė.

Introduction. The major concern of the artificial insemination (AI) programme in boars is production of high quality semen. Fertilisation depends on the availability of a sufficient amount of fertile spermatozoa in the vicinity of the ovum. In turn, the quality of these spermatozoa depends on multiple biological and environmental factors. Some effects, such as insufficient feeding, high ambient temperatures, and aging of the animal have negative effects on sperm production. On the other hand, an extended photoperiod, frequent semen collection, and some genetic factors stimulate positively sperm production (Flowers *et al.*, 1997). Knowledge of factors influencing sperm quality and production is important to AI organisations (Soderquist *et al.*, 1996).

The major goal of the present study was to evaluate semen quality from boars housed in Lithuanian AI centers, depending on different environmental and biological factors.

Materials and methods. In the present work, over 400 records of sperm qualitative parameters from 45 AI boars were included into the analysis. Boars were assigned to five groups according to their age (young, 10 – 18; 18 – 24; 24 – 30; 30 – 36, older than 36 month), and to two groups according to breed (Lithuanian White, LtW; Petren, PI). From each breed and age group 5 to 10 boars were selected. The investigations were made during a one year period, and sperm qualitative parameters were estimated two times per season with a 40–45 day interval. Semen was collected from boars using the gloved hand technique on a phantom mount. The sperm-rich fraction of each ejaculate was evaluated for motile spermatozoa, morphology, and viability. A semen sample was diluted with saline and the percentage of viable sperm was measured by microscopic examination as the percentage viable progressively motile sperm. This was used as an estimate of sperm motility (SM). The percentage of spermatozoa with pathological heads (PH), pathological tails (PT) and total number of pathological spermatozoa (TNPS) was determined according to methods described by Williams (1925) and Hancock (1956). Exclusion of eosin stain was used to evaluate sperm viability as the percentage of viable spermatozoa (VS) (Dott *et al*., 1972).

Statistical analyses were performed using the SPSS statistical package and Analysis of variance used the General linear model (GLM) procedure (Гланц, 1999). The study was conducted from 1997 to 1998. The statistical model included the effects of age, breed and season (the semen-collection frequency was constant: three to two times per two weeks). Differences between the groups and factors were analysed using the Sheffe method of multiple comparison. Differences were regarded to be statistically significant when $P \leq 0.05$. Relationships between dependent variables were assessed using Spearman's rank correlation matrices.

Results. Factors influencing sperm motility (SM)

Season and interactions between boar age and breed, and between boar breed and season, all significantly influenced sperm motility ($P < 0.05$, Table 1). The percentage of motile spermatozoa was lower in summer (7.85±0.66) and spring (8.12±0.93), than that in autumn and winter ($P < 0.05$). The mean differences between sperm motility in summer and spring were significant ($P < 0.01$); the differences between motility autumn and winter were to small to be biologically important (Table 2).

Table 1. **Levels of significance of age, breed, and season on sperm parameters (ns, factors were tested and excluded from the model due to *P*-level ≥ 0.05)**

Factors	Dependent variable				
Individual	SM	PT	PH	TNPS	VS
Boar age (BA)	ns	0.002	0.053	0.004	0.0001
Boar breed (BB)	ns	ns	ns	ns	ns
Season (S)	0.0001	0.0001	0.0001	0.0001	0.0001
Interactions					
BA*BB	0.024	0.046	0.0001	0.01	0.048
BA*S	ns	ns	ns	ns	ns
BB*S	0.003	0.009	0.012	0.003	0.028
BA*BB*S	ns	ns	ns	ns	ns
Number of observations	392.0	396.0	397.0	392.0	392.0

SM, sperm motility
TNPS, total number of pathological spermatozoa, %
PT, percentage of spermatozoa with pathological tails
VS, percentage of viable spermatozoa
PH, percentage of spermatozoa with pathological heads

Table 2. **Estimates of the effects of season on sperm qualitative parameters**

Parameters	Season			
	Summer a	Autumn b	Winter c	Spring d
Percentage of spermatozoa with pathological heads (PH)	2.765±1.904 *bcd*	2.151±2.325 *a*	1.895±1.911 *a*	1.957±2.138 *a*
Percentage of spermatozoa with pathological tails (PT)	10.766±4.064 *bcd*	7.909±3.393 *ad*	4.843±3.912 *ad*	4.115±3.957 *abc*
Total number of pathological spermatozoa (TNPS) %	13.982±5.815 *bcd*	8.669±3.750 *ad*	6.471±4.642 *ad*	6.543±4.638 *abc*
Percentage viable spermatozoa (VS)	78.456±7.558 *bcd*	84.636±6.257 *a*	83.967±7.705 *a*	81.636±6.821 *a*
Percentage of motile spermatozoa (SM)	78.48±0.662 *bcd*	84.55±0.597 *ad*	85.79±0.523 *ad*	81.18±0.927 *a*

a, b, c, d, Means in rows are significant ($P \leq 0.01$)

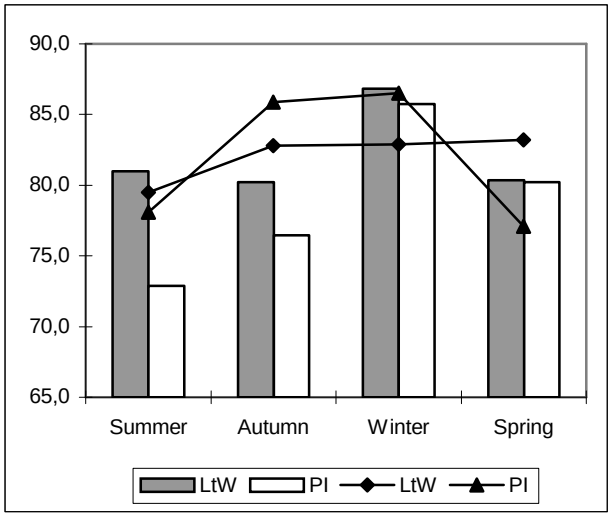

Figure 1. **Percentage of SM (lines) and VS (bars) spermatozoa in ejaculates collected from Lithuanian White (LtW), Petren (PI) in different seasons.**

As can be seen in Figure 1, a significant influence of season on sperm motility was observed for all two breeds of boars. The SM of LtW boars was significantly lower in winter ($P \leq 0.01$), compared with that is PI boars. The PI boars had a significantly lower SM in spring than the LtW boars ($P \leq 0.01$). The differences between LtW, and PI boars SM were not significant in summer and autumn.

Neither age of a boar nor breed alone had a significant effect on sperm motility, but interaction between age and breed effected SM significantly. Young (10 –18 months of age) LtW boars had a significant lower SM ($P \leq 0.05$) than PI boars, but no significant difference between breeds was found for SM in other age groups.

Factors influencing the percentage of spermatozoa with pathological heads (PH), pathological tails (PT), total number of pathological spermatozoa per ejaculate (TNPS) and percentage of viable spermatozoa (VS)

Some significant correlations were found between qualitative semen parameters (Table 3). Due to the high correlations between the percentages of spermatozoa with pathological heads, pathological tails, and total number of pathological spermatozoa, they were analysed in the same statistical model.

Various factors were analysed to determine their effects on PH, PT, TNPS, and VS. The analysis revealed that age, season, the interaction between boar age and breed and interaction between season and breed all had significant effects on sperm morphological characteristics and viability. Levels of significance for these factors are presented in Table 1. Boars within breeds and age-groups varied greatly with respect to all qualitative semen characteristics. PH, PT and TNPS increased with increasing age ($P \leq 0.01$). The percentage of viable spermatozoa decreased as age increased. The mean difference in TNPS between young (10–18 months) and old (30–36, and 36 months and older) boars was significant, and no significant differences were detected in TNPS between other age groups (Table 4)

Seasonal effects on both sperm morphology and viability characteristics were highly significant ($P \leq 0.001$, Table 1). The highest PT and TNPS, and the lowest VS, were observed in summer ($P \leq 0.005$), and autumn ($P \leq 0.001$), compared with spring and winter periods (Table 2). The percentage of spermatozoa with pathological heads did not differ significantly among spring, winter and autumn season. PH, PT, and TNPS were significantly higher, and VS – lower during summer ($P \leq 0.005$). Within breeds, PH and PT differed significantly. Young (10–18 months of age) LtW boars showed higher PT than PI boars of the same age ($P \leq 0.05$; $P \leq 0.01$, respectively). No significant differences were found within breeds in other age groups. Young (10–18 months) LtW boars had significantly lower VS than did PI boars ($P \leq 0.05$). No significant differences in VS were seen within breeds in other age-groups.

Table 3. Correlation coefficients for semen qualitative parameters.

Parameters	SM	PH	PT	TNPS	VS
Sperm motility (SM)	-	-0.573**	-0.170**	-0.574**	0.309**
Percentage of spermatozoa with pathological heads (PH)	-0.573**	-	0.662**	0.811**	-0.577**
Percentage of spermatozoa with pathological tails (PT)	-0.170**	0.662**	-	0.293**	-0.332**
Total number of pathological spermatozoa (TNPS), %	-0.574**	0.811**	0.293**	-	-0.711**
Percentage of viable spermatozoa (VS)	0.309**	0.577**	0.332**	0.711**	-

*$P \leq 0.05$; ** $P \leq 0.001$

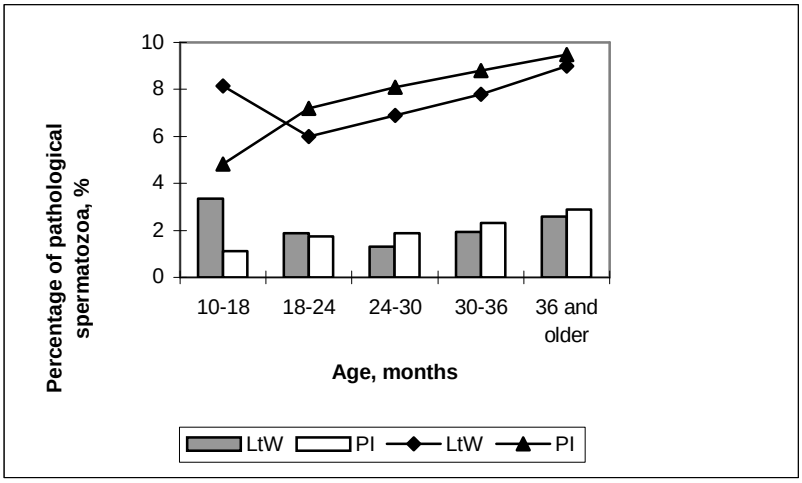

Figure 2. **Percentage of spermatozoa with PH (bars) and with PT (lines) in ejaculates collected from Lithuanian White (LtW), and Petren (PI) in different age groups**

Table 4. **Estimates of the effects of boar age on sperm qualitative parameters.**

	Age, months				
Parameters	**10-18 *a***	**18-24 *b***	**24-30 *c***	**30-36 *d***	**36 and older *e***
Percentage of spermatozoa with pathological heads (PH)	1.950±1.791 *e*	1.797±1.552	2.411±3.761	2.335±1.554	2.681±1.649 *a*
Percentage of spermatozoa with pathological tails (PT)	5.960±4.665 *e*	6.688±3.851	7.087±4.350	7.885±4.150 *a*	8.029±4.815 *a*
Total number of pathological spermatozoa (TNPS), %	8.301±6.579 *e*	8.287±4.269	8.055±5.230	10.083±4.677 *a*	10.400±5.718 *a*
Percentage of viable spermatozoa (VS)	83.050±7.279 *e*	84.022±6.350 *e*	82.698±7.594	80.271±7.471	78.918±7.473 *a*

a, b, c, d, e, Means in rows are significant ($P \leq 0.01$)

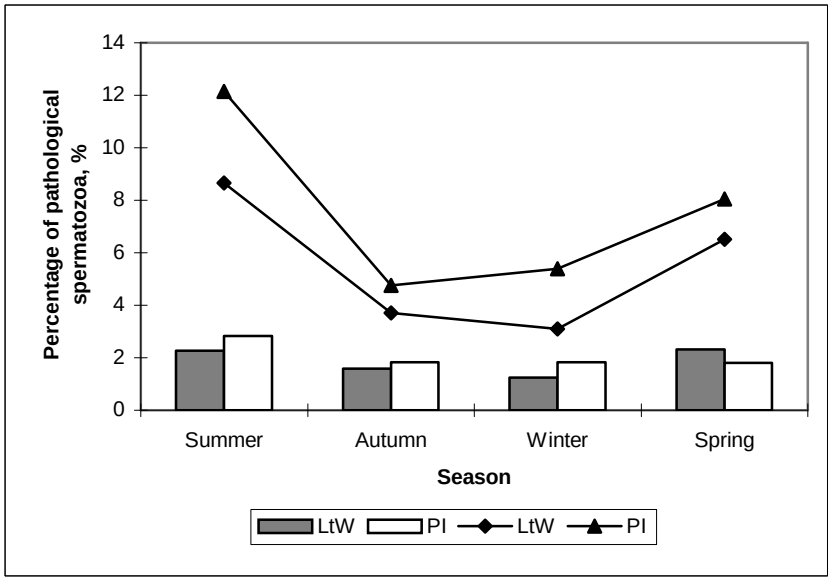

Figure 3. **Percentage of spermatozoa with PH (bars) and with PT (lines) in ejaculates collected from Lithuanian White (LtW), and Petren (PI) in different seasons**

Seasonal variations of sperm morphological characteristics differed significantly among breeds. The highest incidence of abnormal sperm counts was observed for all two breeds in summer (Figure 3). Within breeds, LtW boars showed the lowest incidence of PH and PT in summer, compared with PI ($P \leq 0.01$; $P \leq 0.05$, respectively). No significant difference in TNPS was observed during summer and autumn between PI boars, and during spring and winter between the breeds of boars. The highest incidence of VS was in winter, and the lowest did in summer (Table 2). Within breeds, LtW boars had significantly higher VS in summer than did PI boars in the same period. The percentage of viable spermatozoa in winter, spring and autumn among breeds did not differ significantly.

Discussion. The obtained results revealed that sperm motility was the only sperm parameter analysed that did not significantly differ between age groups of boars. Other qualitative sperm characteristics were significantly influenced by the age. Within the young age group, (10 to 18 months), boars from the LtW breed had the lowest sperm motility, when compared to the PI breed. They also had higher abnormal sperm counts, and their sperm motility values were significantly correlated (r=-0.57) with abnormal sperm morphology results. The incidence of pathological and viable spermatozoa were significantly influenced by the age of the boar. With increasing age, the percentage of pathological spermatozoa and spermatozoa with pathological tails increased, and the percentage of viable spermatozoa decreased. This is in agreement with Greenberg and Mahone (1981) and Kennedy and Wilkins (1984). The significant negative correlation among the above–mentioned sperm characteristics – TNPS, VS and PT (r= -0.71, r= -0.53, respectively) explains these negative interrelations. Young (10 to 18 months) LtW boars had 34–47 % higher pathological sperm count and 5 to 7 % lower sperm viability than PI boars. Comparisons of breed can differ from study to study due to differences in sampling of boars or because interactions between breed of boar and other factors which are not consistently controlled.

Our experiments revealed that the breed of boars had no significant effect on sperm morphological characteristics and sperm viability. These results are in agreement with data from Claus and Weiler, 1985.

Seasonal effects on both male and female pig reproduction have been reported (Claus and Weiler, 1985). In boars, seasonal changes are influenced by the photoperiod (Claus and Weiler, 1985) and temperature (Mazzari *et al.*, 1968). The latter effect is primarily a deleterious effect of high temperature causing germ-cell destruction (Mazzari *et al.*, 1968) and resulting in a temporary decrease in sperm production and fertility (Wettemann *et al*. 1985). Another effect of season can be the so called "heat stress" (Malmgren 1989; Claus and Weiler, 1985) manifested by suppression of spermatogenesis and stimulated positively sperm production (Flowers *et al.*, 1997). Some scientists have found that the season had no significant influence on sperm morphological characteristics (Kunavongkrit and Prateep, 1995; Borg *et al*., 1993). In the present work, during the summer-autumn period, the incidence of pathological spermatozoa increased (54.2 %), while sperm motility and viability decreased (4.1 % and 9.2 %; $P \leq 0.05$, $P \leq 0.01$, respectively). This is in agreement with Kennedy and Wilkins (1984). The tendency of a seasonal increase in abnormal sperm counts and decrease in sperm motility and viability is shown by significant negative correlations among these parameters (r= -0.81, r= -0.71, $P \leq 0.05$). Boars from the LtW breed during the summer–autumn period had a 30–34 % lower incidence of pathological spermatozoa than the PI breed boars, respectively. It is possible, therefore, that reproductive activity is more resistant to the elevation of atmospheric temperature during the summer in Lithuanian White boars than in Petren boars. The scientific of Borg *et al.* (1993) and Harayama *et* al. (1992) may assist in explaining this observation – probably the geographical separation and differences in selection over hundreds of years resulted in the differences in mean abnormal sperm number between Lithuanian White and European (Petren) breed of boars in summer.

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