SEASONAL EFFECT ON SPERM MORPHOLOGICAL PROPERTIES OF EUROPEAN WILD BOAR (*SUS SCROFA* L.) IN LITHUANIA

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Abstract. The aim of this study was to determine the effects of the epididymis sample storage conditions and season on the sperm morphological characteristics of wild boars (*Sus scrofa* L.). The samples were collected from males hunted in northern Lithuania. Straight after shooting the animal and cutting out the both testis, the epididymis was dissected and divided into three parts. One part was placed on a freezing element (4 ± 2^0C), the second one into a 3.0 % sodium citrate and the third one into a preservative solution. For determination of seasonal effects, the samples were placed only into a preservative solution. In the laboratory, all the epididymis were broken up and flushed spermatozoa were dyed with eosin. Morphological characteristics of sperm were evaluated by the accepted methodology.

Sample storage conditions in 20–24 hours period had no effect on the sperm morphological characteristics of wild boar males (n=6). The study of the seasonal effects on the sperm morphological properties of European wild boar indicated that during the breeding season (November and December) the number of intact spermatozoa in the semen of second year males (n=5) and 3 year or older males (n=6) was similar and accounted for 54.0 and 52.0 % respectively. During the off-season (May and June), the average number of intact spermatozoa in the semen of second year males (n=5) and 3 year or older males (n=6) was respectively 16.2 and 13.0 % or by 37.8 and 39.0 % lower ($P<0.001$) than that in the semen of contemporary wild boar males hunted at the time of oestrus. In the summer time, the second-year wild boar males can inseminate the second year gilts in heat of the same group what leads to degeneration due to close inbreeding. Older wild boar males should be preserved in order to preserve the reproductive traits of wild boar groups and their genetic values. The seasonal changes of the sexual cycle might have been influenced by global warming and good nutrition conditions throughout the year.

Keywords: wild boar, season, spermatozoa, sperm defects.

SEZONIŠKUMO ĮTAKA EUROPINIO ŠERNO (*SUS SCROFA* L.) SPERMOS MORFOLOGINIAMS RODIKLIAMS LIETUVOS SĄLYGOMIS

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Santrauka. Tyrimo tikslas buvo nustatyti antsėklidžių mėginių laikymo sąlygų ir sezoniškumo įtaką šernų (*Sus scrofa* L.) spermatozoidų morfologiniams rodikliams. Mėginiai paimti iš Šiaurės Lietuvoje sumedžiotų šernų. Nušovus patiną tuoj pat preparuotos antsėklidės padalintos į tris dalis. Viena dalis padėta ant šaldymo elemento (4 ± 2^0C), antra dalis įdėta į 3,0 proc. natrio citratą, o trečia – į konservuojamąjį tirpalą. Sezoniškumo įtakai nustatyti mėginiai merkti tik į konservuojamąjį tirpalą. Iš laboratorijoje susmulkintų antsėklidžių išplauti spermatozoidai buvo dažomi eozinu, o jų morfologiniai rodikliai įvertinti pagal priimtą metodiką.

Skirtingos mėginių laikymo sąlygos 20–24 h laikotarpiu įtakos šernų (n=6) spermatozoidų morfologiniams rodikliams neturėjo. Tiriant sezoniškumo poveikį nustatyta, kad rujos metu (lapkričio–gruodžio mėnesiais) sveikų spermatozoidų skaičius tiek antramečių (n=5), tiek trečiamečių ir vyresnių (n=6) šernų patinų spermoje praktiškai nesiskyrė ir siekė atitinkamai 54,0 ir 52,0 proc. Gegužės–birželio mėnesiais (ne rujos metu) sveikų spermatozoidų skaičius antramečių (n=5) bei trečiamečių ir vyresnių (n=6) šernų patinų spermoje sudarė atitinkamai 16,2 ir 13,0 proc. arba buvo atitinkamai 37,8 ir 39,0 proc. mažesnis negu spermoje bendraamžių patinų, sumedžiotų rujos metu ($p<0,001$). Taigi vasaros laikotarpiu neatsiskyrę antramečiai patinai gali apvaisinti tos pačios bandos rujojančias antrametes pateles. Pastaruoju metu tokie atvejai pastebėti. Kraujomaiša ar artimas giminingas veisimasis yra nepageidautini. Siekiant šernų gerų reprodukcinių savybių ir bandų genetinio visavertiškumo, būtina išsaugoti vyresnio amžiaus patinus reproduktorius. Pažymėtina, kad šernų lytinio ciklo sezoniškumo silpnėjimą Lietuvoje gali veikti per visus metus susidariusios geros mitybos sąlygos ir globalus klimato atšilimas.

Raktažodžiai: šernas, sezoniškumas, spermatozoidas, spermos defektai.

Introduction. Semen quality of sires is not a fixed and unchangeable value especially regarding the animals with highly expressed seasonal activity. Puberty time of domestic animals is dependent on their species, breed, and gender, environmental, feeding and housing conditions. The animals with a short life span reach puberty earlier than those with a longer one. Females reach puberty earlier than males. All domestic animals reach puberty much earlier than the growth and development of the whole body is completed. As far as wild animals are concerned, physiological and sexual maturity are both reached at the same time because otherwise undeveloped females might die at birth giving or the offspring might be born weak and, thus, the species might disappear. Animals living in warmer climatic conditions reach puberty earlier (Pakėnas, 1968). Mating of wild animals takes place irrespectively of the weather and nutrition conditions but at a certain time every year. Seasonal oestrus is characteristic of the European wild boar (*Sus scrofa* L.) (Mauget and Boissin, 1987). There is only one factor in the nature that does not depend on the temperature or other conditions – and that is the day length that determines the breeding season. The central nervous system reacts accordingly to a certain day length and signals the endocrine glands that are related to the reproductive system. The pregnancy of *Sus scrofa* lasts for approximately four months. The progeny is born from March till May and the birth time partially depends on the climatic conditions at that time and individual features of the animal (Meynhardt, 1978). Mating of wild boar females takes place when the days are shortest, i.e. from November till January, and it is considered that the period of oestrus is the same in the countries with similar climatic conditions. The reflex of sexual attraction for wild boars reveals itself only during the breeding season. However, it is considered that under good nutrition conditions, females start mating at the age of 8 to 10 months (Abraitytė, 1980). The spermatogenesis and sperm quality of wild boar sires can be influenced by numerous factors, i. e. time of the year, age, number of oestrous females (sire usage intensity), adaptation to the environment, nutrition conditions. Kozdrowski and Dubiel (2004) indicated that the qualitative characteristics of wild boar semen are the same as those of domestic boars however for wild boars they tend to be higher in late autumn than in other seasons. Spermatogenesis in young boars begins at 3 months of age; at 5 to 6 months of age males do not yet excrete semen suitable for fertilization and they reach maturity at 10 months of age (Mauget and Boissin, 1987). Furthermore, the quality and amount of semen increase until 1 to 2 years of age, however, later these parameters might start decreasing (Wekerle, 1983; Hovorka and Slechta, 1984; Kennedy and Wilkins, 1985).

The intensity of spermatogenesis is influenced by the changes in the environment temperatures and season (Kemp et al., 1988; Wekerle et al., 1989; Mudra et al., 1991). According to Weiler et al. (1989), the main factor influencing the spermatogenesis is the changing length of the day during the year. Nutritional factor might be also of importance in the process of reproduction because some wild animals that were kept in captivity but had good care and feeding started mating regardless of the time of the year (Pakėnas, 1968). Under lower environmental temperature, offering higher amounts of feeds increases the intensity of sperm production (Kemp and Verstegen, 1990). Wild boars come in heat when the conditions for their nutrition are the worst, however, lower contents of energy and protein in the diets reduce the intensity of semen production too (Kemp et al., 1989, 1990, 1992). All adult wild boar females come in heat almost at the same time in a female group (Baleišis, 2006). First-year and second-year males (before heat) most often live in groups (sounders) that also include a grown-up female (sow) and quite often even several sows when more families join together (Bluzma, 2001).

From 18 months of age, males are driven out of the sounder and start solitary life or still group together for some time (Meynhardt, 1978), sometimes even with the females of the same age. Solitary second-year males can be observed already at the beginning of summer roaming in quite an indefinite area (Tursa, 1974), migrating and, thus, reducing the possibility of kindred mating. Three-year and older males hold themselves only individually (Belova, 2001).

When the heat is over, males leave the sounders however second-year gilts are often fertilized by most probably their contemporaries, i.e. males of the same litter still remaining in the main sounder. Second-year males hunted in summer had no specific meat odour (boar taint) that is acquired in the period of heat. Recently individuals that were hunted in May or June had this specific odour and this allows concluding that two-year old wild boars come in heat also in summer and that seasonal heat has disappeared. It is quite complicated to collect semen from the boars living in the wild and it is inexpedient to collect it from those living in enclosures because of the changes in the behaviour of wild boars and in their semen quality that is affected by numerous factors such as feeding and housing. Therefore, wild boars were hunted, semen collected and the morphological characteristics of spermatozoa evaluated.

The purpose of the study was to determine the effects of the epididymis sample storage conditions on the sperm morphological characteristics, and to investigate the morphological properties of spermatozoa from wild boar males of different ages in the periods of oestrus and anoestrus.

Material and methods. All procedures of animal experiments were approved by the Lithuanian animal care, management and use legislation No. 8-500 (State news, 28 November 1997, no. 108).

Semen collection and analysis. For determining the effects of the sample storage conditions on the sperm morphological characteristics, semen of the second-year wild boar males (n=6) hunted in November–December was used. Momentarily after shooting the animal and cutting out the both testis, the epididymis (Pakėnas, 1968) was dissected and divided into three parts. One part was placed on a freezing element (temperature 4±2 ^{0}C), the

second one into a 3.0 % sodium citrate solution and the third one into a preservative solution composed of Na_2HPO_4 x $2H_2O$ – 6.19 g (4.938 g waterless), KH_2PO_4 – 2.54 g, concentrated formalin – 125.0 ml, NaCl – 5.41 g and distilled water up to 1000 ml. After 20–24 hours at the laboratory of Biological Research Centre of Šiauliai University, all the epididymis were broken up and sperm flushed using 3.0 % sodium citrate solution. The number of intact spermatozoa were evaluated by the below presented methodology.

In order to determine the seasonal effect on the sperm morphological profile of European wild boar (*Sus scrofa* L.) under the conditions in Lithuania, the semen for investigation was collected from different age (second year and three year or older) wild boar males that were shot in northern Lithuania during May–June and November–December, 2010, huntings by battue and watch-tower methods. In the dark, hunter torches may be used to illuminate the desired specimen to be shot (Baleišis, 2006). For investigation 10 second-year (5 during anoestrum and 5 during oestrum) and 12 three-year or older (6 during anoestrum and 6 during oestrum) wild boar males were used. The age of animals was determined visually and by the number of tooth. The snout of three to six-year-old males is crooked, the back line from the middle of the body goes down towards the tail and the tuft of the tail is hanging down lower than the joint of the heel. A tuft of longer hair can be observed on their underbelly. The snout of old males is very crooked, tusks very distinct, and the dorsal line slopes down from the middle. The tail with a big tuft is both larger and longer (Belova, 2001). Momentarily after shooting the animal and cutting out the both testis, the epididymis was dissected. For further studies, the samples were stored in the preservative solution because those stored in sodium citrate solution were spoiled due to high temperatures in summer time. Storage on freezing elements did not guarantee a stable temperature regime. At the laboratory, the epididymis were broken up and sperm flushed using 3.0 % sodium citrate solution.

For determining the effects of the sample storage conditions and seasonality on the sperm morphological properties of wild boars, the flushed spermatozoa were dyed with eosin (1.7 g eosin dissolved in 3.0 % sodium citrate solution) (Pakėnas, 1985). A drop of flushed spermatozoa was put on a glass slide and mixed with the dye to make a thin smear on the whole slide that was afterwards dried in the air. Morphological characteristics of semen were determined using the optical microscope magnifying from 200 to 400 times. 200 spermatozoa were counted in every smear and the percentage of pathologic and normal (intact) spermatozoa determined. The following pathology of spermatozoa was analysed: head, neck and tail pathology, cytoplasmic drops on tails and other pathology (separated, double heads, separate tails, neck and tail separation from the head, etc.).

Statistical analysis**.** The investigation data were processed using statistical package Statistica for Windows version 6.0 (StatSoft, 2001) and following the basic guide to the statistical analysis of biological data by Tucker (2003). The difference was considered significant when $P<0.05$.

Results and Discussion. Sample storage conditions in 20–24 hours period had no effect on the morphological profile of spermatozoa, and the differences between sample delivery methods were statistically insignificant (Table 1). For further studies, semen samples were stored in the preservative solution.

Table 1. **The effect of sample storage conditions on the morphological properties of sperm by evaluation of intact spermatozoa** (n=6)

Sample storage conditions	Intact spermatozoa, %
Temperature 4±2 ^{0}C	47.7 ± 1.02
3.0 % sodium citrate solution	47.3 ± 0.92
Preservative solution	48.0 ± 0.45

During the breeding season, the number of intact spermatozoa found on the average in the semen collected from the epididymis of both testicles of adult 3-year-old or older hunted males was 52.0±1.77 % (Table 2), whereas the percentage of intact spermatozoa in the semen of similar age wild boar males hunted off-season was on the average 13.0±0.45 % or 39.0 % ($P<0.001$) lower. The presence of pathological spermatozoa in the semen is the first symptom indicating degenerative processes undergoing in the spermatogenous epithelium or the disorder of the epididymis functioning (Hafez, 1993; Briz et al., 1996). The highest differences were found for the cytoplasmic drops on tails. During oestrus the number of cytoplasmic drops accounted on the average for 26.0±0.49 %, whereas during anoestrus cytoplasmic drops were detected on all the spermatozoa irrespectively of their pathology type. The presence of a relatively higher number of spermatozoa with cytoplasmic drops on tails during oestrous time can be explained by the fact that all adult wild boar females in female groups come in heat almost simultaneously. The highest difference in oestrus can be 8 days (Baleišis et al., 2003). If the number of older males in the population is sufficient, younger than 3.5-year-old males do not take part in breeding as they lose competition with the older males (Belova, 2001). As a rule, in the group of oestrous females one remaining wild boar male inseminated all the females in heat. The number of females per wild boar male is too high and frequent ejaculation results in higher numbers of immature with cytoplasmic drops, without tails and abnormal head size spermatozoa (Briz et al., 1996). Spermatozoa maturation in the epididymis lasts for 7 to 10 days for both wild boar and domestic pig males. Thus, the epididymis is filled in 6 to 7 days. One ejaculation requires 60 % of the epididymis volume, and after 3 to 4 ejaculations in 12-hour time the epididymis is completely empty. This abnormally speeds up the sperm movement from epididymis head to tail and results in structural changes of spermatozoa (Bonet et al., 1991). The number of morphologically abnormal spermatozoa in the semen might increase up to 35–47 % and sperm motility might decrease as low as 50 %. Frequent semen

collection increases only the number of spermatozoa produced per certain time unit (Du Mesnil de Buisson et al., 1978). The number of pathological spermatozoa with twisted tails and cytoplasmic drops is also higher in the semen of rarely used boars (Larsson, 1995). Cytoplasmic drops were observed on almost all the tails in the semen of wild boar males of 3 to 5 years old and hunted at anoestrum in May or June. In the off-season, males of this age do not take part in female insemination or accidentally inseminate only those females that lost their offspring and might come in heat for late piglets (Baleišis et al., 2003). Besides, it was indicated (Table 2) that the number of spermatozoa with head pathology increased insignificantly from 7.3 ±0.46 to 9.5±0.67 % ($P<0.05$) during anoestrum. The number of spermatozoa with neck pathology during oestrus was on the average 2.5±0.23 % but during anoestrus their number did not change. At anoestrum, the number of spermatozoa with tail pathologies increased up to 29.5±1.57 % ($P<0.001$), while the other pathology accounted for 45.5±0.56 % and exceeded the number of normal spermatozoa by 32.5 % ($P<0.001$).

Table 2. **Spermatozoa morphological evaluation of adult (3 years and older) males hunted during anoestrum** (n=6) **and oestrum** (n=6)

Season	Morphological characteristics, %					
	Head pathology	Neck pathology	Tail pathology	Cytoplasmic drops on tails	Other pathology	Intact spermatozoa
Anoestrus	9.5±0.67	2.5±0.22	29.5±1.57	--------*	45.5±0.56	13.0±0.45
Oestrus	7.3±0.46[a]	2.5±0.23	5.8±0.89[b]	26.0±0.49	6.4±0.68[b]	52.0±1.77[b]

Note:* Drops observed on all spermatozoa tails. [a] $P<0.05$; [b] $P<0.001$.

A comparatively high (44.0±2.75 %, $P<0.001$) number of spermatozoa with cytoplasmic drops was found in the semen of second year males hunted in spring–summer (Table 3). The number of spermatozoa with twisted tails in the semen of second year and 3 to 5-year-old males hunted during anoestrum was respectively 10.3±0.73 % and 29.5±1.57 % or 19.2 % ($P<0.001$) lower. A similar morphological response is observed when testicles are affected by high temperatures. The number of spermatozoa with head pathology and cytoplasmic drops increases and the power of fecundation decreases as low as 33.0 % (Hafez, 1993; Briz et al., 1996). However, the boars of some domestic pig breeds adapt themselves to the influence of high ambient temperature and the quality of their semen is not seriously affected (Flowers, 1997). Wild boars in contrast to domestic pigs are most active after dark and besides, they are able to find natural ways to get cold (mud bathing, day resting in spruce groves and naturally ventilated places). Therefore, the danger for testicle overheating is minimal, and the reduction in the amount of intact spermatozoa can be explained by seasonal effect. During the off-season the semen of the second-year boars contains on the average 16.2±1.91 % intact spermatozoa, i.e. 37.8 % ($P<0.001$) less than that of the semen of the contemporaries hunted in November–December, because the quality of the semen produced off-season is usually lower (Ciereszko et al., 2000). There was actually no difference for the number of intact spermatozoa both in the semen of the second-year and 3 to 5-year-old wild boar males, and this number accounted for 54.0±4.02 and 52.0±1.77 % respectively. In Lithuania, solitary second-year males can be observed already at the beginning of summer. In summer wild boar groups usually consist of a sow and its young; of a sow, piglets and second-year males; of sows, their young and second-year males; of second-year males (Baleišis et al., 2003). Groups of second-year males are often mixed ones, i.e. both males and females from the same group have been hunted. When the same group comprises males with the functioning reproductive system, a second-year female in heat can be inseminated and late litters are an indication of this mating. In October, 2010, a solitary young wild boar sow with three not older than a month old striped offsprings was observed. Stripes usually fade in 3 to 4 months (Baleišis, 2006) and this shows that the offsprings were born in the second half of summer.

Table 3. **Sperm morphological characteristics of second year wild boar males hunted at the time of anoestrus** (n=5) **and oestrus** (n=5)

Season	Morphological characteristics, %					
	Head pathology	Neck pathology	Tail pathology	Cytoplasmic drops on tails	Other pathology	Intact spermatozoa
Anoestrus	6.11±1.03	6.1±0.70	10.3±0.73	44.0±2.75	17.2±1.20	16.2±1.91
Oestrus	10.2±2.43[a]	2.0±0.37[a]	8.1±1.81	12.1±3.97[b]	13.5±2.09[a]	54.0±4.02[b]

Note: [a] $P<0.05$; [b] $P<0.001$.

Two weeks earlier, two striped progeny with their sow were observed keeping apart in the group of seven second-year wild boars. Hunting and studying of one second-year male from the group showed that the

reproductive system of this individual was functioning completely and that it could inseminate the females in the group at any time, though it is considered that a year-old wild boar males are usually driven out of the group (Meynhardt, 1978; Baleišis et al., 2003) and lead a solitary life. Usually the sow gives birth to approx. 4–8 (up to 12) youngsters (Belova, 2001). In the latter case respectively 3 and 2 offsprings were observed what can be explained by the young age of the female (Ruiz-Fons et al., 2006) and high number of pathologic and low number of motile and live spermatozoa in summer and autumn (Šernienė, 2000). Not only small litters but also degenerations are observed when wild boar males from the same group start mating with their contemporary females. In the last two hunting seasons, five young wild boars were hunted with the right front legs reaching only 10–14 cm in length and this might have happened due to inbreeding. In a year, a wild boar can travel up to 150 km from its living place (Tursa, 1974). 10–15 years ago, hunters used to observe and hunt single individuals weighing 300 kg and more. It is probable that these wild boars used to migrate from other forests because, as a rule, they leave the territory occupied during the previous breeding season. Owing to intensive wood cutting, agricultural activities and good nutrition possibilities resulting from global warming, wild boars lose their habit for migration and begin living in a more definite territory. The increased number of large-size and barren wild boar females allows to conclude that young and sexually mature males are not able to inseminate them and, thus, total reproduction performance of the group is worse and cases of degeneration are being observed. In order to preserve the reproductive group, it is considered expedient to hunt wild boar males that are either not older than 2 years or aged males that are incapable of inseminating due to their large size.

Conclusions

1. Sample storage conditions in 20–24 hours period had no effect on the sperm morphological characteristics of wild boar males. The differences between different storage methods were statistically insignificant.

2. During the breading season, the number of intact spermatozoa in the semen of the second-year and 3 to 5 year-old wild boar males was similar and accounted for 54.0 and 52.0 %, respectively. During the off-season (anoestrum), the average number of intact spermatozoa in the semen of the above mentioned age males was respectively 16.2 and 13.0 % or by 37.8 and 39.0 % lower ($P<0.001$) than that in the semen of contemporary wild boar males hunted at the time of oestrus.

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