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Testicular Biometry and Morphological Characteristics of Wild Boar Spermatozoa in Relation to Age during the Anoestrus Period

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Abstract: The aim of the present study is to analyse the biometrical parameters of the testes and morphological characteristics of spermatozoa of different aged wild boars that are hunted outside of the mating season in order to evaluate the expediency of preservation and use of younger males for breeding in the next season. The study indicates that despite obvious biometric differences between the testes of young and mature boars (the weight is 2.6 and the volume is 3.4 times lower in juvenile than in mature specimens) and more effective spermatogenesis of mature boars (the amount of intact spermatozoa is from 2 to 3 times higher), young boars are able to perform reproductive function during the anoestrus period. During this period, the epididymis of both young and mature boars contains 31-35% of spermatozoa with cytoplasmic drops that indirectly indicates comparatively high sexual activity of both groups of boars even during the anoestrous period. Therefore, even the second-year boars can spread, e. g., African swine fever and have to be included into the extermination scheme of the prevention measures.

Key words: African swine fever, spermatogenesis, sexual activity, estrus season, gonadosomatic index

Introduction

In recent years, African swine fever (*Pestis africana suum*) has become one of the major factors influencing wild boar (*Sus scrofa scrofa*) population. On 25 January 2014, a wild boar that had died due to this virus was found in Lithuania. To avoid spreading of the disease, an intensive extermination of the population started. Wild boars were hunted all year round and hunters were paid for killed females (National Food and Veterinary service "Regulation of Payments for killed wild boar females in 2015-2016" November 13, 2015 No. B-1028, Vilnius).

The biological age of wild boars is approximately 13-15 years. Wild boar population can be restored very quickly. The causes of the population increase in recent decades are mainly a combination of ecological and environmental factors (Macchi et al. 2010). Spring is the time for significant increase of their population (Flis 2013, Kniżewska & Rekiel 2015). The European wild boar, which is an ancestor of the domestic pig, is a short-day breeder; its reproduction is stimulated by shortening the day length in autumn (Love et al. 1993, Hälli et al. 2008) and its mating activity is seasonal (Mauget & Boissin 1987, Tast et al. 2001). The most important factor regulating seasonal activity is the photoperiod. This indicates that daylight fluctuations are the main reason for seasonal changes of testicular function and semen quality in the domestic boar (Schopper et al. 1984, Ciereszko et al. 2000). However, the duration of photoperiod does not affect the weights

of the testes and epididymis or the total number of spermatozoa within these tissues when wild boars are still sexually immature (at 6–8 months of age).

Males reach puberty at the age of two years. Mature males, especially older ones, live separately and migrate. A well-developed male forces second-year males to leave the herd and fertilises the females in heat (Delgado et al. 2008). If the herd has a sufficient number of old mature males, they start to breed usually only at the age of 3.5 years. The spermatogenesis and semen quality might be influenced by the season (Kozdrowski & Dubiel 2004), length of natural photoperiod, age (Stone et al. 2013), number of estrus females and the related breeding intensity of the male as a sire. Food supply might have a substantial influence on animal reproduction (Delcroix et al. 1990).

The anatomical position of the testes is unfavourable in terms of thermoregulation and, therefore, temperature changes might have a disturbing effect on spermatogenesis. The presence of pathological spermatozoa in the semen is the first symptom indicating degenerative processes in the spermatogenic epithelium or disorders of the epididymal function. Due to the changing temperature, seasonal changes are followed by changes in spermatogenesis (Kemp et al. 1988). Wild boar spermatogenesis lasts 40–50 days on average and pathological spermatozoa (head and tail pathologies, cytoplasm droplets and acrosome defects) are detected at 20–50 days after the influence of a negative factor. Autumn is the time when the highest number of spermatozoa with pathologic heads and the lowest number of motile and viable spermatozoa in ejaculates is detected. In this respect, autumn is significantly worse than the other seasons, whereas winter is the best (Petrocelli et al. 2015). However, this finding is in contrast with the studies of Rivera et al. (2005) who found no differences in viability of spermatozoa between the seasons.

In order to prevent spreading of the African swine fever, an intensive hunting of wild boars has begun since 2015 and, consequently led to extermination of mature males and the subsequent changes in the structure of the herd. Currently, the increase in the number of second-year males has been observed. These males, being smaller in size, are incapable of breeding large females. The decrease in the numbers of mature breeding males was the reason for a higher number of sterile mature females killed during hunting, triggering a decrease in the wild boar population and its subsequent disappearing.

The purpose of the present study was to analyse the biometrical parameters of the testes and morphological characteristics of spermatozoa of different aged wild boars that were hunted outside the mating season in order to evaluate the expediency of preservation and use of younger males for breeding in the next season. The study was aimed at comparison of the correlations between the different body weight of wild boar and their testicles.

Materials and Methods

The study was carried out in 2015–2016 at the Animal Science Institute of the Lithuanian University of Health Sciences. The samples were collected in northern Lithuania (55°34’ to 55°43’ N; 23°29’ to 23°45’ E) after ordinary hunting that was carried out following the regulations for hunting on the territory of the Republic of Lithuania (approved by the Minister of Environment of the Republic of Lithuania on 27 June 2000 No. 258).

Thirty wild boars were hunted in May-October and were divided into two groups by age and weight determined by weighing of the animals right after hunting. The ages of the hunted animals were determined using the tooth eruption charts. There were two groups of wild boars in the trial. Group 1 (Young) comprised 15 second-year males weighing 64.0 ± 7.6 kg. Group 2 (Mature) comprised 15 third-year or older males weighing 107.3 ± 12.6 kg. African swine fever was not diagnosed for any of the hunted animal.

Immediately after shooting the animal, the two testes were cut, they were placed in a cool box on a freezing element at 4 ± 2 °C (Martinez-Pastor et al. 2005) and delivered to the laboratory. After 15-20 hours at the laboratory, all the epididymis were broken up and sperm flushed using 3.0% sodium citrate solution (Dubost R.: Technique du spermocytogramme. Pharm. Biol. 1979, 13, 133-134.). Spermatozoa were stained using Quick Staining Kit for Spermocytograms ”Kit Spermoscan” according to the protocol included in the kit (Reactif RAL, France). Morphological characteristics of spermatozoa were determined using optical microscope with Sperm Class Analyzer (Microptic S. L., Spain) with 100× immersion lens and program SCA®2005 module Morphology. Two hundred spermatozoa were counted in every smear and the percentage of pathologic and healthy spermatozoa was determined. The following pathology of spermatozoa was analysed: head, neck and tail pathology and cytoplasmic drops on tails. Some of the pathologies were observed visually at a magnification of 200-400× (e.g. separate heads, twisted tails). Spermatozoa without the listed pathologies were classified as intact.

Testes weight with and without tunica albuginea and epididymis were recorded. Testes length, width and thickness were measured using a calliper divided in mm. Volume was measured by dipping testes in volumetric dish divided in millilitres. The gonadosomatic index (GSI – testes mass divided by body weight, see ALMEIDA et al. 2006) was calculated for every animal.

The data were processed using the statistical package Statistica, Version 6.0. The means were presented with standard error (M ± SE) in the tables or with standard deviation (M ± SD) in the text unless otherwise indicated. The difference was considered significant when P<0.05.

Results

The analysis of the biometrical measurements of testes between different age groups of wild boars indicated that there were no significant differences between the mean lengths of right and left testicles of the same individual, between width and weight, weight of testes with tunica albuginea and epididymis and total volume of the testes.

The analysis of the testes parameters of wild boars hunted out of heat season indicated that testes of mature boars weighted on average 2.6 times more than those of young ones. Accordingly, mean volume of the testes of mature boars was 3.4 times higher than that of young males. Thus, the relative increase of the testes volume was higher than the body weight increase during wild boar growth, i. e. when the body weight increased by 67.7%, the testes weight increased 2.6 times. The average weight of testes with tunica albuginea and epididymis were 2.5 times lower for young males. The testes length and width of mature males were on average 52.5% and 57.4% higher, respectively (Table 1).

The results from our study indicated that mature wild boars had less intact spermatozoa by 11.5% in the epididymides of right testicles than left testicles (Table 2). Young wild boars had by two and three times (P<0.005) lower content of intact spermatozoa in the epididymides of, respectively, right and left testicles in comparison with mature wild boars (Tables 2 and 3). Correspondingly, the number of spermatozoa with head pathology in the epididymides of the mature wild boars was from 1.8 (right testicles) to 2.9 (left testicles) times (P<0.005) lower in comparison with young wild boars (Tables 2 and 3).

During the anoestrus period, pathologic spermatozoa made up on average 85.5% and 65.2% in the epididymis of, respectively, young and mature

Table 1. Differences in biometrical measurements of testes of wild male boars of different age.

Item	Young males (n=15)	Mature males (n=15)
Testes mean:		
Length, cm	5.8 ± 0.2	8.8 ± 0.2***
Width, cm	3.3 ± 0.1	5.3 ± 0.1***
Total weight, g	63.1 ± 1.8	226.8 ± 5.1***
Weight with tunica Albuginea and epididymis, g	91.5 ± 2.3	320.5 ± 6.1***
Total volume, cm^3	57.6 ± 1.9	256.0 ± 2.0***

*** P<0.005

Table 2. Morphological characteristics of spermatozoa of mature wild boars hunted at the time of anoestrus (n=15).

Morphological characteristics, %	Left testis	Right testis
Head pathology	16.1 ± 1.4	16.6 ± 1.8
Neck pathology	5.1 ± 1.9	2.0 ± 0.6
Tail pathology	7.0 ± 0.8	12.0.0 ± 1.6**
Cytoplasmic drops on tail	24.2 ± 2.9	37.5 ± 2.8***
Other pathology	11.1 ± 1.8	4.0 ± 1.2***
Intact spermatozoa	36.9 ± 4.5	32.7 ± 4.4

** P<0.01; *** P<0.005

Table 3. Morphological characteristics of spermatozoa of young wild boars hunted at the time of anoestrus (n=15).

Morphological characteristics, %	Left testis	Right testis
Head pathology	46.8 ± 3.4	30.4 ± 3.5***
Neck pathology	5.0 ± 0.8	6.0 ± 0.9
Tail pathology	4.6 ± 0.4	8.2 ± 0.6***
Cytoplasmic drops on tail	31.0 ± 1.4	38.8 ± 1.9***
Other pathology	2.0 ± 0.3	1.8 ± 0.3
Intact spermatozoa	12.4 ± 1.8	16.6 ± 1.9

*** P<0.005

Table 4. Differences in morphological characteristics of spermatozoa between young and mature boars.

Morphological characteristics, %	Young males (n=15)	Mature males (n=15)
Head pathology	38.6 ± 3.4	16.4 ± 1.6***
Neck pathology	5.5 ± 0.8	3.6 ± 1.1
Tail pathology	6.4 ± 0.4	9.5 ± 1.1**
Cytoplasmic drops on tail	34.9 ± 1.5	30.9 ± 2.7
Other pathology	1.9 ± 0.3	7.5 ± 1.4***
Intact spermatozoa	14.5 ± 1.8	34.8 ± 4.4***

** P<0.01; *** P<0.005

wild boars (Table 4). In both age groups, the majority (31-35%) of sperm pathologies were cytoplasmic droplets on tails (only head pathologies were higher (38.6%) in the young-boar group). We recorded an increase of head pathology by 2.4 times on average in the group of young wild boars. Other types of pathologies accounted for no more than 10% in both groups (Table 4).

Discussion

We have found not significant differences in biometric measurements of both testicles of different individuals in different age groups of wild boars. This is in agreement with the findings of Scanlon & Lenker (1983) who have found no significant differences between left and right sides of the reproductive tract of males in terms of testes weights, epididymis weights and spermatozoa contents of both organs.

The length of the testes of a mature boar male usually ranges from 10 to 15 cm, its width – from 5 to 9 cm and weight – from 500 to 800 g. The size of testes in the examined mature wild boars hunted during the anoestrus period, approximately meets the minimum values indicated but the weight is twice lower. According to Mauget & Boissin (1987), testes weight averages 53 g in 30–35 kg boar males, whereas according Costa & Silva (2006) the testes weight of 24.8 ± 3.6 kg boars was 20.2 ± 3.7 g. Thus, GSI are 0.33% and 0.16%, respectively. Almeida et al. (2006) have indicated GSI for boars with average weight of 39.7 ± 2.9 kg is 0.314 ± 0.04% (M ± SE). Our study indicates that the GSI are from 0.10 ± 0.02% for young males to 0.21 ± 0.03% for mature males. Therefore, it can be argued that the experimental matured wild boar testes correspond to an average level of development when young wild boars testes are less developed by about 50%.

Semen quality is influenced by both male weight and its testis weight (Harder et al. 1995). The length of the day has no influence on spermatozoa motility but there is an increase in the number of spermatozoa with morphological changes. Meanwhile, Fraser et al. (2016) have found that seasonal effects on spermatozoa motility are more marked in boars at the age of 19 to 30 months, being significantly higher during the autumn-winter period.

Ciereszko et al. (2000) have reported a very distinct seasonal effect on the qualitative characteristics of semen, i. e. worsening in summer and improvement in autumn-winter. However, Rivera et al. (2005) have not found any natural daylight effects on these characteristics. Lower semen quality in summer could be related with the stress evoked by summer heat. The findings by Briz et al. (1996) indicate that heat stress lowers the semen quality after 15–20 days and the semen fecundation power decreases to as low as 33%. In our study, the method of preparation of samples does not allow to determine spermatozoon motility and vitality in the epididymis. Therefore, the sexual activity of mature and young wild boars, efficiency of spermatogenesis and differences in anoestrus period are evaluated based on morphological characteristics of spermatozoa and biometrical measurements of testes.

Spermatogenesis of ten-month old wild boars is slower than that of domestic pig boars of the same age (Costa & Silva 2006). However, spermatozoa can be detected in the epididymis of ten-month old wild boars. According Strzeżek (2002), this mostly depends on the gonad development. In our study, young wild boars have 2 to 3 times lower content of intact spermatozoa in the epididymides in comparison with mature wild boars and the number of spermatozoa with head pathology of mature wild boars is from 1.8 to 2.9 times lower than those of young wild boars. These differences could be explained by age difference: older males produce semen of higher quality.

Mature wild boars are solitary animals. Separated from their mothers, second-year males and females keep together and form herds. During the anoestrous period, both females and males are mated by their contemporaries. All that stimulates earlier sexual maturation of females and heat exhibition out of season. Several cases of one-year-old females hunted in May–June with signs of heat and some of them even with developing embryos have been observed. Mating also affects the composition of semen plasma due to frequent ejaculation (Strzeżek et al. 1995) and also morphological sperm characteristics. According to Bonet et al. (1991), sperm maturation in the epididymides lasts for 7-10 days in both wild boars and domestic pigs; therefore, the epididymis is filled in 6 to 7 days. One ejaculation empties up to 60% of the capacity of the epididymis and after 3-4 ejaculations in the interval of 12 hours epididymis is completely empty. Due to this, sperm movement from the epididymis head to the tail becomes faster and, as a result, there are changes of sperm structure and increase in the number of cytoplasmic drops on the tail. There is a significant number of spermatozoa with cytoplasmic droplets in the epididymis of both young and older wild boars. Researchers have different opinions but mostly this feature is explained by high sexual activity of males. In our study, the

high amount of cytoplasmic drops in the epididymis is considered to be an important argument that both mature and young wild boars are sexually active even in the anoestrus season. This is in agreement with the findings of Andersson et al. (1998), who reported higher percentage of proximal cytoplasmic droplets in the spring-summer group. In addition, the relatively high mean difference in the number of intact spermatozoa in the right and left testes of mature wild boars (11.5%) may also indicate high level of sexual activity.

The amount of pathologic spermatozoa might increase up to 35-47% due to various reasons, especially the number of spermatozoa with abnormal size heads (Briz et al. 1996). However, our survey period (2015-2016) is characterised by normal weather conditions without extreme temperature changes. The wild boar's nutrition options are also considered as moderate. Therefore, the comparatively high average total number of pathologic spermatozoa in both groups of wild boars may be explained by a higher sexual activity of boars that is atypical for the anoestrus season.

Conclusions

The analysis of biometrical data of testes and that of morphological spermatozoa characteristics of wild boars at different age leads to the conclusion that second-year wild boars produce spermatozoa even when outside of the heat season and can actively reproduce. Local extermination of boars in a restricted territory in order to prevent distribution of African swine fever disease would not be efficient. To prevent African swine fever spreading, not only mature but also all second-year wild boars have to be exterminated. On the other hand, the elimination of adult individuals is not practical because areas that used to be characteristic to the species become free from animals and, consequently, stimulating animal spreading into the living area, which favours the spread of African swine fever. However, if it is intended to restore the population of wild boar in the future, after the end of the epidemic, it is advisable not to hunt well-developed second-year boars because they are capable of reproduction in the estrus season (December–January).

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