

HISTOPATHOLOGICAL FINDINGS IN TESTES AND QUANTITY OF THE SPERM WITHIN DIFFERENT AGE GROUPS OF CULLED BOARS

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Summary. The aim of our study was to determine histopathological changes in testes of sire boars and to evaluate their influence on the quantity of the sperm.

In the present work 47 sire boars were investigated histomorphologically after slaughter. Landrace, Large White, Pjetrens and Duroc breeds of sire boars and their crossbreeds were selected for analysis.

The boars were divided into four groups (Groups 1-4) according to their age: 8-18, 18-30, 30-42 and older than 42 months. Group 1 of young boars included 14 individuals (n=14), while the other three groups (Groups 2-4) consisted of 11 boars (n=11) in each group. The study identified that the most frequent pathology was testicular degeneration, which was estimated in 78.57 % of all tested boars. Degeneration was estimated as low, medium and high degree of affection - 34.09 %, 36.36 % and 29.55 %, respectively, of all tested boars. Fibrosive testicular changes, which resulted in severe degenerative and inflammatory lesions, were diagnosed in 63.8 % of boars. In addition, pathologies such as testicular hypoplasia, calcinosis, stromal hyalinosis, sperm stasis, edema, hyperemia, an increased amount of Leydig cells were diagnosed to a low number of boars. Comparison of the four age groups of boars (Groups 1-4) proved that with age growth the boars showed significantly increased incidence of testes degeneration and fibrosis. The number of Leydig cells was also increased, whereas inflammation was detected most frequent in boars of 18-30 months of age (Group 2). It was also detected that in the case of the testicular fibrosis the volume of ejaculate was lower. In most cases of high level of fibrosis, testis weight and volume of the ejaculate were decreased.

Keywords: testes, histopathology, ejaculate, boars.

HISTOPATOLOGINIAI POKYČIAI IŠBROKUOTŲ VEISLINIŲ KUILIŲ SĖKLIDĖSE IR KIEKYBINIAI SPERMOS POKYČIAI SKIRTINGO AMŽIAUS KUILIŲ GRUPĖSE

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Santrauka. Mūsų darbo tikslas buvo nustatyti histopatologinius pokyčius kuilių reproduktorių sėklidėse ir įvertinti jų įtaką spermės kiekiui.

Tirti 47 išbrokuoti paskersti kuiliai reproduktoriai. Tyrimui atrinkti landrasų, didžiųjų baltųjų, Diuroko veislės kuiliai ir jų mišrūnai.

Kuiliai suskirstyti į grupes pagal amžių: 8–18; 19–30; 31–42 ir vyresni nei 43 mėnesių. Pirmoje grupėje buvo tirta 14 kuilių, kitose trijose grupėse – po 11 kiekvienoje. Statistinė duomenų analizė atlikta „SPSS Windows“ programa. Dažniausiai nustatyta patologija – sėklidžių degeneracija – 78,57 proc. visų tirtų kuilių. 34,09 proc. kuilių nustatyta silpna, 36,36 proc. – vidutinė, o 29,55 proc. – stipri degeneracija.

Kita dažna sėklidžių patologija buvo fibrozė. Fibroziniai sėklidžių pakitimai atsirado dėl stiprių degeneracinių ir uždegiminių procesų. Fibrozės procesas stebėtas 63,8 proc. kuilių. Sėklidžių patologijos, tokios kaip antai hipoplazija, kalcinozė, stromos hialinozė, spermės stazė, edema, hiperemija, padidėjęs Leidigo ląstelių skaičius diagnozuota nedaugeliui kuilių.

Keturių amžiaus grupių kuilių palyginimas parodė, kad su amžiumi sėklidėse dažniau randama degeneracinių ir fibrozinių pakitimų, taip pat didėja ir Leidigo ląstelių kiekis. Tuo tarpu uždegimas dažniau nustatytas produktyviausiame kuilių amžiuje, t. y. 18–30 mėn., o su amžiumi tokių atvejų skaičius statistiškai patikimai ($p < 0,05$) mažėjo. Sėklidžių fibrozė daugiausia įtakos turėjo ejakuliatu kiekiui. Stiprios fibrozės atvejais nustatėme sumažėjusį sėklidžių svorį ir ejakuliatu tūrį.

Raktažodžiai: kuilyls, sėklidės, histopatologiniai pakitimai, ejakuliatas.

Introduction. Today there is substantial amount of scientific literature about sperm quantity, quality variations, spermatozoa pathologies, evaluations of various factors which affect sperm quality. However, there are very few publications about changes in the testes, which tend to have a direct role in sperm production disorders, or qualitative parameters. Acquired testicular pathology has been determined much more frequent than congenital or inherited. Scientists identify hypoplasia, degeneration, orchitis, and fibrosis as the most common cases of testes pathology. According to Lagerlof (1934), inherited or congenital hypoplasia of the seminiferous tubules and degeneration are the two most common lesions which affect disturbance in spermatogenesis. Previous studies (Šernienė et al., 2005) showed that after histopathological examination of culled boars' testes, interstitial orchitis and low degree degeneration were diagnosed. These histopathological findings were associated with pathological heads and tails of spermatozoa and in cases of general sperm pathology, medium degeneration of seminiferous tubules was found. Because of sperm and testicular pathology oligospermia was distinguished as one of the reasons for culling of boars. Semen changes over time do not reflect pathological changes in testes. However, in the boar it takes about 60 days from the first stage of spermatogenesis until sperm release. It is generally accepted, that testis size and weight are correlated with the capacity to produce sperm and that hemiorchidectomy is correlated with sperm production in male animals (Janson and Neaves, 1983; Parcer et al., 1997; Lustra et al., 2002). Mammalian species with relatively large testes produce ejaculates containing more sperm (Pierce et al., 1990) and large testes enhance male reproductive success (Preston et al., 2003; Schulte-Hostedde and Millar, 2004). Testicular structure can be affected by various diseases. In several cases of testicular failure, the problem may be quantitative only, with reduction in the number of one or more of the different types of germ cells (Paulsen, 1968). Quantitative testicular histology has been used to determine daily sperm production in the boar (Kennelly and Foote, 1964).

The aim of this study was to determine histopathological changes in testes of sire boars and to evaluate their influence on the quantity of the sperm.

Materials and Methods. In the present work testes from 47 boars were selected. In years 2006 to 2010 testes of boars were analysed histopathologically strictly from May to September. The animals were kept under similar conditions (single anima/pen, fed the same diet). Semen was collected on bi-weekly and after further processing was used for commercial AI. The boars were of Landrace, Large White, Pjetrens and Duroc breeds of boars and their crossbreds and were culled due to various reasons of reproductive failure such as weakly expressed libido, aspermia, elevated number of pathological spermatozoa. After the slaughter, testes of each boar were weighted, measured and two samples (1x3cm in size) were taken from each testicle for histopathological examination. One sample was taken from caudoventral, another - from craniodorsal part of the bouth testicles.

Histopathological evaluation of testes was carried out after slaughter.

The removed testes samples were placed into Bouin's solution for 24 hours and then stored in 70 % ethyl alcohol. The samples were processed and embedded in paraffin (TES99). Sections – 4 μ m thick were stained routinely with Hematoxylin and Eosin (H&E) and evaluated under a light microscope according to the procedure described by Laurusevičienė A. and Smaliukienė R. 2007.

The boars were divided into four groups according to their age: 8-18, 18-30, 30-42, and older than 42 months. The investigation included 14 animals from the first group of young boars and 11 boars from each of the other three groups.

Statistical analysis was performed with SPSS 13.0 for Windows. Data was analysed using the analysis of variances procedure (Glantz, 1999). Differences among investigated groups were analyzed by LSD method ($\alpha=5\%$). Correlation among dependent variables and strength of the direct relation was evaluated by Pearson correlation coefficients. χ^2 criterion was performed for interactions of age, breed and testicular pathology.

Results. Degeneration was the most frequent testes pathology whose incidence was evaluated in 44 tested boars. Degree of degeneration was estimated as low, medium and high - 34.09 %, 36.36 % and 29.55 % respectively of all tested boars (Fig 1).

Low degree of degeneration morphologically was associated with depletion of spermatogenic epithelium and vacuolization in several tubules, nuclear picnosis, mononuclear or multinuclear giant cells, roughness of the basement membranes were.

In the cases of medium degree of degeneration, more than half of seminiferous tubules were found to be affected. Spermatogenic epithelium was vacuolated, there were more giant cells and tubular basement membrane was thick and wavy in compared to normal tubules (Fig. 2).

In case of high degree of degeneration, Sertoli cells and spermatogonia were found in the seminiferous tubules. Their nuclei were vacuolated and picnotic, giant cells were abundant. Tubuli collapsed tubular basement membrane thick and wavy (Fig. 3).

A high level of degeneration with necrotic changes was recorded in testes of 9 boars. Fibrosis was another frequent pathology which was diagnosed in 30 tested boars.

Inflammation of testes was the third largest pathology diagnosed in 14 tested boars. Inflammation mostly was chronic and interstitial and characterized by proliferation of connective tissue and infiltration by lymphocytes (Fig. 4).

Also, some other pathologies such as testicular hypoplasia, calcinosis, stromal hyalinosis, sperm stasis (Fig. 5), edema, hyperemia, increased amount of Leydig cells (Fig. 6) were diagnosed to a small number of boars. In the boars with hypoplasia lesions the histopathological examination revealed seminiferous tubules of small diameter with absence of germinal cells and lined only by Sertoli cells or Sertoli cells and spermatogonia. The tubular

basement membrane remained solid and smooth. In some of the tubules giant cells were found.

The occurrence among different age groups of boars was statistically significant ($p < 0.05$) and this suggests that

the incidence of various testes pathology increases with advancing age has an increasing impact on boar health and productivity (Fig. 7).

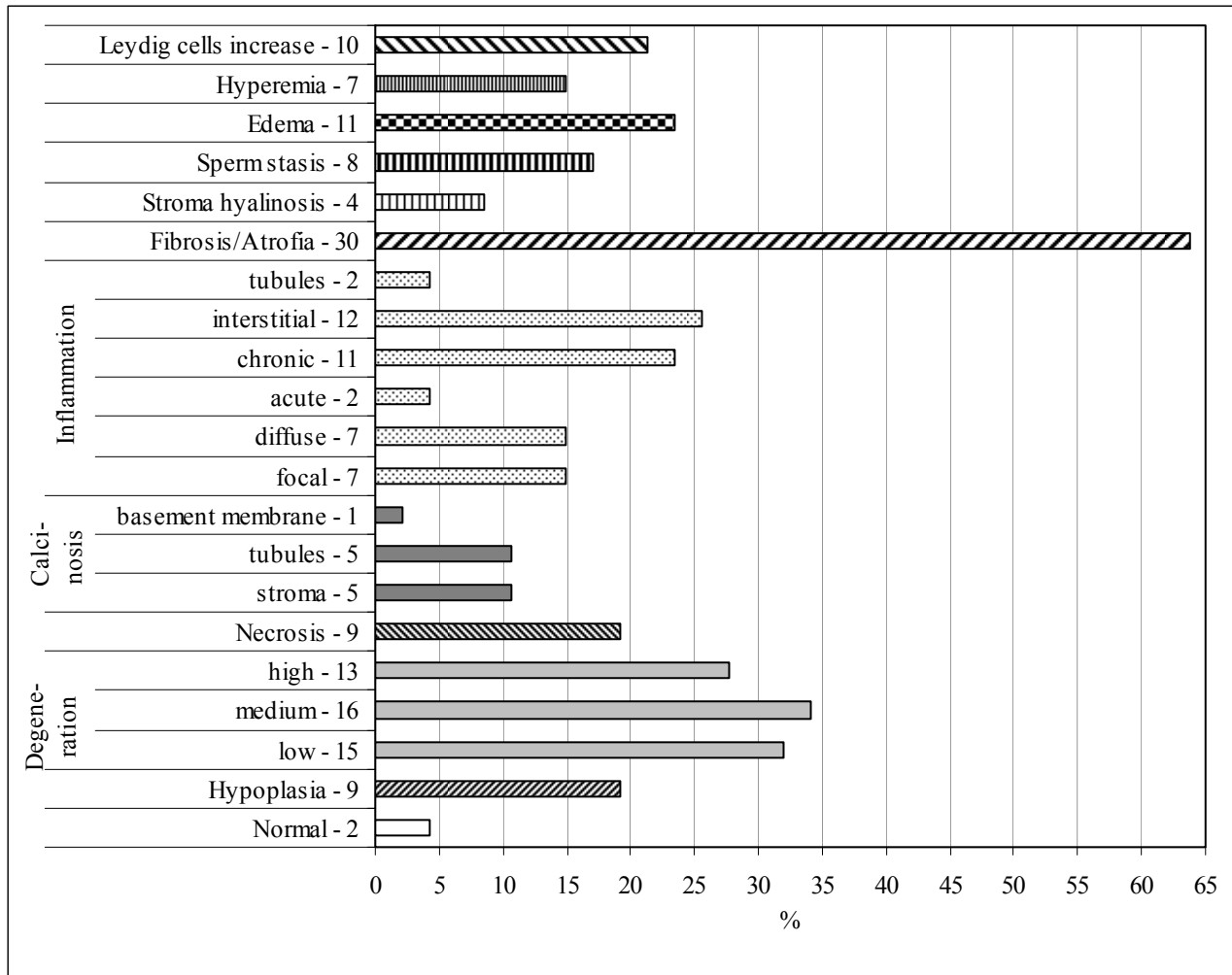


Fig.1. General data of histopathological evaluation of testes

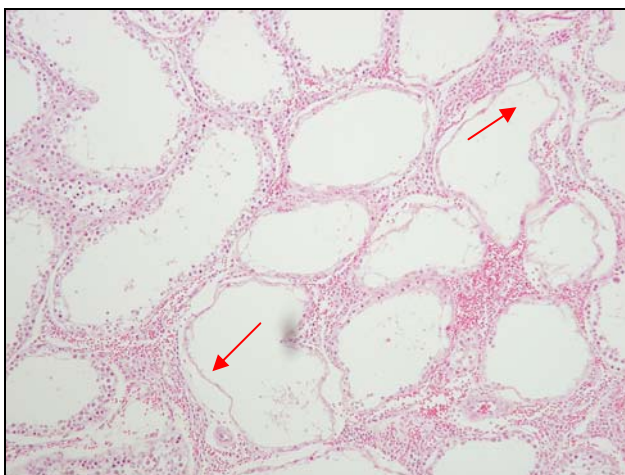


Fig. 2. High degree degeneration of seminiferous tubules (H&E section) X100

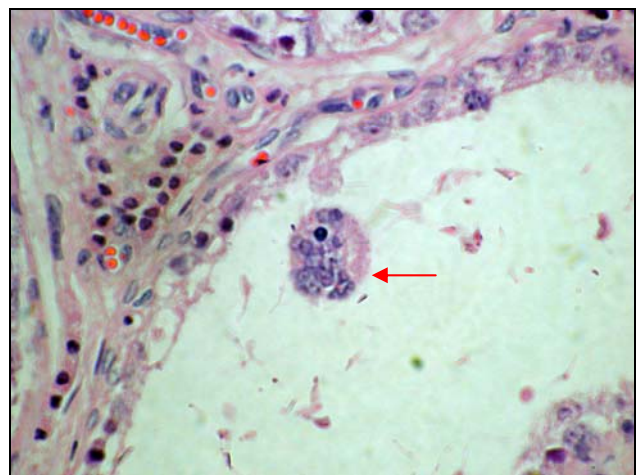


Fig. 3. Giant cells in tubule, nuclear picnosis (H&E section) X400

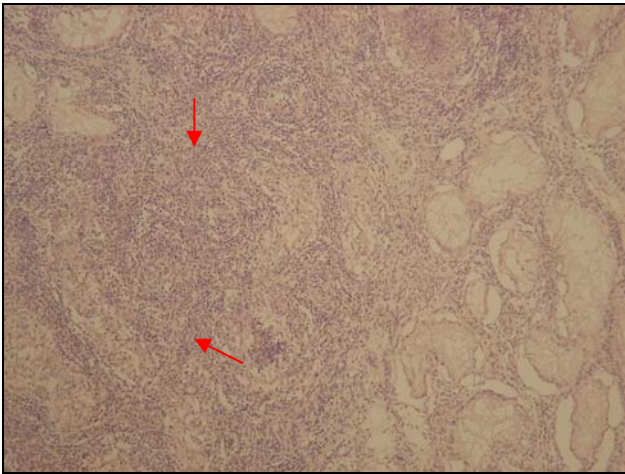


Fig. 4. Acute inflammation, proliferation of connective tissue and infiltration of lymphocytes (H&E section) X40

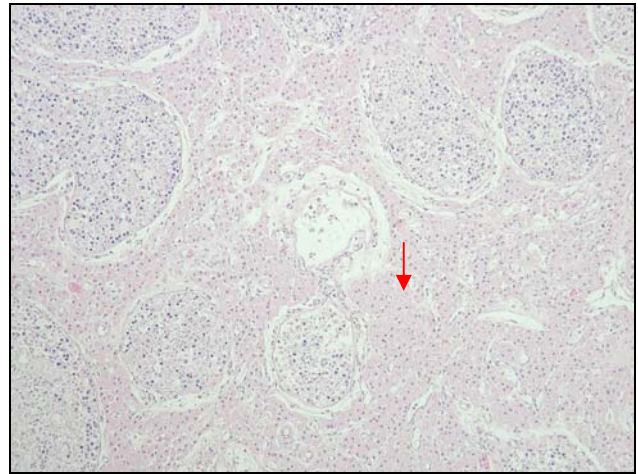


Fig. 6. Increase of Leydig cells amount in stroma of testis (H&E section) X100

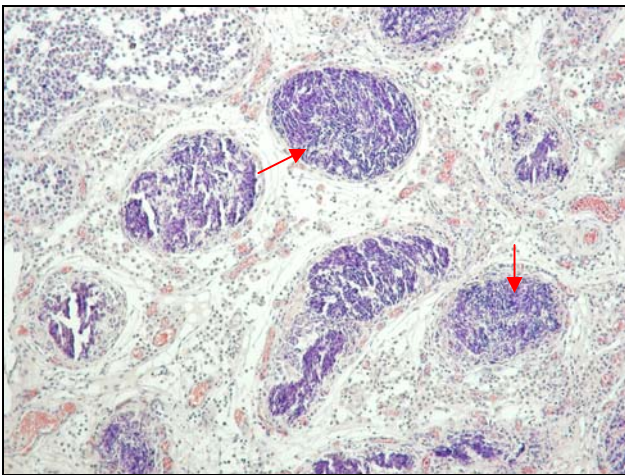


Fig. 5. Sperm stasis in tubules. Degeneration of tubules epithelium, basement membrane is thin (H&E section) X100

In the group of young boars (8-18 month of age), the most common pathologies were degeneration and edema. Calcinos of stroma, seminiferous tubules or basement membrane was estimated in testes of five boars. The study determined an equal number of inflammations, cases of fibrosis and degeneration with necrosis. Signs of low hypoplasia were found in three boars. In this group of age hyalinosis of stroma or hyperplasia of Leydig cells were not recorded, only in one animal there was a sperm stasis in seminiferous tubules.

In the second group of age (18-30 months), the most frequent testes pathologies were degeneration, which was found in all tested boars, and fibrosis of different degree, which was recorded in 8 boars. More than half of the boars in this group had interstitial inflammation of testes, only one of which was acute, and all the other were chronic. Equally often in this group occurred sperm stasis, hypoplasia and degeneration with necrosis. In a small number of boars stromal edema and hyalinosis were diagnosed. Increased amount of Leydig cells in this group of boars was not determined.

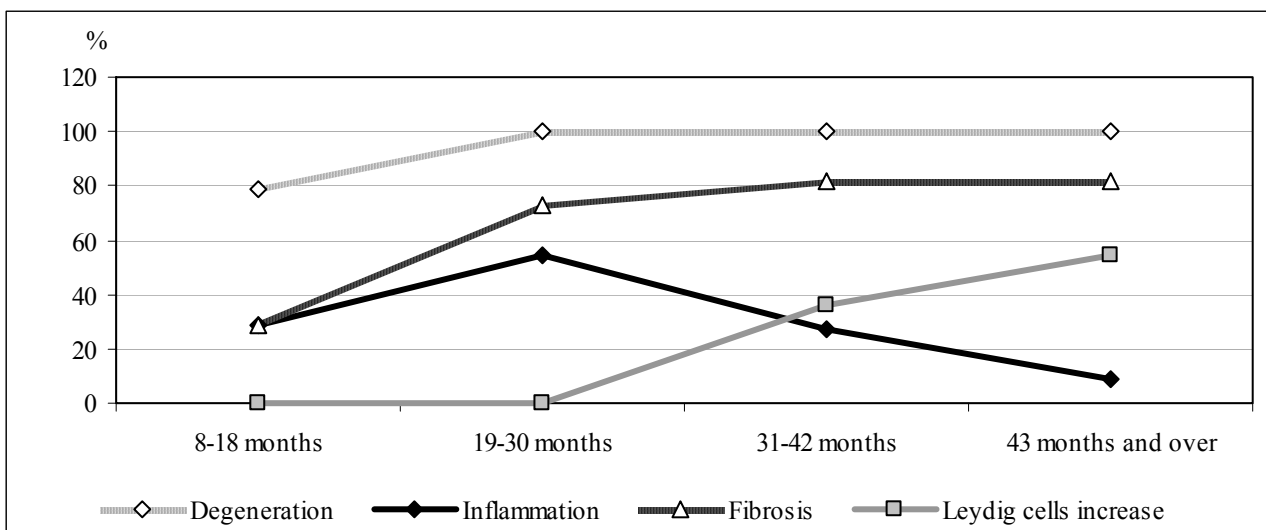


Fig. 7. Range of testes pathology in different age groups of boars

In the third group of age (30-42 months), similar to the second, the prevailing pathologies were degeneration and fibrosis. Inflammation was observed as less common and was as frequent as hypoplasia and degeneration with necrosis. In this group a significant proportion were such pathologies as hyperemia and sperm stasis in seminiferous tubules. The testes of boars showed a significant increase in the number of Leydig cells.

In the fourth group of age (42 months and older) there

were similar pathologies as in the third one. In all tested boars degeneration to a different degree, fibrosis (in 9 boars) and increase in the number of Leydig cells were diagnosed. In this age group, quite frequent cases of stromal and tubular calcinosis were found; equally often stromal edema, hyalinosis and hyperaemia were diagnosed; one case of inflammation was recorded, while sperm stasis and hypoplasia were confirmed as rare.

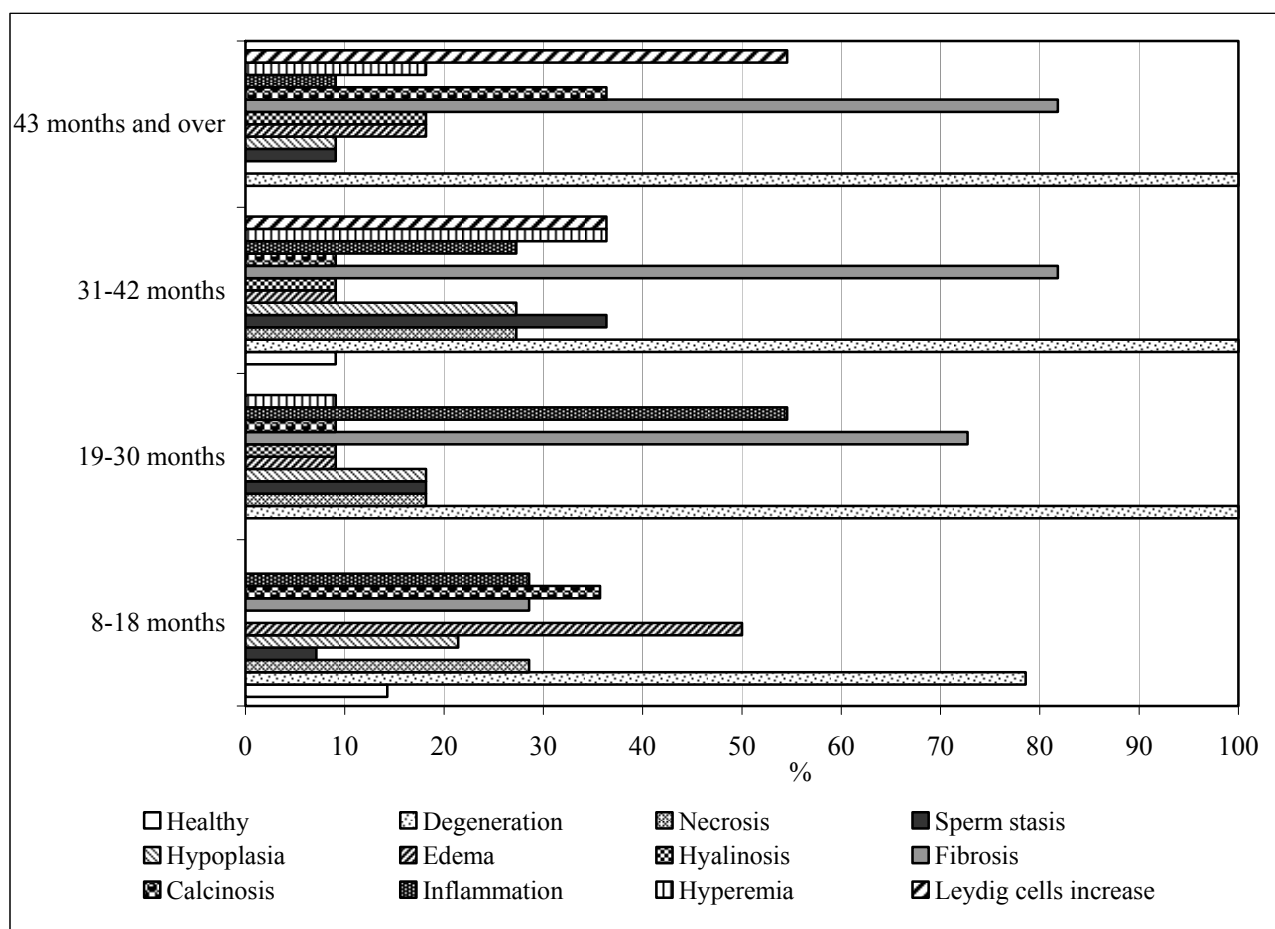


Fig. 8. Testes pathology in different age groups of boars

Table 1. Correlation of the boar's weight, testes size, ejaculate volume and pathologies

Correlations	Testes weight, g	Ejaculate volume, ml
Boar's weight, kg	0,44**	0,42**
Testes weight, g		0,40*
Factors	Influence in percent	
	On testes weight, g	On ejaculate volume, ml
Orchitis	0,7	4,1
Fibrosis	5,9 ^c	7,2 ^c
Degeneration	1,1	5,1
Orchitis * Fibrosis	0,1	7,8 ^c
Orchitis * Degeneration	11,9 ^c	2,2
Fibrosis * Degeneration	7,9 ^c	1,8
Orchitis * Fibrosis * Degeneration	16,7**	2,0

*** - $p < 0,001$; ** - $p < 0,01$; * - $p < 0,05$;

A comparison of the four age groups of boars showed that with the advancing boar age there was increase in the incidences of testes degeneration and fibrosis. The study showed that inflammation was most frequently recorded in 18-30 months of age, and decrease of inflammation with the increase of age was statistically significant ($p < 0.05$), and that inflammation was less frequently diagnosed in young boars' testes. The number of Leydig cells increased with the increase of boars' age (Fig. 8).

We evaluated how such morphological changes as fi-

bro-sis, orchitis, and degeneration were related to the volume of the ejaculate and testes weight.

Linear model was used to establish the effect of pathologies on testes size and ejaculate volume. The same model also includes evaluation of correlation of the boar's weight, testes size and ejaculate volume. This correlation is significant and the influence of pathologies is not big. It was established that a more significant influence on testes weight is that of a general pathology of Orchitis, Fibrosis and Degeneration (Table 1.).

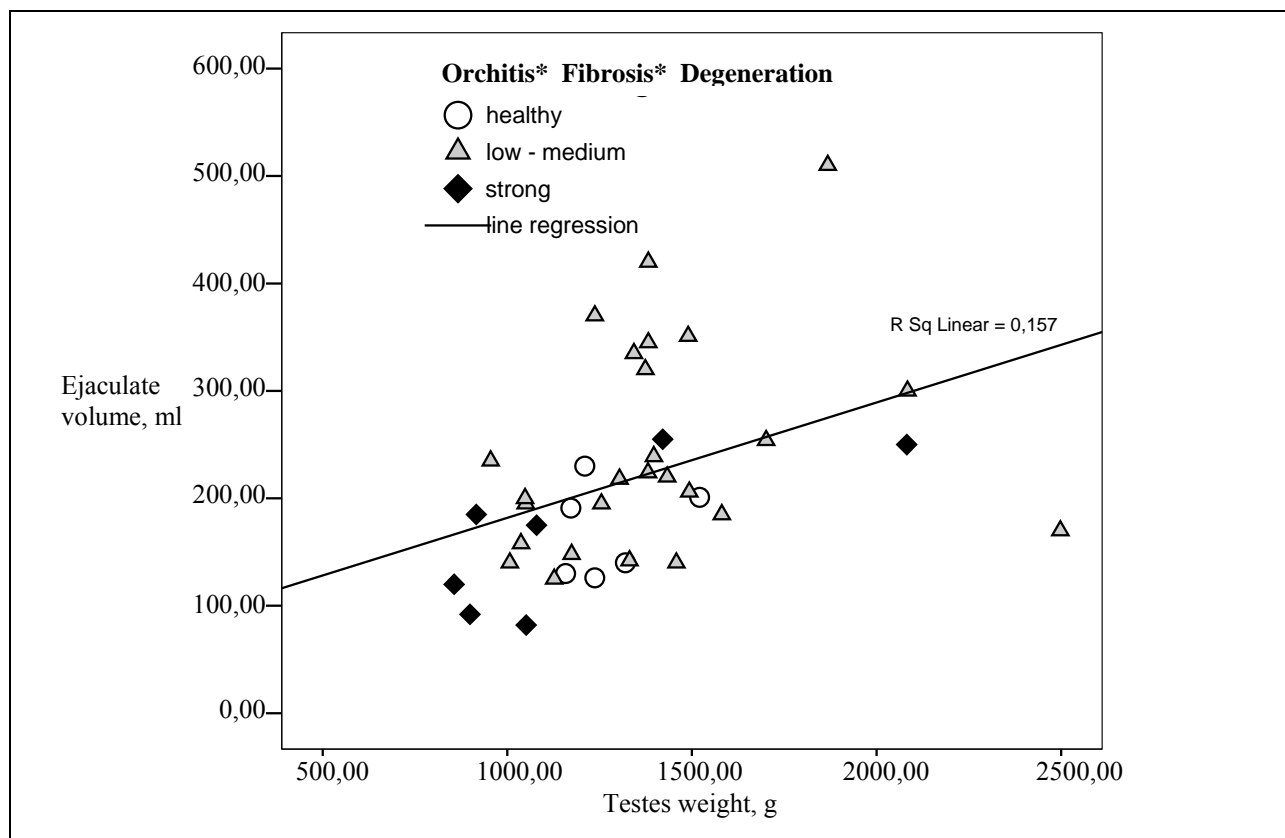


Fig. 9. Correlation of the boar's testes size, ejaculate volume and pathologies

It is statistically significant: the bigger the testes, the larger the ejaculate volume. Correlation is of average strength 0,4 ($p < 0,05$). Correlation of the three pathologies (**healthy** – were healthy in most cases or with no more than two weak pathologies; **strong** – were strong and of medium strength; all the rest were attributed to **low-medium**). It can be said that in case of strong degeneration, the ejaculate volume was measured as average even though the testes were big in size. Meanwhile, the testes of the healthy group measured from 1100 g to 1400 g (Fig. 9.).

Table 2. Linear correlation of testes size and ejaculate volume

Orchitis * Fibrosis * Degeneration	Correlations	Testes weight, g	Ejaculate volume, ml
Strong n=7	Boar's weight, kg	0,70	0,29
	Testes weight, g		0,74
Low – medium n=26	Boar's weight, kg	0,45*	0,53**
	Testes weight, g		0,26
Healthy n=6	Boar's weight, kg	0,66	0,54
	Testes weight, g		0,22

* - $p < 0,05$; ** - $p < 0,01$

A positive relationship of medium strength was established between testes size and ejaculate volume: the bigger the testes, the larger the ejaculate volume. In case of strong pathologies, testes are smaller in size (which also depends on boar's weight and age). Nevertheless, healthy animals and those diagnosed with pathologies had the same relationship between testes size and ejaculate volume. According to our data, boars with diagnosed pathologies had smaller testes than healthy animals (Table 2.).

Discussion. According to Hurtgen (1986), fertility gradually increases and gets to its peak when the boar reaches about 18 months of age and sperm production is maintained at its peak level until a gradual decline sets in at about 5 years of age. As a result of a variety of reproductive disorders and some other reasons, testes of culled boars from 8 months to 5 years of age had pathologies which occurred at different frequencies. Pathological changes in testes found in all age groups of boars could affect their fertility. It is also important to point out that testes examination confirmed not a single pathology but several simultaneous pathologies. In most cases a different degree of degeneration was diagnosed which increased at 19 - 30 months of age, although it was relatively common to culled boars of young age: 8 - 18 months old. According to Williams and Savage (1925) age impact is important in testes degeneration pathogenesis, however testes degeneration can be caused by many factors such as high or low temperature, hormonal, immunological, genetic disorders, the effect of chemical materials, influence of radiation. Degeneration resulting from deficiency of vitamin E and Se is characterized by nuclear homolysis and extensive giant cell formation, poor spermatozoa integrity, sperm with lower motility (Marin-Guzman, 2000). Mycotoxin zearalenone causes the most extensive damage to animal reproductive system as an estrogen antagonist. Fusariotoxicoeses are a major problem in Lithuania (Bakutis, 2002). However, Sutkevičienė et al. (2009) determined that zearalenone at amounts 1 ppm in field exposures for two months did not negatively affect the reproductive potential of mature boars. Occurrence of degeneration is often associated with increasing temperature in case of cryptorchidism. One such case was diagnosed in our investigation, when unilateral cryptorchidism was observed and testicular degeneration of high degree with necrosis and hypoplasia were established. There are many various reasons for temperature changes. The most common of them could be heat during summer time, inflammation process, contusions, decreased level of androgenic hormones and others (Stone, 1982; Tenover, 1997). Hypospermatogenesis, formation of multinucleated giant cells, abundant germ cell depletion and death were observed in boars affected with porcine reproductive and respiratory syndrome (PRRS) (Sur et al., 1997). As several factors affect an individual simultaneously, this could lead to occurrence of degeneration processes in tested boar's testes.

Sertoli cell proliferation was observed in some boars in the background of degenerative changes. Total number of Sertoli cells determines the size of mature testis in males of various mammalian species (Amann, 1970). The

regulatory mechanisms of Sertoli cell proliferation are not yet established. FSH is the major factor responsible for postnatal Sertoli cell proliferation (Heckert et al., 2002). According to Sharpe et al. (2000), Franca et al. (2005), the size of testis and the number of spermatozoa are directly dependent on the number of Sertoli cells per testis and that Sertoli cells contribute to the process of spermatogonial stem cell renewal (McLean, 2005).

The occurrence of degenerative necrotizing and inflammatory processes in the testical evoked the formation of fibrous tissue. Fibrosis characterized by proliferation of connective tissue, which breaks seminiferous tubules into small segments or grows through the entire tubule(s), presses them thus resulting in tubular atrophy (Fig. 10). Some authors consider that fibrosis may also be of insidious or spontaneous onset. The degree of fibrosis varied from a mild local to extreme fibrosis where the testis was hard and smaller grossly visible increasing of stroma.

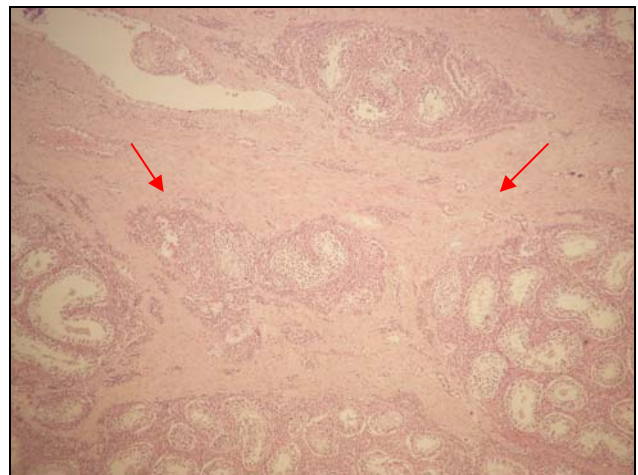


Fig. 10. **Fibrosis. Seminiferous tubules broken into small segments or grown through** (H&E section) X40

Tubular, stromal and basement membrane calcinosis was found in 23.40 % of all boars. Calcinosis usually develops in dead tissue or cells; therefore testicular calcinosis usually accompanies degeneration, sperm stasis, inflammation and fibrosis. Such changes of reproductive organs were recorded in our investigation in many cases.

Our investigation results represent direct correlation between age and the amount of Leydig cells. The greatest amount of Leydig cells in the testes were in the oldest group of the tested boars. According to Goyal and Dingra (1973), the number of Leydig cells increases with age in buffalo testis.

Leydig cells produce testosterone and pheromone which are responsible for male sexuality and secondary male sex characteristics and for the function of accessory glands (Dellmann and Eurell, 1998; Hafez, 2000). Gofur et al., (2008) determined that the number of Leydig cells varied in the testes of bulls of different age groups and even in between left and right testes of the same animal, and that the amount of these cells increases up to puberty and then decreases with the advancement of age associated with an increase of connective tissue fibers in the

intertubular space.

Testes hypoplasia is not a common pathology. Our research showed that from the total number of 47 tested boars, 8 cases with morphological features of focal hypoplasia were established. Since the study included only breeding boars, whose selection was held constant, this result does not show prevalence of hypoplasia among boars in general. Holst's (1949) data were comparable to our results. He diagnosed testes hypoplasia in 8 cases from investigated 30 boars, which all had testes pathology.

Testicular hypoplasia has been reported in boars (Hancock and Darker, 1981) and shows altered spermatogenic activity, decreased fertility or total infertility in bilaterally affected animals, but normal fertility - in unilaterally affected animals.

In our study histological changes in the left and right testes were mostly unequal, which was also proved by other studies (Šernienė et al., 2005). These authors found that the main factor contributing to the culling of boars because of sperm and testes pathology was a single boar affected by various diseases, genetic disorders or other internal factors. Intensive use of boars as semen donors may influence the occurrence of various diseases. Developed testicular fibrosis was of major importance in decrease of ejaculate volume. And this is only natural, because the development of fibrosis affects the body atrophy in which the body usually decreases in its volume and weight, as well as decreases the tubular volume or even their content. Thus, the most common testes pathology for culling breeding boars is degeneration, while the fibrosis of testes was most important for the changes of quantity of the sperm.

Conclusions. The results from performed study demonstrated that for evaluation of the changes in testes tissue of boars it is profitable to rely not only on the exterior characteristics, but to perform morphological examination. Our study showed that the fluctuation of ejaculate volume in boars can indicate pathological changes in testes.

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