RELATIONSHIP BETWEEN SPERM QUALITY AND TESTICULAR LESIONS IN CULLED AI BOARS

Kęstutis Mažeika[1], Neringa Sutkevičienė[2], Henrikas Žilinskas[2], Vita Riškevičienė[1], Albina Aniulienė[1], Nomeda Juodžiukynienė[1]
[1]*Department of Infectious Diseases*
[2]*Department of Infectious Disease, Veterinary Academy, Lithuanian University of Health Sciences*
Tilzes Str. 18, LT-47181, Kaunas; Phone: +370 37 36 28 81; E-mail: mazeika@lva.lt;

Abstract. This study was carried out to assess the relationship between sperm defects and histomorphological changes in the testes of culled AI boars. A total of 23 culled for reproductive disturbances mature boars were included in the analysis. The mean age of the animals was 34.9±10.7 months. The semen was collected once just before the slaughter. Boars were assigned to five groups according to the percentage of total sperm defects in the ejaculate: group I – ≤10 %; group II – 11–20 %; group III – 21–30 %; group IV – 31–50 % and group V – ≥ 50 %. Testicular histomorphological lesions were classified as follows: mild degeneration (DEG1), moderate degeneration (DEG2) and severe degeneration (DEG3), inflammation/orhitis (INFL), fibrosis and atrophia (FIBR). Our results showed the relationship between sperm defects and the major pathologic involvements of the testis: severe degeneration correlated negatively with percentage of motile spermatozoa ($r=-0.50$; $P<0.05$), the total number of normal spermatozoa ($r=-0.67$; $P<0.001$) and positively correlated with the number of spermatozoa presenting simple bent tail ($r=0.68$; $P<0.001$). The results of our study showed the correlation between testis histomorphology and sperm count, morphology and motility of AI boars.

Keywords: boar, testis, sperm quality, degeneration.

BROKUOTŲ VEISLINIŲ KUILIŲ SPERMOS KOKYBĖS IR SĖKLIDŽIŲ PAKITIMŲ TARPUSAVIO RYŠYS

Kęstutis Mažeika[1], Neringa Sutkevičienė[2], Henrikas Žilinskas[2], Vita Riškevičienė[1], Albina Aniulienė[1], Nomeda Juodžiukynienė[1]
[1] *Užkrečiamųjų ligų katedra*
[2]*Neužkrečiamųjų ligų katedra, Veterinarijos akademija, Lietuvos sveikatos mokslų universitetas*
Tilžės g. 18, LT-47181, Kaunas; tel.: +370 37 36 28 81; el. paštas: mazeika@lva.lt

Santrauka. Tyrimas atliktas norint įvertinti brokuotų veislinių kuilių spermatozoidų defektų ir histomorfologinių sėklidžių pokyčių tarpusavio ryšį.

Analizei panaudoti 23 išbrokuoti ir dėl reprodukcijos sutrikimų paskersti veisliniai kuiliai. Gyvūnų amžiaus vidurkis – 34,9±10,7 mėn. Sperma rinkta kartą prieš skerdžiant. Kuiliai suskirstyti į penkias grupes pagal bendrą patologinių spermatozoidų kiekį ejakuliate: I grupė – ≤10 proc., II grupė – 11–20 proc., III grupė – 21–30 proc., IV grupė – 31– 50 proc. ir V grupė – ≥50 proc. Sėklidžių histomorfologiniai pokyčiai buvo šie: silpna degeneracija (DEG1), vidutinio stiprumo degeneracija (DEG2), stipri degeneracija (DEG3), uždegimas/orhitis (INFL), fibrozė ir atrofija (FIBR). Mūsų tyrimo rezultatai parodė, kad spermatozoidų defektai susiję su histomorfologiniais pokyčiais sėklidėse: sunkaus laipsnio degeneracija sėklidėse neigiamai koreliavo su judrių spermatozoidų procentu ($r=-0,50$; $p<0,05$), su bendru normalių spermatozoidų skaičiumi ejakuliate ($r=-0,67$; $p<0,001$) ir teigiamai koreliavo su spermatozoidais paprastai susisukusiomis uodegėlėmis ($r=0,68$; $p<0,001$). Mūsų tyrimo rezultatai parodė, kad veislei naudojamų kuilių sėklidžių histomorfologiniai pakitimai koreliuoja su spermatozoidų morfologija, judrumu ir bendru spermatozoidų kiekiu.

Raktažodžiai: kuilys, sėklidės, spermos kokybė, degeneracija.

Introduction. The quality of spermatozoa is associated with genetic determinant, physiological characteristics, and morphological organization of testes. The sperm cell attains its distinct morphological characteristics in the process of spermiogenesis, which comprises the final phase of spermatogenesis. Any defect in spermiogenesis may lead to abnormalities in the morphology of mature testicular spermatozoa (Yavetz et al., 2001, Chemes and Rawe, 2003). Sperm with intrinsic defects may appear morphologicaly normal upon microscopic evaluation, but in fact, they may have intracellular defects which determine the decreased fertilizing capacity. Histomorphological investigation of testicles monitored in boars culled due to poor reproductive performance directly reflects testicular function. The most common pathologic involvements of the boar‘s testis are hypoplasia, orchitis, fibrosis and degeneration. Inherited and congenital hypoplasia of the seminiferous tubules and degeneration are the two most common lesions which negatively affect spermatogenesis and sperm quality (Foster, 2007).

There is little literature data available about testicular lesions in the AI boars and their relationship with sperm quality parameters. The objective of the present study was

to evaluate the quality of AI boar semen and determine the relationship between sperm quality parameters and testicular histomorphological changes.

Matherial and methods. *Animals:* a total of 23 mature AI boars were recruted for the experiment. The mean age of animals was 34.9±10.7 months. All boars were kept in individual stalls. Water was available ad libitum. All boars were fed the same approved AI boars diet (Jančienė, 2005). All animals enrolled in the present study were culled due to deterioration of the sperm quality and impaired fertility. All boars were assigned to five groups based on the number of pathological spermatozoa in ejaculate: group I (≤10 % total sperm defects in the ejaculate), group II (11–20 % total sperm defects in the ejaculate), group III (21–30 % total sperm defects in the ejaculate), group IV (31–50 % total sperm defects in the ejaculate), group V (≥50 % total sperm defects in the ejaculate).

Semen analysis: ejaculates were collected by the gloved-hand technique. The volume of ejaculate (ml) was recorded by weighting and then semen was extended with the BTS/Androhep (v/v) extender at 37 ^{o}C. Extended semen was used for analysis. Motility of spermatozoa was examined immediately after collection at 37 ^{o}C under phase-contrast microscope Olympus BH2 with a prewarmed 37 ^{o}C stage (Olympus Optical Co., Ltd., Japan) at a ×100 magnification. Motility was analyzed on 5-µl aliquots of fresh semen and was rounded to the closest % of motile spermatozoa. Motility assessment was done by the same person.

Semen aliquots were fixed in buffered formol-saline solution (Hancock, 1956) and used thereinafter for sperm morphological analysis. Sperm tail defects (proximal and distal cytoplasmic droplets, loose heads, acrosome defects, pouch formations, abnormal midpieces and the incidences of tail abnormalities) were determined under the phase-contrast microscope at ×400 magnification. Sperm head defects (pear shape, narrow at base, abnormal countour, undeveloped, loose abnormal head, narrow, big, little normal, short – broad) were determined in dry preparations stained with carbol-fuschin eosin stains as defined by Williams (Williams and Savage, 1925). Sperm concentration was measured in a Goriajev cell counting chamber as described (Pakėnas, 1985).

Testicular morphological analysis: the testes with epididymis were examined macroscopicaly after slaughter, their form and position, colour and consistency of sectional view were estimated. Testicular tissues were immediately dissected into blocks of approximately 1 × 1 × 0.5 cm^3 from the centre of testis and fixed in Bouin´s solution for 24 hours and thereinafter transferred into 70 % ethanol. The samples were then processed and embedded in paraffin. Sections (3–5 µm) were cut and stained with Hematoxylin and Eosin (H&E) on glass sliders according to the routine histological procedure (Laurusevičienė and Smaliukienė, 2007) and evaluated under the light microscopy.

Statistical analysis: statistical analysis was performed using the *SPSS* statistical package (SPSS 9.0 for Windows, SPSS Inc., Chicago, IL, USA, 1989–1995). Data included in the model was analyzed using descriptive statistics (means ±SD). Differences among investigated groups were analyzed by LSD method (α=5 %). The data was considered to be statistically significant when P<0.05. The Spearman rank correlations were used to calculate the relationship between sperm quality parameters and testicular histomorphological lesions.

Results. The volume of ejaculate, sperm concentration and percentage of motile spermatozoa in different boar groups are presented in Table 1.

Table 1. **The volume of ejaculate, sperm concentration and percentage of motile spermatozoa in boar groups** (n=23)

Group	Volume of ejaculate, ml	Sperm concentration, ×10^6 ml	Sperm motility, %	n
I (≤10) a	157.7±33.6	0.6±0.3	71.7±2.9 b, c, d, e	3
II (11–20) b	152.7±87.1	0.6±0.2	66.7±2.9 a, c, d, e	3
III (21–30) c	271.7±90.9	0.6±0.11	61.7±2.9 a, b, d, e	3
IV (31–50) d	189.0±47.8	0.6±0.2	55.0±0.0 a, b, c, e	7
V (≥51) e	207.4±85.6	0.5±0.2	37.1±3.9 a, b, c, d,	7

$^{a, b, c, d, e}$ Means with different superscript in rows are significantly different (P<0.05). Data presented as mean±SD

The results showed significant negative correlation between the percentage of total sperm defects in ejaculate and sperm motility (r=-0.50; P<0.05), but not with the volume of ejaculate (r=0.10; P>0.05) and sperm concentration (r=0.29; P>0.05). The highest percentage of motile spermatozoa (71.7±2.9 %) was found in group I and the lowest motility was (37.1±3.9 %) associated with the highest incidence of abnormal spermatozoa (group V) (Table 1). Percentage of motile spermatozoa negatively correlated with spermatozoa with simple bent tails (r=-0.47; P<0.05) and spermatozoa with tails coiled under the head (r=-0.50; P<0.05).

Testicular morphological lesions were the following: degeneration (DEG), inflammation/orhitis (INFL) and fibrosis/atrophia (FIBR). The degeneration was the most frequent diagnosed pathology whithe the incidence of 65.2 %. Degenerative changes were classified as mild (DEG1), moderate (DEG2) and severe (DEG3), diagnosed respectively in 13.0 %, 26.1 % and 26.1 % of all tested boars. The incidences of testicular lesions and distribution among different groups of boars are presented in Fig. 1.

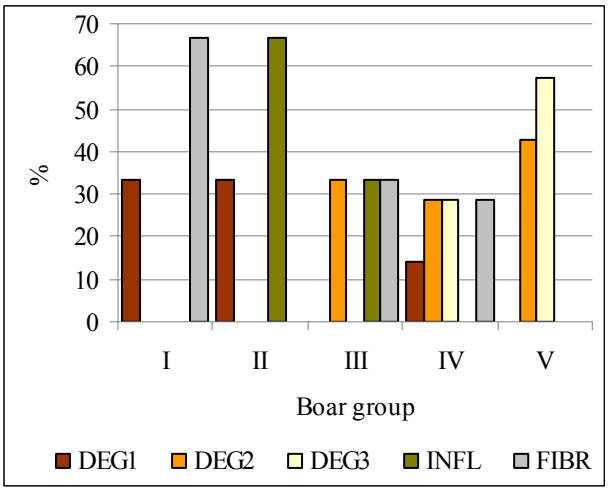

Fig. 1. **The incidences of testis lesions and repartition in different groups of boars**

The most common sperm defects in all boar groups were spermatozoa with proximal and distal cytoplasmic droplets and spermatozoa with simple bent tails. Spermatozoa with tails coiled under the head correlated with spermatozoa with simple bent tails ($r=0.69$; $P<0.001$), spermatozoa with big head correlated with loose abnormal heads ($r=0.55$; $P<0.01$). The volume of ejaculate correlated with loose normal heads ($r=-0.43$; $P<0.01$).

The total number of sperm defects in the first boar group accounted for 7.5±2.8 %. The prevailing sperm patologies in this group were spermatozoa with proximal (2.2±1.0 %) and distal droplets (1.3±1.3 %) and spermatozoa with simple bent tail (2.5±2.0 %). Mild testicular degeneration (DEG1) was diagnosed in 33.3 % of boars in I group. For the mild degeneration, morphological evidence such as depletion of spermatogenic epithelium and vacuolization in several tubules, nuclear picnosis, mononuclear or multinuclear giant cells, and roughness of the basement membranes were attributed (Fig. 2). Fibrosis/atrophia has been also diagnosed in 66.7 % of the boars of this group. Fibrosis is characterized by proliferation of connective tissue which breaks seminiferous tubules into small segments or grows out of all tubules, press them and cause tubule atrophy.

In the second boar group, the total sperm defects accounted for 16.5±3.1 %. The sperm pathologies recorded in this animal group were as follows: spermatozoa with distal droplets (5.5±4.1 %), spermatozoa with simple bent tail (5.3±3.7 %) and spermatozoa bearing pearshaped heads (1.7±2.9 %). Mild testicular degeneration (DEG1) was detected in 33.3 % of the animals, and interstitial inflammation was recorded in 66.7 % of the testes of boars in II group. Acute inflammation was assessed in the boars of this group. The percentage of abnormal loose heads correlated with inflammation in the testes ($r=0.85$, $P<0.001$). The percentage of pearshaped spermatozoa correlated with the incidences of mild testicular degeneration ($r=0.51$, $P<0.05$).

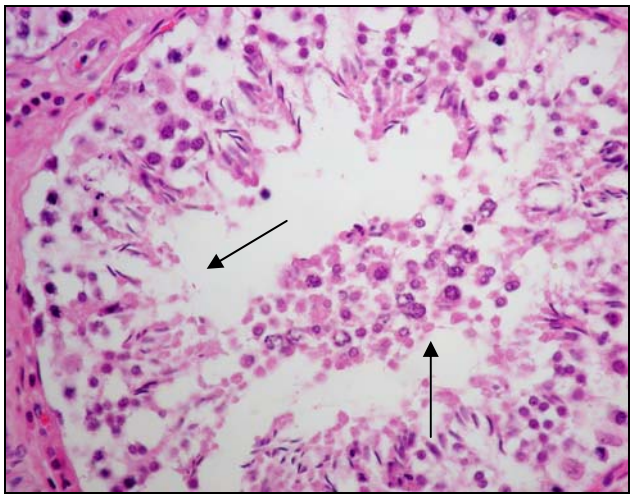

Fig. 2. **Mild degeneration. Weak tubules vacuolization. Appearance of mononuclear or multinuclear giant cells** (H&E section, 400× magnification)

The percentage of total sperm defects in the IIIrd boar group was 23.5±1.8 %. Most of the defects were spermatozoa with simple bent tails (9.5±7.8 %) and spermatozoa bearing proximal cytoplasmatic droplets (9.0±7.0 %). Moderate degree of degeneration, fibrosis and inflammation were diagnosed in 33.3 %, 33.3 % and 33.3 % of boar testes of this group. In the case of moderate degeneration – more than half of seminiferous tubules were affected. Spermatogenic epithelium was vacuolated, spermatozoa – still immature, there were more giant cells, and tubular basement membrane was thick and wavy (Fig. 3).

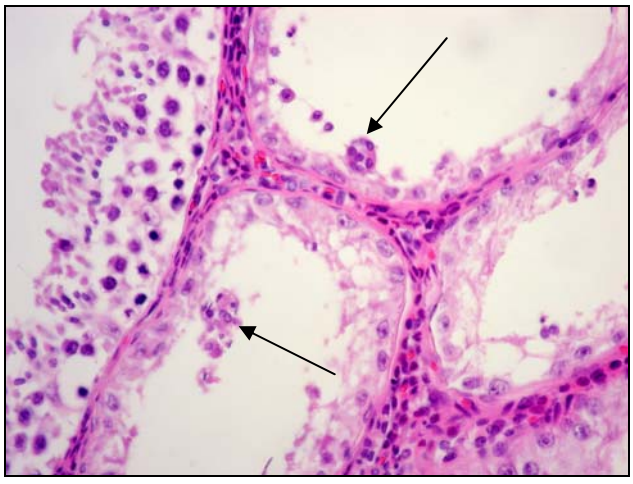

Fig. 3. **Moderate degeneration. Spermatogenic epithelium vacuolated, Giant cells in tubule, nuclear picnosis** (H&E section, 400× magnification)

Animals assigned to the group IV presented 35.6±3.9 % of sperm abnormalities. The mean % of proximal droplets was 11.6±10.9 %, simple bent tails – 10.4±11.2 %. There were also found spermatozoa with loose abnormal heads (4.3±9.3 %), spermatozoa with

abnormaly big heads (0.5±0.4 %), and spermatozoa with abnormal head contour (0.8±0.9 %). Mild, moderate and severe testicular degeneration was diagnosed in 71.4 % animals and fibrosis – in 28.6 % of the testes of boars of this group. The presence of severe degeneration correlated negatively with percentage of motile spermatozoa (r=-0.50; P<0.05), the total number of normal spermatozoa (r=-0.67; P<0.001), and positively with the number of spermatozoa with simple bent tails (r=0.68; P<0.001).

The percentage of total sperm defects found in group V was 74.0±15.3 %. The highest incidences of sperm pathologies were spermatozoa with distal droplets (23.6±21.98 %), spermatozoa with simple bent tail – 35.3±23.1 %, spermatozoa with proximal droplets – 6.6±5.2 % and spermatozoa with tails coiled under the head – 5.5±5.0 %. Pearshaped head spermatozoa were present at 1.0±1.4 % and spermatozoa with abnormal head contour at 0.4±0.8 %. Mild, moderate and severe testicular degeneration was diagnosed in 100 % of the testicles of boars of group V. In the case of severe degeneration, Sertoli cells and spermatogonia were found in the seminiferous tubules only. The latter were vacuolated, their nuclei were picnotic, giant cells were present in large numbers too. The basement membrane of tubules was found thick, wavy and the collapsed (Fig. 4).

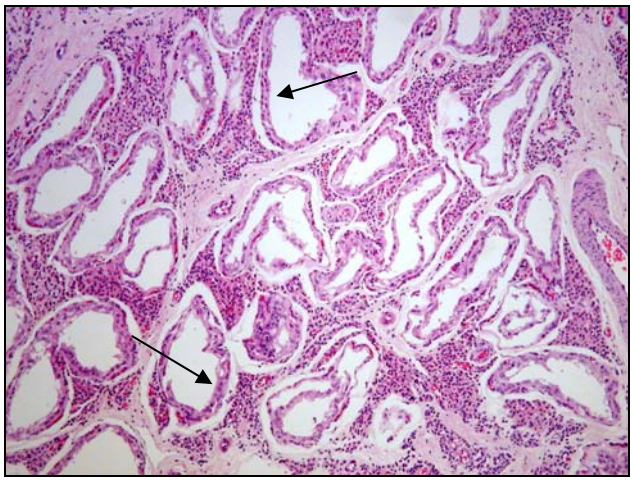

Fig. 4. **Severe degeneration. Empty seminiferous tubules, azospermia** (H&E section, 100× magnification)

Discussion. Testicular function is regularily monitored in males selected for artificial insemination. Assessment of testicular function in males selected for AI is mainly based on total sperm count in the ejaculate, % of motile spermatozoa and sperm morphology. Fertility results indirectly reflect the testicular function (Andersson and Makinen, 1999).

AI boars used in our investigation were culled due to deterioration of the sperm quality and impaired fertility. In order to evaluate the relationship between the pathomorphological changes in the testes of the culled AI boars and the concentration of spermatozaoa, their motility and morphological changes, we investigated the testis and semen of the boars. All tested boars were assigned to five groups according to percentage of total sperm defects in the ejaculate in this study.

Sperm motility is an important characteristic in porcine semen assessment (Britt et al., 1999; Vyt et al., 2004). Many factors are necessary to produce normal sperm motility, not only optimal temperature and pH, but also appropriate levels of reactive oxygen species and adenosine triphosphate (ATP) (Okamura et al., 2009). The normal sperm tail morphology is also necessary for the motion of spermatozoa. Therefore the structural sperm tail abnormalities are responsible for altered/absent sperm motility (Francavilla et al., 2007). Spermatozoa gain motility during ejaculation as pH and bicarbonate concentration increase during mixing of sperm and seminal plasma (Rodríguez-Martínez et al., 1990). Our study showed significant negative correlation (r=-0.50; P<0.05) between the total number of abnormal spermatozoa and sperm motility. These results are in agreement with earlier reports (Jasko et al., 1992; Juonala et al., 1998; Juonala et al., 1999; Kondracki et al., 2006).

The cytoplasmic droplets originate from separation of the sperm cell from Sertoli cell. During the separation process, some of the cytoplasm naturaly remains attached to the sperm cell. As the sperm cell is moved along the seminiferous tubule and into the epididymis, the droplet moves from the proximal position further down the tail and then falls off. In some cases the ejaculate contains higher amounts of these droplets (Cooper, 2005). Infertility characterized by reduced pregnancy rate and litter size is associated with retention of the distal and proximal droplets in boars (Waberski et al., 1994). Retained droplets located proximally or distally also hinder binding to the uterine epithelium (Petrunkina et al., 2001; Kondracki et al., 2006). In our study the, highest incidences of spermatozoa with distal droplets (23.6±21.98 %) was found in V^{th} boar group, where mild, moderate and severe testicular degeneration was diagnosed in 100 % of the testicles of boars of this group. Okamura‘s studies (2009) with rats showed that the increased percentages of broken sperm, inreased the percentage of cytoplasmic droplets, and the percentage with coiled tails is associated with decreased sperm motility, and it might be caused by impaired function of the caput epididymis as suggested by the histopathological alterations in the epithelian cells of the ductus epididymis. However in our study we don‘t study the status of epididymidis.

Little is known about how defective sperms are eliminated during mammalian spermatogenesis. Sutovsky et al. (2001), Baska et al. (2008) described as ubiquitin-dependent sperm quality control mechanism that resides in the mammalian epididymis. If the sperm ubiquitination process is deficient, defective sperm is not phagocytosed by the epididymal epithelial cells and degenerated spermatozoa pass to the ejaculate.

Orchitis is a common clinical diagnosis, but it is often a misleading diagnosis because severe inflammation of the testis is rare (Foster, 2007). There is clinical and pathological evidence that chronic inflammatory conditions of the testis can disrupt spermatogenesis and irreversibly alter sperm number, quality and motility. In

the majority of boars included into the present study, the inflammation was asymptomatic. Only systematic histopathological examination of testis from boars with reproductive disturbances indicated a high (33.3 % to 57.1 %) prevalence of inflammatory reactions. Interstitial inflammation and fibrosis were mostly frequantly diagnosed lesions. Inflammation was characterized by proliferation of connective tissue and infiltration of lymphocytes. A characteristic pattern of inflammatory lesions with focal or multifocal, predominantly peritubular lymphocyte infiltration and concomitant damage of seminiferous tubules is seen in chronic orchitis of various origins. The pattern of lymphocyte infiltration and concomitant damage of seminiferous tubules supports the concept that activation of autoreactive T cells is involved (Schuppe and Meinhardt, 2005). This supports the concept that induction of testicular inflammation is associated with a T-cell-mediated autoimmune response, i.e. disruption of the immune privilege – it could be the reason for the lack of a fully functional blood-testis barrier at the start of spermathogenesis, which may lead to damage of the developing germ sells. Moreover, despite the patchy distribution of the lesions, spermatogenesis may be significantly reduced (Schuppe et al., 2008). For this reason inflammation causes spermatogenic dysfunction, i.e. decreased sperm counts, motility and infertility (Kawakami et al., 2004; Tanıdır et al., 2008).

Moderate and severe degeneration was diagnosed in 100 % testicles of boars of group V in our study. Earlier studies also have showed that the most common testes pathology for breeding boars was the degeneration (Mažeika et al., 2011). Vacuolated with picnotic nuclei Sertoli cells and spermatogonia were found in the seminiferous tubules. Upon collapse of the tubules, their basement membrane was thick and wavy. The degenerative processes in the seminiferous tubules decrease the total number of spermatozoa and lead to the formation of pathological and atypical spermatozoa (Mamina and Zhigal'skii, 2006). Severe degeneration reduces the amount germinal epithelium and eventually normal spermatogenesis stops (Foster, 2007). Negative effect of the testicular degeneration was seen also in the semen picture in the form of increased frequency of sperm abnormalities (74.0±15.3 %) and decreased sperm motility (37.1±3.9 %). Our study showed that incidences of severe degeneration correlated negatively with percentage of motile spermatozoa ($r=-0.50$; $P<0.05$), with the total number of normal spermatozoa ($r=-0.67$; $P<0.001$) and positively with the number of spermatozoa with simple bent tail ($r=0.68$; $P<0.001$).

The results of our study show the correlation between testis histomorphology and sperm count, abnormal morphology and motility parameters of AI boars. The degree of testis degeneration were associated with the number of pathological spermatozoa in the ejaculate.

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