CORRELATION OF HISTOMORPHOMETRICAL PARAMETERS IN RAM TESTES

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Abstract. A lot of research work has been carried out on the spermatogenic process of animals, however, few data on testis morphometry in rams are available in literature. The aim of the present study was to determine histomorphometrical parameters and to analyse the correlation between the parameters in Lithuanian local coarse wool ram testes. Testis of Lithuanian local coarse wool rams (n=12) aged 13 months and weighing 54.50 ± 1,504 kg were investigated. All the animals under investigation were kept in the same conditions. The specimens of testes were selected after slaughter of rams. Bouin´s solution was used as fixative for 24 hours. Paraffin blocks were cut into 4 µm thick sections and were stained with HæE. The morphometrical analysis was carried out during which the diameter of tubules, the height of the seminiferous epithelium, spermatogenic index (SI) developed by Grocock and Clarke (1974), the number of Leydig cells and the ductal epithelium height of the epididymis were measured It was established in the present study that the number of Leydig cells correlated significantly positively with the diameter of testicular tubules and spermatogenesis index ($P<0.05$). The germinal epithelium height of testes revealed the negative correlation with the epididymal height of the epithelium ($P<0.01$) and positive correlation with spermatogenesis index ($P<0.001$). The epididymal height of the epithelium negatively correlated with spermatogenesis index ($P<0.001$). The number of the degenerated tubules negatively correlated with the number of Leydig cells, the diameter of testicular tubules ($P<0.05$), the germinal epithelium height of testes ($P<0.001$) and spermatogenesis index ($P<0.01$), and correlated positively with the epididymal height of the epithelium ($P<0.01$). Diameter of testicular tubules, germinal epithelium height and spermatogenesis index and number of Leydig cells have positive correlations.

Keywords: ram, testes, histopathology, histomorphometry

Introduction. The ram is economically a very important domestic animal. Only few data are available in literature on testis morphometry of this species. Reproductive organs are the most dynamic organs in all animals. Photoperiod and other environmental factors are among many factors affecting reproduction and fertility (Dorostghoal et al. 2009, Young et al. 2000). In such animals with seasonal reproduction as the ram, testicular size, testosterone secretion, sperm production and reproductive behaviour decrease during the non-breeding season; however, the effects of photoperiod on the reproductive activity can be modified to a certain extent by temperature, nutrition, body condition or age of the animal (Gerlach et al. 2000). Spermatogenic efficiency highly correlated with seminiferous tubules volume density, the number of Sertoli cells per testis gram, and the spermatogenic cycle length (Sharpe, 1994, Russell, 1996, Neves, 2001, Leal et al. 2004).

The ratio between the tubular and interstitial compartments varies considerably between species, being responsible for the difference in the efficiency for sperm production. Seminiferous tubules are the main compartment of the testis and accounts for from 70% to 90% of testis parenchyma in most mammals (Russell, 1996, Franca et al. 1998, Hess et al. 2008,). Histological quantification of the testicular parenchyma is the key requirement for studies involving male reproductive parameters (Paula et al. 2002). The average thickness of the seminiferous epithelium in domestic animals is about 60-100 µm (Hess et al. 2008). Leydig cells are of great importance for the interstitial compartment. Besides producing testosterone, Leydig cells secrete other steroids and pheromones that are important to other reproductive functions such as sexual behaviour and maintenance of sexual accessory gland function (Leal et al. 2004).

The qualitative and quantitative study of the spermatogenic process is essential to the understanding of physiological patterns with the help of which parameters for reproductive biology can be established (Morrow et al. 1998, Azvedo et al. 2010, Costa et al. 2011). The Lithuanian local coarse ram is one of the most important native breeds in Lithuania; however, its reproductive organs have not been thoroughly investigated. Therefore, the aim of the present study was to determine histomorphometrical parameters and to analyse the correlation between the parameters in the testes of the Lithuanian local coarse wool ram.

Materials and methods

In the present work the testis of Lithuanian local coarse wool rams (n=12), aged 13 months, 54.50 ± 1,504 kg in weight, were investigated in 2017. The animals were kept in the same conditions at the LUHS Animal Husbandry Institute. All rams had at libitum access to the basal diet. In summer they received grass from grasslands and pasture grass, in winter they were given hay. Additionally the rams were given combined feed. Combined feed was prepared in the LUHS GI. Analytical data /kg feed: dry matter - 872 g, metabolizable energy - 10.1 MJ, crude protein – 160

g, fibre – 93.5 g, calcium 11.7 g, phosphorus – 7.0 g. In summer the animals were sheltered from rain, raw weather and the sun. During winter, the rams were kept in cotes.

The specimens of testes were selected after slaughtering the rams. Bouin´s solution was used as fixative for 24 hours. Specimens were prepared for a histological and histomorphometrical examination using the tissue processor (Shandon Pathcentre, UK, 2004). Paraffin blocks were made with the help of the paraffin block embedding centre ("Tess 99" Medite, USA). The paraffin blocks were cut into 4 µm thick sections by means of the semiautomatic microtome Sukura Accu-Cut SRM". Every specimen of the testes was stained with haematoxylin−eosin (HE). The histological and histomorphometric analyses were performed using the Olympus microscope supplied with the digital Olympus DP72 image camera with CellSensDimension software.

The diameter of 200 tubules was measured in the right and left testis in each animal. The diameter of the round seminiferous tubule was measured across the minor and major axes, and the mean diameter was obtained. The height of the seminiferous epithelium was measured at x400 magnifications in the same section in which the tubule diameter was calculated. Testis tubules were evaluated for their spermatogenic index (SI) by Grocock and Clarke (1974) score. The scores assigned ranged from 1-5. The value of "5"was given to the tubules displaying complete spermatogenesis; score 1 was assigned to the tubules that contained only primary Sertoly cells, spermatogonia, and primary spermatocytes. The number of Leydig cells was measured at 40 fields x400 in the area of 160x220 µm. The ductal epithelium height of 50 tubules was measured in the epididymis.

The statistical analysis was performed using the SPSS program 20.0 for Windows. Experimental data were normally distributed (Kolmogorov-Smirnov test). The results are presented as a mean and standard error of the mean (mean±SEM). The relationship between the traits was evaluated by calculating the linear correlation coefficients and graphically representing the regression line with confidence intervals.

Results. The morphometrical analysis showed that the average of the tubular diameter was 276.1±5.29 µm, the height of the spermatogenic epithelium was 75.4±1.96 µm. The spermatogenesis index (score) was -3.9±0.05. The average number of Leydig cells was 259.8±6.29. The height of the epididymal epithelium was 43±2.51 µm. The histopathologic examination revealed a mild degeneration in the germ line cells without a significant effect on Sertoli cells. The average number of the degenerated tubules was 19.4±2.48 and accounted for 9.7±1.24 per cent. Main changes in the degenerated tubules were vacuolization, disappearance of the seminiferous epithelium, formation of intratubular multinucleate giant cells, spermatogenic arrest at the spermatocyte stage and a decreasing thickness of the germinal epithelium layer. The correlation coefficients between histomorphometric parameters of the examined rams are presented in Table 1. The number of Leydig cells demonstrated a significantly positive relation with the diameter of testicular tubules and spermatogenesis index (P<0,05), the diameter of testicular tubules with the germinal epithelium height of testes and spermatogenesis index (P<0,001). The germinal epithelium height of testes revealed a negative correlation with the epididymal epithelium height (P<0,01) and a positive correlation with spermatogenesis index (P<0,001). The epididymal epithelium height negatively correlated with spermatogenesis index (P<0,001). The number of the degenerated cells negatively correlated with the number of Leydig cells, the diameter of testicular tubules (P<0,05), the germinal epithelium height of testes (P<0,001) and spermatogenesis index (P<0,01), it correlated positively with the epididymal epithelium height (P<0,01).

Table 1. **Correlation between histomorphometric parameters of testes of the examined rams**

	Number of Leydig cells	Diameter of testicular tubules	Germinal epithelium height of testes	Epididymal epithelium height	Spermato-genesis index
Number of degenerated cells	-0,39	-0,49*	-0,73***	0,61**	-0,62**
Number of Leydig cells		0,48*	0,38	-0,22	0,49*
Diameter of testicular tubules			0,84***	-0,34	0,82***
Seminiferous epithelium height of testes				-0,64**	0,84***
Epididymal epithelium height					-0,60**
* - P<0,05; ** - P<0,01; *** - P<0,001					

Calculating relationships between the morphometric parameters of the examined ram group revealed that the tubular diameter of testes had a tendency to increase when the number of Leydig cells increased in accordance with the following equation: $y = 0.3998x + 172.3$; $R^2 = 0.225$ (P<0,05) (Fig.1).

Studies demonstrated that the germinal epithelium height of testes had a tendency to increase with an increase in Leydig cell count, however, the relationship was not statistically significant (Fig. 2).

The analysis showed that when the number of Leydig cell increased significantly (P<0,05), spermatogenic index increased (by the linear regression equation: $y = 0.0035x + 3.003$; $R^2 = 0.241$), (Fig. 3).

Furthermore, the germinal epithelium height of testes increased with an increase in spermatogenetic index according to the linear regression equation P<0.001) (Fig. 4).

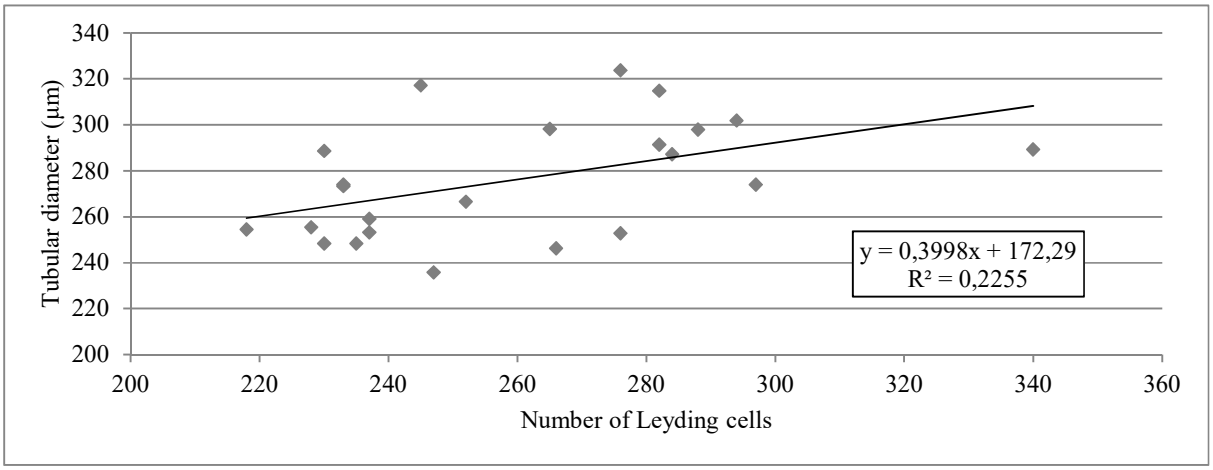

Fig. 1. **Relationship between the number of Leydig cells and the testicular tubular diameter**

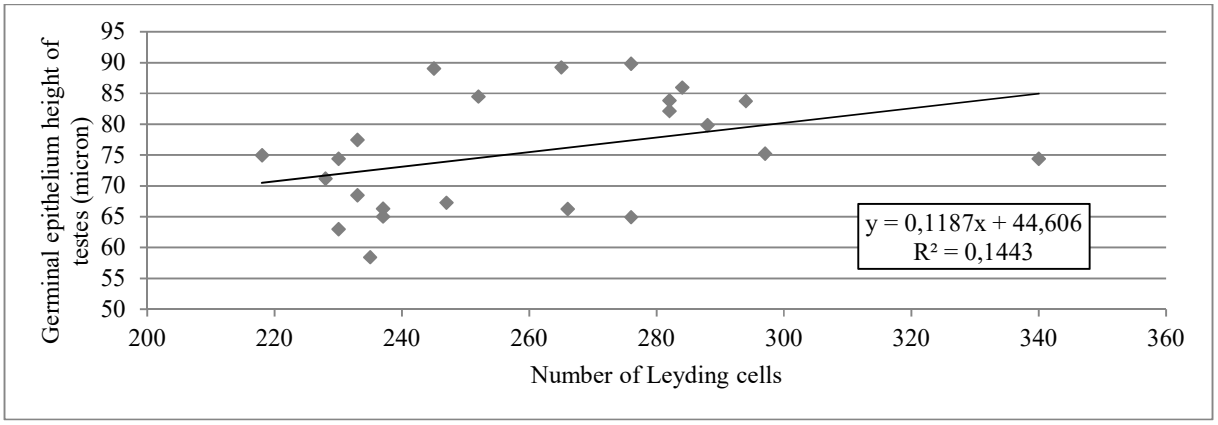

Fig. 2. **Relationship between the number of Leydig cells and the germinal epithelium height of testes**

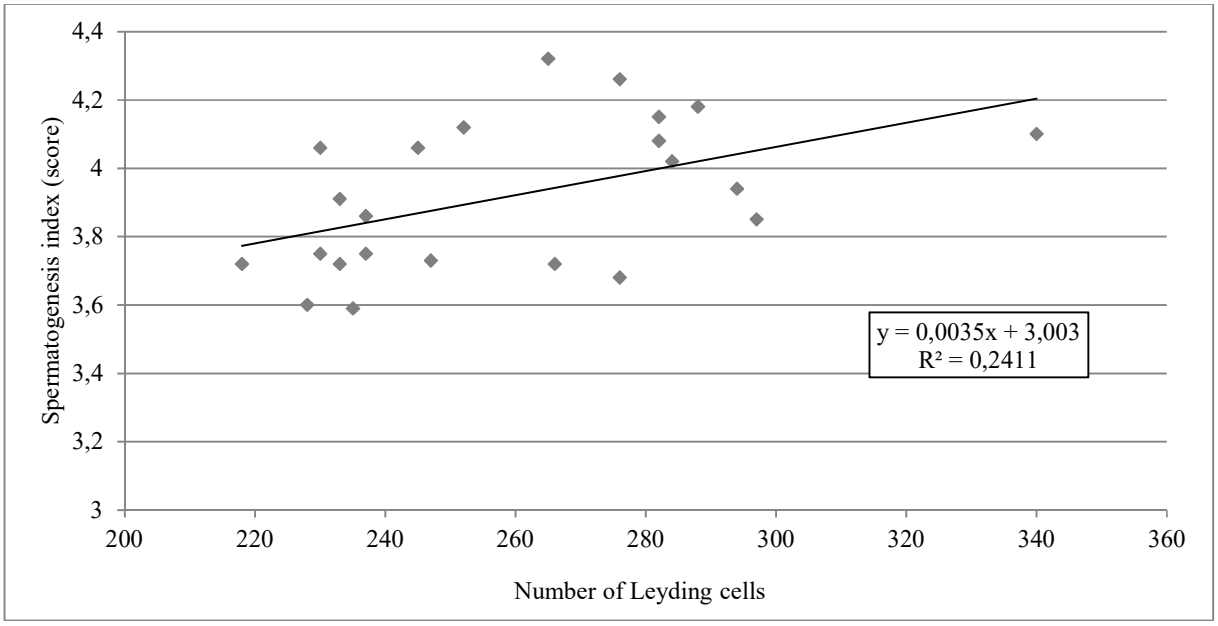

Fig 3. **Relationship between the number of Leydig cells and spermatogenic index**

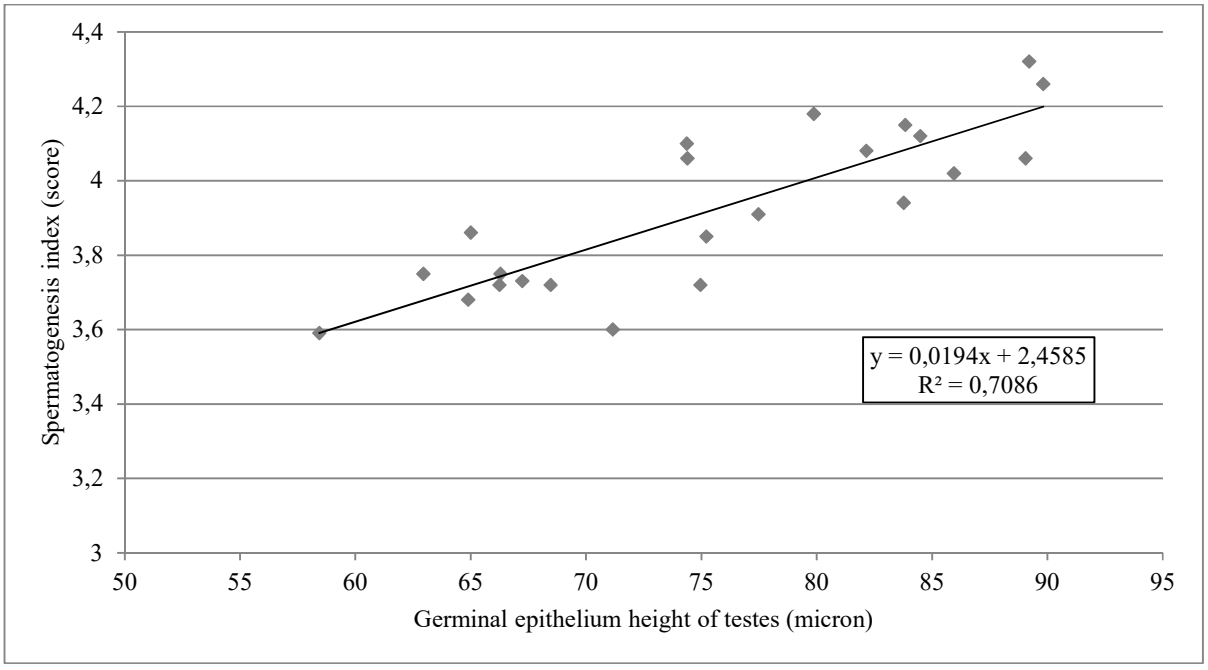

Fig. 4. **Relationship between the germinal epithelium height and spermatogenic index**

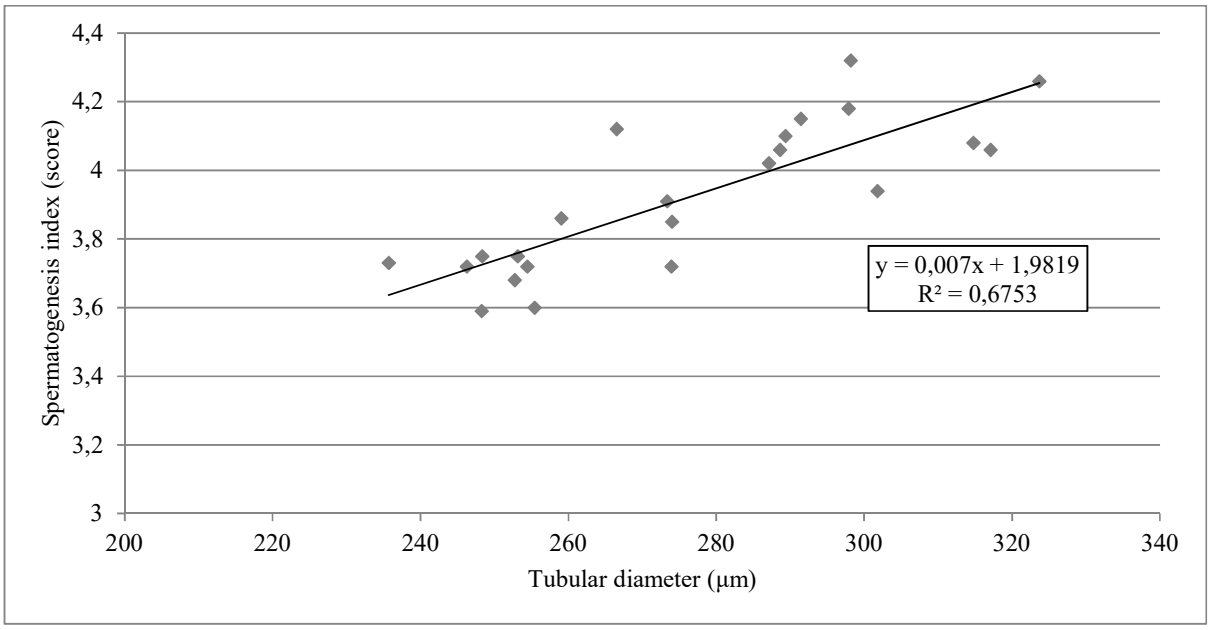

Fig. 5. **Relationship between the tubular diameter and spermatogenic index**

A similar positive linear relationship was established between the tubular diameter and spermatogenic index P<0.001) (Fig. 5).

The dependence of the tubular diameter on the degenerated tubules of rams was calculated. We established a negative relationship - the number of the degenerated tubules increased significantly (P<0,01) with a decrease in the tubular diameter (Fig.6).

Studies showed that spermatogenic index had a tendency to decrease with an increase in the number of the degenerated tubules P<0.01) (Fig.7).

Discussion

The present study is the first morphometric investigation of the testis in Lithuanian local coarse rams, showing correlations between different structures in testis compartments. The consistent pattern is useful in understanding and explaining the functions of different parts and cells of the ram testes.

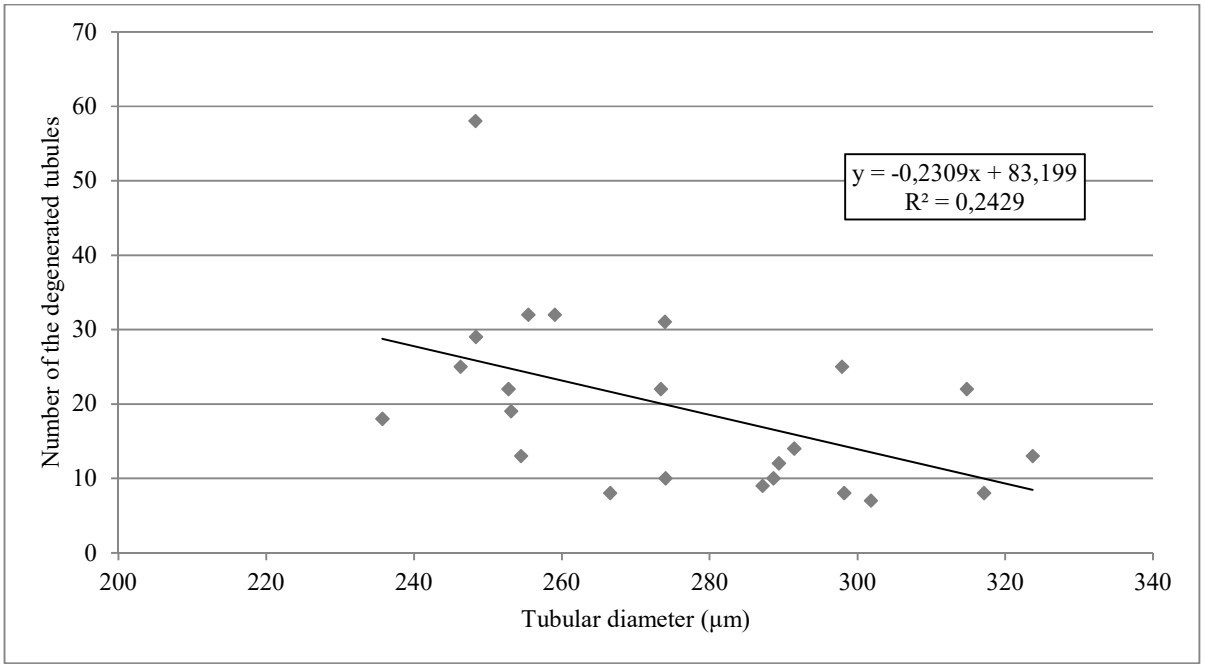

Fig. 6. **Relationship between the number of the degenerated tubules and the tubular diameter**

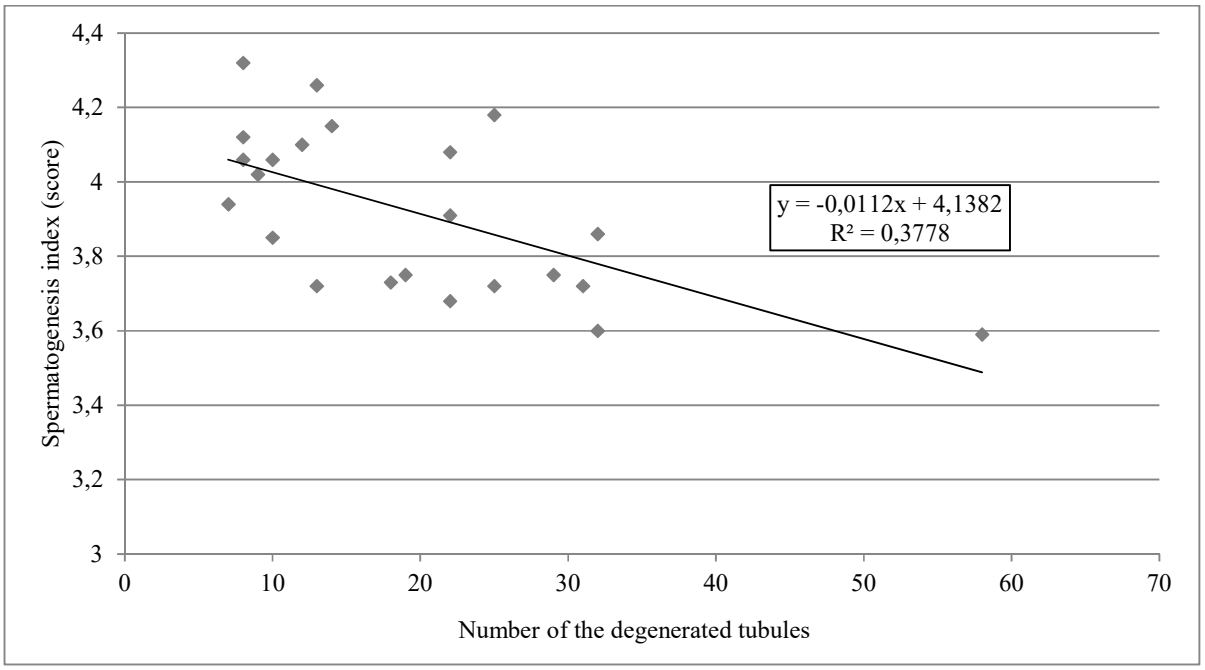

Fig. 7. **Relationship between the number of the degenerated tubules and spermatogenic index**

In our study the mean tubular diameter and the epithelium height in ram testes were 276.1±5.29 μm and 75.4±1.96 μm, respectively. The tubular diameter of the rams under investigation was found to be by about 8% higher than that found in the Arabian ram. According to Dorostghoal *et al.* (2009) the seminiferous tubule diameter of Arabian rams varied significantly during different seasons (P<0,05); the seminiferous tubule diameter was the highest in early winter (220,97 ± 12,15 μm) and the lowest in early summer (June, 186,16 ± 12,16 μm). Total volumes of the seminiferous tubules and the germinal epithelium gradually increased during summer and autumn, with the highest values in early winter. These researchers found significant correlation between the seminiferous tubular diameter and scrotal circumference (r = 0.78; P<0,01). However, on the whole, our results correspond with the

reports about quantitative studies in domestic rams. The average diameter for the majority of amniotes ranges from 180 to 300 μm (Roosen-Runge, 1977), in goats it measures 237.0 (Leal et al. 2004). Sheep are seasonal breeders which are sexually inactive during summer time (Pineda, 2003, Rasooli et al. 2010). The rams that we investigated were slaughtered in early summer (June) and a morphometric evaluation of the testes showed a comparatively high tubular diameter. Gastel et al. (1995) noted that there were some differences in the sexual cycle of ram of northern and southern breeds. Lithuanian local coarse rams belong to the northern breed and their sexual cycle usually occurs during autumn and rarer in summer. The number of the degenerated tubules negatively correlated with the diameter of testicular tubules (P<0,05), the germinal epithelium height of testes (P<0,001) and spermatogenesis index (P<0,01),

The height of the seminiferous epithelium is essential to the evaluation of sperm production (Wing et al. 1982). The results of the present study on the epithelium height (75.4±1.96 μm) are in agreement with those of other studies. In the experiment carried out by Hess and Franca (12) the height of the germinal epithelium was 82,7±1,63 67,5±1,6. In our study the diameter of testicular tubules demonstrated a significantly positive relation with the germinal epithelium height of testes and spermatogenesis index (P<0,001). According to Sharpe (1994), Neves (2001), Leal et al. (2004), the spermatogenic efficiency is highly correlated with the seminiferous tubules volume density, the number of Sertoli cells per gram, and the spermatogenic cycle length.

In our study the rams had a mild degeneration of some testicular tubules and the number of the degenerated tubules negatively correlated with the diameter of testicular tubules (P<0,05), the germinal epithelium height of testes (P<0,001) and spermatogenesis index (P<0,01). High thermal and oxidative stress, physical and chemical agents impaired spermatogenesis by eliminating spermatogonial germ cells in the seminiferous tubules and revealed a severe testicular (germ line, Sertoli and Leydig cells) degeneration and reduced sperm fertility (Gomes et al. 1971, Yaeram et al. 2006, Rasooli et al. 2010, Razi et al. 2012, Gotowiecka et al. 2015).

The number of Leydig cells in our study showed a significantly positive relation with the diameter of testicular tubules and spermatogenesis index (P<0,05). The number of Leydig cells per gram of testis and the volume density accounts for only about 1% in rams. The cells are relatively large, (their individual volume is about 400 μm3 -780 μm3, the volume of the nucleus is approximately 170 μm3); they are polymorphous cells with eccentrically located spherical nuclei, are organized in clusters or distributed individually and do not closely adjoin the walls of blood vessels or the walls of lymphatic vessels (Almeida, 2002, Lunstra et al. 1988, Russell et al. 1996, Leal et al. 2004). Interesting data are presented by Leal (2004) who established that the total number of Leydig cells was significantly and positively correlated with the percentage (r= 0.98 and volume (r=0.99) occupied by lymphatic vessels in the testis parenchyma. They release androgen into extracellular fluid of the interstitial tissue, whence it diffuses to the tubules and to the vessels (Bloom et al. 1986).

The results of this study showed that the seminiferous epithelium height of testes revealed a negative correlation with the epididymal epithelium height (P<0,01) and a positive one with spermatogenesis index (P<0,001) and the epididymal epithelium height negatively correlated with spermatogenesis index (P<0,001). These correlations might be explained by the fact that in case of intensive spermatogenesis tubules of epididymis undergo dilatation and therefore the epithelium height becomes lower. The results obtained in this study indicated that the number of Leydig cells and the degeneration of testes had an impact on the morphometric parameters of seminiferous tubules and spermatogenic index. The number of Leydig cells, the diameter of testicular tubules, the germinal epithelium height and spermatogenesis index had positive correlations.

Conclusion. The diameter of testicular tubules, germinal epithelium height and spermatogenesis index and number of Leydig cells have positive correlations. The number of the degenerated tubules negatively correlated with the number of Leydig cells, the diameter of testicular tubules, the germinal epithelium height of testes and spermatogenesis index and correlated positively with the epididymal epithelium height.

The present study is the first to describe the histomorphometrical parameter correlations of the spermatogenic process in Lithuanian local coarse wool rams. The data will be of value to the future studies.

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