Histological and Morphometric Study of Lactating Mammary Glands in Sows

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Abstract

This work deals with the quantitative histological morphometric description of the mammary glands of sows during the stage of lactation. As experimental animals were used White meat breed pigs (5 animals) kept in standard conditions VÚŽV in Nitra. After killing the animals we sampled mammary glands and have them processed by conventional histological and histochemical methods. Morphometric methods, we found that glandular parenchyma of mammary glands of sows consists of a lobules width 615.28 ± 165.09 μm and length 1827.78 ± 621.43 μm. Glandular parenchyma is 64.84 ± 12.87%, from which secretory epithelial cells of the alveoli are 19.11 ± 4.58 % and alveoli lumen accounts 45.73 ± 13.01 %. Connective tissue stroma is 33.68 ± 10.45 %, of which the collagen tissue accounts for 8.31 ± 9.88% and the loose connective tissue 25.37 ± 9.61%. Adipose tissue forms in this period, only 1.48 ± 4.50%. The work was described and quantified in alveolar differentiation, the average size and frequency of individual types of alveoli and their relative volume of the walls (epithelium) and cavities (lumen), the amount of secretory epithelial cells and the nucleocytoplasmic ratio. The average size of alveoli was 131.06 ± 36.07 μm, and the size of alveoli varied from 77.69 μm to 192.25 μm. Thickness interlobular ducts was 335.00 ± 78.69 μm. The average frequency of the alveoli is 226933.08 ± 161136.27 per 1cm3 of tissue in the mammary glands. Using correlation coefficients, we describe the relationships between structural components of the mammary glands.

Key words: histology, mammary glands, morphometric study, sows.

1. Introduction

Data on the histological structure of mammary glands of pigs are quite rare. Most of the work [1, 2] deals with the influence of of nutrition on mammary development of pigs or deal with lactation insufficiency [3]. [4] observed the effect of caffeine on mammary gland development and lactation in pigs. Morphometric data on glandular parenchyma of mammary glands in lactating cows state [5], goats [6], sheep [7], and [8] and in rabbits [9]. Alveolar differentiation described [10] and [11]. The work also found morphometric data of connective stroma and adipose tissue. Relations between glandular parenchyma, connective tissue and adipose tissue are due to different factors highly variable and also related to interspecies differences in the length of pregnancy, lactation length, number of suckling pups and the intensity of milk production.

2. Materials and methods

For comparative study of the structure of mammary glands as omnivores, we used five experimental animals pig breeds White meat. All experimental animals were kept in standard conditions VÚŽV in Nitra. After killing the animals we did anatomical dissection and mammary glands sampled for histological, histochemical and subsequent morphometric analysis. Samples for histological and histochemical analysis were collected for each animal from three udders (thoracic, abdominal and inguinal) right half carcasses immediately after

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slaughter of the animal. Samples of about 1 cm3 were collected from the glandular parenchyma and were frozen in liquid nitrogen. Then were cut at - 20 ^{0}C by microtome Cryo - Cut (USA) into slices 10 to 15 μm thick. Slides were stained transparent staining with hematoxylin - eosin [12] and Oil red O. We subjectively evaluated on histological preparations of the microscopic structure of the mammary glands, the incidence of glandular parenchyma, adipose, loose and collagen tissue, lobule shape, alveoli and ducts, epithelial cell shape and the shape of adipose cells. For quantitative evaluation, we used the TV - with a camera system Telemic and methodology [13]. We surveyed the relative volume and surface of glandular parenchyma, adipose, loose and collagen tissue, the average thickness of the alveoli and ducts, their frequency and amount of epithelial cells. The paper presents the average values obtained from all glands irrespective of their location. From the obtained data, we calculated basic variation - statistical indicators. Observed differences were tested two-factor analysis of variance, Fisher F - test, Scheffe test, Student t - test and we calculated the correlation coefficients.

3. Results and discussion

Results should be clear and concise. References Structure of mammary glands in sows involved glandular parenchyma 64.84 ± 12.8 7%, which is the epithelium and lumen of alveoli and ducts. Connective tissue stroma occupies 33.68 ± 10.45 % and consists of a loose connective tissue and collagen. Adipose tissue in this period is 1.48 ± 4.50%. Quantity of glandular parenchyma during lactation prevails over the number of connective stroma and adipose tissue and is a main component of the mammary glands. Glandular parenchyma of mammary glands in sows during lactation are oval-shaped lobes, broad 615.28 ± 165.09 μm (v = 26.83%) and length 1827 ± 621.43 μm (v = 34.0%). Lobes are separated by irregular bands of collagen connective tissue, wide average of 67.74 ± 47.01 μm (v = 69.40%). Collagen connective tissue consists of bundles of collagen fibers, going side by side lengthwise. Among them are cells of connective tissue. Broad strips of collagen connective tissue are separating the lobes. Thin strips of loose connective tissue with an average width of 23.22 ± 16.94 μm (v = 72.95 %) surrendered to the interior of lobules. Thin strips of tissue consist of small amounts of collagen, elastic and reticular fibers and a small amount of cells. Loose connective tissue inside the lobule is a layer of coupling, which surrounds the alveoli and ducts of the mammary glands. Loose and collagen connective tissue and thus fulfill the function of the suspension system and also have a maintenance and transportation functions, because they run blood vessels, lymph vessels and nerves. In the vicinity of glandular parenchyma lobules of in lanes collagen tissue is adipose tissue in the form of isolated adipose cells with small size of 70.37 ± 14.84 μm. Only in some few cases, the adipose tissue occurred in the form of small groups of cells. As with other animals, the relative volume of adipose tissue during lactation is very low and has a very high variability, which is caused by interactions of glandular parenchyma and adipose tissue. Relations between glandular parenchyma, connective tissue and adipose tissue of udders in sows can be seen from Table 1. Glandular parenchyma during lactation is a strong negative correlation relationship with the connective tissue and a moderate correlation relationship with adipose tissue, which means that the increase in volume of parenchyma is accompanied by a strong decline in the amount of connective tissue and adipose tissue. This fact also confirms that the relative volume of connective stroma and relative volume adipose tissue are in a moderate positive correlation relation, which means that tissue loss is accompanied by loss of adipose tissue.

Table 1. Correlation coefficients of relationship of glandular parenchyma, connective tissue and adipose tissue of udders in sows at the stage of lactation

	Parenchyma	Connective tissue	Adipose tissue
Parenchyma	1.00000	- 0.94635	- 0.66104
Connective tissue		1.00000	0.38310
Adipose tissue			1.00000

Glandular parenchyma is composed of epithelium and lumen. This means that an increase of glandular parenchyma during lactation is caused by an overall increase in the relative volume of epithelium and lumen of alveoli and ducts. Relative volume of epithelium achieves 19.11 ± 4.58 % and luminal 45.73 ± 13.01%. Relative volume of collagen connective tissue as part of the stroma is only 8.31 ± 9.88 %. The main and largest part of the stroma is loose connective tissue in an amount of 25.37 ± 9.61 %, which is involved in the construction of lobules of mammary glands. Adipose tissue, as already mentioned, is much reduced (1.48 ± 4.50 %) and together with collagen tissue has a very high variability. Mutual relations between structural components of mammary glands show the correlation coefficients in Table 2.

Table 2. Correlation coefficients of relationships epithelium, lumen, loose, collagen connective tissue and adipose tissue of the mammary glands in sows at the stage of lactation.

	Epithelium	Lumen	Collagenous tissue	Loose tissue	Adipose tissue
Epithelium	1.00000	- 0.20764	- 0.49637	0.43501	- 0.25609
Lumen		1.00000	- 0.45680	- 0.52198	- 0.56351
Collagenous tissue			1.00000	- 0.42463	0.53734
Loose tissue				1.00000	- 0.13566
Adipose tissue					1.00000

From Table 2 shows that the epithelium and lumen are in a weak negative correlation relationship, which means that enlarging the relative volume of epithelium is associated with the reduction of the relative volume of the lumen or vice versa. Both of these happening, we observed in the alveoli of mammary glands during lactation and associated with secretory cycle. With the increasing of the relative volume of epithelium is also related to reducing the relative volume of collagen tissue (moderate negative correlation dependence) and adipose tissue (weak negative correlation dependence), where the expense of the glandular parenchymal component grows. But at the same time generates an increase in the relative volume of loose connective tissue, which is with the epithelium in a moderate positive correlation relationship. Increase in the relative volume of the lumen is a moderate negative correlation relationship associated with loss of collagen, loose and adipose tissue. The relative decrease in collagen tissue in the mammary glands associated increase loose tissue, because loose tissue is stroma of glandular parenchyma lobules. Simultaneously with the decline in the relative volume of collagen tissue and decreases adipose tissue, because they are in a moderate positive correlation relationship. Loose connective tissue with adipose tissue is a very weak negative correlation relationship, so we can conclude that the increasing of loose tissue caused only very small relative decline of adipose tissue. Glandular parenchyma of mammary glands of lactating pig consists of a large number of alveoli and ducts. Alveoli have an elliptical shape. On the surface they form basement membrane, inside the boot and saddle by secretory epithelial cells that surround the lumen. The average size of alveoli is 131.06 ± 36.07 μm (v = 27.52%) and interlobular ducts 335.00 ± 78.69 μm (v = 23.49%). We found that in 1 cm^3 of the mammary gland is 226933.08 ± 161136.27 alveoli, while fluctuations of empirical values were 75732.05 - 855548.80, because not all alveoli are the same size. Their size ranges from 77.69 μm to 192.25 μm, with one lobe alveoli are approximately the same size, but the alveoli adjacent lobes differ in size respectively may vary. Between size and frequency of alveoli there is a very strong negative correlation relationship (- 0.71608), which means that the zoom alveoli associated reduction in their number in a unit volume. We found that in sow mammary glands during lactation there are three types of alveoli. Alveoli with small size to 100 μm, with intermediate size of about 120 μm and a large alveoli with larger size than 120 μm. Percentage of different types of alveoli representing 26.25 % small, 28.75 % intermediate and 45 % of large alveoli. Alveolar size is affected by the presence or. absence of intraluminal secretion, which is especially evident in the lumen of the intermediate and large alveoli. Accumulation of secretions in the lumen of the alveoli causes flattening of epithelial secretory

cells and thereby enlarging the lumen. Relative volume of epithelium is 39.53 ± 5.88 % and luminal 60.47 ± 5.88 % in small alveoli, while the height of epithelial cells reached 17.56 ± 4.30 um (v = 24.49%). Relative size of epithelium is 34.94 ± 5.12 % and luminal 65.06 ± 5.12 % of their total volume in the intermediate alveoli, the height of epithelial cells reached 14.89 ± 3.10 μm (v = 20.82%) . Relative size of epithelium is 26.21 ± 6.62% and luminal 73.79 ± 6.62% in large alveoli, while the height of alveolar epithelial cells is the lowest, reaching 9.18 ± 2.30 μm (v = 25.05%). Secretory epithelial cells, whose average height is 13.88 ± 3.50 μm to put on the basal membrane of the alveoli expanded its base. Limiting its evident cytoplasmic membrane, this encloses the apical poles of fat droplets in the cytoplasm of cells undergoing apocrine secretion into the lumen of alveoli.

In the middle of the cell nucleus is a round shape. In some cells, lipid droplets were pushed to the basal pole and have an oval shape. Inside the nucleus under the nuclear membrane there are interchromatín and perichromatín granules present. Because the volume ratio of nucleus and cytoplasm during physiological cycles vary depending on the synthetic processes taking place in the nucleus and also in the cytoplasm, we surveyed the medium ground (1 μm thick) sections of the nucleocytoplasmic ratio. We found that the relative volume of nuclei reached 21.95 % and 78.05 % of cytoplasm, it follows that the nucleocytoplasmic ratio is 1: 3.55. The value of cores varied from 12.07 % to 36.07 % and the volume of cytoplasm from 87.93 % to 63.93 %. This means that the nucleocytoplasmic ratio fluctuated in the range from 1: 1.77 to 1: 7.29. Although the histological structure of mammary glands in sows does not show too much difference in comparison with other species, we found some differences concerning the quantitative representation of individual structural components of the mammary glands. Relative volume of glandular parenchyma of mammary glands of pigs reached 64.84 %, which is 1.49% less than in rabbits [9] and 4.72% less than in sheep [14]. In accordance with the findings in cows [5] also in sows consists of enclosed lobuloalveolar structures surrounded by little amount of connective tissue. Lobes of glandular parenchyma epithelium in the mammary glands of the pig is approximately equivalent to the volume of are larger compared with rabbits [9] and also in sheep [14]. We surveyed the number of alveoli in one lobe. Structure and shape of the alveoli of mammary glands of sows do not differ from the alveoli of mammary glands of other species as described by [15] in mice, [16a, 16b] in rats, [17] in bat, [11] in cows, [6] in goats, [7] and [9] in sheep, [18] for heifers, [19] for females, [20] in kangaroos and [9] in rabbits. The shape and size of alveoli varies considerably depending on the state of secretory activity and stage of lactation. According to [7] differentiation is also caused by suckling or milking. We found substantial alveolar differentiation, which means that the lobes of mammary glands of pigs contain three types of alveoli, which represent various stages of secretion. We conclude that thick-walled small alveoli are transient and pig thin-walled large alveoli with large lumens are generally larger than found in cows [11], and in sheep and in rabbits [9, 14]. Lactation comes to raising the number of alveoli [20]. On average in 1 cm^3 mammary glands of pigs is 226933 alveoli, which is much less than found [31] in cows (800000), [9, 14] in the rabbits (603,860) and sheep (1 295 724). These data correspond to comparing the average size of alveoli in each species, suggesting that the mammary glands of pigs consist of a smaller number but larger alveoli. Changes in shape and size of alveoli also associated with accumulation of secretions in the lumen of alveoli, which crushed them and also affects the amount of secretory epithelial cells of alveoli. The apical regions of secretory cells, we observed lipid droplets and apocrine secretion into the lumen of alveoli. Given that in the nuclei of secretory epithelial cells, transcription takes place, changes in the nucleocytoplasmic ratio may be as [21] due to the fact that in later stages of lactation, the ratio of DNA relative to the amount of protein decreases, which means that the volume of cytoplasm increases while the concentration of DNA in the secretory cells is relatively stable, RNA concentration varies depending on physiological processes and under certain authors increases during pregnancy and early lactation. After lactation, according [21] reduces the concentration of mRNA in secretory epithelial cells, or secretory cells grown in lactation procedure. Relative volume of epithelium in rabbits [9] and cows [11]. However, it is lower in comparison with the

volume of the epithelium, which was detected in sheep [14, 7], which is probably related to interspecies differences in milk production. Relative size of the lumen is almost identical with those in rabbits and sheep, which is slightly higher than in cows, which may be related to the size of alveoli.

The ratio between the secretory tissue and connective tissue is the influence of various factors very variable and fluctuates with a periodicity of secretory activity. This same fact was also detected in rabbits [9]. Relative volume of collagen tissue did not lessen due to absorption but due to compression of the getting bigger of alveoli. Adipose tissue is absorbed and adipose cells during lactation reduce and maintain only a small group of cells or isolated cells. When alveolar absorption of fat from adipose cells to apply the system and local effect, which means that if the adipose tissue in immediate contact with the alveoli and the absorption of fat is much higher than when adipose tissue in immediate contact with the alveoli is not. Scope of pumping oil is dependent on a number of suckling pups and the duration of lactation [22]. These changes are also linked to levels of certain hormones, especially insulin and thyroid hormones. Control growth connective stroma provides relaxin and estradiol [23]. Major influence on milk production has prolactin and growth hormone, also glucocorticoids [24] and ACTH [25]. [26] states that the effect of prolactin is stimulated by glucocorticoids and insulin and inhibited by progesterone. According to [27] the maintenance of milk secretion during lactation also affects thyroxine. Lactation is also influenced by parathyroid hormone. According to several authors [28, 29] interaction of secretory epithelial cells of mammary glands with adipocytes leads to a striking increase in epithelial proliferation. According [30] estrogen regulation of mammary epithelial growth takes place under the influence of adipose and connective tissue, the cells have been found in increased concentrations of DNA [31].

4. Conclusions

Our histological observations and also the correlation coefficients shows that the amount of collagen tissue and adipose tissue is also in mammary glands very reduced, because of their expense is growing robustly glandular parenchyma. Along with the growth of alveolar epithelium is a relatively lower volume of lumen, collagen tissue and adipose tissue, but increases the volume of loose connective tissue, which is part of glandular parenchyma lobules.

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