Histological and Morphometric Study of Regressive Changes in the Mammary Glands of Pigs during Regression

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Abstract

The work deals with the histological and morphometric description of regressive changes in the mammary glands of swine after lactation. Samples of mammary glands of thoracic, abdominal and pubic udders were taken soon after they were killed. Samples were processed by histological and histochemical methods and evaluated by subjective and quantitative morphometrical methods. It was found that post-lactation increases the relative volume of connective stroma up to 75.10 ± 14.76 % of collagen in the tissue falls to 74.13 ± 14.87 % and the loose connective tissue, which was part of glandular parenchyma lobules accounts for only 0.97 ± 2.13%. Adipose tissue is 18.93 ± 14.93% of the udders volume.. Relative of glandular parenchyma fell to 5.97 ± 4.95% and mainly consists of a interlobular and lobar ducts and atrophic alveoli. Epithelium is generally in the mammary glands 4.22 ± 3.56% and lumen 1.75 ±1.81%. 1 cm^3 of tissue in the mammary glands located 190745.52 tubuloalveolar structures. Alveoli reach an average size of 36.80 ± 11.57 μm. It was found relative volume of the walls (epithelium) and cavities (lumen) of each alveolus, the amount of epithelial cells and the nucleocytoplasmic ratio. The individual building components mammary glands were calculated correlation relations.

Keywords: histology, morphometric study, mammary glands, pigs, regression.

1. Introduction

After the lactation as with other animals also in pigs gradual regression (involution) of udders and mammary glands occurs. Regression changes are reflected in a decline of lobulus glandular parenchyma [1, 2] and an increase in connective stroma and adipose tissue [3, 4, 5]. At the same time reduces the number and size of alveoli [6, 2] and comes to the atrophy [7, 8, 4], resulting in a gradual reduction in secretory activities of the mammary gland [9].

2. Materials and methods

To study of regression changes in the mammary glands, we used five pig breeds White meat aged 22 months. All pigs were in the process of post-lactation (two months after weaning). Immediately after the pigs were killed, samples of glandular parenchyma were taken from mammary glands thoracic, abdominal and pubic udders for histological and histochemical analysis. Samples great about 1 cm^3 were frozen in liquid nitrogen and to cut Microtome Cryo - Cut (USA). Rough cuts from 10 to 15 mm were dyed in a transparent color hematoxylin - eosin and oil red to demonstrate neutral lipids [10]. Preparations were evaluated subjectively and quantitatively. Morphometric data were obtained through the TV - the camera TELEMIC and the methodology employed and developed by [11]. We measured the relative volume of glandular parenchyma, epithelium, lumen, as a whole tissue, collagen, fat and loose connective tissue. We measured the abundance of tubuloalveolar structures, the average size of alveoli and ducts, the relative volume of the walls (epithelium) and cavities

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(lumen) of alveoli, the amount of epithelial cells and the nucleocytoplasmic ratio. The paper presents the average values from all glands irrespective of their location. From the data we have calculated the basic variation - statistical parameters and correlation coefficients.

3. Results and discussion

In the post-lactation increases the relative volume of connective stroma to 75.10 ± 14.76 %. The most important part of it to 74.13 ± 14.87 % is collagen tissue. This tissue occurs very broad areas in which progress of bunches collagen fibers in different directions with a large number of tissue cells. These areas are more or less interrupted by large and smaller irregular lobules of adipose tissue, which consists of univacuolar fat cells, with an average size of 86.22 ± 27.77 μm and reticular and elastic fibers. Adipose tissue is up to 18.93 ± 14.93% of the udders volume. Quantity of glandular parenchyma strongly reduced as can be seen from Table 1.

Table 1. Relative volume (%) of glandular parenchyma tissue and adipose tissue in pigs after lactation

	$\bar{x}$	± s	v %
Glandular parenchyma	5.97	4.95	82.94
Connective tissue	75.10	14.76	19.66
Adipose tissue	18.93	14.93	78.87

Glandular parenchyma consists mainly from interlobular ducts, which are of different shape and place in the collagen tissue and isolated remnants of glandular parenchyma lobules width 169.23 ± 44.97 μm (v = 26.57%) and a length of 319.29 ± 104.16 μm (v = 32.62%). Relations between glandular parenchyma, connective tissue and adipose tissue we can see from the table 2.

Table 2. Correlation coefficients of relationships of glandular parenchyma tissue and adipose tissue in udders after lactation period.

	Parenchyma	Connective tissue	Adipose tissue
Parenchyma	1,00000	- 0,13494	- 0,19828
Connective tissue		1,00000	- 0,94442
Adipose tissue			1,00000

Table No. 2 shows that glandular parenchyma tissue had only weak negative correlation relation with connective and adipose tissue which means that the relative reduction in volume is accompanied by only slight increases in the relative volume of both tissues. Connective tissue and adipose tissue in comparison, show a strong negative correlation relationship, implying that the increase in the volume of connective tissue is accompanied by a very strong depletion of adipose tissue or vice versa.

Glandular parenchyma is composed in this period of epithelium and lumen of ducts and small amounts of persistent alveoli. Total is the mammary epithelium in this period, only 4.22 ± 3.56 %. The relative luminal volume is only 1.75 ± 1.81 %. The relative volume of collagen and adipose tissue we have mentioned. So reducing the size and quantity glandular parenchyma lobules is related also with a relative decrease in the volume of loose connective tissue up to 0.97 ± 2.13%.

Generally we can say that the greatest variability in this period reaches loose connective tissue and lumen of alveoli and ducts.

Table 3. Correlation coefficients relating epithelium, lumen, collagen, adipose and loose connective tissue in pig udders after lactation.

	Epithelium	Lumen	Collagenous tissue	Loose tissue	Adipose tissue
Epithelium	1,00000	0,66284	- 0,19392	0,57057	- 0,20723
Lumen		1,00000	- 0,20964	0,44894	- 0,13449
Collagenous tissue			1,00000	- 0,12108	- 0,90732
Loose tissue				1,00000	- 0,21249
Adipose tissue					1,00000

From Table No. 3, we see that the relative volume of epithelium and lumen is a strong positive correlation relationship, so the loss of epithelium in this period is also accompanied by loss of lumen. Moreover, the epithelium and lumen are moderately strong positive correlation relationship with loose connective tissue, so at this time of loss is also accompanied by a loose tissue loss. Epithelium and lumen weakly negatively correlated with collagenous connective tissue with adipose tissue, which means that the relative increase in the volume of tissues to cause slight reduction in the relative volume of epithelium and lumen. The rise of the relative volume of collagen tissue is associated with a very small reduction in the relative volume of loose connective tissue but strongly affects the loss of adipose tissue. The loss of the relative volume of loose connective tissue is associated with a weak increase in the relative volume of adipose tissue.

Glandular parenchyma is made up of alveoli, intralobular, interlobular and lobar ducts. 1 cm^3 of tissue in the mammary glands is approximately 190745.52 tubuloalveolar structures. Alveoli are small groups appearing sporadically in the broad areas of collagen tissue. The alveoli through a very small number of loose connective tissue that forms the border between them. Alveoli are very small, reaching an average size of 36.80 ± 11.57 μm (v = 31.43%). Some are oval, but many of them are irregular in shape. Alveoli have either little or no lumen and only consist of clusters of cells. In the alveoli lumen there is no liquid. Individual alveoli epithelium is 87.40 ± 8.09% and lumen 12.60 ± 8.09 % of their volume. Epithelial cells surrounding alveoli are low, amounting to 6.76 ± 1.80 μm. Nuclei are flattened and located near the basal cell membrane. In cells, we found the presence of intracellular secretion. Nucleocytoplasmic average ratio was 1: 1.83. The nuclei accounted for 35.34 % and cytoplasm 64.66%. The relative amount of nuclei ranged from 29.59 % to 43.86 % and cytoplasm from 70.41% to the 56.14%, which means that the nucleocytoplasmic ratio varied from 1: 1.28 to 1: 2.38. We can say that it is inactive alveoli. Ducts are of various shapes and are like a group of alveoli in the broad areas of collagen tissue. Achieve an average thickness of 45.74 ± 31.46 μm (v = 68.79%). Duct epithelium is two to multi-layered and covers 63.75 ± 10.26 %, while the lumen accounts for 36.25 ± 10.26 %. In the lumen in some cases found intraluminal liquid, probably containing a variety of cell fragments, but did not contain lipid droplets. Ducts are sequentially together and pass into milk cisternae, which wall consist of connective tissue layer and dual-layer cylindrical epithelium. Teat canal cross-section is round and is covered by multilayer squamous epithelium.

According to [8] mammary gland involution occurs very quickly in sow. It starts with the accumulation of milk in the alveoli in the first 2-3 days after weaning, mice by [12] has 24 to 48 hours after weaning. At that time most of the alveoli filled with milk and there have been some signs of degeneration. In some alveoli collapse occurred. Similar changes were also observed [4] in cows. While in mice 72 hours after weaning, most of the alveoli was reduced or rent [12], sows found [8] destruction of alveoli in the 3 – 9 day. In contrast, [13] argue that the omission of sucking in sheep for 5 days has little influence on the secretory cells of the mammary glands of alveoli. After 15 days of the omission of sucking found that secretory cells are overfilled by lipid droplets, glandular parenchyma is reduced and connective tissue stroma was increased. Recovery of adipose tissue begins involving a large number of multivacuolar and later univacuolar cells [12]. In the first two stages in the alveoli and intratubular connective tissue found many granulocytes and mononuclear leukocytes [8, 1] and their infiltration into involuting tissue is associated with

heterophagocytosis of degenerating cells [14]. During the involution are the predominant type of cells in the mammary gland macrophages and lymphocytes [4, 3] and also in many emerging multinuclear giant cells, which play resorbtion role in the insoluble components of milk and moved amyloid [3]. Our results show that reducing the amount of glandular parenchyma and epithelial and luminal tubuloalveolar structures in pigs is greater than rabbits [2]. In contrast, the quantity of adipose tissue formed in this period in pigs 18.93 ± 14.93 % in rabbits enacted whereas only 3.22 ± 7.01 % [2]. In goats found [5] that the amount of interstitial tissue and the number of mononuclear cells is greatly increased after the 50th days from the start of involution of the mammary gland.

4. Conclusions

Reggression differences may be due to interspecies differences in the sexual cycle and length of pregnancy, when rabbits in a shorter period of regression of left over epithelium, respectively. Glandular parenchyma and store less adipose tissue in their mammary glands compared with pigs in a period of regression which lasts much longer. These differences may also be associated with interspecies differences in the composition of milk in rabbits and pigs.

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