

# An Ultrastructural Study of Mitochondria of Mammary Secretory Cells in Rabbits During Lactation and Regression Stages

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## Abstract

The aim of this study was ultrastructural study of mitochondria in epithelial secretory cells in mammary glands of rabbits during lactation and regression stages. Our results using conventional transmission electron microscopy has shown that the mitochondria are relatively changed during the various mammary gland development stages. We found that the average relative volume of mitochondria achieves 12.530±3.127% of cytoplasm volume in the stage of lactation. During regression mitochondrial relative volume decreased to 3.583±1.626% of the cytoplasm. This difference compared to lactation is statistically significant ( $p < 0.05$ ). Relative surface of mitochondria during lactation reaches 2.092±0.550  $\mu\text{m}^2/\mu\text{m}^3$  of cytoplasm. We found a decrease of relative surface of mitochondria to 0.556±0.184  $\mu\text{m}^2/\mu\text{m}^3$  of cytoplasm during regression stage. After the end of lactation, we found that the average size of mitochondria is 0.376±0.074  $\mu\text{m}$ . Differences in the average size during lactation and regression are statistically significant, suggesting that mitochondria retain their size, regardless of the physiological state of activity of the mammary gland. Abundance of mitochondria during lactation is 49.34±15.2 mitochondria per 100  $\mu\text{m}^3$  of cytoplasm. Abundance of mitochondria positively correlated with abundance of all cytoplasmic structures except the abundance of fat droplets, where we found a very strong negative correlation. We found 10.70±7.0 mitochondria per 100  $\mu\text{m}^3$  of cytoplasm after the end of lactation. Average volume of individual mitochondria reaches 0.196±0.008  $\mu\text{m}^3$ . Average volume of individual mitochondria reaches 0.239±0.142  $\mu\text{m}^3$  during regression. Although there is a certain tendency to increase in volume, which is also linked with an average size of mitochondria, the difference compared with the period of lactation is not statistically significant.

**Keywords:** electron microscopy, mammary glands, mitochondria, secretory epithelial cells

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## 1. Introduction

The changes of mitochondrial structure in the lactating and involution mammary epithelial cell are poorly understood. Mitochondria are a kind of organelles existing widely in cytoplasm [1]. They have an oval shape and consist of two membranes and the base matrix [2]. They play a vitally important role of ATP formation through the

operation of electron transport chain located in the membranes, and generate reactive oxygen species as a by-product in the process of ATP production. As mitochondria are fusing or dividing depended on cell cycle, their morphology is continuously changing [3]. Comparative studies of structure related to function offer a promising means of understanding the significance of differences in cytoarchitecture. Mitochondrial crista structure is linked tightly to mitochondrial function [4]. Also their size and number vary widely [2]. The intensity of their activity also varies according to the stage of the cycle, and these changes are reflected in their structure and are subject to the

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energy needs of the cells in that stage [5]. Hollman [6] found that the number of mitochondria is increased in cells of the mammary gland during pregnancy and early lactation [7]. The aim of this study was to describe changes of mitochondria in epithelial secretory cells of lactating and involuting mammary glands in rabbits.

## 2. Materials and methods

In this work samples of mammary glands of 10 rabbits were used. Depending on the stage of the physiological activity of the mammary glands rabbits were divided into two groups: lactation and the regression stage. All animals were kept in standard conditions at Research Institute of Livestock Production in Nitra. Samples for electronoptical examination were collected for each of the three mammary glands (thoracic, abdominal and pubic) the right half of the body of rabbits immediately after killing the animal. A sample size of 1 to 1.5 mm<sup>3</sup> were collected from glandular parenchyma and processed according to the methodology prepared by the Mráz and Polónyi [8]. Electronograms were made by electron microscope JEOL 100MX on the Elektronen Platte EU 2 ORWO at the Department of reproduction (Research Institute of Livestock Production). We used a 7200-fold magnification, in some cases 3600, 10000 and 14000-fold. For quantitative assessment methodology was used microscopic system Nikon Eclipse E 600 and camera Pixelink (PL-A642) in connection with software for image analyse Lucia 4.8. Basal statistical indicators and correlations were calculated from obtained data using Statgraphics statistical software. Basic variational-statistical characteristics and the observed differences by ANOVA, F-test and Scheffe test were tested.

## 3. Results and discussion

### *Lactation stage*

Mitochondria are irregularly distributed in the cytoplasm of all secretory cells of the mammary glands in rabbits during lactation. The mitochondria show more or less regular spherical and elliptical shape. Their sizes also vary from small through medium to large. Mitochondria

were compact (dense) with clearly distinct double membrane and a lot of crista extending into the interior of mitochondria. Matrix appeared compact and darker with fine granules in smaller mitochondria. Matrix of larger mitochondria was less compact with lighter sections.

### *Regression stage*

Mitochondria had a few density and inner membrane form short and irregular crista, in most cases lacked crista during regression stage. Matrix was light, fine granular and fibrillar material was located at the periphery of the inner mitochondrial membrane. In consideration of the small volume of cytoplasm, mitochondria were located close to the nucleus.

### *Quantitative assessment*

We found that the average **relative volume** of mitochondria achieves 12.530±3.127% of cytoplasm volume in the stage of lactation. Relative volume of mitochondria positively correlated with the relative volume of rough endoplasmic reticulum and fat droplets. Negatively correlations were calculated with the other structures of cytoplasm. During regression mitochondrial relative volume decreased to 3.583±1.626% of the cytoplasm. This difference compared to lactation is statistically significant ( $p<0.05$ ).

**Relative surface** of mitochondria during lactation reaches 2.092±0.550  $\mu\text{m}^2/\mu\text{m}^3$  of cytoplasm. Surface of mitochondria positively correlated only with the surface of Golgi apparatus cisternae and multivesicular vacuoles. We found a decrease of relative surface of mitochondria to 0.556±0.184  $\mu\text{m}^2/\mu\text{m}^3$  of cytoplasm during regression stage. This difference was statistically significant ( $p<0.05$ ). Surface of mitochondria during this period negatively correlates with the surface of lysosomes, fat droplets and the surface of smooth membranes.

The **average size** of mitochondria during lactation is 0.360±0.004  $\mu\text{m}$ . The average size of mitochondria negatively correlates with an average size of rough endoplasmic reticulum cisternae, Golgi apparatus cisternae, ( $r=-0.99858$  +), lysosomes, fat droplets and smooth endoplasmic reticulum cisternae. Positive correlations were found with the average size of secretory vesicles, empty vesicles and multivacuolar vesicles.

After the end of lactation, we found that the average size of mitochondria is  $0.376 \pm 0.074 \mu\text{m}$ . Differences in the average size during lactation and regression are statistically significant, suggesting that mitochondria retain their size, regardless of the physiological state of activity of the mammary gland. The average size of mitochondria during this period only negatively correlates with an average size of lysosomes. With the other components of the cytoplasm shows a positive correlation.

**Abundance** of mitochondria during lactation is  $49.34 \pm 15.2$  mitochondria per  $100 \mu\text{m}^3$  of cytoplasm. Abundance of mitochondria positively correlated with abundance of all cytoplasmic structures except the abundance of fat droplets, where we found a very strong negative correlation. We found  $10.70 \pm 7.0$  mitochondria per  $100 \mu\text{m}^3$  of cytoplasm after the end of lactation. This decrease compared to lactation is statistically significant ( $p < 0.05$ ). Abundance of mitochondria negatively correlated with the abundance of lysosomes, vesicles, fat droplets and vesicles with a fine grained content.

**Average volume** of individual mitochondria reaches  $0.196 \pm 0.008 \mu\text{m}^3$ . We found that the volume of individual mitochondria negatively correlated with the volumes of individual lysosomes, fat droplets and smooth endoplasmic reticulum and significantly positively correlated with the volume of individual secretory vesicles ( $r = 0.99879+$ ). Average volume of individual mitochondria reaches  $0.239 \pm 0.142 \mu\text{m}^3$  during regression. Although there is a certain tendency to increase in volume, which is also linked with an average size of mitochondria, the difference compared with the period of lactation is not statistically significant. Average volume of individual mitochondria negatively correlated only with the volume of individual lysosomes, strongly positive correlations were found with other components of the cytoplasm. Mitochondria, which were observed in the mammary glands of rabbits had similar shape as mitochondria, which described Lee et al. [9], Uhrín and Kliment [5] in lactating cows and Hluchý [10] in lactating pigs. Its oval and spherical shape, however, differed from the long and slender shape of mitochondria in mammary glands in bat [1] and bitch [11]. Differences in density and amount of crista during lactation and regression correspond to the data of Bóznér [2] on the functional dependence.

Observations of two distinct types of mitochondria [5] also support the idea of Rosano [7], which wrote about the existence of two distinct populations of mitochondria one of which has a regression characteristic. Relative volume of mitochondria in rabbits during lactation is about 0.92 to 4.63% higher than found [12, 5] in cows but lower when comparing our data with observations, which describes [2] in liver cells (16-30%) and rat heart muscle cells (46-53%), guinea pig (38-41%) and rabbit (35-42%). Relative volume of mitochondria in pig at the stage of lactation is only  $8.137 \pm 2.66\%$  [4], which is approximately equal to the relative volume of mitochondria in small mammary alveoli in cow [5] and in mouse [6, 4, 13]. Relative volumes of mitochondria in the cytoplasm were significantly reduced in rabbits during regression stage. Relative surface of mitochondria is rather difficult to objectively comparable due to the variation in shapes. Bóznér [2] indicated the surface of the outer mitochondrial membrane of the epithelial cells of the small intestine in man  $1.6 \mu\text{m}^2/\mu\text{m}^3$ . Uhrín and Kliment [5] indicate the surfaces of mitochondria in small, medium and large alveoli in cows, but which are larger compared to our findings in rabbits. The relative surface is related to a relative volume of mitochondria and their amount. Relative surface of mitochondria also depends on functional burden of mitochondria and possibly with type of animals. These findings correspond with recent reports [14, 3, 15]. The average size is highly variable and depends on the cell type and the intensity of features that cell performs [16, 2]. Bóznér [2] indicates the length of mitochondria from 0.5 to 20  $\mu\text{m}$  and a width of 0.1 to 3  $\mu\text{m}$ . These data did not correspond with our findings 0.360  $\mu\text{m}$  in rabbits during lactation. At the end of lactation would be expected, the regression of the secretory activity and reduced energy needs, the average size of mitochondria becomes smaller. However, our results show that the average size of mitochondria changed insignificantly, mitochondria retain their size even after lactation. According to several authors [16, 14, 3, 4] abundance of mitochondria is also variable and is influenced by the same factors as average size. Bóznér [2] indicates 55 mitochondrias per  $100 \mu\text{m}^3$  of cytoplasm of liver cells, 76 in neurons and 24 in mouse heart muscle. Number of mitochondria vary from 16.5 to 22.8/00  $\mu\text{m}^3$  of cytoplasm in secretory epithelial

cells of cows [16], in mice from 10.8 to 15.8 mitochondria/100  $\mu\text{m}^3$  cytoplasm [14]. We found up to 49.34 mitochondria/100  $\mu\text{m}^3$  of cytoplasm in rabbits, but only 14.59 in pigs [4]. This difference is statistically significant. While number of mitochondria in rabbits decreased statistically significantly at the end of lactation to 10.70 mitochondria/100  $\mu\text{m}^3$  of cytoplasm, in pigs have not been observed such a decrease [4]. Average volume of individual mitochondria is not statistically significantly changed after lactation, which is related to the unchanged average size. We found 0.19  $\mu\text{m}^3$  (rabbit) and 0.415  $\mu\text{m}^3$  (pig) [4]. Bóznér [2] found average data from 0.411 to 1.370  $\mu\text{m}^3$  and in rat myocardium 0.57  $\mu\text{m}^3$ . Average volume of individual mitochondria in mammary glands of mice reach 0.47 to 0.71  $\mu\text{m}^3$  [14].

#### 4. Conclusions

Our study shows that the stages of mammogenesis are accompanied by changes in cytoarchitecture of organelle such as mitochondria of the mammary epithelial cells. The results showed clear differences between lactation and regression stage. Although it is possible that these changes were influenced by various processes, we propose that the mitochondrial adjustment in epithelial secretory cells might help to spare substrates and therefore energy for milk production in the mammary gland.

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