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This paper should be cited as follows:

Hluchy, S. and Toman, R. (2016). **"Transmission Electron Microscopic Analysis of Smooth Endoplasmic Reticulum of Mammary Secretory Cells in Rabbits during Lactation and Regression Stages",** Athens: ATINER'S Conference Paper Series, No: **AGR2015-1823.**

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URL Conference Papers Series: www.atiner.gr/papers.htm

ISSN: 2241-2891
10/02/2016

Transmission Electron Microscopic Analysis of Smooth Endoplasmic Reticulum of Mammary Secretory Cells in Rabbits during Lactation and Regression Stages

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Abstract

The aim of the research was to describe and analyze changes of smooth endoplasmic reticulum (SER) of mammary epithelial secretory cells in rabbits during lactation and regression stages. Typical short tubules, thinned cisternae and elliptical vesicles without ribosomes were found in the middle and apical parts of cells during lactation stage. The average relative volume of SER reaches 0.439 % of cytoplasm volume. Negative correlations were calculated with the relative volume of granular endoplasmic reticulum (GER), mitochondria and lipid droplets. The relative surface of SER reaches 0.09 μm^2 / μm^3 of cytoplasm, while negative correlations with relative surface of GER, mitochondria, empty vacuoles, lipid droplets and multivesicular bodies were found. The average size of tubules and vesicles of SER was 0.172 μm. Negative correlations with an average size of mitochondria, secretory vesicles, protein granules, empty vacuoles, lysosomes, lipid droplets and multivesicular bodies were found. An average size of SER positively correlates with an average size of Golgi apparatus cisternae. The relative volume of each single SER vesicle reaches 0.022 μm^3. The presence of tubules and vesicles of SER during regression stage were not found, however smooth membranes can be considered as remains of granular endoplasmic reticulum (GER). Our observations using transmission electron microscopy, quantitative and statistical analysis have shown the functional relationship of these structures with synthetic and transport processes in the cell.

Keywords: correlations, quantitative analysis, smooth endoplasmic reticulum (SER), transmission electron microscopy

Acknowledgements: This work has been supported by the VEGA project No. 2/0006/12 and the VEGA project No. 1/0292/14 (Ministry of Education, Slovak Republic).

Introduction

Smooth endoplasmic reticulum (SER) occurs in a small amounts at the cytoplasm of secretory epithelial cells (Mettler et al. 1984). SER forms short, slightly dilated smooth membrane tubules and vesicles (Uhrín and Kliment 1982; Jimenez et al. 1984). The membranes of smooth endoplasmic reticulum (SER) can interact with the membranes of granular endoplasmic reticulum mainly during proteosynthesis, but did not interact with the perinuclear space. While granular endoplasmic reticulum (GER) is involved in proteosynthesis, protein folding, quality control and despatch, smooth endoplasmic reticulum (SER) is associated with the production and metabolism of lipids and steroid hormones. SER is providing transport processes, glycogenolysis and detoxification (Valivullah et al. 1986; Schinko et al. 1990; Ghosal et al. 1994; Reinhardt and Lippolis 2009; Invernizzi et al. 2012; Monks and McManaman 2013).

The aim of this study was to describe changes of smooth endoplasmic reticulum (SER) in epithelial secretory cells of lactating and involuting mammary glands in rabbits.

Materials and Methods

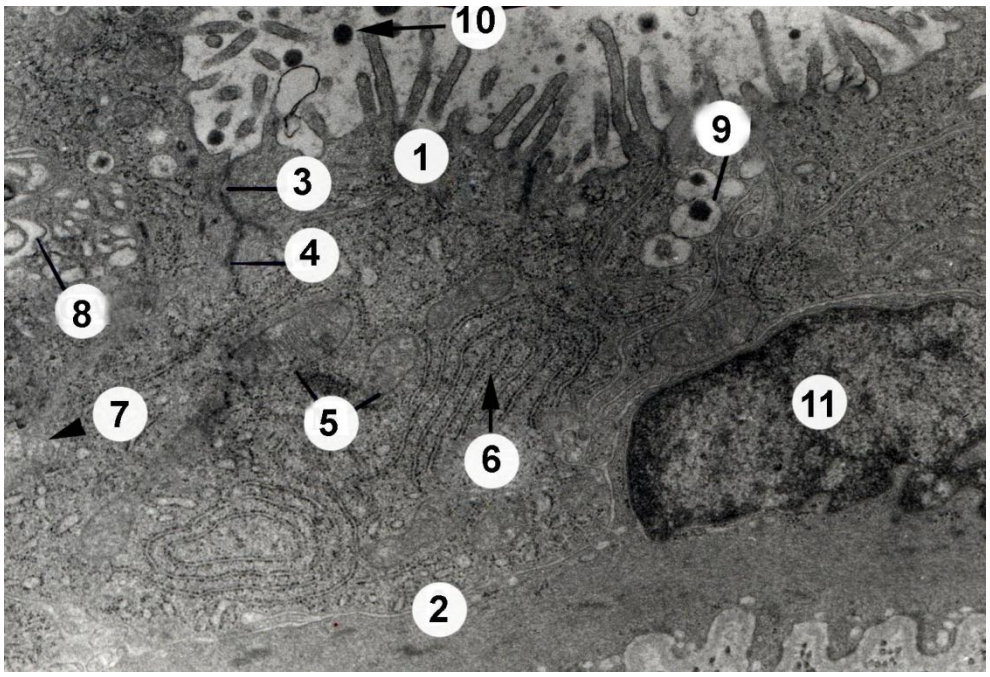
In this work samples of mammary glands of 10 rabbits were used. Depending on the stage of the physiological activity of the mammary glands rabbits were divided into two groups: lactation and the regression stage. All animals were kept in standard conditions at the Research Institute of Livestock Production in Nitra. Samples for electron optical 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^3 were collected from glandular parenchyma and processed according to the methodology prepared by Mráz and Polónyi (1988). Electronograms were made by an 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, a microscopic system Nikon Eclipse E 600 and camera Pixelink (PL-A642) in connection with software for image analyze Lucia 4.8, was used. 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.

Results and Discussion

Typical short tubules, thinned cisternae and elliptical vesicles without ribosomes were found in the middle and apical parts of cells **during lactation stage** (Figure 1).

The **average relative volume** of SER reaches 0.439 % of the cytoplasm volume. Negative correlations were calculated with the relative volume of granular endoplasmic reticulum (GER), mitochondria and lipid droplets. The **relative surface** of SER reaches 0.09 μm^2 / μm^3 of cytoplasm, while negative correlations with relative surface of GER, mitochondria, empty vacuoles, lipid droplets and multivesicular bodies were found. The **average size** of tubules and vesicles of SER was 0.172 μm. Negative correlations with average size of mitochondria, secretory vesicles, protein granules, empty vacuoles, lysosomes, lipid droplets and multivesicular bodies were found. The average size of tubules and vesicles of SER positively correlates with the average size of Golgi apparatus cisternae. The **relative volume of each single** SER vesicle reaches 0.022 μm^3 and the negative correlations with each single relative volume of other organells were calculated.

Figure 1. *Secretory Epithelial Cell of Mammary Gland of Rabbit during Lactation (magnification 16 350x)*

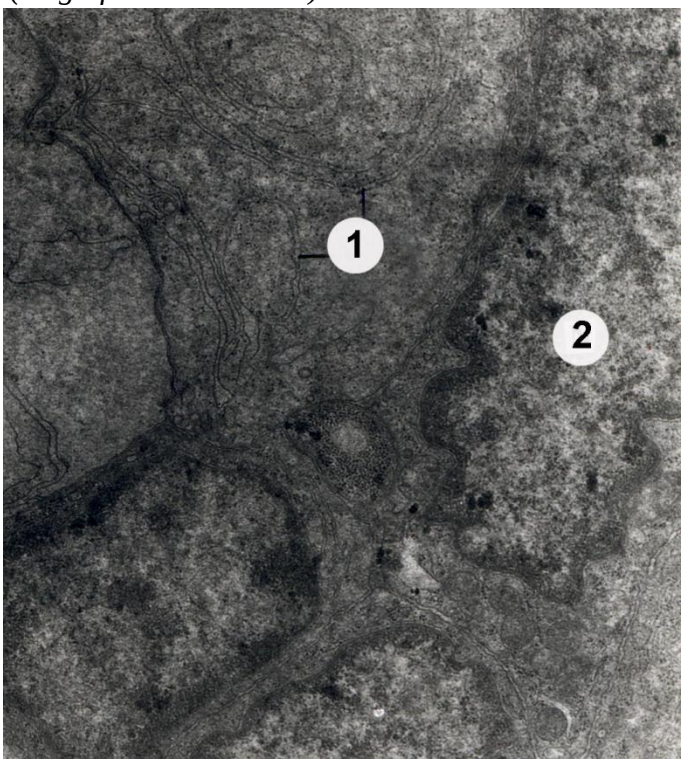


1 – apical surface with microvilli , 2 – basal lamina, 3 – zonula occludens, 4 – zonula adhaerens, 5 – mitochondria, 6 – granular endoplasmic reticulum (GER), 7 – smooth endoplasmic reticulum (SER), 8 – Golgi apparatus cisternae, 9 – secretory vesicles with protein granules, 10 – alveolar lumen with protein granules, 11 - nucleus

Presence of longitudinal flattened cisternae and vesicles composed of a smooth membrane and resembling remains of granular endoplasmic reticulum

cisternae in cytoplasm of mammary secretory cells during **regression stage** were found (Figure 2). On the surface, however, they are not studded with ribosomes. Desintegration and quantitative changes of GER were described by Hluchý and Toman (2014).

Figure 2. *Secretory Epithelial Cell of Mammary Gland of Rabbit during Regression (magnification 16 350x)*



1 – longitudinal flattened cisternae, 2 – nucleus with invaginations of nuclear envelope

The **average relative volume** of smooth membranes (SM) reaches 2.323 ± 0.968 % of cytoplasm during the regression stage. Negative correlations were found with relative volume of remains of granular endoplasmic reticulum (GER) and vacuoles with firm granular content (V). A statistically significant negative correlation with relative volume of lysosomes ($r = - 0.99869^{+}$) was calculated. Positive correlations with relative volume of mitochondria and lipid droplets were found (Table 1).

Table 1. *Correlation Coefficients of Relative Volumes of Secretory Epithelial Cells Structures of Mammary Glands in Rabbits during Regression Stage*

	GER	M	L	LD	SM	V
GER	1.00000	1.00000	1.00000	-1.00000	-1.00000	1.00000
M		1.00000	- 0.53995	0.57642	0.49615	0.23569
L			1.00000	- 0.99903^{+}	-0.99869^{+}	0.69073
LD				1.00000	0.99547	-0.65828
SM					1.00000	-0.72684
V						1.00000

ER – remaibs of endoplasmic reticulum, M – mitochondria, L – lysosome, LD – lipid droplets, SM – smooth membrane, V – vacuoles

The **relative surface** of smooth membranes reaches 0.524 ± 0.271 μm^2 / μm^3 of cytoplasm. Negative correlations with a relative surface of remains of GER, mitochondria, vacuoles and lysosomes were found. However, smooth membranes relative surface positively correlated with relative surface of lipid droplets (r = + 0.99341). The **average size** of flattened cisternae formed by smooth membranes was 0.149 ± 0.055 μm. Positive correlations with the average size of other structures were found, except the average size of lipid droplets (r = - 0.09160).

Our observations of shape, arrangement and location of endoplasmic reticulum (ER) in rabbit´s epithelial secretory cells do not differ from the observations of other authors. Only a few authors deal with ultrastructural morphometry changes in the mammary glands (Uhrín and Kliment 1982; Mettler et al. 1984; Qu et al. 2012; Hluchý and Toman 2014; Hluchý et al. 2014). There are no differences in the cellular structure of mammary tissue, but significant differences in the relative volume of mitochondria and vacuoles between transgenic and non-transgenic mammary gland epithelium were observed (Dragin et al. 2006). Some authors (Chanat et al.1999; Ahlem et al. 2008; Stiening et al. 2008) had studied the changes of ultrastructure of mammary epithelial cells dependent upon the synthesis of casein micelles and their transport inside the cell and into alveolar lumen. Also the synthesis of triacylglycerols in the smooth endoplasmic reticulum (SER) and formation into cytoplasmic lipid droplets has been studied (Smoczynski et al. 2012; Invernizzi et al. 2012). The analysis of milk fat globule membrane proteins has revealed their origin from the Golgi apparatus and endoplasmic reticulum of mammary epithelial cell (Wu et al. 2000; Murgiano et al. 2013). After the end of lactation, the regression of the secretory activity and reduced energy needs caused an increase of the smooth membrane relative surface and the average thickness of cisternae and tubules becomes smaller. Mechanisms responsible for the disruption in mammary epithelium function during involution were described (Baldassarre et al. 2011; Ren et al. 2014).

Conclusions

Our results concerning of low occurrence of SER and smooth membranes in cells correspond with the findings of several authors. Their relative volume, surface, average thickness and volume of individual tubules and their co-relationship with fat droplets, secretory vesicles and vacuoles indicate the functional dependence of these structures with synthetic and transport processes in the cell.

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