**Original Paper**

# The morphological changes of *oviduct*al mucose in oestral cycle of sows

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The aim of this work was to describe microscopic and submicroscopic changes in *uterine tube* of 40 sows in the estral cycle. Samples of the *uterine tube* were obtained for histological studies by vivisection from three sections of *uterine tube*. Samples were fixed for light microscopy (LM) in formaldehyde and in glutaraldehyde paraformaldehyde for scanning (SEM) and transmission (TEM) electron microscopy. They were subsequently processed in the usual manner in the LM and electron microscopic studies laboratories. We did not detect progressive changes in the length of the *uterine tube*. Unlike the sows' weight (2.57 ±1.12 g or 2.26 ±0.96 g), the length of the *uterine tube* was virtually unchanged depending on the stage of the cycle (30.2 ±2.51 cm in FF or 30.1 ±2.39 cm in LF). The largest relative volume of the epithelial layer was at the follicular stage of the cycle along the entire *uterine tube*. The difference varied from 4.99% – *isthmus* to 13.62% *infundibulum* between each part. Significant changes were seen between the ciliary and secretory cells during the estral cycle in the various parts of the *uterine tube*. Ciliary cells dominated throughout the cycle in infindibulum and *ampulla*, whereas secretory cells in *isthmus*. Their changes and differentiations are the manifestations of hormonal changes that direct the estral cycle. Submicroscopic changes of cells in the estral cycle have also been described.

**Keywords:** sows, *oviduct – uterine tube*, histology

## 1    Introduction

The *uterine tube* is a tubular organ that ensures transitory survival of gametes and embryos. Series of precisely initiated processes requiring full completion take place in the *uterine tube* (Besenfelder et al., 2012). Oviduct plays a key role in sperm fertilization, development of a zygote, capacitation and transport of a zygote to the uterus (Lauschova 2003, Sangha et al. 2003, Tienthai et al., 2009, Sharma et al., 2013).

The *uterine tube* is composed of the *infundibulum*, the *ampulla* and the *isthmus*, connecting *uterine tube* to the uterus (Hafez, 1987; Sharma et al., 2013). The *oviduct* wall is made up of muscle layers arranged lengthwise and circularly and of the *mucous membrane*. The mucouse is organized into a number of folds (Kenngott and Sinowatz, 2007), consisting of ciliary and non ciliary secretory cells and lamina propriae mucosae layer (Uhrín,1992).

These structures provide the conditions for a transport, a survival, a capacitation of sperm and fertilization of an ovulated oocyte (Koelle et al., 2010). The secretory cells play an important role in the developmental changes taking place in the *uterine tube* (Prichard et al., 1992). The ciliary cells fulfill the transport role in transporting the immobile oocyte through the *uterine tube*.

The importance of the *uterine tube epithelium* and its secretions has been described in various studies during the estrus, or menstrual cycle in mammals, including humans, monkeys, cows, sheep and pigs (Verhage et al., 1979; Brenner, 1969; Bjorkman and Fredricsson, 1961; Hadek, 1955). The fastest growing part of the *uterine tube* is the *mucous membrane*, which plays an essential role in the physiologyIn general, the *infundibula*ry and *ampulla*ry regions have more ciliary cells than the isthmic region.

The ciliary cells are predominant in the *uterine tube* region with a prevalence of the folds. The quantity of the secretory cells gradually increases towards the istmus (Abe, 1994; Senger, 2003). In the period of ovulation the quanity of higher ciliary cells is increased. Steinhauer et al. (2004) describes a slightly coloured ciliary cells with

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localized apical nuclei differing from basofil secretory cells with apical projections. Cell height and percentage of ciliary cells were significantly higher than in the anestrus. High levels of P4 during the middle phase of the luteal phase is associated with differentiation and dedifferentiation of cells, as well as with regression of cells that are visible on the lining of the folds in the *uterine tube*. The percentage and amount of the ciliary cells were significantly lower than during the late follicular phase.

Shirley and Reeder (1996) describe large amounts of the secretory cells in rats. The ciliary cells are smaller with a shorter cilium in an *ampullar*y region during the estrus and metestrus. In the course of the estrus the secretion of the secretory cells accumulates in the apical end of the cells. This causes the cell protrusion to the lumen of the *uterine tube* at the time of diestrus (Shirley and Reeder, 1996).

The longest part of the *uterine tube*, the *ampulla*, is the extended tubular area where the process of fertilization is completed (Bosch and Wright, 2005), and its lining is made up of primary and secondary folders (Abe, 1994) and tertiary folders are created as well in the 9 month old sows during estrus (Šťastný and Lacková,1987). Bullón et al. (1980) describes epitelium cells in the *oviduct*, which he named as "basal", "storage", or as "indifferent" cells. Those are localized in the basal layer of the *epithelium*, are small, round, or oval in shape and having the heterochromatic nuclei. The cells are specified as non-differentiated and can be transformed into a secretory or ciliary cells.

Between the ciliary cells are the plug cells that contain the apical granules and create the secretions of the fallopian tube. Progesterone increases the number of plug cells, whereas estrogen increases their height and secretory activity (Shirish and Chakravarti, 2011; Lacková and Šťastný, 1987). The secretory cells of the *epithelium* contain a considerable amount of intra-cytoplasmic granules, which are considered to be released by the mechanism of the exocytosis (Abe,1994). These granules are distinctly smaller in the cells of the *ampulla* during the luteal phase. These granules were present in a smaller quantities also in the cytoplasm of the *isthmus* cells during the luteal phase of the cycle (Abe and Hoshi, 2007). The structure of the fallopian tube is primarily affected by the influence of hormonal activity as evidenced by changes in the mucous of the fallopian tube during the estral cycle (Hackett and Hafs,1969; Shirley and Reeder, 1996; Abughrien and Dore, 2000; Ulbrich et al., 2010, Lewis and Berardinelli, 2001; Steinhauer et al., 2004). It has also been found that the process of ciliation and deciliation of the *oviduct epithelium* depends also on steroid hormones (Verhage et al., 1979). Shirley and Reeder (1996) describe changes in the number of secretory and ciliary cells in rats during the estral cycle.

## 2 Material and methods

### 2.1 Animals

Tissue samples were taken from the fallopian tube of 40 sows (crossbreds Slovak Large White and Landrace breeds). Animals (primipara) with a small litter were excluded from further breeding. Sows were divided into 2 groups according to the stage of the estral cycle (the follicular phase and the luteal phase, with each group consisting of 20 animals). Age of the pigs at the time of slaughter was 368–382 days. The body weight of sows was in the range of 123–141 kg. The ovarian tube changes in the estral cycle were assessed according to the individual cycle control and according to the post mortem image on the ovaries. The follicular stage (FS) of the estral cycle was limited by the 20th day of the estral cycle (beginning) and the first day of reluctance to mate (end). The luteal stage (LS) of the estral cycle was limited by the third day from the reluctance to mate (start) and the 17th day of the estral cycle (end). All the animals have been slaughtered in a slaughterhouse in the usual way and immediately after bleeding their reproductive organs were remove.

### 2.2 Material

The samples from *oviduct* were taken from *infundibulum*, *ampulla* and *isthmus* for light (LM), transmission (TEM) and scanning electron microscopy (SEM). Samples for LM were fixed in 10% formol (Merck Millipore), dehydrated by a sequence of alcohols and sealed in paraffin 8–10 μm thick slices were made from blocks, which were coloured by haemalaun eosine (Merck Millipore) and by greens trichrome (Merck Millipore). For histochemical proof of glycogen and PAS – positive substances we have used samples fixed in Gendres solution (Vacek, 1974) with PAS reaction (Schiff´s reaction periodic acid-Schiff, Merck Millipore). Sections were evaluated on LM (Olympus Provis AX) with Image ProPlus (Spectra Services Inc, NY) program designed for assessment of individual morphological structures and MS Excel 2000. Samples from the same parts and regions of *oviduct* were taken for electronmicroscopic studies (TEM, SEM). Samples were fixed in 4% solution of glutaraldehyde paraformaldehyde (pH 7.4 Merck Millipore) with 0.08 M – cacodylate buffer (pH 6.9–7.1). For post fixation for TEM we used 1% osmiumoxid (Merck Millipore) with phosphate buffer (Milloning, 1962), samples were rinsed by Milloning´s phosphate buffer and sucrose. They were dehydrated by ascending sequence of ethanols, rinsed by propylene oxide (Merck Millipore) and deluged in the compound Durcupan ACM (A Fluka A. G., Buchs.

Switzerland-Registered Trademark). Semi-thick (1 µm) and ultra-thin slices were made on ultramicrotome (LKB 8800 III). Semi-thick slices were coloured by Toluidine blue (Merck Millipore) and assessed on (Olympus Provis AX). Samples for SEM were rinsed and dehydrated after fixation (3 hours) in ascending sequence of acetones and desiccated with $CO_2$ (CPD Polaron, England). Dried samples on fixtures were then vacuum-coated with 20 nm thick layer of gold (All Chemie LTD, US). Ultra-thin slices were contrasted with lead citrate (Reynolds, 1963) and uranyl acetate (SPI Supplies and Structure Probe, Inc). Electronograms were made on TEM (TESLA BS 500) and SEM (TESLA BS 301). Morphometric methods were used for objectification of results (Weibel et al., 1966; Mráz and Polónyi,1988).

## 3    Results and disscussion

Macroscopically *uterine tube* is quite simple organ, in the cyclic gilts is 16.0 cm to 27.0 cm long with weight from 0.86 g to 2.33 g (Šťastný and Lacková, 1987). In sows in the estrusl cycle its length is virtually unchanged (30.2 cm or 30.1 cm, Table 1) in contrast to the weight (2.57g or 2.26 g, Table 1). In the gilts (Šťastný and Lacková, 1987) as well as in the case of heifers, Šťastná et al. (2013) found that the length of the *uterine tube* changed with the estrus cycle change. Significant gradual shortening has passed from estrus to diestrus (*P* <0.01). The *uterine tube* is divided into three parts: *infundibulum*, *ampulla* and *isthmus* (Menezo and Guerin, 1997; Ellington, 1991).

**Table 1**    Weight and length of the *uterine tube* (*n* = 40)

|  | FF | LF |
|---|---|---|
| Weight (g) | 2.57 ±1.12 | 2.26 ±0.96 |
| Length (cm) | 30.2 ±2.51 | 30.1 ±2.39 |

FF – follicular phase; LF – luteal phase

Sperm and oocyte enter the *uterine tube* from opposite ends. In order to meet, they use the *oviduct*al countercurrent system for transfer. Its structure is also adapted for this purpose. The *oviduct* consists of three parts (*infundibulum*, *ampulla* and *isthmus*) that have a longitudinal and circular muscular layer and a *mucous membrane* (Besenfelder et al., 2012; Menezo and Guerin, 1997; Ellington, 1991). These parts are characterized by polymorphic folds and ridges of varying sizes. The folds are longitudinally different, forming secondary – to – tertiary folds, forming irregular net-like structures forming different troughs, vesicles and crypts (Yaniz et al., 2000; Kenngott and Sinowatz, 2007). Sharma et al. (2015) describes characteristic pattern of variations in the *ampulla*ry segment of the *uterine tube* during follicular and luteal phases of estrous cycle in goat. The *tunica mucosa* of *ampulla* was characterized by presence of longitudinal mucosal folds throughout the length with extensive secondary and tertiary branches in follicular phase, whereas the luteal phase was characterized by primary and secondary branching patterns. Similar differences can also be seen when comparing the *ampulla* mucosa and the *isthmus* of sows in the same phase of the estrus cycle (Figure 1).

The epithelial layer fold volume varied depending on the ongoing phase of the estrus cycle of cycling heifers (Šťastná et al., 2013). The size of the epithelial cells is minimal in the proestrus (Hackett and Hafs, 1969 presented as well by the relative volume of the epithelial layer in the follicular phase of sows (Figure 2), which is dominant in all parts of the *oviduct* (80.96%, 74.64%, 71.55%, respectively.). Also according to Uhrin (1992) in cows after the follicular phase the volume of the mucosa gradually decreases in the diestral stage of the cycle. Natarajan et al. (2003) believes that increasing level of estrogen in the follicular phase is responsible for increasing the height of the mucosa that
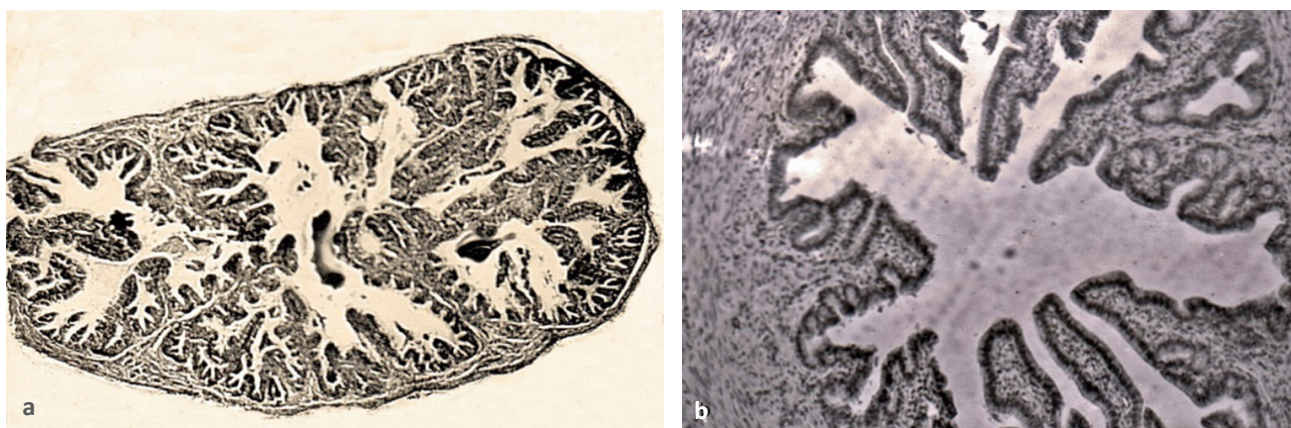


**Figure 1**    Pronounced primary and secondary folds leading to the centre of the duct can be seen on the transverse section of the *ampulla* of the *oviduct* in the follicular phase of estrus cycle (a) and transverse section of *isthmus* (b) has mainly primary folds in the follicular phase, HE, mag. ×330
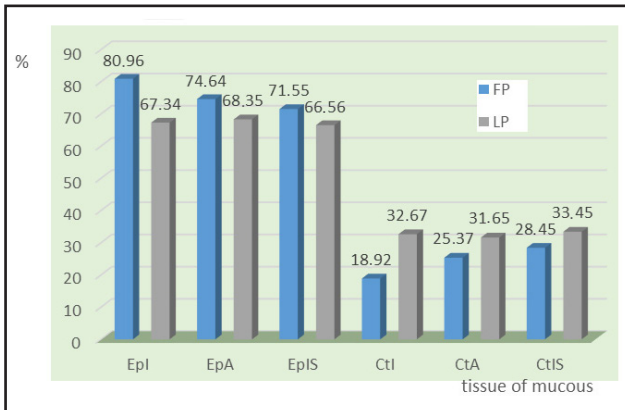
**Figure 2** Epitelial and conectical tissue percentage in the different parts of the *uterine tube*
FP – follicular phase; LP – luteal phase; EpI – epitel of the *infundibulum*; CtI – connective tissue of the *infundibulum*; EpA – epitel of the *ampulla*; CtA – connective tissue of the *ampulla*; EpIS – epitel of the *isthmus*; CtIS – connective tissue of the *isthmus*
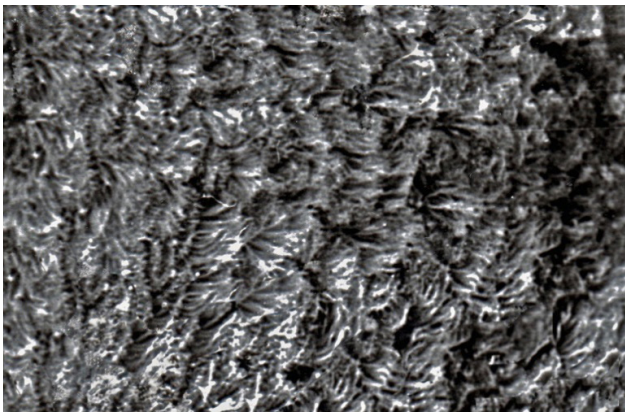


**Figure 3** Scanning electron micrograph of infundibullary region of *oviduct* during follicular phase revealing a richly ciliated *epithelium* with uniformed cilia length, coated in gold, mag. ×6,320
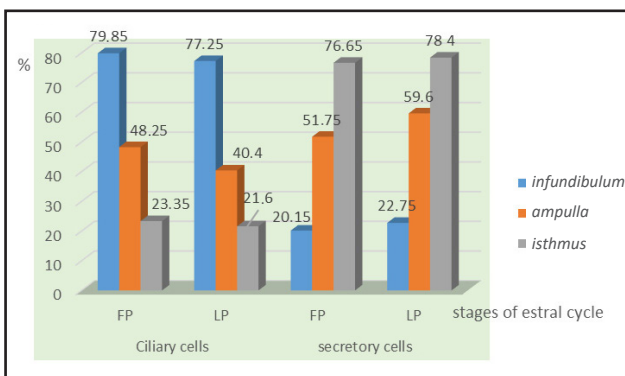


**Figure 4** Ciliary and secretory cells precentage in different parts of the *uterine tube*
FP – follicular phase; LP – luteal phase

decreases during the luteal phase. The epithelial layer is composed of ciliary and non-ciliary secretory cells. Their representation varies in the individual parts of the *oviduct* depending on the stage of the estrus cycle. *Infundibulum* and *ampulla* usually have more ciliary cells than *isthmus*. The ciliary cells are slim, cylindrical in shape, attached to the basal membrane and covered by dense and relatively long cilia. They may be lighter and darker with condensed chromatin in the nucleus (Uhrin, 1992). The *infundibular* ciliary cells virtually overlapped the secretory cells in the follicular phase (Figure 3), which formed only 20.15% in this part of the *oviduct* (Figure 4). This ratio varies mainly in *ampulla* (*P* <0.01) and *isthmus* in the luteal phase of the estral cycle when the predominance of secretory cells is evident (Figure 5). In contrast, the ratio of secretory
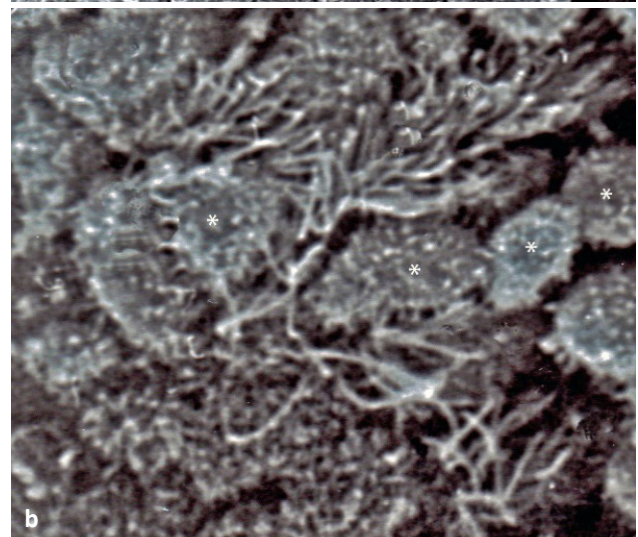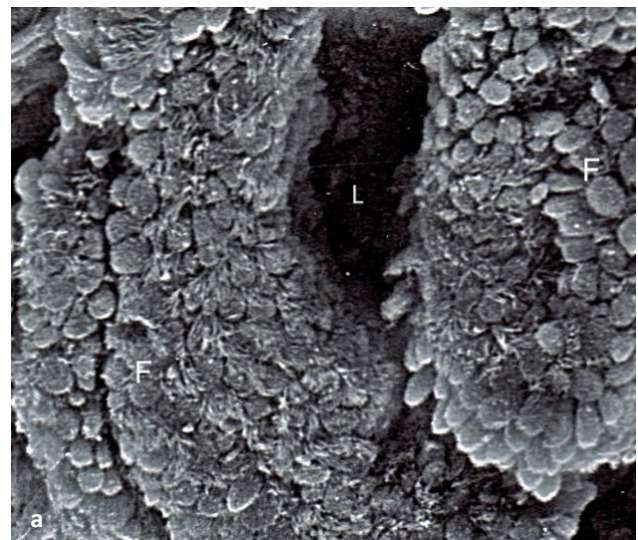


**Figure 5** Scanning electron micrograph of *ampullary* region of *oviduct* during luteal phase of estral cycle revealing a richly evident secretory cells on mucous lines (a), coated in gold, mag. ×5,000, enlargement (b) mag. ×16,200
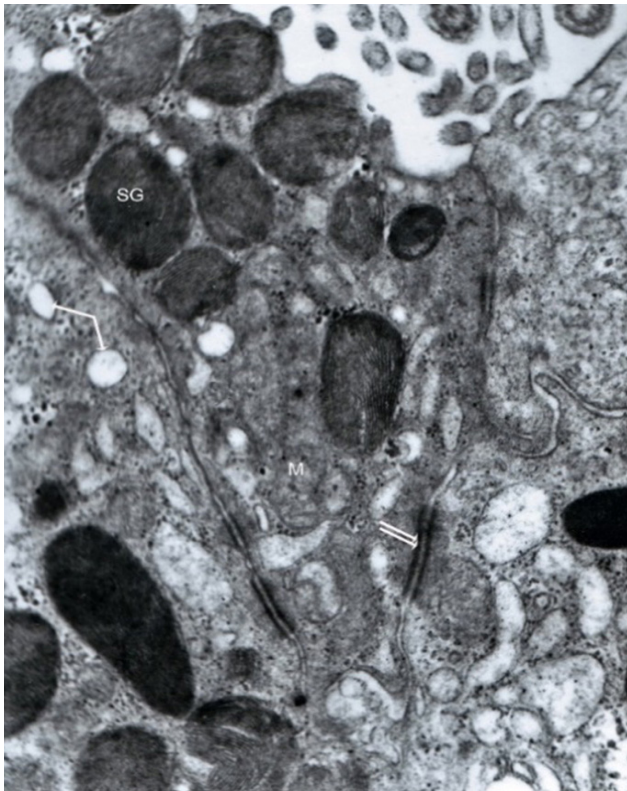L – lumen, F – folds, * – secretory cells

**Figure 6** Transmission electron micrograph of *ampullary* region of *oviduct* during follicular phase showing quantum of the secretory granules in the apically ends of the secretory cells
SG – secretory granules, arrows – lipidic drops, double arrows – desmosome), mag. ×14,000



**Figure 7** Transmission electron micrograph of *ampullary* region of *oviduct* during luteal phase showing apical protrusions (P) of secretory cells with the secretory granules (arows)
N – nucleus, M – mitochondria, G – Golgi, mag. ×19,920
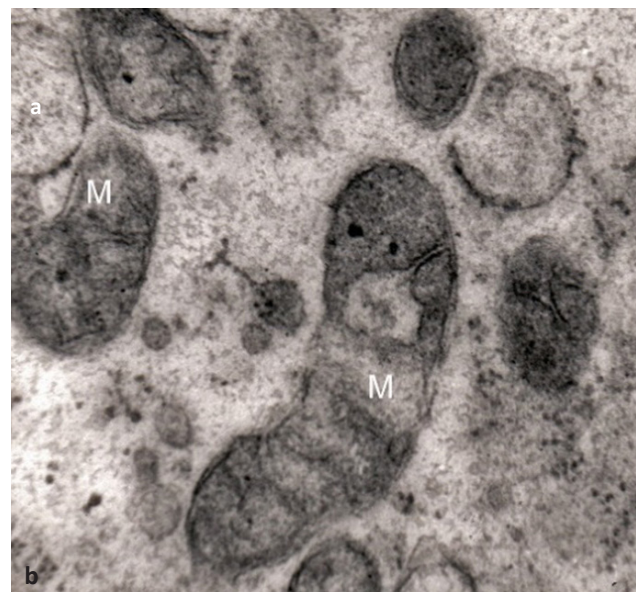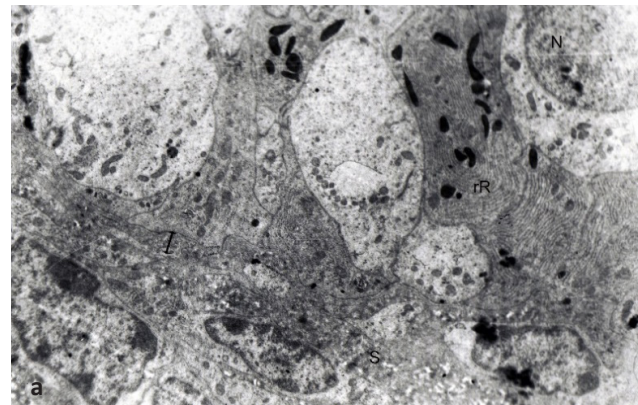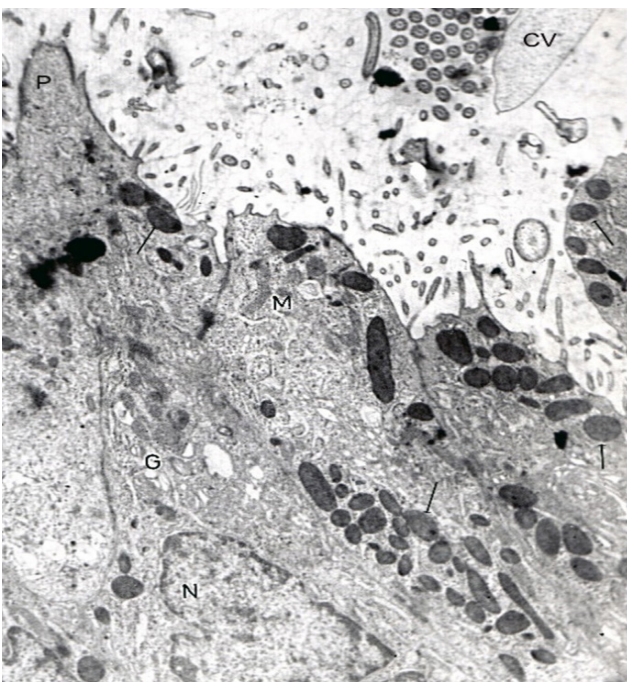


**Figure 8** Transmission electron micrograph of *ampullary* region of *oviduct* during luteal phase showing the basal cells with the presence of numerous ribosomes, a large rough endoplasmic reticulum (a) and well-developed mitochondria (b), mag. ×9800 (a), ×76,000 (b)
N – nucleus, R – rough endoplasmic reticulum, S – submucous, arrows – basal membrane, M – oval mitochondria

and ciliary cells according to Abe et al. (1999) in the goat *oviduct* is not different in the luteal and follicular phases, but reduction in cell size, especially ciliary cells in the luteal phase of the cycle has been found. Similar findings are reported by Abe and Oikawa (1992) in sows and by Abe and Oikawa (1993) in cows. These cells are characterized by a decreased number of secretory granules, the presence of numerous ribosomes, a large and rough endoplasmic reticulum and a well-developed mitochondria (Figure 8a, b). These changes can also be seen in basal cells. The most characteristic property of secretory cells are the secretory granules. Their amount and size vary depending on the stage of the cycle (Abe et al., 1999). It is found in the follicular phase in the *ampulla* and less in the *infundibula* and the least in the
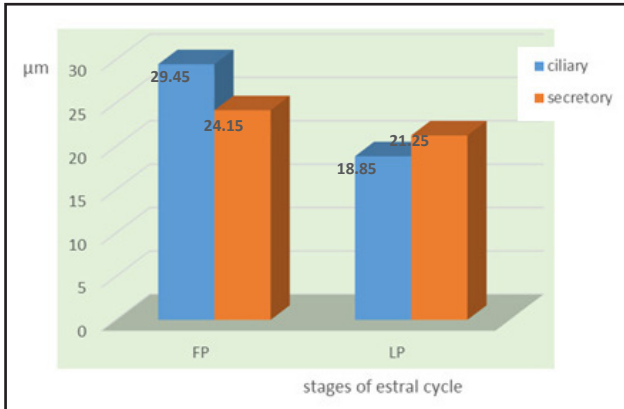
**Figure 9** The heigth of the epitelial cells of the *uterine tube* mucosa
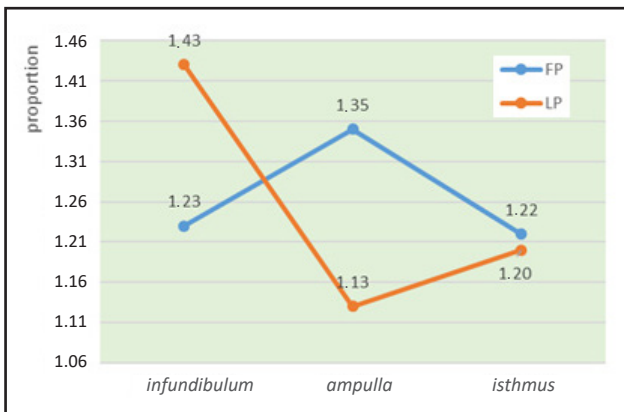FP – follicular phase; LP – uteal phase



**Figure 10** Nucleo-cytoplasmatic proportion of cells of the *uterine tube* mucous (nucleus = 1)
FP – follicular phase, LP – luteal phase

*isthmus*. This also assumes a different secretory activity of cells (Abe et al., 1999). These changes in secretory cells should be the result of the steroid hormones action on epithelial cells and the different reaction of different sections of the *oviduct* to these hormones (Abe et al., 1999). A number of intracytoplasmic granules and lipid droplets (Figure 6) are seen in the secretory cells of the *epithelium*, that are released from the cell by the mechanism of exocytosis (Figure 7). Secrete granules are located primarily in apical cell sections as densely oval forms. They occur the most in the later follicular phase of the cycle (Figure 6), followed by their intense excretion and the decrease in the luteal phase (Uhrin, 1992). Density structures that are similar in size and shape to the cell core have often been extruded from the epithelial layer of the *oviduct*. These granules significantly decreased in *ampulla*ry cells in the luteal phase. Many granules were observed throughout the ovarian cycle in the cytoplasm of isthm cells, with the exception of the luteal phase, where granules were reduced (Abe and Hoshi, 2007). The epithelial cells are higher in the estrus and in the time of incoming ovulation and are reaching the peak when compared to diestrus (Yaniz et al., 2000; Abe and Hoshi, 2008; Ulbrich et al., 2010; Nakahari et al., 2011). Findings of Steinhauer et al. (2004) that the cells in the follicular phase are higher than in the luteal phase were also confirmed in sows (Figure 9). The size of the epithelial cells varies according to Mc Daniel Scalzi and Black (1968) due to the progesterone action. Relative changes in volume of cells alter also the nucleocytoplasmic ratio (NCR) in both cell types in estral cycle. The highest NCR (1 : 1.43) is in the luteal phase of the estral cycle in the *infundibulum* region and the lowest (1 : 1.13) in the *ampulla* of the
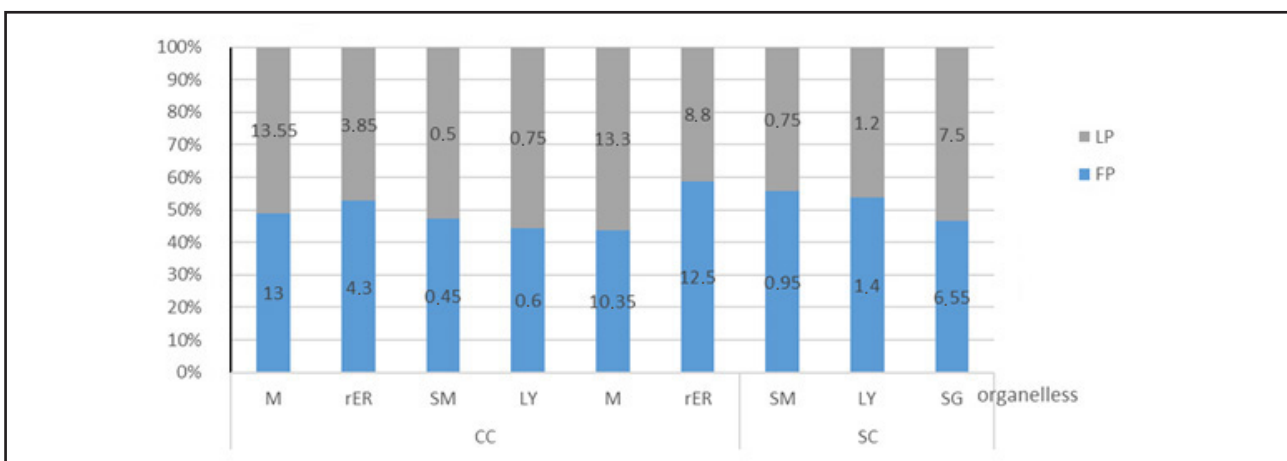


**Figure 11** The volume of the organelles in the ciliary and the secretory cells of the *uterine tube* during estral cycle (% of the volume of cytoplasm)
Cc – ciliary cells; Sc – secretory cells; M – mitochondria; rER – rough endoplasmatic reticulum; SM – smooth membranes; LY – lysosomes; SG – secretory granules

*oviduct* in the luteal phase of the cycle (*P* <0.01, Figure 10). The increase in secretory activity in the cytoplasm also changes NCR (Uhrin, 1992). In the follicular phase of the estral cycle, the highest ratio was found in the *uterine tube* (1 : 1.35, *P* <0.05). Šťastná et al. (2013) found the lowest NCR in the proestrus of both cell types in the heifers (1 : 1.16 or 1 : 1.36) since cell nuclei enlarge at this time, but the volume of the cytoplasm does not change over the diestrus. Approximately the same volume of mitochondria was present in ciliary and secretory cells in the luteal phase of the cycle (13.55 vs. 13.3%, Figure 11). Follicular phase was dominated by mitochondria in ciliary cells. In contrast, the rough endoplasmic reticulum and smooth endoplasmic reticulum had a significantly higher volume in secretory cells throughout the cycle (*P* <0.01). The cell nuclei were localized at different cell levels, depending on the phase of the cycle, with predominantly granulated density nucleoplasm.

## 4 Conclusions

The thesis describes structural quantitative and qualitative changes in the ovary of sows in the estral cycle. Progressive changes in mucosal structures, particularly the epithelial component of the mucosa have been confirmed. Different qualitative and quantitative changes were observed in ciliary and secretory cells under steroid control. Quantitative and qualitative changes in the estral cycle also indicate that they are under hormonal control and are differentiated at different stages in the cycle. This is related particularly to the secretory cells undergoing more intense changes at both microscopic and submicroscopic level. The numerical differentiation of ciliary and secretory cells in individual parts of the sows' *oviduct* was confirmed as well. Photo documentation supports the description of detected microscopic findings.

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