

## Morphological changes of oviduct in postnatal development and in oestrous cycle of heifers

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The aim of the work is to describe microscopic and sub-microscopic changes of oviduct of 40 heifers of Pinzgau breed in post-natal development (3<sup>rd</sup>, 6<sup>th</sup> and 9<sup>th</sup> month of age) at subclinical experimental hypoglycaemia (the value of glucose in blood plasma was 1.9 – 2.2 mmol) and in oestrous cycle. The oviduct samples for histological study were gained by vivisection from three parts of oviduct. Samples were fixed for light microscopy (LM) in formaldehyde and for scanning electron microscopy and transmission electron microscopy in glutaraldehyde paraformaldehyde. Subsequently they were processed by usual method which is use in laboratory for LM and electron microscopical studies. We discovered progressive changes in the length of oviduct in post-natal period (+7.9 cm up to 9<sup>th</sup> month) and in comparison with sexually mature animals retarded growth at hypoglycaemic 15 months old heifer (18.0±1.8 cm). The length of oviduct in cycling heifer varied depending on the stage of cycle (21.7±1.9 in diestrus or 24.8±2.5 in oestrus). At the same time we have discovered progressive changes in relative volume of epithelium during development depending on the part of oviduct and age. This volume was on the level of 3<sup>rd</sup> – 6<sup>th</sup> month of age at hypoglycaemic animals. The most significant changes were observed in ampulla and infundibulum during the development as well as during oestrous cycle. The same tendencies were observed as representation and character of epithelium cells. There were no ascertained significant changes between ciliary and secretory cells, with the exception of hypoglycaemic heifers. This cannot be assumed about these cells during oestrous cycle. In their representation there were hormonal changes regulating individual stages of cycle. The sub-microscopic changes of cells in post-natal development as well as in oestrous cycle were also described. The energy deficit appeared in retardation of growth and development changes at animals with subclinical form of hypoglycaemia.

**Keywords:** heifers, oviduct, histology, hypoglycaemia

### 1. Introduction

Oviduct is a canal organ which creates conditions for temporal existence of gametes and embryos. A series of activities, which have to be precisely and in time initiated and fully completed, take place in oviduct (Besenfelder et al., 2012). The oviduct plays an important role in reproduction in such processes as sperm capacitation, fertilization and early embryonic development. It has three parts – infundibulum, ampule and isthmus by which it connects to uterus. The oviduct wall is composed of muscular layer arranged lengthwise and circularly, and from mucous. The mucous is arranged into numerous folds (Kenngott and Sinowatz, 2007) made by ciliary and non ciliary epithelial cells and the layer of lamina propriae mucosae (Uhrín, 1992). These structures should secure all necessary conditions for transport, survival, sperm capacitation and successful fertilization of ovulated oocyte (Koelle et al., 2010). Secretory cells play an important role in reproduction and in developmental changes which take place in oviduct (Prichard et al., 1992). Ciliary cells fulfil an important role at transport of immovable oocyte into the oviduct.

The prenatal development of oviduct is accompanied by creation of mucous fimbriae at the time when the foetus crown – rump length (CRL) has the length only 29 cm (Kenngott and Sinowatz, 2007). The differentiation of epithelial cells of heifer oviduct into secretory and ciliary happens in the prenatal development too (Kenngott et al., 2008). Cilliogenesis and cells with complete ciliation were discovered at the CRL foetus length 55 cm. The secretory cells with numerous secretory granules in supranuclear area differentiated at the CRL length 94 cm. The development of oviduct continues even in the postnatal period by quantitative and qualitative changes of its tissue structures. At birth, the oviduct is 12.4 cm long (Salisbury et al., 1985), in time of genital maturity it has 20.9 cm (Bielaňski, 1972) and mature cows have oviducts 25 cm long (McDonald, 1975). The mucous is the most dynamically developing part of the oviduct. It has an important function in its physiology. In general, the infundibular and ampullary part have more ciliated cells than the isthmus part. It is generally known that the ciliated cells dominate in the part of oviduct with the majority of fimbriae and the number of secretory cells

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gradually increases towards isthmus (Abe, 1994; Senger, 2003). But in the ovulation period there are much more higher ciliated cells. As regards rats, Shirley and Reeder (1996) talk about large number of secretory cells. Ciliary cells are smaller with shorter ciliaries in ampullare part during estrous and metestrus. During estrous the secretion of secretory cells concentrates in the apical end of cell, due to what the cell protrusion into lumen of the oviduct in time of diestrus is created (Shirley and Reeder, 1996).

Secretory cells have maximum height in diestrus (Ulbrich et al., 2010). Based on the findings of Hackett and Hafs (1969) the height of epithelium during oestrous cycle varies between 20.7  $\mu\text{m}$  (20<sup>th</sup> day) up to 25.6  $\mu\text{m}$  (7<sup>th</sup> day). Bullón et al. (1980) in epithelium describes cells which he named as “basal”, “reserve” or “indifferent” cells. They are located in the basal layer of epithelium, they are small, have round or oval shape, they have heterochromatic nucleus. The cells are described as non-differentiated and can be transformed into secretory or ciliary cells. The most conservative part of the oviduct wall is the muscular layer which does not change during the oestrous cycle (Uhrín, 1992).

Koelle et al. (2009) studied the frequency of beat movement of cilia and state that the cilia activity differs in proximal, medial and distal part of the oviduct. They assume that the ciliary activity is independent on the phase of oestrous cycle and gravidity stage, but the local detection system, which is initiated by the presence of embryo, modulates the ciliary activity, vascularisation and forming of secretory cells in the near surroundings.

Secretory cells of epithelium have significant number of intra cytoplasmatic granule, which are considered to be released by the exocytosis mechanism. These granules are clearly smaller in the cells of ampullare during luteal phase. These granules were also located in isthmus cell cytoplasm and there was lower number of them during luteal phase of the cycle (Abe and Hoshi 2007).

The oviduct structure is primary influenced by the effect of hormonal activity which is shown by the mucous changes of the oviduct during the estrous cycle (Hackett and Hafs, 1969; Shirley and Reeder, 1996; Abughrien and Dore, 2000; Ulbrich et al., 2010), but the changes can appear secondary at the change of metabolic status. Haase et al. (2012) studied the effect of long-term negative energy balance (NEB) on the mRNA manifestation in oviduct. These results show that the NEB can have long-term effect on the mRNA manifestation rules, which could lead to very poor fertility.

## 2. Material and methods

The samples of tissue were taken from oviducts of 40 heifers of Pinzgau breed, which were divided into 8 groups based on their age, phase of sexual cycle and metabolic status. The postnatal development of oviduct was monitored in 3<sup>rd</sup>, 6<sup>th</sup> and 9<sup>th</sup> month of age (in every group of 5 animals). Changes

of oviduct in oestrous cycle were monitored after individual oestrus check of 20 heifers (5 animals/phase of cycle) at the age of 15 months and live weight of 310 – 340 kg. In case of 5 heifers a subclinical form of hypoglycaemia (the value of glucose in blood plasma was 1.9 – 2.2 mmol-using commercial spectrophotometric kits, plasma was analyzed for glucose – Sigma Tech, Bull.; Sigma Chemical, St. Louis, MO at 14 day intervals) was experimentally induced in 8<sup>th</sup> month of age and was maintained until 15<sup>th</sup> month, while at that time these animals had average live weight of 212.3 kg. All animals were slaughtered in line with the current state by usual method and the reproductive organs were removed immediately after draining of blood. The samples from oviduct were taken from infundibulare, ampullare and isthmus for light (LM), transmission (TEM) and scanning electron microscopy (SEM). Samples for LM were fixed in 10 % formol (Merck Millipore), dehydrated by sequence of alcohols and deluged into paraffin. From blocks were made 8 – 10  $\mu\text{m}$  thick slices, which were coloured by haemalauneeosine (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 program bound for assessment of individual morphological structures Image ProPlus (Spectra Services Inc, NY) and MS Excel 2000.

Samples from the same parts and places of oviduct were taken for electronmicroscopic studies (TEM, SEM). They were fixed in 4 % solution glutaraldehyde paraformaldehyde (pH 7.4 – Merck Millipore) with 0.08M – cacodylate buffer (pH 6.9 – 7.1). For post fixation for TEM we used 1 % osmiumoxid (Merck Millipore) with phosphate buffer (Millonig, 1962), samples were rinsed by Millonig’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  $\mu\text{m}$ ) 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 after fixation (3 hours) rinsed and dehydrated in ascending sequence of acetones and desiccated with the help of CO<sub>2</sub> (CPD Polaron, England). On the fixtures, the dry samples were then by vacuum steaming metalized with 20 nm thick layer of gold (All Chemie LTD, US).

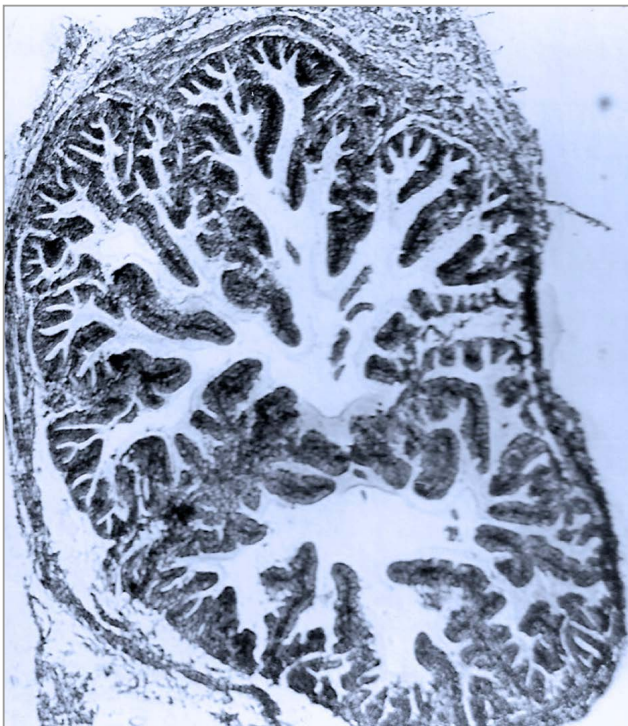
Ultra-thin slices were contrasted with lead citrate (Reynolds, 1963) and uranyl acetate (SPI Supplies and Structure Probe, Inc). Electronograms were made on TEM (TESLA BS 500) and SEM (TESLA BS 301).

Morphometric methods were used for objectification of results (Weibel et al., 1966; Mráz and Polónyi, 1988).

### 3. Results and discussion

The oviduct is in terms of macroscopy rather simple organ, with approximate length 21 – 28 cm. It is divided into three parts: infundibulum, ampulla and isthmus (Menezo and Guerin, 1997; Ellington, 1991). Stated dimensions are gradually reached in postnatal development by progressive growth changes (table 1).

At the beginning of sexual maturity (9<sup>th</sup> month of life) it does not reach such parameters despite cycle manifestation as with heifer with repeated cycle in time of breeding maturity (15<sup>th</sup> month of life). In contradistinction to that, 15 months old heifer which were in the state of subclinical experimental hypoglycaemia had evidentially shorter oviduct ( $P < 0.01$ ) in comparison to regularly cycling 15 months old heifer and shorter in comparison to 9 months old animals ( $P < 0.05$ ; table 1). The level of nutrition in pre-pubertal age of heifer development influences the beginning of effective hormone production for the growth of follicles and production of steroid hormones on forming of genitalia. Intensive nutrition in post-natal period of life of heifer accelerates the transition



**Figure 1** The mucous of the ampulla oviduct of 3 month old heifer formed only primary fimbriae. The identical picture provides also mucosa of the oviductus ampulla of 15 month old heifer with subclinic hypoglycaemia, HE, mag.  $\times 330$

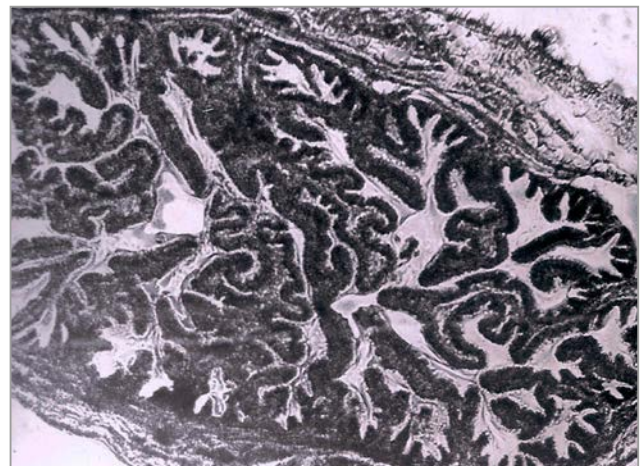
into puberty in comparison with normal nutrition (Gasser et al, 2006), although the development of genitalia is not completely finished until the 12<sup>th</sup> month of life. On the other side Monteiro et al. (2003) did not discover any difference in histological structure of oviduct between heifer and cows, but they do not specify the heifer age or cycle stage.

The length of oviduct changes with the change of oestrus cycle (table 1). The gradual significant shortening went from oestrus to diestrus ( $P < 0.01$ ) and although the oviduct was shorter in comparison to values in other phases of the cycle, these differences were not proved.

The progressive tendency in post-natal development of oviduct can be seen in the change of relative volume (RV) of epithelial component of mucous (table 2).

In 6 months the RV of epithelial layer of infundibulum mucous increased of 5.9 % and of ampulla of 7.3 % ( $P < 0.01$ ). It is obvious that by gradual increasing of secondary and tertiary fimbriae (figure 1, 2) of oviduct the epithelium layer was formed. There were no ascertained yellow bodies (CL) and pre-ovulatory follicles in 9<sup>th</sup> month of age at the observed heifer. There were only follicles with the size up to 10 mm. The size of epithelium of uterus and infundibulum of oviduct is according to Larson et al. (1971) much lower in young calves than in older calves, so the pre-pubertal organs react to progesterone (Spilman et al., 1970).

The volume of epithelium layer at sexually mature, cycling animals changes depending on the current phase of the oestrous cycle (table 3).



**Figure 2** The mucous of the ampulla oviduct of 9 month old heifer formed numerous fimbriae with rich branchiness (tertiary fimbriae too), HE, mag.  $\times 330$

**Table 1** Length of the oviduct (in cm) in a postnatal development, in a hypoglycaemia and in an oestrous cycle

	Age in month			Estrous cycle*				Hypoglyc. (15 M)
	3	6	9	P	E	M	D	
$\bar{x} \pm s$	11.8 $\pm$ 0.3	15.4 $\pm$ 0.7	19.7 $\pm$ 0.7	23.2 $\pm$ 1.8	24.8 $\pm$ 2.5	24.1 $\pm$ 2.0	21.7 $\pm$ 1.9 <sup>a</sup>	18.0 $\pm$ 1.8 <sup>ab</sup>

<sup>a</sup> –  $P < 0.01$  (15H: P, E, M); <sup>b</sup> –  $P < 0.05$  (15H: D); 15M: 15 months old; \*P – proestrus; E – estrus; M – metestrus; D – diestrus

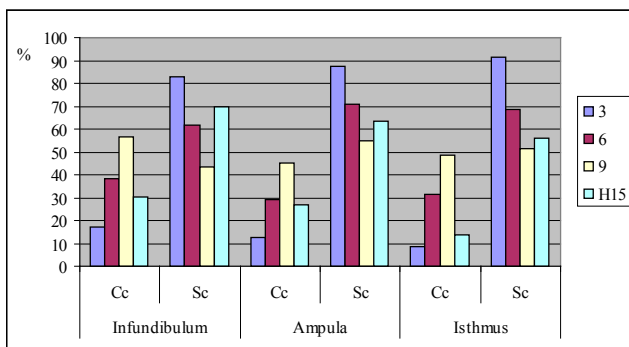


**Table 2** Relative volume of the oviducts` epithelium in a postnatal development and in a hypoglycaemia (% of a mucosal volume)

	Age in month			Hypoglycaemia (15M)
	3	6	9	
Infundibulum	54.1 ±4.2	60.0 ±3.0	61.1 ±5.4	61.9 ±65.2
Ampula	52.7 ±6.5	59.9 ±5.8a	60.0 ±4.5	58.6 ±82.3b
Isthmus	53.3 ±3.6	59.4 ±3.2	57.3 ±3.4	48.5 ±33.7a

<sup>a</sup> –  $P < 0.01$  (6: 3 amp.; 15 H: 3, 6, 9 istm.); <sup>b</sup> –  $P < 0.05$  (15H: 9 amp.); 15 M: 15 months old

The size of epithelium cells is minimal in proestrous (Hackett and Hafs, 1969) which is represented by relative volume of epithelium layer (table 3). It gradually increases through oestrous up to 7<sup>th</sup> day of the cycle and again decreases into diestrous and new proestrous stage (Uhrín, 1992). In contrary to these data, the size of epithelium cells has progress according to McDaniel et al (1968) due to the effect of progesterone. Hackett and Hafs (1969) stimulated the size of oviduct epithelium in the early luteal phase of oestrous cycle, and not during oestrous. In comparison to 9<sup>th</sup> month of age the cycling animals had higher RV of the epithelium layer during the whole cycle, but the values were significantly higher of 8.8 % in the oestrous phase ( $P < 0.05$ ). The smallest RV of epithelium during oestrous cycle was ascertained in the isthmus of oviduct ( $P < 0.05$ ). At animals with subclinical form of hypoglycaemia, despite their age, which represents the period of full reproduction activity of heifers, on their oviducts there were identified only follicles of medium size, similarly as in 9 months old heifers, and this complies with the RV of epithelium layer, which was in ampulla lower than at 6 months old (-1.3 %) and in isthmus lower of 4.8 % than in 3 months old ( $P < 0.01$ ). In comparison to the heifer of the same age in cycle the lowest value of RV measured in isthmus was 14.9 % lower than the lowest value in proestrous of cycling animals ( $P < 0.01$ ; table 2 and 3). There were not ascertained significant changes in RV of epithelium during cycle with the exception of the oestrous stage with the highest RV ( $P < 0.05$ ).

**Figure 3** The ratio of the ciliary and the secretory cells of the oviduct in postnatal development and in a hypoglycaemia of heifers in %**Table 3** Relative volume of the oviducts` epithelium in oestrous cycle (% of a mucosal volume)

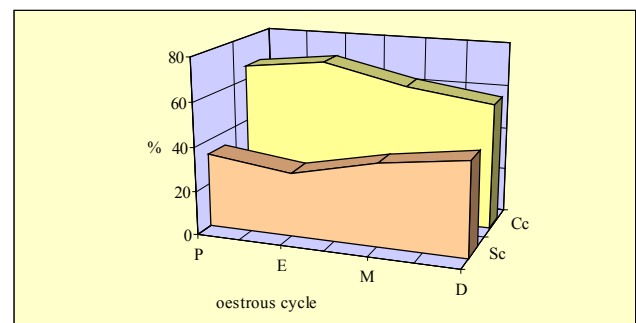
	Estrous cycle			
	P	E	M	D
Infundibulum	65.2 ±3.3	68.8 ±7.9b	65.4 ±4.5	64.9 ±4.9
Ampula	63.7 ±4.1	65.6 ±1.2	65.3 ±1.2	64.0 ±1.9
Isthmus	63.4 ±3.1	65.6 ±2.1b	65.2 ±2.6	60.5 ±2.8

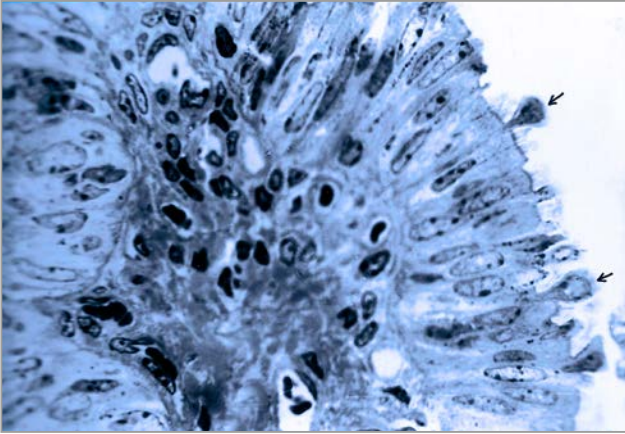
<sup>b</sup> –  $P < 0.05$  (E: P, M, D inf.; E: P, D istm.), P – proestrous; E – estrus; M – metestrus; D – diestrus

Low prepubertal oestrogenous activity was shown in the presence of ciliary (Cc) and secretory (Sc) oviduct epithelium cells in the first 9 months of postnatal development and at hypoglycaemia of heifer (figure 3).

After the 6<sup>th</sup> month of age the secretory cells dominate and the ratio between SC and Cc equalizes in the 9<sup>th</sup> month of age. In 3<sup>rd</sup> month of age the percentage of Cc varies from 8.4 % in isthmus to 17.4 % in infundibulum, but in 9<sup>th</sup> month they have proportionate representation which refers to the increasing ovarian activity with forthcoming puberty. Similar ratio of Cc as was ascertained in 6<sup>th</sup> month of age was at heifer which suffered from hypoglycaemia. Unlike this state in all stages of oestrous cycle dominated mainly Cc (figure 4, 5) as their ratio did not decrease below 53 %.

During development it is possible to observe this ratio in 9<sup>th</sup> month of age in infundibulum ( $56.7 \pm 1.8$ ;  $P < 0.01$ ). Hormonal ratio on cell ciliation is obvious from their increased presence in oestrous ( $P < 0.05$ ), which probably relates to their activity and motility of the whole oviduct. The ratio of secretory and ciliary cells is according to Abe et al. (1999) not different in the oviduct of goats in luteal and follicular phase, but there was ascertained reduction of cell size, mainly ciliary cells in luteal phase of the cycle. Similar findings were published by Abe and Oikawa (1992) at sows and Abe and Oikawa (1993) at cows. Cilia are present at the whole oviduct but the number of ciliary cells significantly increases towards the infundibulum (Stalheim et al., 1975). The oviduct succumbs in ciliation to cyclical changes, mainly in infundibulum and ampulla, where the number of cilia significantly increases during the follicular phase of the cycle (Abe et al., 1993; Abe and Oikawa, 1992;

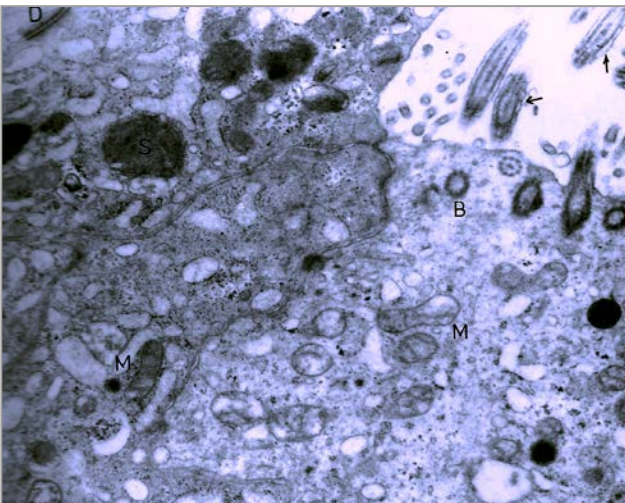
**Figure 4** The ratio of the secretory and the ciliary cells of the oviduct in oestrous cycle in %  
Sc – secretory cells, Cc – ciliary cells



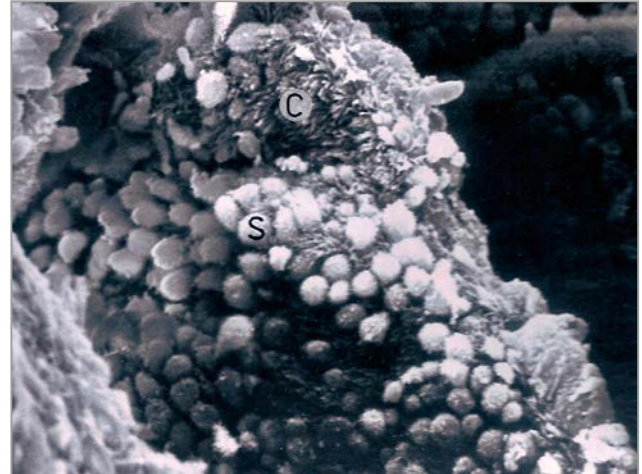
**Figure 5** The fimbria of the ampulla of 6<sup>th</sup> month's heifer; cuneiform cells (arrows) are disseminated between the ciliary cells

Abe and Oikawa, 1993; Abe et al., 1999) and these are not present during the luteal phase. The findings of Abbe (1994) were proved, since he explains the increased presence of Cc in infundibulum and ampulla as a result of increased presence of fimbriae in these parts of oviduct. And, on the contrary, higher ratio of Sc increases towards isthmus where we have discovered the ratio of 91.6 % in 3<sup>rd</sup> month of age ( $P < 0.01$ , figure 6).

We often meet with the description of types of oviduct cells as ciliary and non-ciliary, which, according to our opinion, suits to the description of cells during the development from puberty when the non-ciliary cells do not fulfil their future secretory function. It would be probably more suitable for simplification of terminology to use terms



**Figure 6** Apical part of the ciliary cell (lights) and secretory cell (dark) of 6 month's heifer. Ciliary cell is in the ciliogenesis stage – it can see more basal bodies (BT) and growing cilia too (arrows), in cell it can see more active mitochondria. Active is also secretory cell, with secretory dense granules (S) a mitochondria (M), desmosome (D), TEM,  $\times 7200$



**Figure 7** Proestrus – a fimbria of the isthmus contain more secretory (S) than ciliary (C) cells scattered between secretory cells, SEM, covered by gold,  $\times 3712$

“ciliary” and “secretory”, which expresses their physiological status (figure 7).

Ciliary cells are high, thin, they have columnar shape, they lay on the basement membrane and are covered by thick, rather long cilia.

They can be lighter and darker with condensed chromatin in the nucleus (Uhrín, 1992).

The secretory cells are present disseminated or as aggregates among ciliary cells. They are attached to the basement membrane by wide base. There are secretory granules (figure 8) in apical parts of cells (very rare in early stages of postnatal development) and their number related to the phase of oestrous cycle.

Apart from this stamens and cuneiform cells occur (Uhrín, 1992). Cuneiform cells (figure 9) rise above the level of apical epithelium layer but they do not reach the basement membrane by their base and create holocrine secretion.

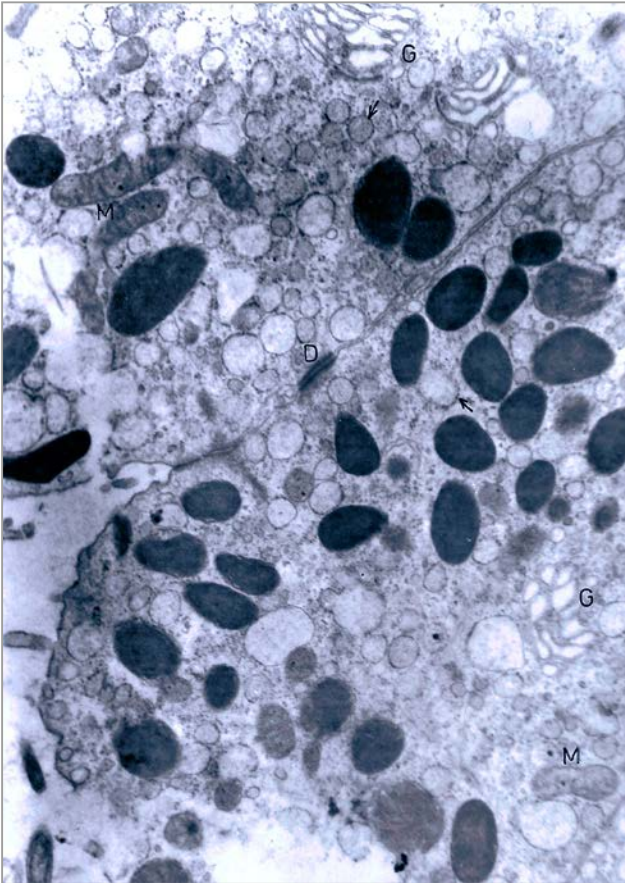
Secretory granules are the most characteristic sign of secretory cells (13, 14). Their number and size varies depending on the age of heifer and the stage of cycle (Abe et al., 1999). Most of them are in follicular phase in ampulla (figure 10, 11), less in infundibulum, and the least in isthmus. This assumes also different secretory activity of cells (Abe et al., 1999).

These changes of secretory cells should be the result of effect of steroid hormones on epithelium cells and with different reaction of various parts of oviduct on these hormones (Abe et al., 1999).

With the change of relative volume of cells the nucleocytoplasmic ratio (NCR) changes as well in both types of cells in the period of postnatal development (figure 12) and in oestrous cycle (figure 13).

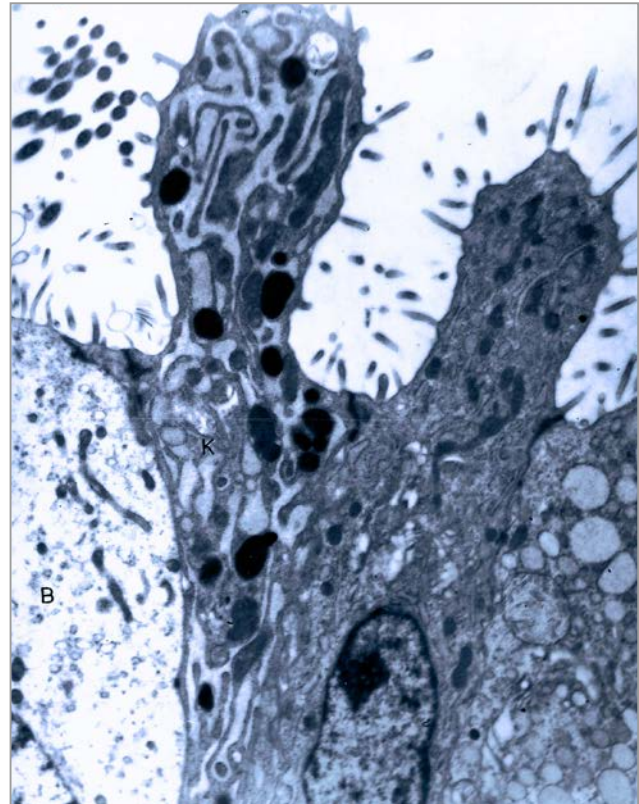
The highest NCR in Cc (1 : 1.34) is at animals at the age of 3 months, on the contrary to Sc which is in this age NCR the lowest (1 : 1.96) which represents the percentage of nucleus from the volume of the cell. Subsequently with





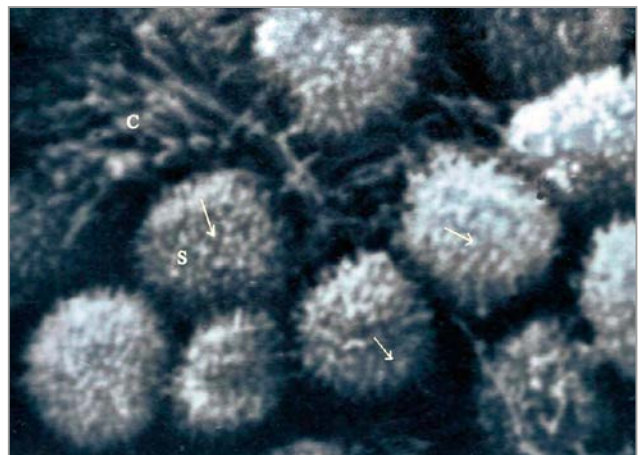
**Figure 8** In the estrus, the secretory cells contained a lot of secretory granules, cell's activity is expressed by clear mitochondria (M), which have lamellar structures, good developed Golgi aparats (G), rER is formed into numerous circled forms (arrow); desmosom (D), TEM,  $\times 7200$

age the NCR in Cc decreases and it is almost unchanged up to 9<sup>th</sup> month of age, but in Sc it increases (1 : 1.24), which probably relates to the changes in activity of cells towards puberty. The growth of secretory activity in cytoplasm changes also NCR. In oestrous cycle in proestrous the NCR is the lowest in both types of cells (1 : 1.16 resp. 1 : 1.36), since the nucleus of cells become bigger at this time, but the volume of cytoplasm compared to diestrous does not change (Uhrín, 1992). The NCR decreases even more in the time of oestrous (figure 4). Nucleus start to get bigger and the secretory activity despite growing cytoplasm does not reach the peak. This is realized in post-ovulatory period when the activity of ciliary cells reaches its peak and secretory cells reach the biggest volume because the secretory granules were not excreted yet. In this stage it is possible to observe the obvious difference of NCR Cc and Sc cells (1 : 1.28 or 1 : 1.88). At 15 months old heifer with subclinical hypoglycaemia was ascertained the lowest NCR (1 : 1.03) in Cc and the same as in 6<sup>th</sup> month of age in Sc (1 : 2.41) while cycling animals of the same age had the highest NCR in diestrous 1 : 1.81 in Sc.

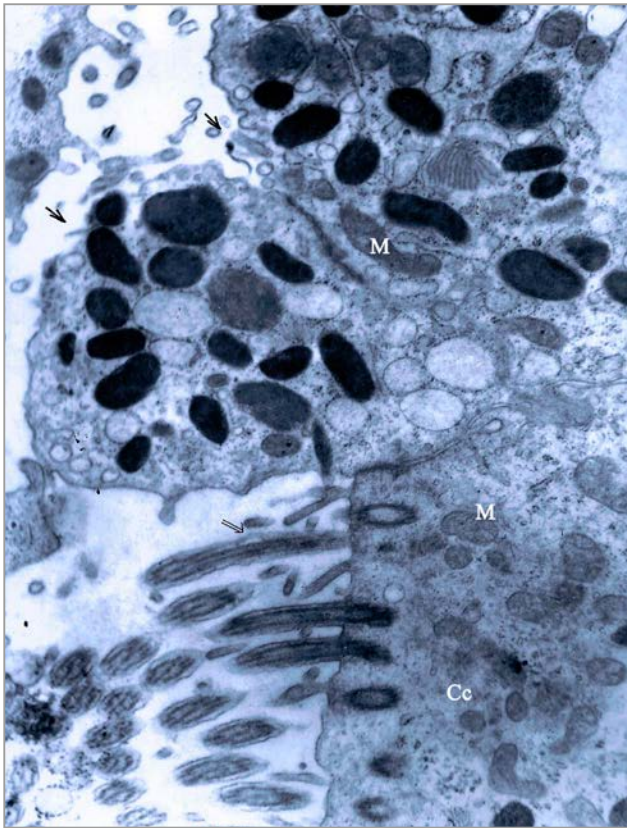


**Figure 9** Bleb secretory's cell contains in the proestrous except rER secretory granules too; pale ciliary cell (B), TEM,  $\times 8450$

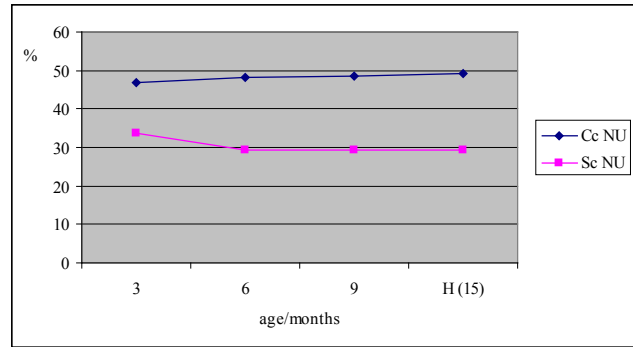
During the postnatal development and oestrous cycle there are quantitative and qualitative changes in ciliary cells of oviduct. Until the 9<sup>th</sup> month of age the RV of all observed cytoplasmatic organelles decreases with the exception of lysosomes mitochondriae by 3.8 %, rough endoplasmatic reticulum by 0.4 %, smooth membranes by 0.2 %. The RV of lysosomes increases until the 9<sup>th</sup> month of age by 0.2 %. At hypoglycaemic animals these values are on the level of animals in 6<sup>th</sup> to 9<sup>th</sup> month of age (table 4, figure 14).



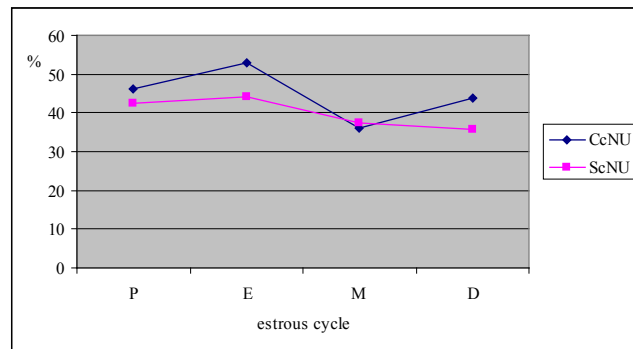
**Figure 10** In estrus the secretory cells (S) have already on their surface the released granules (arrows); ciliary cells (C), SEM, covered by gold,  $\times 21000$



**Figure 11** On the surface of the secretory cells it can see formation of the protrusions (arrows) and secretions at metestrus. On the ciliae it can see colaps, scarification of the ciliae, detachment of plasmolema from axonema of ciliae (twin arrow); ciliary cell (Cc), mitochondria (M),  $\times 7200$



**Figure 12** The ratio of the nucleus of the secretory cells (ScNU) and the ciliary cells (CcNU) of the oviduct in postnatal development and the hypoglycaemia (H15) in %



**Figure 13** The ratio of the nucleus of the secretory cells (ScNU) and the ciliary cells (CcNU) of the oviduct in oestrous cycle (% from cells volume); P – proestrus; E – estrus; M – metestrus; D – diestrus

During oestrous cycle there is the highest RV of organelles in ciliary cells (M, HM) in oestrous and metestrus, while the rER is in proestrus more by 1.2 % and it is possible to observe dilatation of its tubules (figure 15) (Uhrín,

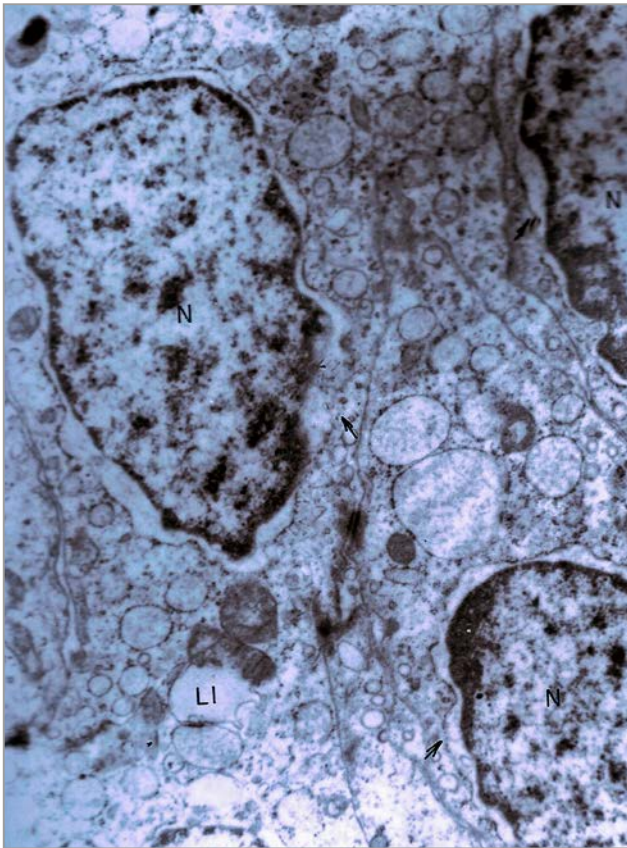
1992). The highest number of lysosomes was observed in diestrus stage. In proestrus the mitochondriae are less differentiated, oval with lower number of lamellae.

**Table 4** The relative volume of the organel of ciliary and the secretory cells of oviduct in postnatal development, hypoglycaemia and the oestrous cycle (% of cytoplasm volume)

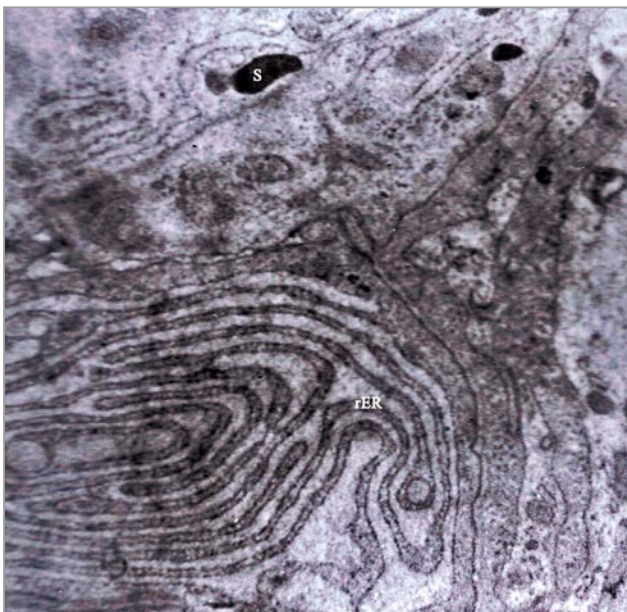
	Cc				Sc				
	M	rER	HM	LY	M	rER	HM	LY	SG
<b>Age/MM-cycle</b>									
3	13.7±5.3	4.3±1.1	0.7±0.4	0.7±0.6	14.7±3.7	4.6±2.1	0.9±0.6	0.7±0.3	0
6	10.3±3.7	3.3±0.7	0.6±0.5	0.6±0.5	11.1±5.8	3.4±0.6	0.7±0.5	0.5±0.5	1.8±0.6
9	9.9±4.4	3.9±1.4	0.5±0.3	0.9±0.4	11.4±2.4	3.5±0.8	0.7±0.6	0.9±0.6	1.9±1.1
H(15)	8.9±2.8	3.2±1.3	0.7±0.4	1.3±0.8	10.2±5.1	3.2±0.4	0.8±0.3	1.2±0.9	1.8±0.7
<b>Oestrus cycle</b>									
P	11.8±5.1	4.4±0.3	0.4±0.2	0.6±0.6	9.3±3.4	12.5±5.2	0.9±0.3	1.4±0.4	3.0±0.9
E	14.2±3.9	3.2±0.6	0.5±0.4	0.6±0.4	11.4±2.6	8.7±3.7	1.0±0.6	1.4±1.0	10.1±3.4
M	14.8±4.7	2.3±0.6	0.5±0.2	0.7±0.5	17.2±6.3	5.6±1.9	0.8±0.4	1.0±0.7	10.4±5.2
D	12.3±4.2	3.5±0.8	0.5±0.5	0.8±0.7	9.5±1.8	6.0±2.4	1.0±0.3	1.3±0.4	10.1±4.7

Cc – ciliary cells, Sc – secretory cells, M – mitochondria, rER – rough endoplasmatic reticulum, HM – smooth membranes, LY – lysosomes, SG – secretory granules, H(15) – hypoglycaemia





**Figure 14** In heifers with subclinical hypoglycaemia the cells of the ampulla contains a lot of vacuolar structures, mitochondriae formed from external membrane vacuoles (LI) and arisen a collapse with interruption of karyolema (arrows), nucleus (N), TEM,  $\times 29600$



**Figure 15** In the proestrus began in the ampullary cells massive development of rER, arisen wide cisternae, it means start of a cell's secretory activity, TEM,  $\times 20040$

RV of organelles Sc is higher than in Cc even though the tendency of their movement is the same, the RV up to 9<sup>th</sup> month decreases (table 4). During cycle the quantitative changes of Sc were similar to the tendency of changes as in Cc (table 4). Sc is characterized by secretory granules which appear in postnatal development in 6<sup>th</sup> month of age and gradually increase their number. They are located mainly in apical parts of cells as dense, mainly oval structures. The highest number of them during cycle is in oestrous and metestrous, where their intensive excretion starts with subsequent decrease (Uhrín, 1992). Densite structures which are similar to cell nucleus in size and shape were often extruded from the epithelium layer of the oviduct. Desjardins and Hats (1969) describe that these structures were most frequent at heifer after their first oestrous and were missing at non-treated heifer before their 6<sup>th</sup> month of age (Larson et al., 1971). The dynamics of Sc in cycle is more considerable than in Cc. It is shown by significant RV rER in Sc, which proves increased synthetic activity in the cell. In oestrous the majority of endoplasmic reticulum is dilated with the highest number of visible polyribosomes (Nayak and Ellington, 1977).

#### 4. Conclusion

This work describes structural quantitative and qualitative changes of heifer oviduct during their post-natal development until the pubertal period and in oestrous cycle. Changes in the heifer oviduct structure with induced subclinical experimental hypoglycaemia from 8<sup>th</sup> month of age up to 15<sup>th</sup> month were also assessed. Progressive development changes in mucous structures, especially epithelium mucous component were confirmed. We discovered different development and quantitative changes in ciliary and secretory cells which are under steroid pre-pubertal control. In this period there are small changes in both types of cells in comparison with changes in the cycle. Based on the changes in hypoglycaemic animals we can assume the importance of sufficient energy subsidy during development, because these animals based on comparison with other observed animals and despite their age 15 months (reproduction age) had the development of oviduct structures on the level of animals in pre-pubertal age. Quantitative and qualitative changes in oestrous cycle show that they are under hormone control and that they are differentiated in individual phases of cycle. This concerns mainly secretory cells in which more intensive qualitative and quantitative changes on the microscopic and sub-microscopic level take place.

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