FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 48, No. 3, 2010
pp. 148-156

The tunica mucosa of the oviduct in case of ovarian cysts presence in sows

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Abstract: The unfavorable morphological changes in the oviductal mucosa may lead to infertility in females and be one of the reasons for slaughtering of farm animals. The aim of study was to investigate the morphological changes in the epithelium of oviductal mucosa of sows with ovarian cysts. The oviducts of 18 sows were obtained after slaughter. Sows were divided into three groups: 1st group – 6 sows with polycystic ovaries, 2nd group – 6 sows with single cysts, 3rd group – 6 sows without ovarian cysts. The epithelium was examined by light microscopy, SEM and TEM. Ciliated and secretory cells were count on 150 μm segments in apical and basal zone of folds both in ampulla and isthmus. We analyzed the number of cells in 5 folds in both these oviductal parts in dexter and sinister oviduct. We have noted unfavorable changes in oviductal mucosa consisting in increase of the secretory cells with simultaneous decrease of ciliated cells. The correlations between the general occurrence of ovarian cysts and the morphological state of epithelium of oviductal tunica mucosa were determined. The changes in proportion of cells occurred both in ampulla and isthmus. The excessive secretion covering epithelium promotes agglutinations and adhesions of the tubal folds and occlusion of the oviduct. These alterations may create problems in the migration of gametes and prevent the movements of the zygote towards the uterus and cause some disturbances in conceptus development in its early stages. Results suggest that COD is connected with unfavorable morphological and functional changes within epithelium of the oviductal tunica mucosa.

Key words: Oviduct, tunica mucosa, ovarian cysts, sows

Introduction

Reproductive disorders are an overwhelming problem in the farms and are one of the reasons for culling of sows [1]. Cystic ovarian degeneration (COD) is a common endocrinopathy affecting approximately 10% of sows, moreover it is the reason for infertility and a serious problem in the breeding of pigs [2-6]. The clinical symptoms of ovarian cysts in sows are not specific or pathognomic. COD is accompanied by an irregular or prolonged estrous cycle, permanent anoestrus and infertility, so it is very difficult to diagnose especially in farm conditions and can be wrongly recognized as a different abnormality within the reproductive system [5,7,8]. It is difficult to diagnose ovarian cysts through serological methods because serum concentrations of progesterone, estradiol, luteinizing hormone and cortisol are similar to sows in dioestrus [9]. However, it is now possible to diagnose ovarian cysts by ultrasound [10-12].

Pig reproductive organs are known to undergo dynamic changes during the oestrus cycle and pregnancy [13]. In mammalian reproduction the oviducts are the site of crucial processes that occur before the implantation and the initial stages and subsequent phases of embryo development [14].

Cell growth and regeneration of the reproductive tissues are closely correlated with ovarian steroid hormone levels. The mammalian oviduct, like other organs of the female reproductive tract, is under the influence of peripheral and local steroids, especially estradiol from the ovarian follicles and progesterone from the corpora lutea. Sensitivity of the structural components of the oviduct to ovarian steroid hormones was by several authors [15-19].

The oviductal mucous membrane, the endosalpinx, consists of simple columnar epithelium and sub epithelial connective tissue. The epithelium generally is composed of two major cell types i.e. ciliated cells and

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Folia Histochem Cytobiol. 2010:48(3): 148 (141-147)
10.2478/v10042-010-0032-0

secretory (non ciliated) cells. The former have a luminal surface covered with cilia. Besides these two types of cells we can detect parabasilaris cells situated between ciliated and secretory cells. These cells occur in a lesser amount than ciliated and secretory ones. The mucosal layer forms longitudinal folds along the tube [16].

The morphological changes within the oviductal mucosa occur periodically with the phases of estrous cycle [3]. A precise and functionally related synchronization of all parts of the reproductive system is essential for the fertilization and embryonic development. The oviduct, especially the epithelium together with its secretion, serves as an optimal environment for gametes transport, final sperm maturation (capacitation), fertilization and in case of pregnancy creates a suitable environment for the development of the conceptus [7,18-21]. After ovulation the oocytes are transported through the infundibulum and the ampulla to the ampullaryisthmic junction (AIJ) by ciliary beating in combination with an interaction (adhesion) between cumulus cells of the oocytes and extracellular matrix on the cilia. Isthmus has a special role in the reproductive tract as the fertilization takes place in the AIJ and the early embryo stays in the isthmus at least 48 h before entering the uterus [20,22-24]. At this time the zygote is exposed to and influenced by oviduct epithelial cells contacts and secretions providing a micro-environment beneficial to embryo development [25].The oviduct is capable of transudating substances from the circulation into the oviductal lumen as well as the novo synthesizing and releasing molecules like lipids, enzymes and growth factors next to a variety of oviduct specific proteins like oviductin [15]. Produced by the oviductal tunica mucosa fluid plays a facilitatory role in the fertilization and embryos development before they reach the uterus on days 2-3 [9,16,26,27]. The immersion in the intra luminal fluid and contact with the lining epithelium maintain normal ultra structure, viability and potential fertilizing ability of spermatozoa before the time of fertilization. The pattern of oviductal secretion coincides with the changes of the oestrus cycle [28,29].

Reproductive organs and their physiology depend upon a complex sequence of events of neuroendocrinological nature, disturbances of which may lead to infertility [30,31]. Because cystic ovarian disease is an endocrine disorder, the hormonal milieu could create a favorable environment for the development of changes in the proliferation and secretory activity of cells of oviductal tunica mucosa. The endocrinological status of the cystic ovarian disease affects oviductal functions [2]. Of all conditions causing female infertility, the potential involvement of disturbances in oviduct cellular physiology is the least understood [25]. This segment of reproductive system is very difficult to clinical examination therefore oviduct is rarely considered during research of infertility causes [31,32]. Accordingly, we can suppose that the incidence of pathological states of the oviduct is more frequent than we expect. The pathological changes within the oviduct may cause disturbances in reproduction. The effect of ovarian cysts presence on morphological changes of sow endosalpinx has not been thoroughly investigated. Therefore, the aim of the present study was to investigate the qualitative and quantitative changes in the histological structure of oviductal tunica mucosa in case of ovarian cysts presence in sows.

Material and methods

Animals and sample collection. The experiment was performed with 18 purebred, 2-5 years old multiparous Rodone sows from a commercial pig breeding farm located in the Region of Leszno, Poland. On the farm the sows were on a commercial diet, fed twice a day and had *ad libitum* access to water. Experimental females derived from animals culled from the farm mainly due to low fecundity, overweight, hoof diseases and other ailments after the 5 weaning period. Immediately after the slaughter reproductive organs of 238 sows were remove and examined macroscopically in order to assess ovarian status. Females were divided into three groups: PCO (polycystic ovaries) group – 6 sows, SOC (single ovarian cysts) group – 6 sows and – a NO (normal ovaries) – 6 sows without ovarian cysts which were culled only due to diseases of the hoofs) (control group). Females were estimated into two schemes. On the first one they were divided into 3 groups (scheme 1) for PCO, SOC and control animals. In the second one they were divided into w groups (scheme 2) for cystical and control sows. The border number between polycystic ovaries and simple ovarian cysts was 5 cysts on one ovary as described by Bosted and Ebbert (1999). Tissue samples of oviducts were collected immediately after slaughter of sows. After preparation, the oviducts (dexter and sinister) were divided into ampulla and isthmus. 1cm fragments of central parts of oviductal ampulla and isthmus were collected.

Histological and histometrical analysis. The fragments of ampulla and isthmus were fixed in 4% formalin for 48 hours and embedded in paraffin. Then organs were cut at 6-7 μm, fixed on slides, dried, deparaffinized and rehydrated in ethanol solutions. The microscopic sections were stained with hemotoxylin/eosin. Quantitative and qualitative examinations of oviductal epithelium were performed by light microscopy. A light microscope with objective ×40 and eyepiece ×10. With 400× magnification was used, ciliated cells (CC) and secretory (nonciliated) cells (SC) were count on 150 μm segments in apical and basal zone of oviduct folds both in ampulla and isthmus. To quantify the cells in tunica mucosa, 5 oviductal folds were analyzed in both ampulla and isthmus in dexter and sinister oviduct of each sow.

Scanning electron microscopy. The fresh material was fixed in 2.5% glutaraldehyde on phosphate buffer of pH 7.4. The specimens were then dehydrated in a graded series of acetone 5-100%. Next the specimens were mounted on stages and sputtered with gold using a Scancoat 6 sputter (Edwards, London, England). The ultra structure of the studied material was analyzed using a LEO ZEISS 435VP scanning microscope (Zeiss, Oberkochen, Germany).

Transmission electron microscopy. Samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). After several rinses in the same buffer the material was post-fixed for 2 hours in 2% osmium tetroxide in the buffer. Following dehydratation in an acetone series (30-100%), the material was embedded in Epon 812.

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10.2478/v10042-010-0032-0

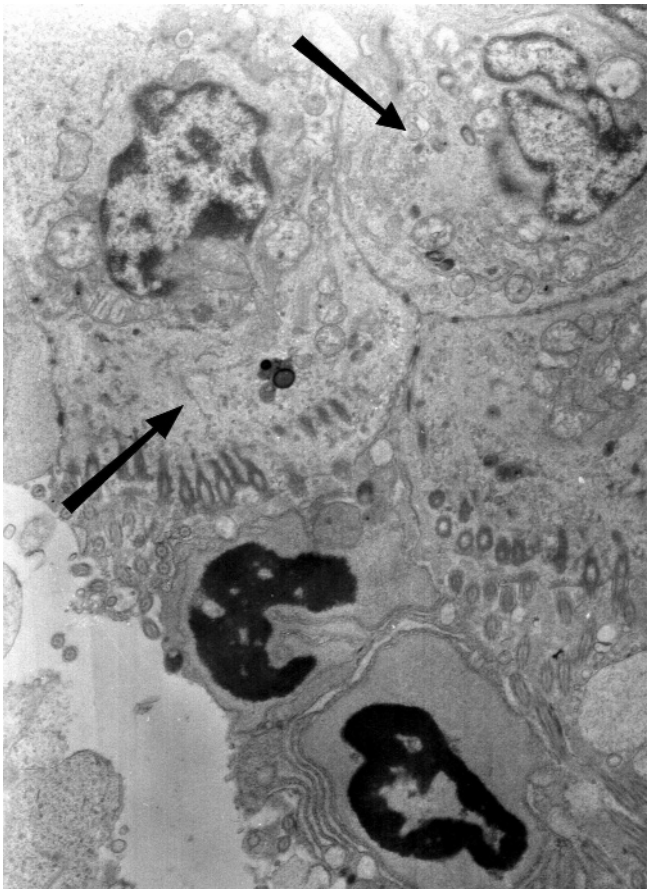

Fig. 1. Epithelium of oviductal tunica mucosa of sows with ovarian cysts, numerous secretory granules in secretory cells, ciliated cells on the left. TEM, original magnification ×10000.

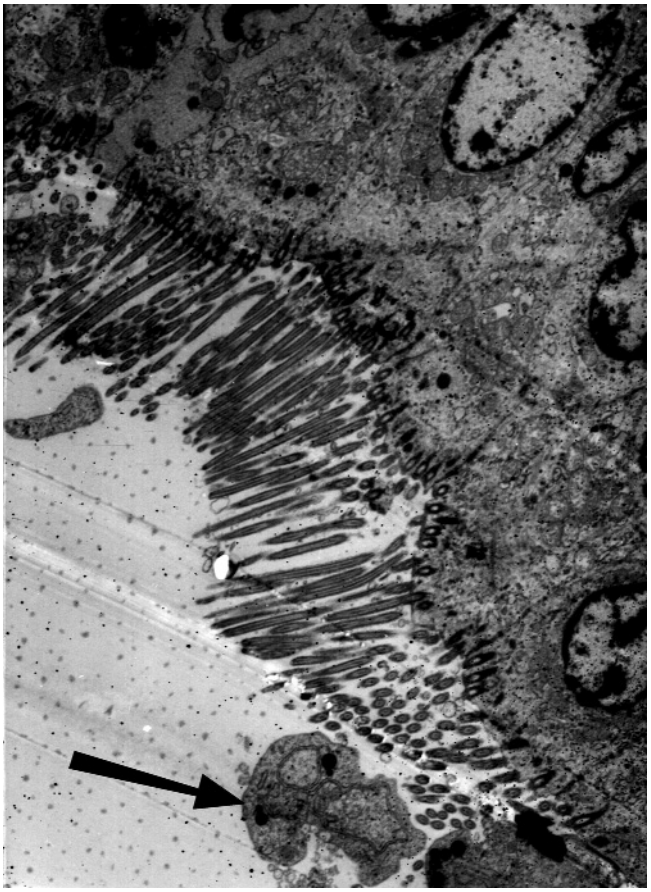

Fig. 2. Ciliated cells of oviductal ampulla. TEM, original magnification ×6000.

Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in transmission electron microscopy (Tesla BS500).

Statistical analysis. Treatment comparisons were made in the analysis of variance procedures for a completely randomized design using STATISTICA 8 statistical package. The statistical differences between analyzed means were determined by Duncan test when analyses were conducted for three groups (scheme 1) for PCO, SOC and control animals, and by Tukey test when the analyses were conducted for two groups (scheme 2) for cystical and control sows. The differences were considered significant if the p value was ≤0.05 (small letters) or high significant if the p value was ≤0.01 (capital letters). Pearson correlation between general occurrence of ovarian cysts and number of secretory (SC) and ciliated cells (CC) dependent on oviduct' region were calculated. All the values are presented in tables as average and standard deviation (Mean ±SD).

Results

Histological examination

The histological analysis showed an excessive proliferation of secretory cells (SC) and a decreased number of ciliated cells (CC) within oviductal epithelium. The differences in number of SC and CC between investigated groups occurred both in ampulla and isthmus of the oviduct. Not only the proportion in number of these two types of cells changed but also their structure. The CC were small, whereas the SC were elongated. Slender secretory cells demonstrated apical plasma protrusion. The apices of SC bulge into a lumen and over the apical surface of CC. The epithelial surface was covered by an excess of secretion. Additionally we observed desquamation of epithelial cells what indicates that there are advanced degenerative processes of oviductal mucosa. We noted an almost complete atrophy of the epithelial cells and hyperplasia of subepithelial connective tissue. In transmission electron microscopy we observed a significant accumulation of cytoplasmic vesicles in pars apicalis of SC. Abundant amount of granular endoplasmic reticulum was noticeable in this cytoplasmic area. We noted various extent of secretion of epithelial cells.

In scanning electron microscopy we observed predominance of SC in both oviductal ampulla and isthmus of sows with ovarian cysts. On their surface we noted numerous secretory granules. The secretion from granules covered cells surface. The sparse CC were pressed by higher secretory cells and often only their cilia were noticeable.

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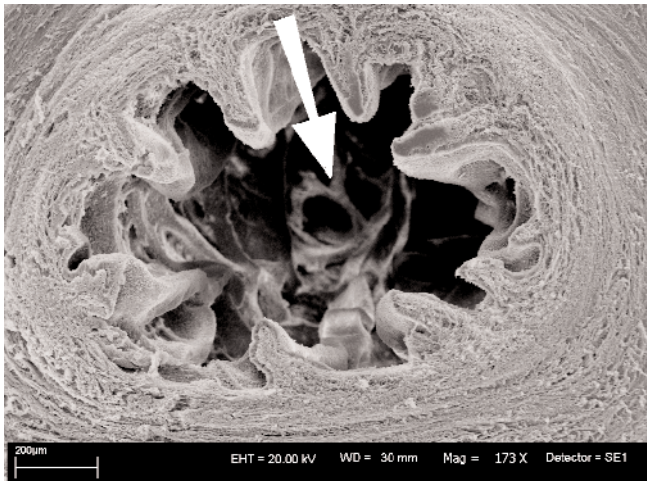

Fig. 3. Cross-section of oviductal isthmus of sows with ovarian cysts. SEM, original magnification ×200.

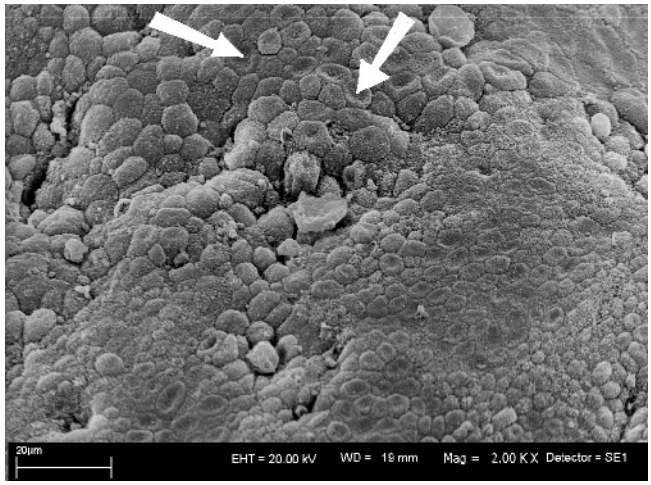

Fig. 5. The excess of secretion covering oviductal folds. Numerous secretory cells. SEM, original magnification ×2000.

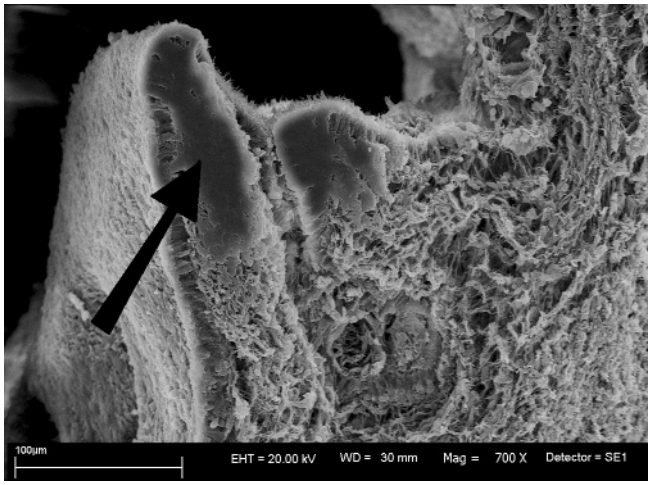

Fig. 4. The remaining secretion on oviductal folds in sows with ovarian cysts. SEM, original magnification ×700.

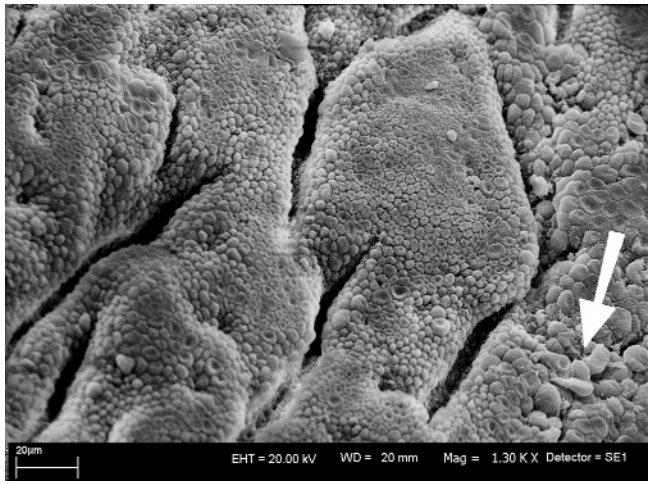

Fig. 6. The excess of secretion covering oviductal folds. Numerous secretory cells. SEM, original magnification ×1300.

Histometrical analysis

Table 1 presents findings of the histological examination of the oviduct ampulla from 18 sows divided into 3 groups. In ampulla we noted a significant difference in mean number of ciliated cells (CC) in zone basilaris of folds between all groups i.e. PCO (Polycystic Ovaries), SOC (Single Ovarian Cysts) and NO (Normal Ovaries) sows (11.28 *vs.* 12.72 *vs.* 17.23, respectively). The PCO females showed a significant decrease in number of CC in zone apical of folds in comparison with SOC and NO sows (16.53 *vs.* 18.93 and 19.73, respectively) (p≤0.01). Significant differences were detected in the number of SC in zone basal of folds between all investigated groups. In PCO and SOC was significantly higher (30,20 and 25,68) than in the healthy sows (21.90) (p≤0,01). The number of SC in zone apicalis of folds was significantly higher in PCO in comparison with SOC and NO (33.15 *vs.* 30.65 and 30.93) (p≤0.01). There was no significant difference in this parameter between group SOC and control sows.

Table 2 presents histological analysis of the epithelium of oviductal tunica mucosa in oviduct ampulla of sows divided into 2 groups: group 1- all sows with ovarian cysts (OC), group 2 – sows with normal ovaries (NO). Interestingly, statistical analysis did not confirm differences in the number of CC both in zone basilaris and apicalis of fold between OC and NO. Whereas the number of SC both in zone basal and apical of fold was significantly higher in OC then in control females (27.94 and 32.04 *vs.* 21.90 and 30.65) (p≤0,01).

Table 3 presents results obtained after the examination of epithelial cells number in the oviduct isthmus from18 experimental sows. In our studies we observed that the number of CC both in basal and apical zone of

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Table 1. Histometrical analysis of epithelium of oviductal tunica mucosa on 150 μm segments in ampulla of oviduct (x±SD).

			Group 1 n=6	Group 2 n=6	Group 3 n=6
Pars basilaris of folds	Number of ciliated cells	x ±	11.28 C 0.94	12.72 B 1.25	17.23 A 1.41
	Number of secretory cells	x ±	30.20 A 0.84	25.68 B 1.27	21.90 C 1.06
Pars apicalis of folds	Number of ciliated cells	x ±	16.53 B 0.48	19.73 A 1.10 a	18.93 A 0.85 b
	Number of secretory cells	x ±	33.15 A 1.12	30.93 B 1.21	30.65 B 0.78

Group 1 – PO (Polycystical Ovaries)
Group 2 – SOC (Single Ovarian Cysts)
Group 3 – NO (Normal Ovaries)

Table 2. Histometrical analysis of epithelium on 150 μm segments of oviduct ampulla (x ±SD).

			Group 1 n=12	Group 2 n=6
Pars basilaris of folds	Number of ciliated cells	x ±	14.26 3.26	12.72 1.25
	Number of secretory cells	x ±	27.94 A 2.54	21.90 B 1.06
Pars apicalis of folds	Number of ciliated cells	x ±	18.13 1.83	18.93 0.85
	Number of secretory cells	x ±	32.04 A 1.61	30.65 B 0.78

Group 1 – OC (Ovarian Cysts)
Group 2 – NO (Normal Ovaries)

Table 3. Histometrical analysis of epithelium of oviductal tunica mucosa on 150 μm segments in isthmus of oviduct (x ±SD).

			Group 1 n=6	Group 2 n=6	Group 3 n=6
Pars basilaris of folds	Number of ciliated cells	x ±	11.38 B 0.71	11.92 B 1.05	26.73 A 0.75
	Number of secretory cells	x ±	42.15 A 1.19	40.40 A 0.79	34.37 B 2.57
Pars apicalis of folds	Number of ciliated cells	x +	1318 B 0.67	14.03 B 1.46	25.37 A 0.92
	Number of secretory cells	x ±	40.80 A 0.74	39.15 B 1.61	33.58 C 1.11

isthmus folds was significantly lower in PCO (11.38 and 13.18) and SOC (11.92 and 14.03) groups in comparison with control sows (26.73 and 25.37) (p≤0.01). There was a significant increase in the number of SC in zone basilaris in PCO and SOC groups compared with the animals from NO group (42.15 and 40.40 *vs.* 34.37) (p≤0.01). Whereas the number of SC in zone apicalis was significantly different between all groups and considerably higher in PCO and SOC sows then in control ones (40.80 and 39.15 *vs.* 33.58, respectively) (p≤0,01). The number of SC in zone basilaris of folds was notably increased in PO and SOC groups compared with control females (42.14 and 40.40 *vs.* 34.37).

Table 4 presents the results of the statistical analysis of the differences in the number of SC and CC in

Table 4. Histometrical analysis of epithelium of oviductal tunica mucosa in isthmus of oviduct on 150 μm segments (x ±SD).

			Group 1 n=12	Group 2 n=6
Pars basilaris of folds	Number of ciliated cells	x ±	11.65 B 0.92	26.73 A 0.75
	Number of secretory cells	x ±	41.28 A 1.33	34.37 B 2.57
Pars apicalis of folds	Number of ciliated cells	x ±	13.61 B 1.19	25.37 A 0.92
	Number of secretory cells	x ±	39.98 A 1.49	33.58 B 1.11

Capital letters p≤0.01

Table 5. Correlations between general occurrence of ovarian cysts and morphological state of oviductal epithelium.

Morphological features of oviductal epithelium		General occurrence of ovarian cysts
Zona basilaris of ampulla folds	number of CC number of SC	-0.939^{A} 0.871^{A}
Zona apicalis of ampulla folds	number of CC number of SC	-0.937^{A} 0.587^{a}
Zona basilaris of isthmus folds	number of SC	0.695^{a}
Zona apicalis of isthmus folds	number of SC	0.635^{a}

^{A}p≤0.01. ^{a}p≤0.05

the oviduct isthmus between OC and NO groups. There were significant differences in all examinated parameters between these groups. The number of SC both in zone basilaris and apicalis of folds was significantly higher in OC sows in comparison with the control group (41.25 and 39.98 *vs.* 34.37 and 33.58, respectively) (p≤0.01). Whilst the number of CC in sows with pathological ovaries was significantly lower, both in zone basilaris and apicalis of folds, than in the healthy sows (11.65 and 13.61 *vs.* 26.73 and 25.37, respectively, p≤0,01).

Table 5 presents the correlations between the general occurrence of ovarian cysts and the morphological state of epithelium of oviductal tunica mucosa in sows. The general presence of ovarian cysts was high significantly negatively correlated with CC number both in zone basilaris (-0.939, p=0.01, n=18) and apicalis (-0.937, p=0.01, n=18) of ampulla folds and high significantly with SC number in zone basilaris of ampulla folds (0.871, p=0.01, n=18). We also noted a positive correlation between the general occurrence of ovarian cysts and number of SC in zone apicalis of ampulla folds (0.587, p=0.05, n=18), and number of SC in both zone basilaris(0.695, p=0.05, n=18) and apicalis (0.635, p=0.05, n=18) of isthmus folds.

Discussion

Here we performed the histological and histometrical investigation of the oviductal tunica mucosa in the case of ovarian cysts presence in sows and found that this ovarian pathology is connected with unfavorable morphological changes within the oviductal mucosa. Our study demonstrates that in both ampulla and isthmus of sows with ovarian cysts presence, especially with polycystic ovaries, the oviductal surface epithelium consisted of increased number of secretory cells with a simultaneously decreased number of ciliated cells. Changes in cells proportion of these two types may affect the oviductal functions like movement of gametes and embryos.

Our current observations also demonstrate proliferation of connective tissue in place of damaged epithelium. This irreversible pathological state leads to permanent oviductal mucosa damage. The examinations by SEM and TEM confirm the excess of secretory cells and hypersecretion of these cells.

In a preceding study on the same material on morphological changes within ovarian cortex, we showed that cystic ovarian disease is connected with an advanced stage of degenerative changes in ovarian cortex which could be a reason of persistent infertility of pigs. On the basis of our previous studies we noted that there is a correlation between the ovarian cysts presence and the changes within the ovarian cortex (not published), what is consistent with Kuryszko studies of ovarian cysts in cows [33]. Examinations showed that cystical degeneration of ovaries is connected with a significant increase of atretic ovarian follicles number and decrease of the number of normal follicles of all generations [34]. COD has a harmful effect not only on the ovarian cortex state but also on the oviductal tunica mucosa [33].

A precise and functionally related synchronization of all parts of the reproductive system is essential for the fertilization and embryonic development. Disturbances of endocrinological mechanisms regulating physiology of reproductive organs may lead to infertility [30,31]. The oviduct is one of target organs that undergo morphological and physiological changes under the influence of ovarian steroid hormones [13,17,36]. Oviductal tunica mucosa is a dynamic tissue changed cyclically during normal estrus cycles [13]. Studies of other authors showed minimal differentiation of epithelial lining during anoestrus and maximal differentiation during late follicular phase leading to high percentage of ciliated cells and clearly distinguishable secretory cells. In this case organelles like cilia, mitochondria, endoplasmic reticulum, Golgi complexes and secretory granules are well developed. During mid-luteal phase, there is an onset of focal epithelial dedifferentiation and regression [19]. Jiwakanon *et al*. in his study on the sow oviducts, found morphological differences both between the oestrus cycle stage and between the segments of the oviduct. Pseudo stratification of the epithelial cell layer in ampulla and infundibulum was high at pro oestrus and oestrus, i.e. the stages when also the estradiol plasma level was high [18]. Most secretory granules were found at oestrus. Cystic ovarian disease, an endocrine disorder, is connected with various hormonal interrelations between sex steroids. We can suspect that there are some structural changes in the configuration of oviductal which is a target tissue for the abnormal steroid environment present in sows with ovarian cysts. The development of these morphological changes may not be solely regulated by ovarian steroid hormones but other factors involving sensitivity of oviductal tissue and receptors' binding affinity and capacity are undoubtedly involved [8,34,36]. Studies showed that ovarian cysts produce steroid hormones [3,33]. We can suspect that morphological and functional changes within oviductal mucosa in case of COD in pigs are connected z endocrinal activity of cysts.

The development of the conceptus needs a suitable environment which is created by secretory cells by producing and secreting oviductal fluid. The oviduct secretes specific proteins and growth factors during the periovulatory period that play a role in the maturation of gametes, fertilization and embryonic viability [37-40]. But the intraluminal fluid may have both beneficial and detrimental effects on the gametes and zygotes. Many studies support a functional role for the oviduct and its secretions in fertilization, regulating processes such as sperm-zone pellucida binding, the establishment of species-specific zone pellucida barriers, and early embryonic development [2,14,16,17,41-47]. Quantitive and qualitive changes of the oviductal fluid may have negative effects on sperm motility, gametes fusion and affect the early reproductive events that take place in the oviducts. The changed biochemical composition of the intraluminal fluid may have a harmful influence on the gametes or zygotes/conceptuses vitality or damage cellular structures like plasmalemma. The excess of secretion has a direct harmful effect on the viability of the gametes (sperms and egg cells) resulting in their inability to fertilization. Abnormalities concerning composition and the amount of the oviductal fluid may lead to lack or improper changes of gametes before fertilization and incorrect conceptus development in its early stages [13]. In our studies we noted excessive proliferation of secretory cells and increase their activity (hypersecretion). The excess of secretion may constitute the barrier which prevents moving of gametes and zygotes/conceptuses descent. Additionally the retained secretion promotes the development of and adhesions of mucosal folds and the occlusion of oviductal lumen especially within ampulla. This state causes disturbances connected with the transport of the gametes and prevents the movement of the zygote towards the uterus [33,34]. Unfortunately an advanced pathological state of oviductal mucosa is irreversible and involves elimination of the sow in question from reproduction.

During the ovulation the egg cell gets into the oviduct where the fertilization and first stage of conceptus development take place. The necessary condition for these processes is a correct state of oviductal mucosa. There are numerous ciliated cells in infundibulum and ampulla of oviduct which ciliary movement promotes egg cell and embryo descend [20,28,48-50]. We noted significant decrease of ciliated cells number in case of ovarian cysts presence in sows. Therefore, we can suspect that this structural change within oviductal mucosa may complicate mentioned processes.

In conclusion, we can confirm that COD is connected with unfavorable morphological changes within epithelium of the oviductal tunica mucosa which may have some influence on oviductal functions. This will be an important subject for future investigations.

Acknowledgments: This study was supported by grant KBN 7/14-W/2008/G.

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Submitted: 20 January, 2010
Accepted after reviews: 25 May, 2010