Original Articles

A Scanning Electron Microscopic Study of Oviduct Epithelium in Culling Repeat Breeder Gilts

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Abstract

In the porcine reproductive tract, the oviduct is the vital organ for fertilization and early embryonic development. Repeat breeder (RB) gilts have previously shown the abnormal histological changes and deviant hormonal levels throughout estrous cycle. The aim of present study was to examine the oviduct epithelium of gilts culled due to RB (n = 9) in comparison to normal sows (controls, n = 6) by scanning electron microscope (SEM). The reproductive organs were collected after sending the RB gilts to local abattoir and their ovaries were kept to classify the estrous cycle. The ultrastructural details of control oviduct epithelium composed of ampulla, isthmus and uterotubal junction (UTJ) at follicular (n = 3) and luteal (n = 3) phases were similar to earlier studies. The surface of lining epithelium differed in appearance between controls and RB gilts. In all segments of gilt oviducts, the cilia were fused together with mucous-like substance and disarrangement; microvilli were sparse and almost disappeared. The epithelial surface was obviously covered with mucous-like debris and inflammatory cells. In the ampulla, the secretory cells were smaller and less cytoplasmic protrusions including sloughing of epithelial cells. The findings indicate that the abnormal ultrastructure presented in the RB gilts affected the oviductal environment and function resulting in failure of gamete transports, fertilization and early embryonic development.

Keywords : gilt oviduct, epithelium, repeat breeder, ultrastructure

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บทคัดย่อ

การศึกษาเยื่อบุท่อนำไข่สุกรสาวที่ถูกคัดทิ้งเนื่องจากผสมไม่ติดโดยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด

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ท่อนำไข่เป็นส่วนที่สำคัญของอวัยวะสืบพันธุ์สุกรเพศเมีย เนื่องจากมีหน้าที่หลักในการปฏิสนธิและการเจริญของตัวอ่อนในระยะแรก การศึกษาที่ผ่านมาพบลักษณะที่ผิดปกติทางเนื้อเยื่อวิทยาและความบกพร่องของฮอร์โมนเพศเมียในสุกรสาวที่มีปัญหาผสมไม่ติด วัตถุประสงค์ของการศึกษานี้คือศึกษาลักษณะเยื่อบุท่อนำไข่สุกรสาวที่ถูกคัดทิ้งจากปัญหาผสมไม่ติดจำนวน 9 ตัว เปรียบเทียบกับท่อนำไข่แม่สุกรปกติจำนวน 6 ตัว โดยใช้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด เก็บอวัยวะสืบพันธุ์ของสุกรสาวที่โรงฆ่าสัตว์ ทำการแยกท่อนำไข่และรังไข่ ตรวจสภาพภายนอกด้วยตาเปล่า โดยสภาพของรังไข่นำไปประเมินระยะของวงรอบการเป็นสัด จากผลการศึกษาพบว่า รังไข่สุกรสาวอยู่ในระยะฟอลลิคูล่าจำนวน 3 ตัว และระยะลูเทียลจำนวน 3 ตัว เยื่อบุท่อ นำไข่ของสุกรปกติในส่วนแอมพูลล่า อิสมัส และรอยต่อของปีกมดลูกกับท่อนำไข่ เมื่อศึกษาภายใต้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดมีลักษณะเหมือนกับเยื่อบุท่อนำไข่ส่วนต่างๆ ของแม่สุกรจากรายงานการวิจัยที่ได้ศึกษาผ่านมา แต่มีความแตกต่างอย่างชัดเจนเมื่อเปรียบเทียบกับเยื่อบุท่อนำไข่สุกรสาวที่ผสมไม่ติด การเปลี่ยนแปลงทั่วไปที่พบในเยื่อบุท่อนำไข่สุกรสาวทุกส่วนได้แก่ ลักษณะการยึดติดกันและการเรียงตัวที่ไม่เป็นระเบียบของซีเลีย ปริมาณของไมโครวิลไลที่ลดน้อยลงจนเกือบหายไป พบสารคล้ายเมือกและเซลล์เม็ดเลือดขาวปกคลุมอยู่บนผิวของเยื่อบุ นอกจากนี้ในท่อนำไข่ส่วนแอมพูลล่า พบลักษณะ cytoplasmic protrusion ของเซลล์คัดหลั่งมีขนาดเล็กลงและจำนวนน้อยกว่าปกติ รวมทั้งการลอกหลุดของเซลล์เยื่อบุ จากผลการศึกษาบ่งชี้ว่า เยื่อบุท่อนำไข่ภายใต้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดในสุกรสาวที่ถูกคัดทิ้งจากปัญหาผสมไม่ติดพบโครงสร้างที่ผิดปกติ โดยความผิดปกตินี้ส่งผลกระทบต่อการทำหน้าที่ของท่อนำไข่ซึ่งอาจส่งผลให้เกิดความบกพร่องในระหว่างการเคลื่อนที่ของเซลล์สืบพันธุ์ การปฏิสนธิ และการเจริญของตัวอ่อนในระยะแรก

คำสำคัญ : ท่อนำไข่สุกรสาว เซลล์เยื่อบุ ผสมไม่ติด โครงสร้างระดับจุลทรรศน์อิเล็กตรอน

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Introduction

The series of critical reproductive events have to occur under the specific conditions in the oviduct before the final establishment of pregnancy in the uterus (Buhi et al., 1997). The gamete transport, sperm capacitation, final oocyte maturation, fertilization and early embryonic development are taking place in the direct contact with the mucosal epithelium and its secretions of the oviduct (Hunter, 1988; Rodriguez-Martinez et al., 2001). The details of scanning electron microscopy (SEM) in the porcine oviductal epithelium during follicular and luteal phases have been observed for decades (Stalheim et al., 1975; Wu et al., 1976; Abe and Oikawa, 1992) to explain the changes of morphological features for the functions in the reproductive processes. The interactions of ciliated and secretory cells in the oviduct with gametes both *in vitro* and *in vivo* obviously support the normal growth of early embryos and the maintenance of sperm functions (Abe, 1996). These previous studies demonstrated that there are regional variations in the morphological changes and biological functions of the epithelial cells in oviduct portion that regulated by the ovarian steroid hormones. Thus, the complexity of physiological effects or the impaired functions on the spermatozoa, oocytes and embryos could be described by morphological changes in segmental differences of the oviduct associated to the estrous cycle.

In the swine industry, gilt fertility influences herd performance because they are the largest farrowing group and about 50% of the sow herd is replaced by gilts each year (D'Allaire and Drolet, 1999). Reproductive failures are the major reason for culling sows and gilts in commercial swine herds, and the most frequent of these is repeat breeding (RB) problem (D'Allaire et al., 1987; Dijkhuizen et al., 1989). Any disturbance along the pathway of reproductive events, for instance, the impaired tubal motility, reduction in the beat frequency of cilia and disorder in absorption due to the scarce of microvilli may modify conditions of normality and implied a risk for pregnancy, giving rise the case of RB (Pope et al., 1990; Kauffold et al., 2006) and the deviating ultrastructure of the lining epithelium was found in the RB heifer oviducts (Bage et al., 2002). The multiple disturbances of histological changes in the oviduct and hormonal levels have recently been reported in RB gilts (Tienthai et al., 2006). These observations suggested that the gametes and early embryonic development occurred under undesirable conditions in the oviduct and it proposed that the tubal functions should also be affected. To our knowledge, a comprehensive well-illustrated SEM study is not available regarding the morphological changes in the oviduct epithelium of RB gilts. The objective of the present study was, therefore, to determine the SEM ultrastructure of the oviductal epithelium collected from RB gilts, compared with that of cyclic sows at the follicular and luteal phases.

Materials and Methods

Animals and tissue collection

Nine cross-bred (Landrace x Yorkshire) replacement gilts culled due to RB from five commercial swine farms in the central region of Thailand were sent to a local abattoir. After slaughter, the genital organs were removed and transported to the laboratory within 4-6 h by keeping in the ice box. The ovaries were macroscopically observed the stage of estrous cycle as described by Knox (2005). The oviducts were dissected from the fold of broad ligament and 10-mm. sections were transversally removed from UTJ, isthmus and ampulla parts. The oviducts from healthy cyclic sows (parity 1-2) during follicular (n =3) and luteal (n = 3) phases were used as the control group.

Tissue preparation for SEM study

The pieces of tissue samples were open longitudinally with tiny scissors and totally immersed in 2.5% (v/v) glutaraldehyde in 0.1 M phosphate buffer saline (PBS, pH 7.4) at 4^{o}C for 24 h. Afterwards, the tissues were rinsed in distilled water, post-fixed for 1 h in 1% osmium tetroxide (Merk, Darmstadt, Germany) in 0.1 M PBS and washed again. Fixation and washing were carried out at 4^{o}C and the specimens were dehydrated in graded ethanol (30-100%) and substituted with acetone. Tissue samples were then subjected to critical point drying using liquid CO_2 substitution. Dehydrated samples were mounted on stubs, coated with gold-palladium in a sputter coater, and examined using a JEOL 5800 LV (JEOL, Tokyo, Japan) SEM at the accelerating voltage of 15 kV.

Results

Based on the ovarian macroscopic appearance, six gilts presented luteal phase and three animals demonstrated follicular phase. The lining epithelium of the oviduct segments differed in feature between control sows and RB gilts both at follicular and luteal phases (Fig. 1 compares to Figs. 2, 3). The ultrastructure of sow oviducts had the expected characteristics of a porcine oviduct (Fig. 1), whereas the secretory and ciliated cells in all oviductal segments of the RB gilts presented atypical structure (Figs. 2, 3). At follicular phase, the epithelial surface in the ampulla from cyclic sows revealed to be abundantly ciliated with many bulbous processes of secretory cells located among the cilia (Fig. 1a). The cilia were thin, smooth, equitable uniform in length, quite evenly distributed and did not adhere to each other.

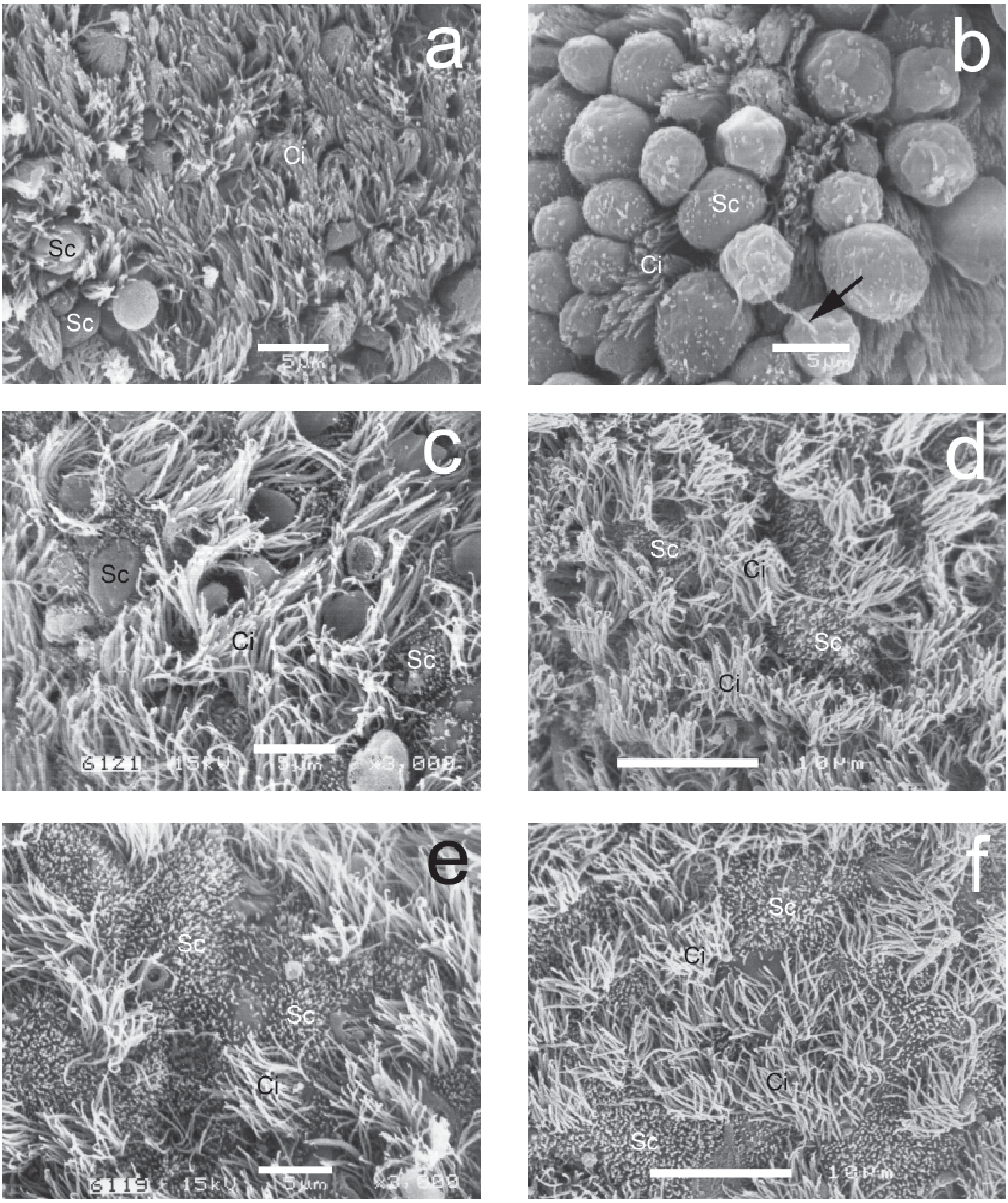

Figure 1 Scanning electron micrographs of the lining epithelium of the ampulla (a,b), isthmus (c,d) and UTJ (e,f) of representative first parity sows (controls). Samples were removed at the follicular (a,c,e) and luteal (b,d,f) phases. Sc, Secretory cells; Ci, ciliated cells. Note the bulbous and protruding of secretory cells on the entirely epithelial surface of ampulla during luteal phase and found solitary cilia (arrow) on apical surface. Bars represent on figures a,b,c,e = 5 μm and represent on figures d,f = 10 μm.

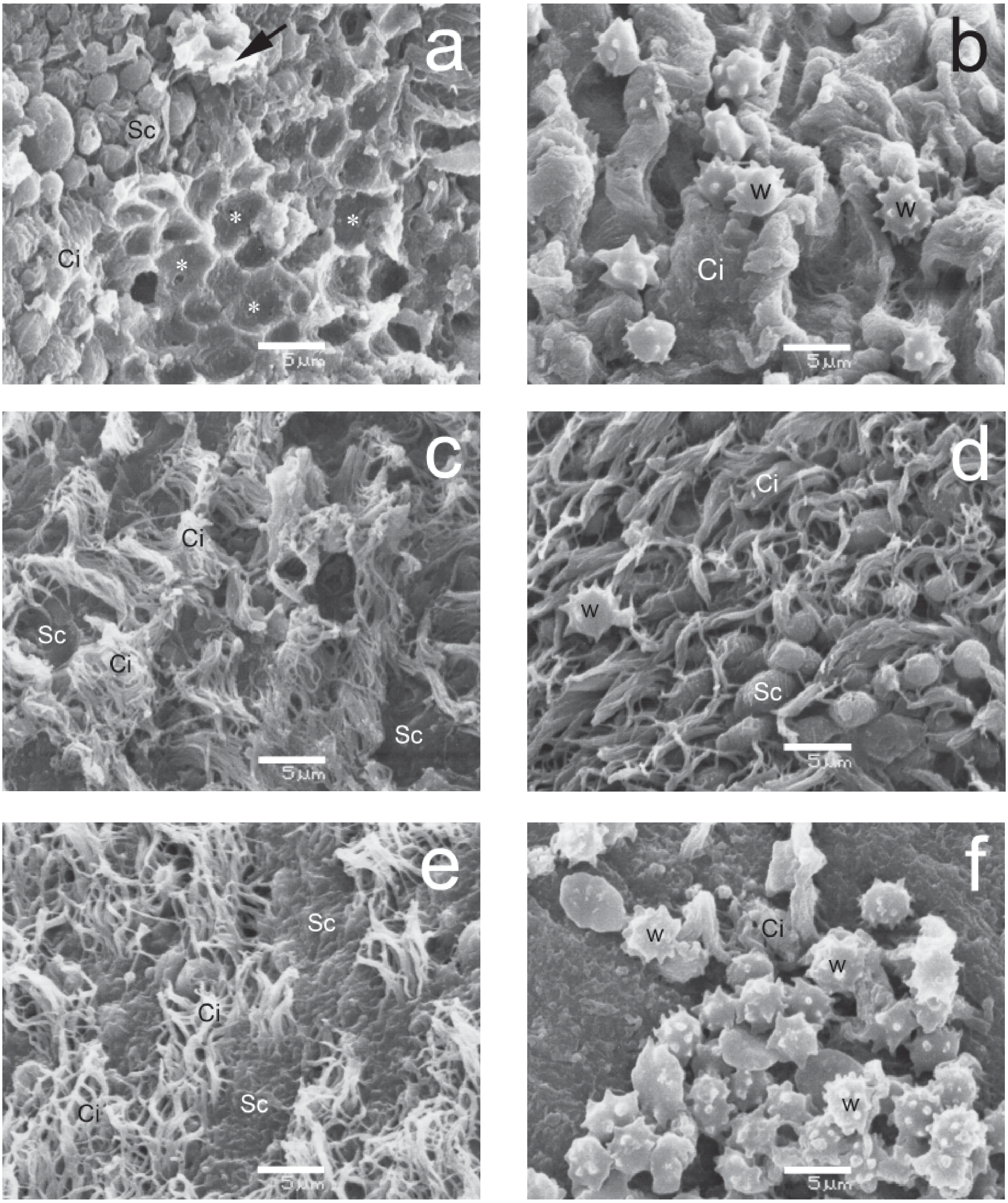

Figure 2 Scanning electron micrographs of the lining epithelium of the ampulla (a,b), isthmus (c,d) and UTJ (e,f) of repeat breeder gilts collected at the follicular phase. Sc, Secretory cells; Ci, ciliated cells; W, white blood cells. Note the presence of mucous-like debris covering the epithelial cells (arrow) and shallow craters (stars) on the luminal surface. The bars indicate the original magnifications. Bars = 5 µm.

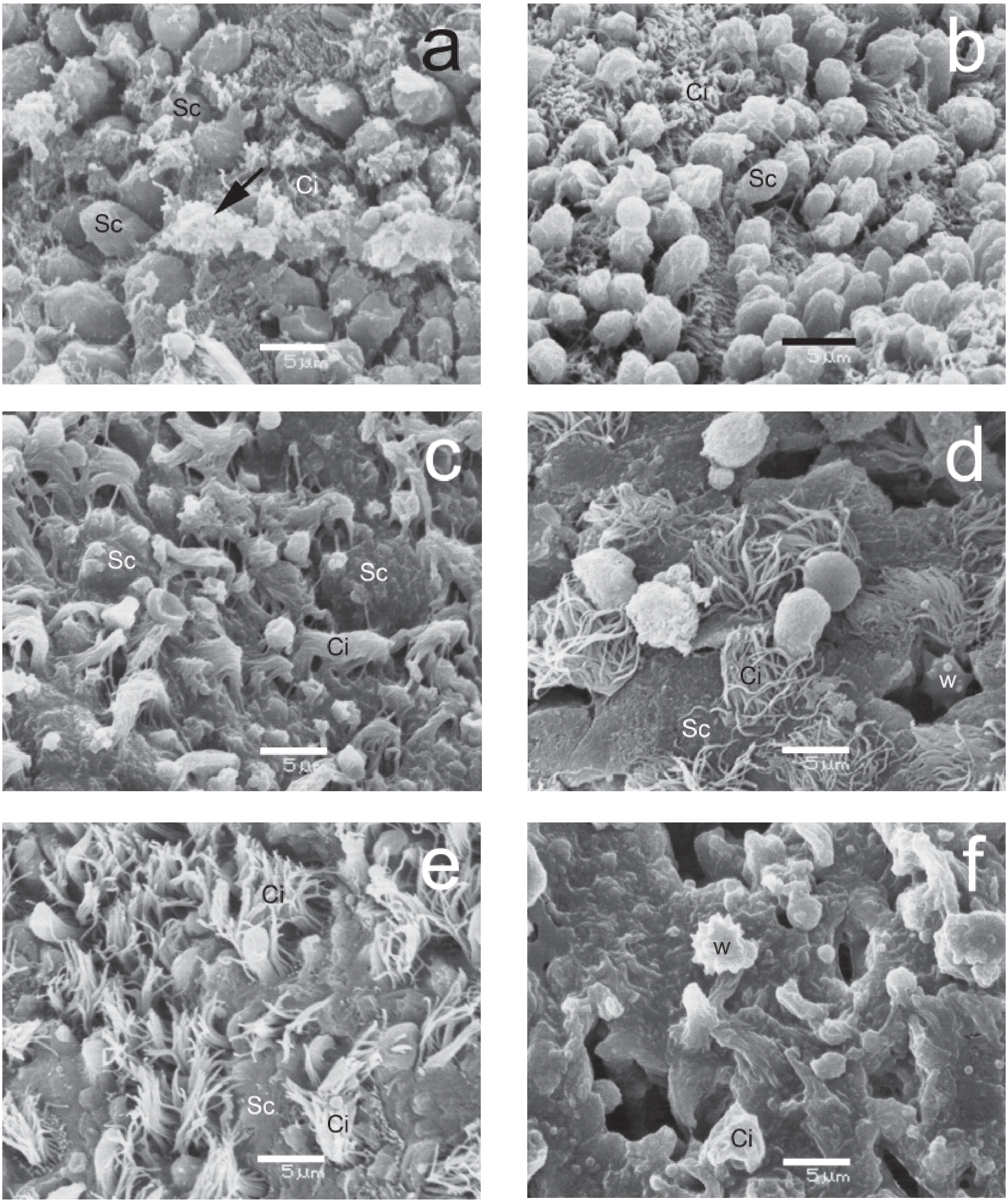

Figure 3 Scanning electron micrographs of the lining epithelium of the ampulla (a,b), isthmus (c,d) and UTJ (e,f) of repeat breeder gilts colleted at luteal phase. Sc, Secretory cells; Ci, ciliated cells; W, white blood cells. Note the presence of the amorphous mucous-like debris covering the cilia and secretory cells (arrow) particularly in the ampulla (a) and the damage between epithelial cells in isthmus (d) and UTJ (f). Bars = 5 µm.

The cilia usually extended above the apical surface of secretory cells which they concealed to some extent. The secretory cells appeared a variety in shape from completely flat and covered with cilia to distinctly protruding. In isthmus and UTJ, the ciliated and secretory cells were equally distributed over the epithelium (Figs. 1c, e) and the secretory cells appeared a bit bulge at their apical surfaces and obviously covered with short microvilli. In the gilt ampulla, the various shallow depressions, the debris and inflammatory cells were found on the epithelial surface (Figs. 2a, b). The cilia were coated and attached together by mucous-like substance (Fig. 2b). In the isthmus (Figs. 2c, d) and UTJ (Figs. 2 e, f), the cilia were fused each other and declined on the lining surface, whereas the microvilli seemed sparse and less distinct on the apical surface of secretory cells compared with controls. A cluster of leukocytes were also seen on the epithelial surface, particularly in the UTJ.

At the luteal phase, the lining epithelium of sow ampulla was entirely covered with the bulbous apical processes of secretory cells (Fig. 1b). The cilia were concealed by the processes of secretory cells. Stubby microvilli extended from secretory cells and occasionally solitary cilia were found on their apical surface (Fig. 1b). The epithelium of isthmus and UTJ presented a few changes in ciliation and resemble that in the follicular phase (Figs. 1d, f compares to Figs. 1c, e). The secretory cells of the gilt ampulla became less distinctly bulbous apical processes, lacking of microvilli, without solitary cilia on the apical surface and the size of these cells were smaller than controls (Figs 3a, b). The cilia showed atypical orientation which fused with the epithelial surface and the amorphous debris covered the epithelial cells (Figs 3a, b). In the isthmus and UTJ of RB gilts, the ultrastructure of the epithelium conspicuously presented abnormal compared with controls. The cilia were packed together and did not evenly distributed, whereas the secretory cells were shown no microvilli and covered with the mucous-like substance (Figs. 3c-3f). The heavily damaged epithelium following with the inflammatory cells infiltration and unidentified droplets were seen (Figs. 3d, f)

Discussion

The purpose of the study was to use SEM to investigate the morphology of the oviduct lining epithelium of RB gilts in comparison to normal cyclic sows during estrous cycle. The SEM photomicrographs demonstrated the morphological changes of ciliated and secretory epithelial cells found in RB gilt oviducts both the follicular and luteal phases. The present investigations and the previous studies about the histological defects in RB gilt oviducts (Tienthai et al., 2006), suggesting that one possible risk factor of the reproductive disturbances is associated with the inadequate oviductal function and this might lead to the cause of RB in gilts.

The knowledge about the complicated SEM topography of mucosal epithelium, not only gamete transport or embryonic growth was interacted but also the secretion movement which is essential for creation of regional microenvironments along the oviduct (Leese et al., 2001). The general results of control sows in this study are in agreement with those of earlier SEM studies of the lining epithelium of the ampulla, isthmus and UTJ of the normal cyclic pigs. The epithelial morphology of porcine oviductal segments was characterized by two types of cells, ciliated and non-ciliated but microvilli covered so called secretory cells (Stalheim et al., 1975; Wu et al., 1976; Abe and Oikawa, 1992). In general, the porcine infundibulum and ampulla were heavily ciliated during the follicular phase, but large numbers of cilia were hidden during luteal phase. By contrast to the ciliated cells of the infundibulum and ampulla, the ciliated cells of the isthmus and UTJ demonstrated little changes between follicular and luteal phases (Abe and Oikawa, 1992). The regular cycle of ciliogenesis and deciliation by epithelial cells found in the infundibulum and ampulla is depended on the female hormonal levels, i.e. the ciliogenesis in the follicular phase occurs under the influence of estrogen, whereas the appearance of progesterone inhibits this regulation and leads to deciliation (Nayak et al., 1976; Verhage et al., 1979). In the same time, this process showed a few changes in UTJ and isthmus suggesting that there are cellular differences associated with the hormonal responses occurred in ciliated epithelial cells of the various pig oviduct segments.

Normally in mammals, the cilia in the infundibulum and ampulla are considered to be primarily responsible for transport newly oocytes and the presence of a normally ciliated epithelium during follicular phase and at ovulation is very important for reproductive process (Odor and Blandau, 1973; Hunter, 1988). On the contrary, the cilia of the isthmus and UTJ that showed few changes during follicular phase have not been clarified the exact function. However, there is evidence that the caudal isthmus and UTJ is the site of sperm reservoir (Hunter, 1988), where spermatozoa usually bind to the cilia and maintain their viability, motility and fertilizing capacity after detachment (Suarez et al., 1991). The investigations of the sperm ultrastructure resided in the sperm reservoir during estrus found that most of spermatozoa apposed to cilia and some sperms attached to the microvilli of epithelial cells (Mburu et al., 1997). We also found that these spermatozoa were largely viable during pre- and peri-ovulation as detected by flow cytometry (Tienthai et al., 2004). Meanwhile, the function of microvilli covered on secretory cells is absorption and the secretions from these cells varied along the estrous cycles provide the necessary environments for fertilization processes and early embryonic development (Hunter, 1988). The above investigations suggest that the complex interaction between ciliary activities, microvilli absorption and the flow of tubal secretion in the pig oviduct are essential for achievement of pregnancy (Rodriguez-Martinez et al., 2001).

The secretory cells presented cyclic changes in all oviduct regions but marked changes of these cells were observed as the cytoplasmic protrusions in the ampulla at luteal phase. It has been known that the secretory cells actively produce and secrete specific glycoproteins into the lumen and their secretions form the oviduct fluid (Leese et al., 2001). Some kind of the glycoproteins found in the infundibulum and ampulla associate with ovulated oocytes and developing embryos and play important roles in fertilization and early embryonic development (Hunter, 1994). Furthermore, glycoproteins and glycosaminoglycans secreted in the caudal isthmus and UTJ become associated with the surface of spermatozoa (Rodriguez-Martinez et al., 2001) and might affect other functions of spermatozoa to progress fertilization (Tienthai, 2005). The secretory cells of the porcine oviduct epithelium have been recently studied by transmission electron microscopy (TEM) suggesting that the number of secretory granules was highest in the secretory cells of ampulla, isthmus and UTJ at follicular phase, whereas the number of granules was less in the cells of these segments and almost free in the cells of infundibulum at luteal phase. Importantly, the moderately electron-dense granules were observed only in the ampulla, while the electron-lucent granules were found in the isthmic and UTJ (Abe and Hoshi, 2007). Therefore, the differences in SEM secretory cell features between the estrous stages were distinctly shown only in the ampulla indicating the sensitive response of the secretory cells in this region to female hormonal levels. By the TEM data, however, there are some differences in the secretory activity and the contents of the secretory granules in each region of the pig oviduct adjusting for normal functions which occurred during fertilization process and estrous cycle. In addition, the secretory cells in the sow ampulla of luteal phase usually displayed the solitary cilia has also reported in the pig (Abe and Oikawa, 1992), pig-tailed monkey (Rumery et al., 1978) and human (Barberini et al., 1994). However, the solitary cilia were not observed in the RB gilt ampulla and several studies suggested that the appearances of solitary cilia may be closely related to fluctuations in levels of ovarian hormones (Odor and Blandau, 1985; Abe and Oikawa, 1989). Although, the function of solitary cilia was not clearly studied, the absence of these solitary cilia may be involved in the deviating hormonal pattern during estrous cycle of RB gilts.

Reproductive failures, in particular RB, were the second dominating problem in the farms and were one of the reasons for post-mortem examination of genital organs of culled gilts (Ehnvall et al., 1981). Based on our study, all gilts culled because of this cause unrelated to the suspected diagnosis because the macroscopic examination of the ovaries in these animals was shown cycling. Their ovaries were active but they presented failed to conceive. Tummaruk et al. (2001) reported that two main biological components causing the RB problem were fertilization failure and embryonic loss. Except for the ovaries and uterus, therefore, the oviduct might consider being the cause of this problem. In the present study we found the atypical differences in ultrastructure of epithelium, for instance, the disarrangement of cilia, less distinctly microvilli, less bulbous protruding and damage of secretory cells which has also been observed in earlier study of RB heifer oviducts (Bage et al., 2002). We have known that the cilia are very important for oocyte and early embryonic transport as well as the intraluminal fluid movement. The dysfunctions of cilia referred as the reduction of frequency beat, low density, short or damage and loss of cilia were found in salpingitis and some disease (Halbert et al., 1997; Leng et al., 1998). The accumulation of the cellular or mucous debris on the oviduct epithelial surface in the present study could have result from the inadequate ciliary function. Considering to the microvilli, we found in the RB gilts that microvilli were scarce on the surface of secretory cells. Normally, the main function of the microvilli is absorption and could be involved in the retention of the oviduct fluid (Hunter, 1988), thus the dysfunction of microvilli might case the hydrosalpinx and interfered the normal microenvironment (Ajonuma et al., 2005). Although the morphological differences could be observed only at SEM, it could be deleterious to fertility which has already been found in RB heifers (Linares, 1981; Gustafsson, 1985) as an impaired developmental competence in the early embryo, and a reduced morula or blastocyst rate. Therefore, further studies on the secretory patterns and characteristic of early embryos in RB gilts are highly activated.

Furthermore, the numerous inflammatory cells have been presented on the epithelial surface of RB oviducts corresponded to the recent study which demonstrated the tendency of intraepithelial immune cell infiltration being higher in RB gilts than controls (Tienthai et al., 2006). These observations might be deleterious to fertility and increasing the risk of RB problem and such aspects have already been established in RB heifers (Linares, 1981; Gustafsson, 1985). In the normal pig oviduct, the main type of immune cells presented in the intraepithelium were lymphocytes which were high variation in the distribution numbers among regions and stages found in individual pigs, while the neutrophils and macrophages were also presented in a small number (Jiwakanon et al., 2005). These immune cells found in the oviduct were involved in the unspecific and specific defense mechanisms (Morris et al., 1986). The appearance of the inflammatory cell infiltration has been recorded in RB gilt oviducts indicating, at least, the viral or bacterial infections occurred in the female genital organs and caused the damaged epithelium (Busch et al., 2000; Mengeling et al., 2000). In addition, it is possible that the subclinical inflammations by microbial infection in oviduct could not be examined by macroscopic examination. Therefore, the presence of immune cells infiltration on the surface epithelium referred as the damages of gilt oviduct epithelium, e.g. the sloughing of the epithelial cells found in the present study, might be the reason of oviduct dysfunction.

In conclusion, the ultrastructural changes representing in the RB gilt oviducts cause a reduction in the capability for ciliary beat, microvilli absorption and secretion of epithelial cells and impair the potency of the pig oviducts. In order to better understand physiological changes and type of microorganisms infected oviduct during the pig pathological RB, the expression of estrogen and progesterone receptors as well as the bacteriogical examination from the oviducts of RB gilts needs to be investigated in the further study.

Acknowledgements

The present study has been supported by the Ratchadaphiseksomphot Endowment Fund, 2005, Chulalongkorn University, Bangkok, Thailand. The excellent technical support of Miss Bang-orn Wattana-umpai is gratefully acknowledged.

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