

## Morphological Changes in the Oviduct of Culling Replacement Gilts

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### Abstract

The aim of this study was to investigate the morphological changes of the oviduct from 30 culling replacement gilts and 6 control sows. Blood samples and female genital organs were taken immediately after slaughter. Post-mortem examinations were made on the genital organs and oviductal samples, i.e. utero-tubal junction (UTJ), isthmus and ampulla, were collected and fixed for histological analysis. Observations indicated that the reasons for culling the gilts were 'no estrus' (68%) and 'repeat breeding' (32%). The greatest pathological changes (67%) were found in repeat breeding gilts, whereas 52% of no estrous gilts were at prepubertal phase. The female hormonal levels detected from the majority of gilts corresponded to ovarian appearances. Microscopic findings of the gilt oviductal epithelium revealed that characteristics of secretory and ciliated cells similar to the sows and pathological abnormalities were found in some gilts. The cytoplasmic protrusions and periodic acid-Schiff (PAS) staining revealed variable results. The distribution of intraepithelial leukocytes was significant higher in no estrous/prepubertal gilts than follicular sows ( $p \leq 0.05$ ). A significant decrease in epithelial cell height was observed in the UTJ and ampulla of culling gilts compared with sows ( $p \leq 0.05$ ). In conclusion, the epithelial cell height was the essential parameter for indicating the morphological changes of culled gilt oviducts, whereas the distribution of intraepithelial leukocytes needs to be further studied. The results suggested that reproductive failures, i.e. no estrus and repeat breeding, were found in culled replacement gilts of some swine farms in Thailand and were involved in the proper functions of the oviduct.

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**Keywords :** Pig, morphology, oviduct, repeat breeding, no estrus.

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## บทคัดย่อ

### การศึกษาการเปลี่ยนแปลงทางสัณฐานวิทยาของท่อนำไข่สุกรสาวทดแทนที่ถูกคัดทิ้ง

ไฟศาด เทียนไทย<sup>1</sup> เกรียงยศ สังเจริญพงษ์<sup>1</sup> เพศีชา ธรรมรักษ์<sup>2</sup>

วัตถุประสงค์ของการศึกษานี้ ต้องการทราบถึงการเปลี่ยนแปลงทางสัณฐานวิทยาในท่อนำไข่ของสุกรสาวทดแทนที่ถูกคัดทิ้งจำนวน 30 ตัวเปรียบเทียบกับแม่สุกรล่าดับครอคที่ 1 ถึง 2 จำนวน 6 ตัว เจาะเลือดและเก็บอวัยวะสืบพันธุ์ หลังจากสุกรถูกส่งไปยังโรงฆ่าสัตว์ ตรวจลักษณะทางพยาธิสภาพและเก็บท่อนำไข่ (รอยต่อของปีกมดลูกกับท่อนำไข่ อิสมัสและแอนพูลล่า) เพื่อศึกษาทางเนื้อเยื่อวิทยา ผลการศึกษาพบว่า สุกรสาวมีปัญหาไม่เป็นสัดร้อยละ 68 และผสมไม่ติดร้อยละ 32 รอยโรคร้อยละ 67 พบในสุกรสาวกลุ่มผสมไม่ติด ขณะที่สุกรสาวกลุ่มไม่เป็นสัดร้อยละ 52 อยู่ในระยะก่อนการเจริญพันธุ์ ระดับออร์โนนเพค เมียของสุกรสาวส่วนใหญ่สอดคล้องกับสภาพการทำงานของรังไข่ เมื่อศึกษาด้วยกล้องจุลทรรศน์แสดงว่าพบว่าเซลล์คัดหลัง และเซลล์ที่มีชีวีเลี้ยงของเยื่อบุท่อนำไข่สุกรสาวคล้ายกับสุกรกลุ่มควบคุม และพบความผิดปกติบางอย่างของเยื่อบุในสุกรสาวบางตัว ผลการประกูลของ cytoplasmic protrusions หรือ secretory granules และการย้อมด้วย PAS ในการศึกษาครั้งนี้มีความแปรปรวน การกระจายของเซลล์เม็ดเลือดขาวในชั้นเยื่อบุมีความแตกต่างอย่างมีนัยสำคัญในสุกรสาวกลุ่มไม่เป็นสัดระยะก่อนการเจริญพันธุ์เปรียบเทียบกับแม่สุกรระยะฟอลลิคูล่า ( $p \leq 0.05$ ) ความสูงของเซลล์เยื่อบุรีเวณรอยต่อของปีกมดลูกกับท่อนำไข่และแอนพูลล่าของสุกรสาวมีความแตกต่างทางสถิติ เปรียบเทียบกับแม่สุกร ( $p \leq 0.05$ ) สรุปได้ว่าพารามิเตอร์ที่ใช้เป็นเกณฑ์ในการตรวจความผิดปกติของท่อนำไข่สุกรสาวคือ ความสูงของเยื่อบุ ขณะที่การกระจายของเซลล์เม็ดเลือดขาวในชั้นเยื่อบุจำเป็นต้องศึกษาเพิ่มเติม ผลการศึกษายังชี้ว่าปัญหา ระบบสืบพันธุ์สัมภาระ ซึ่งประกอบด้วยการไม่เป็นสัดและการผสมไม่ติด สามารถพบได้และเป็นสาเหตุหนึ่งที่สำคัญของการคัดทิ้งสุกรสาวทดแทนในฟาร์มสุกรบางส่วนของประเทศไทย ซึ่งส่งผลกระทบต่อการดำเนินการที่อย่างสมบูรณ์ของท่อนำไข่สุกร

**คำสำคัญ:** สุกร สัณฐานวิทยา ท่อนำไข่ การผสมไม่ติด การไม่เป็นสัด

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### Introduction

Gilt fertility impacts overall herd performance since these are often the largest farrowing group in the swine industry and approximately 35-55% of the sow herd is replaced by gilts each year (D'Allaire and Drolet, 1999). The largest proportion of female

pigs is removed as a result of reproductive disorder (Dijkhuizen et al., 1989) and it is the most frequent reason for culling gilts in commercial herds in several countries (Einarsson et al., 1974; Ehnnvall et al., 1981; D'Allaire et al., 1987; Heinonen et al., 1998).

Reproductive disturbances occur on farms in many different forms and the main causes are no estrus, repeat breeding and failure to farrow (Koketsu et al., 1997; Heinonen et al., 1998). When female pigs are culled due to reproductive failure, post-mortem examination of the genital organs, including histological examination of the uterus and ovary, is a useful diagnostic tool (Karlberg et al., 1981; D'Allaire et al., 1987; Dalin et al., 1997). The genital tracts of slaughtered gilts in Thailand have been investigated and have represented abnormalities of the uterus 0.3%, the oviduct 4% and the ovary 5.6% (Kunavongkrit et al., 1986). Other studies have usually focused on the uterus and ovary though the data indicates that the incidence of the oviduct disorders is high and might be the cause of the reproductive failure in gilts. In Thailand, investigations into the causes of reproductive failure in gilts are scarce and no comprehensive evaluation of the gilt oviduct has been carried out. Therefore, the morphological changes of the oviduct collected from replacement gilt removal because of no estrus and repeat breeding from commercial swine farm could be the important data to suggest preliminary causes of reproductive failure in gilts.

The oviduct plays an important role in reproductive events, such as gamete transport, sperm capacitation, ovulated oocyte maturation, fertilization and embryo development (Hunter, 1998; Rodriguez-Martinez et al., 2001). The epithelium, subepithelial tissues, internal muscular layer, and intraluminal fluids of specific oviductal segments favor and support these processes (Hunter, 1988). The basic morphological changes which follow with the female hormonal levels in blood plasma have been studied to determine the functions of the porcine uterus (Kaeoket et al., 2001). These parameters have been applied to examine the cyclic sow oviduct (Jiwakanon et al., 2005) suggesting that the morphology of each oviductal segment differs with the estrous cycle. To better understand the

differences of reproductive physiology and pathology in the pig oviduct, more information is needed regarding the morphological studies of culling gilt oviducts. The objective of this study was to characterize the morphological changes of oviductal mucosa at different estrous stages associated with the ovarian features and the plasma hormonal levels of culling replacement gilts removed from the commercial farms in Thailand.

## Materials and Methods

### *Animals, tissue collection and macroscopic findings*

Thirty cross-bred (Landrace × Yorkshire) replacement gilts with individual farm histories culled due to no estrus ( $n = 21$ ) and repeat breeding ( $n = 9$ ) from commercial swine farms in central and eastern regions of Thailand were slaughtered at local abattoirs. Immediately after slaughter, the genital organs were removed and kept in plastic bags at a temperature of 4 °C and transported to the laboratory within 4-6 hours. The genital tracts comprising the cervix, uterus, oviducts and ovaries, were macroscopically observed for pathological lesions and recorded. The ovaries and oviducts were dissected for further evaluation. The morphology of the ovary was studied to determine the phase of the ovarian cycle in the gilts, according to Knox (2005). The oviducts from healthy cyclic sows with parity number of 1-2 during follicular ( $n = 3$ ) and luteal ( $n = 3$ ) phases were used as the control group.

### *Blood collection and hormone assays*

Blood samples were collected from the jugular vein of a restrained animal less than 1 hour prior to slaughter, using vacutainer heparin tubes. The blood samples were centrifuged at 3,000 rpm, for 10 min and the plasma was stored in plastic tubes at -20 °C until analyzed. Both estradiol and progesterone levels

were determined by a Chemiluminescent Microparticle Immunoassay (CMIA, Abbott Laboratories, IL, USA) at Bangkok RIA Laboratory (Bangkok, Thailand). The minimum assay sensitivity of estradiol was 33.03 pmol/L and the maximum assay sensitivity of progesterone was 146.8 nmol/L.

#### ***Preparation of oviduct samples and histological studies***

The oviducts from the gilts: no estrus at follicular phase (NF); no estrus at luteal phase (NL); no estrus at prepubertal phase (NP); repeat breeding at follicular phase (RF); repeat breeding at luteal phase (RL) and control animals: at follicular phase (CF); at luteal phase (CL), were separated into UTJ, isthmus (IST) and ampulla (AMP), fixed in 10% (w/v) buffered formalin and embedded in paraffin. Sections were cut to 5-7  $\mu$ m thickness and stained with hematoxylin-eosin (H&E) for evaluation of their general morphology and distribution of intraepithelial immune cells. The presence of glycoprotein was demonstrated by PAS reaction.

Microscopic evaluation of the oviductal samples was done by the same person who was also unaware of the identity of the animals, i.e. all slides were coded before examination. A light microscope was used with objective 20 and eyepieces 10. The samples were firstly examined for normal or pathological changes and photographs were captured from selected sections with a Moticam 2000 digital microscopic camera (Motic Incorporation Ltd., Hong Kong). The morphological changes occurring in the gilt oviduct were examined by the characteristics of the epithelium composed of pseudostratified (with low or high columnar) and simple columnar. The cytoplasmic protrusions and secretory granules of the secretory epithelial cells including the intensity of PAS-positive staining in different compartments of UTJ, IST and AMP were semi-quantitatively evaluated.

The height of epithelium was determined by using Motic Image 2.0 Software and a Moticam 2000 digital microscopic camera (Motic Incorporation Ltd.). For this measurement 100 cells in different locations for each region were selected only if the plane of section clearly passed through the cell nucleus, and the section was parallel to the longitudinal axis of the cell and the apex and base of the cell could be easily distinguished (Verhage et al., 1979).

#### ***Immune cell evaluation***

A count of numbers of intraepithelial immune cells were performed by using an ocular reticule with 25 small squares placed in one eyepiece of the light microscope with 200 $\times$  magnification. In each section, counts were conducted by movement along the length of the epithelial layer in a non-overlapping manner. In the UTJ and IST sections, counts were conducted by movement of the entire area in a non-overlapping manner, while the fifty arbitrarily chosen microscopic fields were counted in the AMP. Only the immune cells found in the surface epithelium were counted as the number of cells per 25 small squares.

#### ***Statistical analysis***

Data of epithelial cell height and the number of immune cells was handled and statistically analyzed using the SAS statistical package (version 9, SAS Institute Inc., Cary, NC, USA). The differences between means (as least squares means) were determined by analysis of variance (ANOVA) using the MIXED MODEL procedure. The significance level was set at  $p \leq 0.05$ .

## **Results**

#### ***Reproductive history data, macroscopic findings and hormonal analysis***

The 30 gilts from the commercial swine farms in the central and eastern parts of Thailand over a 4-month period (July-October, 2005) were

culled due to the reproductive problems as summarized in Table 1. Approximately 68% of the total number of gilts slaughtered during the study period resulted from no estrus. Based on ovarian macroscopic appearance, the greatest number of animals with no estrus demonstrated prepubertal phase (52%), whereas the majority of gilts with repeat breeding presented a luteal phase (67%). About 53% of culling gilts showed pathological changes (Table

1) of the ovaries, uterus and oviduct. According to the temporary data on these gilts, the common pathological lesions were par-oviduct cysts, uterine edema and par-ovarian cysts, respectively (Table 2). The mean plasma levels of estradiol and progesterone on the day of slaughter relating to the estrous stages are presented in Table 3. We also found that the hormonal levels of some gilts in both repeat breeding and no estrus groups did not corresponded to the ovarian macroscopic findings.

**Table 1** Percentages of culled gilts with pathological abnormalities in relation to ovarian status based on macroscopic appearance.

Reason for culling	No. of culled gilts (%)	Pathological changes (%)	Estrous stages		
			Follicular (%)	Luteal (%)	Prepubertal (%)
No estrus	21 (67.6)	10 (43.5)	3 (14.3)	7 (33.3)	11 (52.4)
Repeat breeding	9 (32.4)	6 (66.7)	3 (33.3)	6 (66.7)	0 (0.0)
Total	30 (100.0)	16 (53.3)	6 (20.0)	13 (43.3)	11 (36.7)

**Table 2** Number of culled gilts and detail of pathological changes.

Reason for culling	No. of gilts	Number of gilts with pathological changes (%)					
		Endometritis	Uterine edema	Par-ovarian cysts	Par-oviduct cysts	Hydro-salpinx	Ovarian adhesion /like tumor
No estrus	10*	1 (10.0)	5 (50.0)	2 (20.0)	4 (40.0)	0 (0.0)	0 (0.0)
Repeat breeding	6*	1 (16.7)	2 (33.3)	3 (50.0)	4 (66.7)	2 (33.3)	2 (33.3)

\*some gilts presented more than one pathological changes

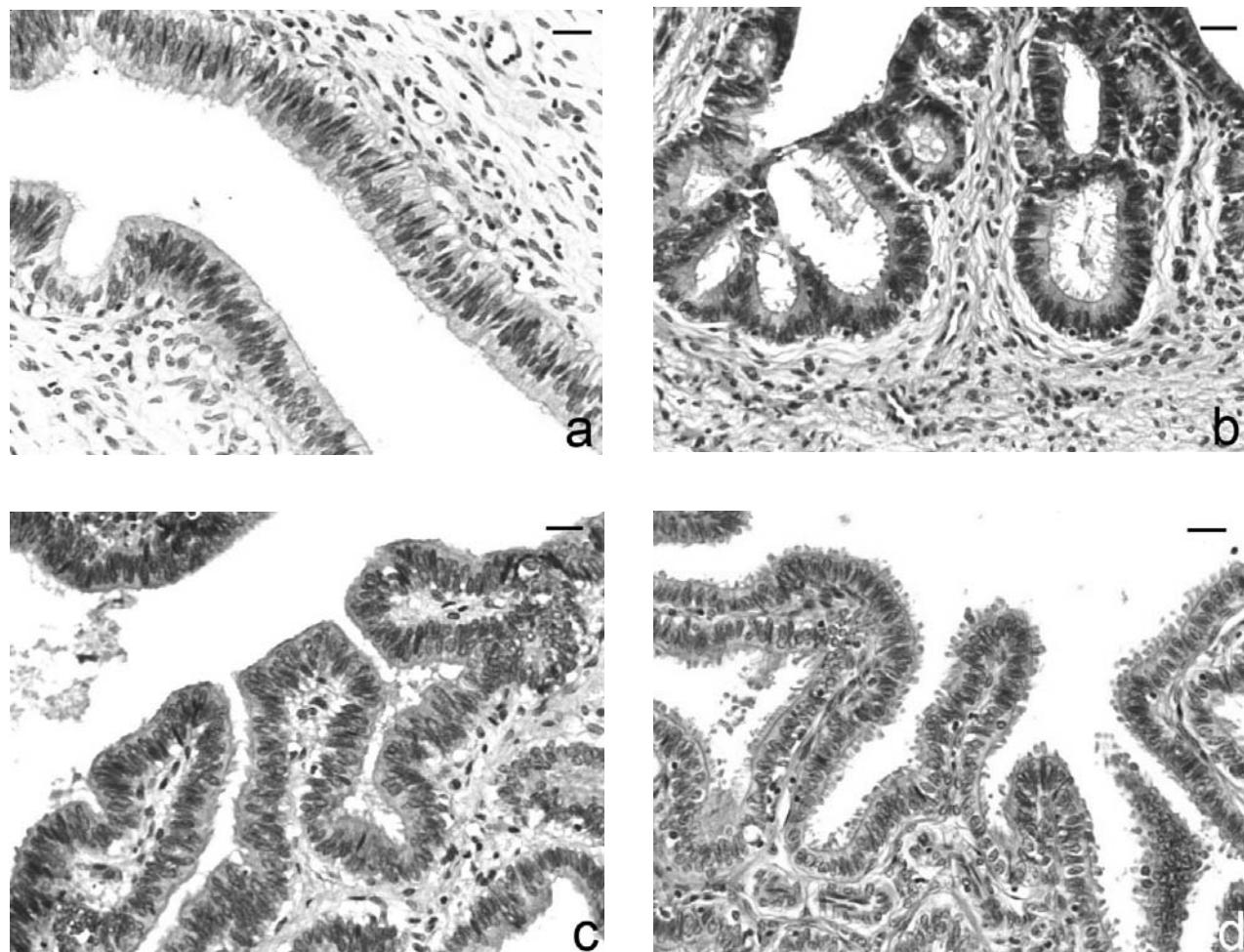
**Table 3** Estrous stages by ovarian macroscopic appearances and female steroid hormones (mean  $\pm$  S.D.) in replacement gilts with reproductive failures.

Reason for culling	Stages (n)	Estradiol (pmol/l)	Progesterone (nmol/l)
No estrus	Follicular (3)	79.6 $\pm$ 42.4	6.7 $\pm$ 4.7
	Luteal (7)	44.5 $\pm$ 20.1	93.1 $\pm$ 38.4
	Prepubertal (11)	33.7 $\pm$ 2.2	8.5 $\pm$ 11.1
Repeat breeding	Follicular (3)	111.3 $\pm$ 30.5	4.1 $\pm$ 1.6
	Luteal (6)	37.9 $\pm$ 10.3	103.6 $\pm$ 48.8

### ***Histological and intraepithelial leukocyte examinations***

The epithelial cells of UTJ and IST were consistently the same pattern at both estrous stages and slightly increased in height at the follicular phase (Fig. 1a, b). In the AMP, the epithelia demonstrated pseudostratification with high columnar at the follicular phase (Fig 1c) and pseudostratified low

columnar or simple low columnar at the luteal phase (Fig. 1d). The evaluation of cytoplasmic protrusions and secretory granules in different segments of the oviducts is presented in Table 4. The protrusions and some extruded nuclei of epithelial cells were clearly observed in the AMP at the luteal phase (Fig. 1d) and also found in the AMP of the no estrus/prepubertal gilts.



**Figure 1** Morphology of the culled gilt oviductal mucosa by light microscopy. (a) uterotubal junction, (b) isthmus and (c) ampulla, at follicular phase, (d) ampulla at the luteal phase. H&E staining, bar = 20  $\mu$ m.

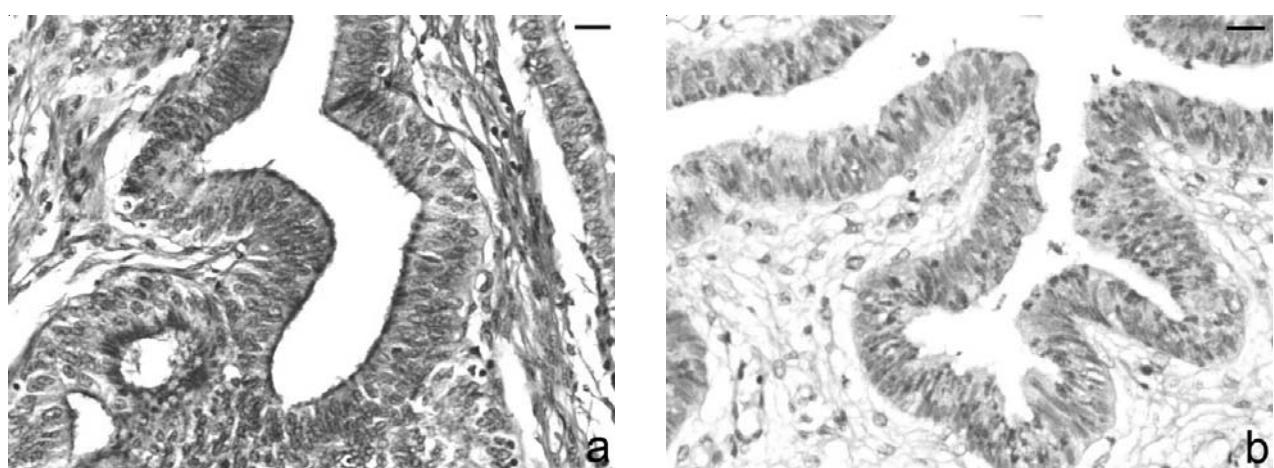
**Table 4** Estimation of cytoplasmic protrusions and secretory granules of the secretory epithelial cells of reproductive failure gilt oviducts.

Group of animals	UTJ	IST	AMP
<b>Control/Follicular</b>	-/+	-/+	-/+
No estrus/Follicular	-/+	-/+	-/+
Repeat breeding/Follicular	-/+	-/+	-/+
<b>Control/Luteal</b>	-/+	-/+	++/+ ++
No estrus/Luteal	-/+	-/+	++/+ ++
Repeat breeding/Luteal	-/+	-/+	++/+ ++
No estrus/Prepubertal	-/+	-/+	++/+

The presence of secretory granules and cytoplasmic protrusions of the epithelial cells was determined as follows: - = none; + = few; ++ = moderate and +++ = high amounts.

Histochemical reactions of the glycoprotein from the oviduct epithelium of culling gilts and control sows are summarized in Table 5. At the follicular phase, secretory cells with high PAS-positive were found in the AMP (Fig. 2b) of all animal groups while PAS staining in the UTJ (Fig. 2a) and IST was shown to be abundant on the surface of the epithelial cells and variably appeared in the apical part of the

secretory cells depending on samples. At the luteal phase, the intensity of secretory cells stained with PAS was decreased in the AMP and PAS staining on the surface epithelium of UTJ and IST was also diminished. In prepubertal gilts, the pattern of PAS staining in the UTJ and IST was demonstrated to be similar to the follicular phase and in the AMP was observed as the luteal phase of other animal groups.



**Figure 2** Sections of uterotubal junction (a) and ampulla (b) during follicular phase of culled gilts showing the PAS-positive intensity on the surface epithelium and supranuclear regions of the secretory cells. Bar = 20  $\mu$ m.

**Table 5** PAS staining of the secretory epithelial cells and the secretions on the surface epithelium of culling gilt oviducts.

Group of animals	UTJ	IST	AMP
<b>Control/Follicular</b>	++	++/++	++/++/++
No estrus/Follicular	++	++	++
Repeat breeding/Follicular	++/++/++	++/++/++	++
<b>Control/Luteal</b>	++	++	+
No estrus/Luteal	+	++/++	-/+
Repeat breeding/Luteal	+	+	-/+
No estrus/Prepubertal	++/++	++/++	-/+

The intensity of PAS staining the oviductal epithelium was determined as follows:

- = absent; + = weak; ++ = moderate and +++ = intense staining.

The epithelial cell heights in the three different portions of the oviducts in all animals groups were measured (Table 6). Significant differences ( $p < 0.05$ ) of epithelial cell heights were observed in the UTJ

and AMP of control groups compared to the reproductive failure groups while the height of IST epithelium in both gilts and sows did not show any difference.

**Table 6** Epithelial cell height of the culled gilts in relation to the reproductive problems, the stage of estrous cycle and the site of the oviduct (LSM SEM).

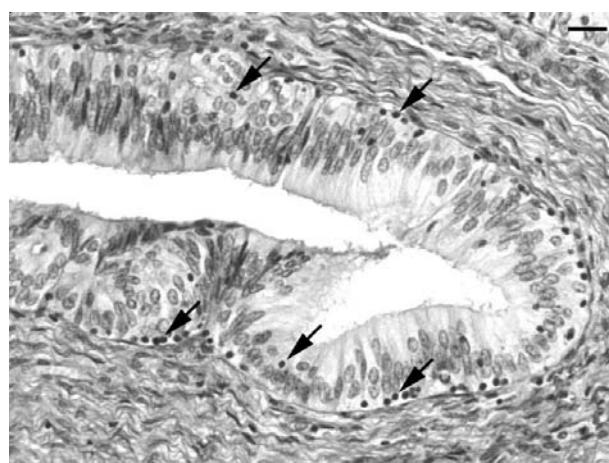
Group of animals	UTJ		IST		AMP	
<b>Control/Follicular (CF)</b>	38.09	3.08	24.01	2.41	34.57	2.07
No estrus/Follicular (NF)	28.93	3.56	19.40	1.97	27.95	3.41
Repeat breeding/Follicular (RF)	29.67	3.57	25.61	3.31	28.19	3.55
Statistics	a,b		-		a,b	
<b>Control/Luteal (CL)</b>	35.51	4.43	20.27	1.69	25.76	1.84
No estrus/Luteal (NL)	27.60	3.65	21.56	2.22	18.58	1.53
Repeat breeding/Luteal (RL)	28.55	2.82	24.67	2.28	19.41	2.18
Statistics	d,e		-		d,e	
No estrus/Prepubertal (NP)	26.34	1.91	23.48	2.01	20.49	1.76
Statistics*	g,h		-		g,h	

Data within a column differ significantly ( $p < 0.05$ ) as follows: a, CF vs NF; b, CF vs RF; c, NF vs RF; d, CL vs NL; e, CL vs RL; f, NL vs RL.

\*NP group compared to both control groups as follows: g, CF vs NP; h, CL vs NP

Intraepithelial leukocytes of the pig oviduct that are mainly located in the basal lamina of the epithelium or between the epithelial cells (Fig. 3) were counted as shown in Table 7. Because of high variation among animals, the numbers of leukocytes in the control and reproductive failure groups counted in most sections were not significant within segments

of both estrous stages. However, the numbers of immune cells found in all portions of no estrus gilts at the prepubertal phase were significantly higher than in the control group at the follicular phase ( $p < 0.05$ ) and tended to be higher ( $p > 0.05$ ) when compared to the control group at the luteal phase.



**Figure 3** Section of uterotubal junction epithelium from culled gilt showing the round shaped nucleus and location of the intraepithelial immune cells (arrows). PAS staining, bar = 20  $\mu$ m.

**Table 7** Distribution of immune cells (number of cells per 25 small squares) in the epithelium of the culled gilt oviduct in relation to the reproductive problems, the stage of estrous cycle and the site of the oviduct (LSM  $\pm$  SEM).

Group of animals	UTJ	IST	AMP
<b>Control/Follicular (CF)</b>	$4.35 \pm 0.71$	$1.51 \pm 1.12$	$1.92 \pm 0.23$
No estrus/Follicular (NF)	$4.18 \pm 0.91$	$2.55 \pm 0.63$	$3.36 \pm 1.13$
Repeat breeding/Follicular (RF)	$3.98 \pm 1.02$	$2.61 \pm 0.59$	$3.35 \pm 0.98$
Statistics	-	-	-
<b>Control/Luteal (CL)</b>	$6.64 \pm 1.16$	$3.14 \pm 0.67$	$2.07 \pm 0.26$
No estrus/Luteal (NL)	$5.90 \pm 1.33$	$4.18 \pm 1.10$	$3.78 \pm 0.42$
Repeat breeding/Luteal (RL)	$4.64 \pm 1.15$	$3.88 \pm 1.13$	$5.98 \pm 1.12$
Statistics	-	-	e
No estrus/Prepubertal*(NP)	$7.26 \pm 0.83$	$3.86 \pm 0.61$	$4.56 \pm 0.51$
Statistics	g	g	g,h

Data within a column differ significantly ( $p < 0.05$ ) as follows: a, CF vs NF; b, CF vs RF; c, NF vs RF; d, CL vs NL; e, CL vs RL; f, NL vs RL.

\*NP group compared to both control groups as follows: g, CF vs NP; h, CL vs NP

## Discussion

In this study, we confirmed that the reproductive failures, i.e. repeat breeding and no estrus problems, were found to be the essential cause for culling replacement gilts on some commercial swine farms in Thailand. Post-mortem of the genital organs together with a microscopic examination of the oviducts was needed to investigate the problem. The results indicated that the replacement gilts culled due to reproductive failures demonstrated deviant functions of the oviduct.

Impaired fertility was found to be the most frequent cause for culling sows in several countries and it was also the main reason for culling gilts which had never produced piglets (Heinonen et al., 1998). Based on herd records and slaughterhouse material in Sweden and Denmark, a large proportion of culled gilts were slaughtered with the cause of reproductive failure comprising no heat 11% and repeat breeding 9% (Ehnvall et al., 1981). The percentage of gilts culled because of no estrus and repeat breeding in the present study was 67% and 33%, respectively. Therefore, the results demonstrated in the two investigations tend to be comparable. Interestingly, 52% of the culled gilts with no estrus presented the prepubertal stage, according to the macroscopic appearance of the ovaries and lower female hormonal levels; thus indicates that poor farm management may influence these replacement gilts' delayed puberty. This information corresponds to Einarsson et al. (1974) and Karlberg (1979) suggesting that delayed puberty was the dominant reason for gilts being slaughtered due to failure to exhibit estrous signs. Considering another reproductive problem, Tummaruk et al. (2001) suggested that repeat breeders were identified in gilts that were rebred between 18 and 100 days after first mating and two main biological components causing this problem were fertilization failure and embryonic loss. However, other factors such as cystic ovaries, abortion (Martinez et

al., 1992) and missing first estrus detection (Tummaruk et al., 2001) may also be involved. The repeat breeder gilts investigated in this study were at the luteal phase and the ovarian cysts were not observed indicating a normal function of the corpus luteum after ovulation for maintaining pregnancy. It is possible that the embryonic death due to various factors could be the cause of these repeat breeder gilts.

The present study has attempted to elucidate the morphological changes of reproductive failure in gilt oviducts at all estrous stages compared with the sows. It was shown that cytoplasmic protrusions and the number of secretory granules on the surface epithelium were found to be high in the AMP of gilts and sows at the luteal phase as well as of prepubertal gilts. Similar results were found in cyclic sows by light microscopy (Jiwakanon et al., 2005) and gilts by scanning electron microscopy (Abe and Oikawa, 1992). They observed the cytoplasmic protrusions and bulbous processes of secretory cells in the AMP and infundibulum (INF) at stages with high progesterone levels. The cause of cytoplasmic protrusions in the oviduct was not clear but several authors suggested it to be a part of the process of cell death (Hollis et al., 1984; Murray, 1995). Furthermore, we used PAS-staining which is the normal method for determining the proportion of secretory (PAS-positive) and non-secretory (PAS-negative) cells to indicate the modification of epithelial cells in different parts and at different cycles related to the functions of oviduct (Anzaldua et al., 2002). Because of technical problems in the present study, the PAS-staining sections of gilt oviducts could not be evaluated by counting the number of secretory and non-secretory cells. Additionally, variable semi-quantitative results were found in the present finding. These criteria (cytoplasmic protrusions and PAS-staining), therefore, could not be appropriated for examining the changes of culled gilt oviducts.

The epithelial cell heights of the UTJ and AMP were significantly different between animal groups, while the numbers of intraepithelial immune cells in all segments increased more in prepubertal gilts than in follicular sows. The histological changes and distribution of leukocytes of the sow oviduct composed of IST, AMP and INF throughout the estrous cycle were studied (Jiwakanon et al., 2005) but no report has been done on gilts. This is the first report describing the histological changes of replacement gilt oviduct which includes the UTJ segment. In the sow oviduct, the morphology differed only in the AMP and INF at the estrous cycle stage (Jiwakanon et al., 2005). In other species, the cell heights of the AMP and INF epithelium were higher during the follicular phase than the luteal or anestrous phases indicating the influence of estrogen, whereas lower degree of cellular variations was observed in the UTJ and caudal IST (Baracat et al., 1991; Steinhauer et al., 2004). Considering the results among segments and stages, the findings above were in accordance with the pig oviducts in this study. Comparing the changes within the segment and estrous stage, we found that epithelial cell heights measured in the UTJ and AMP of culling replacement gilts were lower than in the control sows. Morphometric data in the ewe oviduct and uterus demonstrated that the cell height of the AMP and uterine epithelium were lowest in prepubertal ewes but did not differ in first-estrus, third-estrus and mature ewes (Lewis and Berardinelli, 2001). Furthermore, a study of the subfertile heifer oviducts by Bage et al. (2002) reported that deviating hormone patterns affect the ultrastructural changes of the oviductal epithelial cells corresponding to this study that suggested that the female hormonal levels in some gilts were not related to the ovarian macroscopic appearance. These changes found in the culling gilts might be related to the inadequate morphological and physiological characteristics of gilt oviduct for

facilitating the gametes or normal embryonic development.

A significant difference in the numbers of intraepithelial leukocytes within segments (UTJ, IST and AMP) was observed in prepubertal gilts compared with sows at the follicular phase and the AMP of repeat breeding gilts compared with sows at the luteal phase. The most common leukocyte in the porcine oviductal epithelium of all segments was lymphocyte and numbers of intraepithelial lymphocytes did not differ among segments and estrous stages (Jiwakanon et al., 2005). This suggested that the lymphocyte infiltration in the oviduct of sows is less influence by ovarian steroid hormones. There is no study involving the distribution of intraepithelial leukocytes in the gilt oviduct. However, the increase in the numbers of intraepithelial immune cells examined in repeat breeding gilt oviducts might be induced by the pathological changes found in the individual animals as shown in the infertility heifer oviduct (Kubar and Jalakas, 2002). Furthermore, the number of intraepithelial lymphocytes in the uterus of prepubertal gilts (Jiwakanon et al., 2006) was approximately two times higher than in cyclic sows (Kaeoket et al., 2001). Therefore, lymphocytes may be involved in the essential regulation of the reproductive tracts during the prepubertal stage of gilts. Of course, the increase of immune cells in the UTJ, IST and AMP of these gilts might disturb normal functions of the oviduct.

An understanding of the morphological changes in the oviduct with the farm history of culled gilts and healthy sows is implicit to our understanding of the physiological mechanisms related to the cause of reproductive disorders. The present study confirmed that the gilts culled due to 'no estrus' and 'repeat breeding' showed differences of cell height and infiltration of immune cells compared with cyclic sows, which indicates poor management and some infections occurred in the replacement gilts found in

Thailand. To better clarify the functional changes of the oviduct in the replacement gilts, the female hormone receptors and ultrastructure of the oviductal mucosa need to be further investigated.

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### References

Abe, H. and Oikawa, T. 1992. Examination by scanning electron microscopy of oviductal epithelium of the prolific Chinese Meishan pig at follicular and luteal phases. *Acta. Anat. (Basel)* 233: 399-408.

Anzaldua, S.R., Camacho-Arroyo, I. and Cerbon, M.A. 2002. Histomorphological changes in the oviductal epithelium of the rabbit during early pregnancy. *Ant. Histol. Embryol.* 31: 308-312.

Bage, R., Masironi, B., Sahlin, L. and Rodriguez-Martinez, H. 2002. Deviant pre-ovulatory hormone patterns affect the epithelium of uterine tube in repeat breeder heifers. *Reprod. Fertil. Dev.* 14: 461-469.

Baracat, E.C., Simoes, M.J., Novo, N.F., Juliano, Y., de Lima, G.R., de Lima Filho O.A. and Kulay Junior, L. 1991. Morphologic and morphometric aspects of the uterine tube epithelium during the menstrual cycle. *Rev. Paul. Med.* 109: 204-212.

D'Allaire, S., Stein, T.E. and Leman, A.D. 1987. Culling patterns in selected Minnesota swine breeding herds. *Can. J. Vet. Res.* 51: 506-512.

D'Allaire, S. and Drolet, R. 1999. Culling and mortality in breeding animal. In: *Diseases of Swine*. 8<sup>th</sup> ed. B.E. Straw, S.D. D'Allaire, W.L. Mengeling and D.J. Taylor (eds.) Iowa: Iowa State University Press. 1003-1016.

Dalin, A.M., Gidlund, K. and Eliasson-Selling, L. 1997. Post-mortem examination of genital organs from sows with reproductive disturbances in a sow pool. *Acta. Vet. Scand.* 38: 253-262.

Dijkhuizen, A.A., Krabbenborg, R.M.M. and Huirne, R.B.M. 1989. Sow replacement: a comparison of farmer's actual decisions and model recommendations. *Livest. Prod.* 23: 207-218.

Ehnvall, R., Blomqvist, A., Einarsson, S. and Karlberg, K. 1981. Culling of gilts with special reference to reproductive failure. *Nord. Vet. Med.* 33: 167-171.

Einarsson, S., Linde, C. and Settergren, I. 1974. Studies of the genital organs of gilts culled for anestrus. *Theriogenology*. 2: 109-113.

Heinonen, M., Leppavuori, A. and Pyorala, S. 1998. Evaluation of reproductive failure of female pigs based on slaughterhouse material and herd record survey. *Anim. Reprod. Sci.* 52: 235-244.

Hollis, D.E., Frith, P.A., Vaughan, J.D., Chapman, R.E. and Nancarrow, C.D. 1984. Ultrastructural changes in the oviductal epithelium of Merino ewes during the estrous cycle. *Am. J. Anat.* 171: 441-456.

Hunter, R.H. 1988. *The Fallopian Tubes: Their roles in Fertility and Infertility*. Berlin: Springer-Verlag. 184 pp.

Hunter, R.H. 1998. Have the Fallopian tubes a vital role in promoting fertility? *Acta. Obstet. Gynecol. Scand.* 77: 475-486.

Jiwakanon, J., Persson, E., Kaeoket, K. and Dalin, A.M. 2005. The sow endosalpinx at different stages of the oestrous cycle and at anoestrus: studies on morphological changes and infiltration by cells of the immune system. *Reprod. Domest. Anim.* 40: 28-39.

Jiwakanon, J., Persson, E. and Dalin, A.M. 2006. The endometrium of the anoestrous female pig: studies on infiltration by cells of the immune system. *Reprod. Domest. Anim.* 41: 191-195.

Kaeoket, K., Persson, E. and Dalin, A.M. 2001. The sow endometrium at different stages of the oestrous cycle: studies on morphological changes and infiltration by cells of the immune system. *Anim. Reprod. Sci.* 65: 95-114.

Karlberg, K. 1979. Post-mortem examination of genital organs from sows slaughtered due to reproduction failure. *Norsk. Veterinaertidskrift*. 91: 731-741.

Karlberg, K., Rein, K.A. and Nordstoga, K. 1981. Histological and bacteriological examination of uterus from repeat breeder gilt and sow. *Nord. Vet. Med.* 33: 389-365.

Knox, R.V. 2005. Recruitment and selection of ovarian follicles for determination of ovulation rate in the pig. *Domest. Anim. Endocrinol.* 29: 385-397.

Koketsu, Y., Dial, G.D. and King, V.L. 1997. Returns to service after mating and removal of sows for reproductive reasons from commercial swine farms. *Theriogenology*. 47: 1347-1363.

Kubar, H. and Jalakas, M. 2002. Pathological changes in the reproductive organs of cows and heifers culled because of infertility. *J. Vet. Med.* 49: 365-372.

Kunavongkrit, A., Chantaraprateep, C., Prateep, P. and Poomsuwan, P. 1986. Ovarian activites and abnormalities in slaughtered gilts. In: *Proceedings 9<sup>th</sup> IPVS Congress*, July 15-18, Barcelona, Spain. 34.

Lewis, A.W. and Berardinelli, J.G. 2001. Gross anatomical and histomorphometric characteristics of the oviduct and uterus during the pubertal transition in sheep. *J. Anim. Sci.* 79: 167-175.

Martinez, E., Vasques, J.M., Roca, J. and Ruiz, S. 1992. Use of the real time ultrasonic scanning for the detection of reproductive failure in the pig heard. *Anim. Reprod. Sci.* 29: 53-59.

Murray, M.K. 1995. Epithelial lining of the sheep ampulla oviduct undergoes pregnancy-associated morphological changes in secretory status and cell height. *Biol. Reprod.* 53: 653-663.

Rodriguez-Martinez, H., Tienthai, P., Suzuki, K., Funahashi, H., Ekwall, H. and Johannisson, A. 2001. Involvement of oviduct in sperm capacitation and oocyte development in pigs. *Reprod. Suppl.* 58:129-145.

Steinhauer, N., Boos, A. and Gunzel-Apel, A-R. 2004. Morphological changes and proliferative activity in the oviductal epithelium during hormonally defined stages of the oestrous cycle in the bitch. *39: 110-119.*

Tummaruk, P., Lundeheim, N., Einarsson, S. and Dalin, A.M. 2001. Repeat breeding and subsequent reproductive performance in Swedish Landrace and Swedish Yorkshire sows. *Anim. Reprod. Sci.* 67: 267-280.

Verhage, H.G., Bareither, R.C., Jaffe, R.C. and Akbar, M. 1979. Cyclic changes in ciliation, secretion and cell height of the oviductal epithelium in women. *Am. J. Anat.* 156: 505-522.