Immune Cell Population in Oviducts in Relation to Ovarian and Progesterone Status of Anoestrous and Repeat Breeding Gilts

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Abstract

The aim of the present study is to determine the distribution of immune cells within the endosalpinx related to the ovarian function in gilts culled due to repeat breeding and anoestrus. A total of 30 reproductive organs from culling crossbred Landrace x Yorkshire gilts were collected from commercial swine herds in Thailand and categorized into 3 groups, i.e. anoestrus, repeat breeding and non-involved in reproductive disorder gilts (control group). The ovaries and oviducts were separated from the reproductive tracts and macroscopically investigated. The oviducts were divided into the utero-tubal junction (UTJ), the isthmus, the ampulla and the infundibulum. The gilts were classified according to ovarian appearance into the follicular and luteal phases. The results showed that lymphocytes were the most dominant immune cells found in all segments and in both phases of the control and culling gilt oviducts. In the epithelium, the numbers of lymphocytes in UTJ and the isthmus of anoestrous and repeat breeding gilts were significantly greater than the control group at luteal phase ($p<0.05$). The neutrophils in UTJ epithelium of repeat breeding gilts were higher than the control gilts at both the follicular and luteal phases ($p<0.05$), whereas, the macrophages in the repeat breeding gilts were found significantly higher than the control gilts only at follicular phase ($p<0.05$). In the subepithelial layer, the numbers of lymphocytes in the infundibulum of anoestrus gilts were significantly greater than the control group at both stages ($p<0.001$), whereas, the plasma cells in the infundibulum of repeat breeding gilts were higher than the control group at both stages ($p<0.001$) as well. Furthermore, the other immune cells, i.e. neutrophils, macrophages and eosinophils, in the UTJ also had the unusual numbers in both culling gilts compared to the control gilts ($p<0.05$). In conclusion, the irregular infiltration of various immune cells appeared in the epithelial and subepithelial layers of the oviduct, especially in the site of the sperm reservoir, possibly indicating improper oviductal functions in both anoestrus and repeat breeding gilts.

Keywords: Anoestrus, gilt, leukocyte, oviduct, repeat breeding

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บทคัดย่อ

ปริมาณเซลล์ระบบภูมิคุ้มกันภายในท่อนำไข่ของสุกรสาวคัดทิ้งเนื่องจากปัญหาไม่เป็นสัดและผสม ไม่ติดสัมพันธ์กับสภาพของรังไข่และระดับของฮอร์โมนโปรเจสเตอโรน

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การศึกษานี้ มีวัตถุประสงค์เพื่อศึกษาการกระจายตัวของเซลล์ระบบภูมิคุ้มกันที่พบภายในชั้นเยื่อบุและชั้นใต้เยื่อบุของท่อนำไข่ สัมพันธ์กับการทำงานของรังไข่ในสุกรสาวคัดทิ้งเนื่องจากไม่เป็นสัดและผสมไม่ติด เก็บอวัยวะสืบพันธุ์ของสุกรสาวคัดทิ้งพันธุ์ผสม แลนด์เรซ x ยอร์กเชียร์ (LY) จากฟาร์มสุกรจำนวนทั้งหมด 30 ตัว แบ่งกลุ่มสุกรออกเป็น 3 กลุ่ม ได้แก่ สุกรสาวไม่เป็นสัด สุกรสาวผสมไม่ติด และสุกร สาวที่ไม่พบความผิดปกติทางระบบสืบพันธุ์ (กลุ่มควบคุม) แยกรังไข่และท่อนำไข่ออกจากอวัยวะในระบบสืบพันธุ์เพศเมียเพื่อตรวจสอบทา งมหกายวิภาค ตัดแบ่งท่อนำไข่สุกรสาวออกเป็น ส่วนรอยต่อระหว่างปีกมดลูกกับท่อนำไข่ อิสธ์มัส แอมพูลลา และปากแตร คัดแยกสุกรสาว ตามลักษณะของรังไข่ โดยแบ่งเป็นระยะฟอลลิคูลาร์และลูเทียล ผลการศึกษาพบว่า ลิมโฟไซต์เป็นเซลล์ระบบภูมิคุ้มกันส่วนใหญ่ที่พบได้ในท่อ นำไข่สุกรสาวทุกส่วน และทุกระยะของวงรอบการเป็นสัด ในชั้นเยื่อบุผิวของท่อนำไข่ จำนวนลิมโฟไซต์ในส่วนรอยต่อระหว่างปีกมดลูกกับท่อ นำไข่และอิสธ์มัสของสุกรสาวที่ไม่เป็นสัดและผสมไม่ติดสูงขึ้นในระยะลูเทียลอย่างมีนัยสำคัญ (*p*<0.05) เมื่อเปรียบเทียบกับกลุ่มควบคุม ยัง พบว่านิวโทรฟิลในชั้นเยื่อบุผิวของส่วนรอยต่อระหว่างปีกมดลูกกับท่อนำไข่ในสุกรสาวผสมไม่ติดทั้งระยะฟอลลิคูลาร์และลูเทียล สูงขึ้นอย่างมี นัยสำคัญ (*p*<0.05) เมื่อเปรียบเทียบกับกลุ่มควบคุม ขณะที่แมคโครฟาจในสุกรสาวผสมไม่ติดพบจำนวนที่มากกว่ากลุ่มควบคุมอย่างมี นัยสำคัญ (*p*<0.05) เฉพาะในระยะฟอลลิคูลาร์ สำหรับเนื้อเยื่อชั้นใต้เยื่อบุผิว พบว่า ลิมโฟไซต์มีจำนวนเพิ่มสูงขึ้นในท่อนำไข่ส่วนปากแตร ของสุกรสาวไม่เป็นสัดอย่างมีนัยสำคัญ (*p*<0.001) ขณะที่พลาสมาเซลล์ที่พบในชั้นใต้เยื่อบุผิวส่วนปากแตรของสุกรสาวผสมไม่ติดทั้งสอง ระยะมีจำนวนสูงขึ้นอย่างมีนัยสำคัญ (*p*<0.001) เช่นเดียวกัน สำหรับเซลล์ภูมิคุ้มกันอื่นๆ ในชั้นนี้ คือ นิวโทรฟิล แมคโครฟาจ และอีโอซิโน ฟิล ต่างก็พบจำนวนที่ผิดปกติในรอยต่อระหว่างปีกมดลูกกับท่อนำไข่ของสุกรสาวคัดทิ้งเมื่อเปรียบเทียบกับสุกรสาวควบคุม (*p*<0.05) จาก การศึกษานี้สรุปได้ว่า การเคลื่อนที่สะสมของเซลล์ระบบภูมิคุ้มกันชนิดต่างๆ ที่เกิดขึ้นอย่างผิดปกติในชั้นเยื่อบุผิวและชั้นใต้เยื่อบุผิวของท่อนำ ไข่โดยเฉพาะในส่วนกักเก็บเซลล์อสุจิบ่งชี้ถึงความไม่สมบูรณ์พร้อมในหน้าที่ของท่อนำไข่ทั้งกลุ่มสุกรสาวที่ไม่เป็นสัดและผสมไม่ติด

คำสำคัญ: การไม่เป็นสัด สุกรสาว เซลล์เม็ดเลือดขาว ท่อนำไข่ การผสมไม่ติด

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Introduction

Female reproductive tract disorders are the most common unplanned removal reason in sows and gilts (D'Allaire and Drolet, 1999). It is known that the main reproductive failure reasons for culling gilts in Thailand are anoestrus (44%) and repeat breeding (16%) which cause economic loss in commercial pig farms (Tummaruk et al., 2009). The anoestrus problem usually occurs because of ovarian hormonal imbalances leading to delayed puberty in gilts whereas fertilization failure and embryonic loss mainly occur because of repeat breeding problems (Tummaruk et al., 2001). Furthermore, the reproductive function of female pigs is difficult to investigate under field conditions but of course, post-mortem examination of the reproductive organs might be a useful tool in obtaining a potential source of information on infertility problems (Karlberg et al., 1981; Dalin et al., 1997; Heinonen et al., 1998). Previous studies macroscopically examined culling gilt reproductive tracts in Thailand and represented the abnormalities of the uterus 0.3%, oviduct 4.0% and ovary 5.6% (Kunavongkrit et al., 1986). The ovaries and uterus in culling gilts have been thoroughly explored both in terms of histological changes and distribution of immune cells (Teamsuwan et al., 2010). In addition, only basic morphological changes (Tienthai et al., 2006) and ultrastructural features by scanning electron microscopy (Tienthai and

Sajjarengpong, 2007a; 2007b) have been performed in the oviducts of gilts culled due to anoestrus and repeat breeding, respectively. However, the infiltration of immune cells in the oviduct of these culling gilts has not yet been well-scrutinized.

It is known that the animal oviduct plays a vital role in reproductive processes, such as in gamete transport, sperm reservoir, sperm capacitation, oocyte maturation, fertilization and early embryonic development before implantation in the uterine horns (Rodriguez-Martinez et al., 2001). The utero-tubal junction and the adjacent isthmus in the mammalian oviduct are considered to function as a sperm reservoir which maintains the viability and fertilizing capacity of the spermatozoa prior to fertilization occurring (Hunter, 1998; Rodriguez-Martinez et al., 2005). Therefore, the environment within the oviduct should be suitable for the spermatozoa, oocytes and early embryos as well as free from those microorganisms that intermittently may invade the upper reproductive tract (Ellis et al., 1986). The distribution of immune cells in the porcine endosalpinx, composing of isthmus, ampulla and infundibulum, throughout the oestrous cycle has been comprehensively evaluated (Jiwakanon et al., 2005) and thus has indicated the different distributions in each segment of the oviduct and immune functions within each segment of the oviduct. Therefore, the infiltration of immune cells within both epithelial and sub-epithelial layers of oviducts collected from anoestrus and repeat breeding gilts at different phases of oestrous cycle might imply the abnormality of oviductal functions related to these reproductive problems. Therefore, the objective of the present study is to investigate the infiltration of selected immune cells in the endosalpinx of gilts culled due to the most frequent reproductive failures, i.e. anoestrus and repeat breeding, in relation to the ovarian status and serum progesterone.

Materials and Methods

Animals and tissue collection: Thirty Landrace × Yorkshire crossbred gilts from commercial swine herds in Thailand were used in the present study and the selected gilts were culled due to anoestrus (n=16), repeat breeding (n=8) and non-reproductive problems (control group, n=6). After slaughter, the female reproductive tracts were collected, kept on dry-ice and transported to the laboratory within 12 hrs. The ovaries and oviducts were separated and examined to assess the stages of the oestrous cycle and gross pathology. The appearance of corpora lutea, corpus albicans and follicles on both ovaries were used for categorizing phases of the ovaries into the follicular and luteal phases. The ovaries with large follicles (7-12 mm in diameter) together with corpus albicans were classified into the follicular phase. The luteal phase was characterized by ovaries that contained corpora lutea with or without small follicles and/or corpus albicans. The oviducts were dissected into 4 segments, i.e. utero-tubal junction (UTJ), isthmus, ampulla and infundibulum. The tissue samples, fixed in 10% neutral buffered formalin, were embedded in paraffin and cut into 5 μm thickness. The slides were deparaffinized and stained with haematoxylin and eosin (H&E) for examining under a light microscope (LM).

Serum progesterone assays: Prior to the slaughter, blood samples were collected from the external jugular vein and were centrifuged at 3,000 rpm for 10 min. The serum was collected and stored at -20°C until analyzed. The serum progesterone level was determined by Enzyme-immunoassay (EIA) as described earlier by Joyce et al. (1977).

Immune cell evaluation: The UTJ, isthmus, ampulla and infundibulum sections from each group were coded before examination and each section of oviductal segment was divided into two compartments composed of epithelial and subepithelial connective tissue layers. Immune cell counts were performed under LM with 400× magnification as previously performed by Jiwakanon et al. (2005) using an ocular micrometer (ocular reticule) with 25 squares corresponding to 15,625 μm² of the real tissue placed in one eyepiece. Counting was accomplished by movement along the length of the epithelial layer and in the large area of the subepithelial layer in a non-overlapping manner. Twenty microscopic fields were arbitrarily selected for evaluation of each layer and section. Five types of immune cell, comprising of the lymphocytes, neutrophils, eosinophils, macrophages and plasma cells, were counted in the subepithelial connective tissue whereas the lymphocytes, neutrophils and macrophages were evaluated in the epithelial layer.

Statistical analyses: Data was analyzed using the SAS statistical package (version 9.0, SAS Institute Inc., Cary, NC, USA). Least squares means were determined by analysis of variance (ANOVA) using the General linear model procedure (GLM) and compared by use of the least significant difference test. A value of $p \leq 0.05$ was regarded as statistically significant.

Results

Reproductive parameters, macroscopic findings and hormonal analysis: Reproductive data, the pathological changes and the serum progesterone levels of gilts in this study are summarized in Table 1. The age at culling, interval between entering farm and culling and body weight were significantly different between the groups ($p < 0.001$). In addition, approximately 50% of the anoestrus gilts and 62.5% of the repeat breeding gilts displayed a pathological abnormality in the uterus. An abnormality of ovaries was noticeably found in about 31% of the anoestrus whereas pathological oviducts were found to be higher in repeat breeding gilts (37.5%). Serum progesterone in all groups showed lower levels at the follicular phase and higher levels at the luteal phase. However, the progesterone levels in both anoestrus and repeat breeding gilts at the luteal phases were a bit lower than the control gilts at a similar stage (Table 1).

Table 1 Age at culling, Body weight at culling and the length of the oviducts (mean±SEM), pathological abnormalities (%), progesterone levels (nmol/l; mean±SEM) of control, anoestrous and repeat breeding gilts

Parameters/Group (n)	Control (6)	Anoestrus (16))	Repeat breeding (8)
Age at culling (d)	249.3±3.0[a]	285.6±6.7[b]	340.1±12.0[c]
Periods from entry to culling (d)	44.2±2.8[a]	39.1±3.0[a]	106.1±18.9[b]
Body weight (kg)	135.0±5.7[a]	152.3±3.3[b]	177.2±6.6[c]
Average daily gain (g/d)	537.8±2.1	530.4±11.9	519.0±15.9
Number of ovulation (n)	13.0±2.1	14.3±1.6	16.8±1.5
Oviductal length (cm)	29.4±2.2	30.7±1.5	31.5±2.1
Ovarian abnormalities (%)	0	31.2	12.5
Oviduct abnormalities (%)	0	25.0	37.5
Uterine abnormalities (%)	0	50.0	62.5
P4 at follicular phase (nmol/l)	2.8±1.1 (n=3)	8.4±5.5 (n=6)	7.9±4.2 (n=4)
P4 at luteal phase (nmol/l)	102.9±32.9 (n=3)	63.1±11.1 (n=10)	64.9±24.9 (n=4)

[a, b] different superscripts within the same row differ significantly ($p<0.05$)

***Distribution of immune cells in the epithelial layer***: The three types of immune cells found in the epithelium of gilt oviducts composed of lymphocytes, neutrophils and macrophages (Figure 1). The lymphocytes were the predominant immune cells, which were found in all groups and all segments of the gilt oviduct (Figure 2A). The intraepithelial lymphocytes (IELs) were mainly located above the basement membrane of the epithelial layer (Figure 1a, b). The numbers of IELs in the UTJ and isthmus (the site of sperm reservoir), especially at the luteal phase, showed a significant difference between the control and the culling groups ($p<0.05$) while IELs in ampulla and infundibulum did not differ between groups. At the follicular phase, the lymphocytes counted in UTJ of repeat breeding gilts were significantly higher than the other groups ($p<0.05$). For the other immune cells, the numbers of neutrophils and macrophages in the UTJ of repeat breeding gilts were higher than the other groups ($p<0.05$).
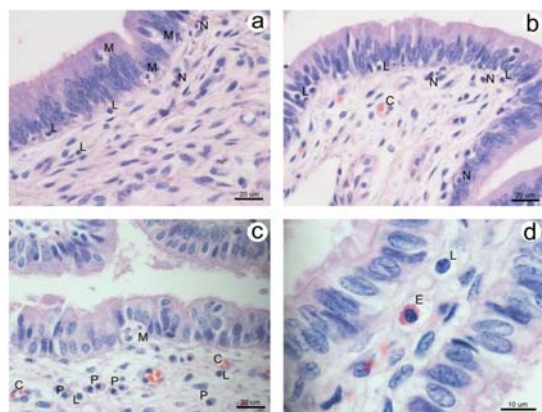
Figure 1. Characteristics of the oviductal endosalpinx and immune cells in the epithelial and sub-epithelial layers of UTJ (a), isthmus (b), ampulla (c) and infundibulum (d) of culling gilts by light microscopy. L: lymphocyte, M: macrophage, N: neutrophil, P: plasma cell, E: eosinophil: C: capillary, H&E staining.

Distribution of immune cells in the subepithelial connective tissue layer: Lymphocytes and plasma cells which are immune cells, were most commonly
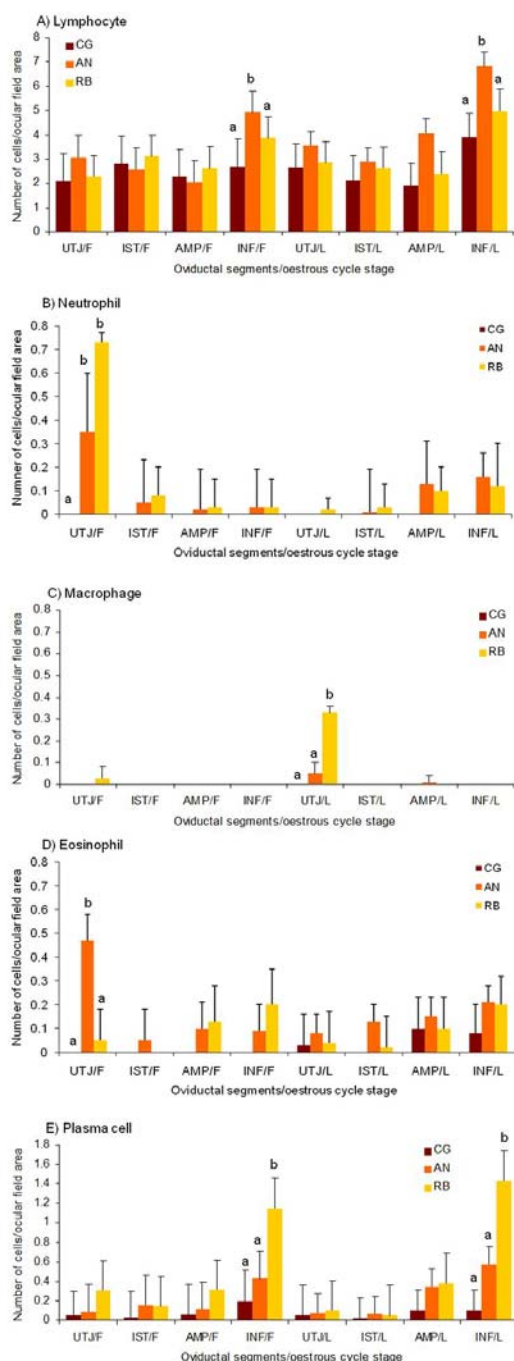
found within the subepithelial layer in all groups and
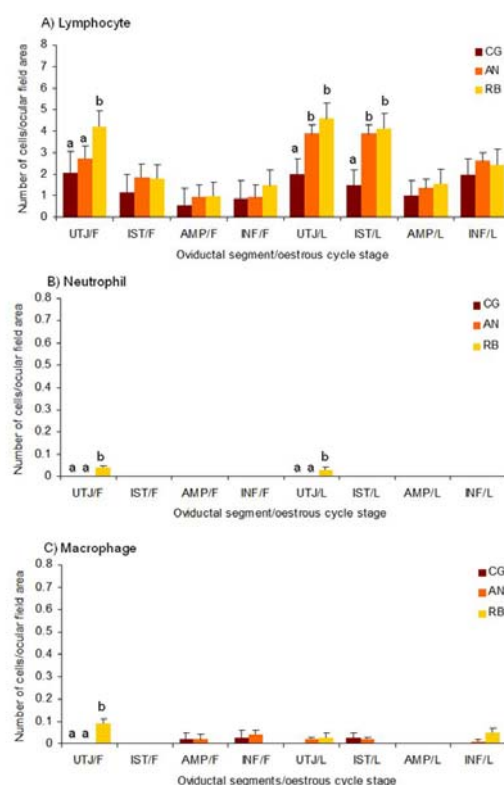
Figure 2 Numbers of intra-epithelium immune cells (mean ± SEM) in different segments of control (CG), anoestrus (AN) and repeat breeding (RB) gilt oviducts in relation to oestrous cycle stages. UTJ: utero-tubal junction, IST: isthmus, AMP: ampulla, INF: infundibulum, F: follicular phase, L: luteal phase. [a,b] different superscripts differ significantly ($p<0.05$).

all portions of the gilt oviducts (Figure 1 and Figures 3A, E), whereas neutrophils, macrophages and eosinophils were not found in all segments of the gilt oviducts and were observed in very low numbers (Figures 1c, d and Figures 3B, C, D). Noticeably, a higher number of lymphocytes was significantly observed in the infundibulum of anoestrus gilts at the follicular and the luteal phases than in the control and repeat breeding gilts ($p<0.05$ and $p<0.001$). The plasma cells also showed significant differences in the

infubulum similar to the lymphocytes but these cells were observed to have a higher degree in the

Figure 3 Numbers of immune cells (mean ± SEM) within the sub-epithelial layer in different segments of control (CG), anoestrus (AN) and repeat breeding (RB) gilt oviducts in relation to oestrous cycle stages. UTJ: utero-tubal junction, IST: isthmus, AMP: ampulla, INF: infundibulum, F: follicular phase, L: luteal phase. [a,b] different superscripts differ significantly (*p*<0.05).

repeat breeding gilts than control and anoestrus gilts (*p*<0.05). The neutrophils were usually found close to the basement membrane of the lining epithelium (Figures 1a, b). A higher number of neutrophils was significantly found in the UTJ at the follicular phase of anoestrus and repeat breeding gilts compared to the control group (*p*<0.05). Contrastly, the eosinophils were observed in higher numbers in the UTJ at the

follicular phase of anoestrus gilts than other groups (*p*<0.05). In the UTJ of repeat breeding gilts at the luteal phase, the macrophages were obviously in higher numbers than the similar segment of other gilts (*p*=0.001).

Discussion

The reproductive performances in this study showed that the age and weight at culling including the number of days from when the gilts entered the farm until the culling of repeat breeding gilts, was significantly higher than other groups. It is well-known that repeat breeding gilts were categorized among gilts that were culled because they returned to oestrus after insemination was repeated for ≥ 2 consecutive oestrous cycles, whereas, the anoestrus gilts were culled because no behavioural oestrus could be observed (Tummaruk et al., 2001). With this definition, it is clear that repeat breeding gilts take a longer period because of fertilization failure or embryonic loss until it is considered to cull on the pig commercial farms. Recently, Tummaruk et al. (2009) reported that the mean age of culling gilts because of reproductive failure in Thailand is about 321.0±51.0 days which was not different from the gilts' culling age in this study (285.6±6.7 for anoestrus and 340.1±12.0 for repeat breeding). Although we found that three groups of gilts used in this study were significantly different in ages, there have not yet been any reports monitoring the effect of the gilts' age affecting on the immune cell infiltration in the oviduct at different stages of estrous cycle. However, a previous work in the endometrium of anestrous sows and prepuberty gilts by Jiwakanon et al. (2006) reported that the infiltration of lymphocytes and macrophages demonstrated higher in anestrous sows than prepuberty gilts of both epithelial and subepithelial layers. This finding indicated that the conditions of pregnancy and parturition were able to induce these immune cells, thereby the distribution of immune cells in different ages of normal gilts that had never been pregnant could be a similar situation and did not affect the results of this research study.

Considering the hormonal level, progesterone is generally highest (86.6±18.9 nmol/l) at dioestrus (luteal phase) and lowest (0.8±0.8 nmol/l) at pro-oestrus (the follicular phase) in cyclic pigs (Kaeoket et al., 2001; Jiwakanon et al., 2005). In this study, the progesterone levels in all groups at the luteal phase corresponded to previous studies except the hormonal levels at the follicular phase of anoestrus and repeat breeding gilts, which were a bit higher than that of the control gilts and the standard level. Noticeably, we found that about 23.5% of the anoestrus and 12.5% of repeat breeding gilts had pathological changes in the ovaries, i.e. ovarian cysts. We know that cystic ovarian disease is a reproductive disorder and its high incidence in pigs is a cause of infertility in pigs, for example, anoestrus, irregular oestrus and complete or partial infertility (Wrathal, 1980). Furthermore, Babalola and Shapiro (1990) reported that a significantly higher concentration of progesterone had been found in pigs with cystic

ovaries containing no corpora lutea (CL) compared with normal pigs. The previous studies in the uterus suggested that the variation of steroid hormones throughout estrous cycle influenced the distribution of immune cells (Kaeoket et al., 2001). Recently, the progestin treatment in the human endometrium corresponded to the increase in subpopulations of lymphocytes, especially cytotoxic T-cells (Witkiewicz et al., 2010). These findings may imply the changes of progesterone which abnormally appear in culling gilts, especially in anoestrus gilts and can be associated with the atypical infiltration of immune cells.

Recently, a basic morphological and scanning electron microscopic (SEM) study were thoroughly performed in the epithelial oviduct of gilts culled due to anoestrous and repeat breeding (Tienthai et al., 2006; Tienthai and Sajjarengpong, 2007a; 2007b) indicating the abnormal structural changes and the atypical increasing of immune cells in the culled gilt oviducts. Generally, the most frequent leukocytes within the epithelial and subepithelial layers of all segments and stages in pig oviducts were lymphocytes, whereas other types of immune cells were observed in very low numbers (Jiwakanon et al., 2005; 2006) corresponding to the present investigation. Importantly, there are significant differences in the numbers of lymphocytes in this study, between lymphocytes in both anoestrus and repeat breeding gilts within the epithelial layers of the UTJ and isthmus which was the site of sperm reservoir, while the lymphocytes were in the subepithelial layer were significantly observed only in the infundibulum of anoestrus gilts compared to the control group. Previous studies reported that the lymphocytes in the oviductal epithelium mainly composed of T-cells and natural killer (NK) cells and these cells did not depict any significant difference between segments and estrous stages (Jiwakanon et al., 2005; 2006). Hence, the pathological finding in the ovaries of both culled gilts, i.e. cystic ovaries, may not be involved in the atypical increasing of these lymphocytes. We know that T-cells compose of T-helper and T-cytotoxic cells which function in different ways. During antigenic stimulation, the T-helper cells will release cytokines to promote the proliferation and differentiation of T-cytotoxic cells, NK cells and macrophages. Both T-cytotoxic and NK cells are capable of inducing the death of tumor cells and infected cells, particularly cells infected by viruses (Roitt et al., 1998). Up to now, porcine reproductive and respiratory syndrome virus (PRRSV) antigens have been detected in the macrophage-like cells within the subepithelial layer of the endometrium of gilts culled due to various reproductive disturbances including anoestrus and repeat breeding (Olanratmanee et al., 2009). As it is shown in the present data (Table 1), the pathological conditions (mild degree) observed in both the uterine horns and oviducts of culling gilts may be caused by the PRRSV which can circulate and be re-infected in vaccinated and non-vaccinated swine herds (Tummaruk and Tantilertcharoen, 2009). These recent studies support the abnormal infiltration of lymphocytes found in the culled gilt oviductal

epithelium, especially in the site of the sperm reservoir (UTJ and isthmus) which is connected to the uterine horn. The atypical increasing of lymphocytes in the infundibulum, found only in subepithelial layer (Figure 3A), may explain that this portion of the oviduct is outlying from the uterine horn.

Furthermore, we also found significantly increasing numbers of neutrophils and macrophages within the UTJ epithelium of repeat breeding gilts compared to the control group. In the tissue, neutrophils migrate along the chemotactic factors toward the site of infection and these cells seem to be the first line of defence for capturing and destroying foreign material via phagocytosis (Roth, 1999). After phagocytosis, neutrophils attract macrophages to the site of invasion to destroy certain type of bacteria which cannot be killed by neutrophils (Tizard, 1996). However, the migration of both neutrophils and macrophages in the pig oviduct is normally found in very low numbers (Jiwakanon et al., 2005). Although the characteristics of the pathological gilt uterus and the bacteriological examination did not emerge in the present study, earlier studies suggested that the endometritis was the most common post-mortem abnormality found in the culled gilts especially repeat breeding (Tummaruk et al., 2009) and several types of bacteria were present in the gilt uterus with signs of endometritis (Karlberg et al., 1981). Additionally, Dalin et al. (1997) reported that about 50% of the culled pigs with endometritis were classified as mild degree corresponding to the present study. Undoubtedly, the rising of neutrophils and macrophages in the UTJ can be related to the abnormalities of the culled gilt uterus. Therefore, the irregular increasing numbers of lymphocytes, neutrophils and macrophages could interrupt normal functions in the sperm reservoir, i.e. maintaining sperm viability and modulating sperm capacitation, leading to varieties of reproductive failure; particularly repeat breeding in gilts (Cho and Dee, 2006). However, it may not be clear whether these conditions are certainly the main cause of anoestrus in gilts and there needs to be more study in the near future.

In the subepithelial connective tissue layer, the numbers of lymphocytes and plasma cells found in this study were significantly higher in the infundibulum of anoestrus and repeat breeding gilts, respectively. Moreover, the differences in the numbers of the other immune cells in this layer were observed only in the UTJ. The immune cells were found in various manners, i.e. neutrophils were higher in both reasons for culling gilts; macrophages were abnormally raised in repeat breeding gilts, and eosinophils were obviously depicted in high numbers in the anoestrous gilts. In the first aspect, the lymphocytes and plasma cells were normally found in very high numbers in the infundibulum of the porcine oviductal subepithelial layer and the lymphocytes were significantly highest in sows at weaning (non-active ovaries), but the plasma cells were not different at the oestrous stages (Jiwakanon et al., 2005). Subepithelial lymphocytes can be shown in anoestrous gilts with the inactive ovaries, however, the samples we used in the present study were

anoestrous gilts with the ovarian functions. Therefore, it is possible that the appearance of follicles and corpora lutea in these anoestrous gilts may not be related to the proper ovarian functions causing these gilts to show no signs of oestrus. Also the infectious agents, as we assumed by the pathological oviducts, might induce irregular patterns of subepithelial lymphocytes. Considering the plasma cells, B-cells were found only in the subepithelial layer and could be classified as plasma cells after activation and plasma cells that locally produced antigen-specific antibodies in the tissue (Calame, 2001). Unsurprisingly, the numbers of subepithelial plasma cells in this study showed a significant difference in the repeat breeding gilts, which had been suspected to have been infected by viruses and/or bacteria. Therefore, the distribution of lymphocytes and plasma cells in the subepithelial layer may be involved in both physiological (via the improper ovarian functions) and pathological conditions. In the second aspect, the subepithelial neutrophils and macrophages were not normally found or found in a very low number in the UTJ and isthmus (Rodriguez-Martinez et al., 1990; Jiwakanon et al., 2005), thus these immune cells presented in the subepithelial layer of this study might be involved in the endometritis, which invades the site of the sperm reservoir and functions in the defense mechanism of the gilt oviducts. Finally, a large number of eosinophils in the subepithelial layer were found only in the UTJ of the anoestrous gilts at the follicular phase, whereas these immune cells were usually very low and did not vary between the oviductal segments and oestrous phases in healthy sows (Jiwakanon et al., 2005). Because the study on the distribution of immune cells in the pig oviduct by Jiwakanon et al. (2005) did not cover the UTJ, it is possible that the infiltration of UTJ, which is connected to the uterine horn, may have to be considered instead. In the gilt uterus, a large number of eosinophils appeared during days 10-14 of pregnancy (Bischof et al., 1995) and these cells in the non-pregnant sow uterus were present in large amounts at dioestrus (Kaeoket et al., 2001). Recently, our co-workers studied anoestrus gilts and reported a significant increasing of eosinophils in the uterine horns during the luteal phase (Teamsuwan et al., 2010). These investigations indicate the high concentration of progesterone influencing the infiltration of eosinophils. The appearance of eosinophils in the UTJ of anoestrus gilts in this study could be involved in the highly specific function of these cells in elaborating inflammatory mediators against micro-organisms and vascular remodeling (Costa et al., 1997) that may occur owing to pathological or irregular hormonal effects in some anoestrus gilts. Furthermore, we have known that the aseptic micro-environment have to be maintained in the oviduct for the achievement of fertilization and initial embryonic development, thereby the proportion of various immune cells in this organ must be appropriated for these critical events (Jiwakanon et al., 2005). The atypical numbers of immune cell population could impact the main function in each segment of the pig oviduct, particularly in the sperm reservoir (UTJ) which could be free from the leukocytes that attempt to destroy the survival spermatozoa such as neutrophils (Rodriguez-Martinez et al., 1990).

In conclusion, the infiltration of various immune cells appearing in the epithelial and subepithelial layers of the oviduct, especially in the site of the sperm reservoir, might indicate the improper oviductal functions in both anoestrus and repeat breeding gilts. However, the irregular patterns of immune cell distribution in these culling gilts require more studies to prove the atypical ovaries and types of infectious diseases.

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