Infiltration of Immune Cells in the Endometrium of Gilt Culled due to Anoestrus in Relation to the Ovarian Appearance, Oestradiol-17β and Progesterone

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Introduction
In general, the annual removal rate of sows in swine commercial herds is between 30-60% (1). Reproductive disturbances are the most common unplanned removal reason in both sows and gilts (2, 3). In Thailand, 51% of the culling gilts were classified as anoestrus (3). Factors that cause reproductive failure alter physiological status of the sow’s endometrium in different pathways (4). The reproductive function of the female pigs is difficult to examine under field condition. Post-mortem examination of the reproductive organs is a useful tool to obtain a potential source of information on infertility problems (5). The infiltration of immune cells in cyclic gilts and sows was influenced by the oestrous cycle and hormones (6-8). To our knowledge, the infiltration of the immune cells in the impaired endometrium of anoestrous gilts has not been completely elucidated. The objective of the present study was to investigate the infiltration of immune cells in the endometrium of gilts culled due to anoestrus in relation to the ovarian appearance and the serum oestradiol-17β (E2) and progesterone (P4).

Materials and Method
Animals and tissue: Genital organs from 27 Landrace×Yorkshire crossbred gilts from 5 herds were used in the present study. All of the gilts were culled due to anoestrus and none of them has been mated. The average age at culling was 301±34 d and body weight was 149±21 kg. The genital organs were examined to assess the stage of the oestrous cycle and gross-pathology. Numbers of follicles, corpora lutea (CL) and corpora albicantia (CA) in the ovaries were counted. The gilts were classified according to the ovarian appearance into three groups, i.e., pre-pubertal (n=10), follicular (n=7) and luteal phase (n=10). Seven tissue samples per gilts were collected, from proximal, middle and distal part of each uterine horn and, from the uterine body. Blood collection and hormonal assays: Blood samples were collected from the jugular vein prior to slaughter. The blood samples were centrifuged at 3000 rpm for 10 min. The serum P4 level was determined by a solid-phase radioimmunoassay (Coat-A-Count®, CA, USA). This method had earlier been validated for P4 analyses in the pig (9).

The serum E2 level was determined by the electrochemiluminescence immunoassay (Cobas® IN, USA) according to the method previously described (10). Tissue section: The uterine samples were fixed in 10% neutral buffered formalin for at least 24 h and embedded in paraffin. The sections were cut (5 μm), placed on glass slides and stained with hematoxylin and eosin (H&E).

Histological examination: The sections were divided into three layers for histological examination, i.e., epithelium, subepithelial and glandular layers. Immune cells, i.e., lymphocyte, neutrophil, eosinophil, macrophage and plasma cell in each layer were quantified under light microscope (400x) (Fig. 1a-c). For each section and each layer, 20 microscopic fields were arbitrarily selected for investigation. Ocular micrometer with 25 squares corresponded to 15,625 μm² (400x) of real tissue area was used for counting the number of immune cells.

Statistical analyses: Data were analyzed using SAS version 9.0 (SAS Inst., Cary, NC, USA). Numbers of cells were presented as the total number of cells per 20 ocular fields area (312,500 μm²). The data were log transformed and were analyzed using general linear mixed model procedure. The model included groups as fixed effect and included the animal and section nested within animal as random effects. Pearson’s correlation was used to analyze the association among E2, P4 and the number of immune cells. p≤0.05 was regarded to have statistical significance.

Results and Discussion
The average diameter of the follicles in the ovaries was 5.4±1.1 mm. The small size follicles (diameter ≤ 5 mm) were observed in 7 pre-pubertal and 9 luteal gilts. The weights of the uteri in the follicular, luteal and pre-pubertal groups were 422±244, 663±170 and 160±99 g. The surface epithelium: Lymphocytes were Predominant cells in all groups. Number of neutrophil in the follicular phase was higher (p<0.05) than other groups. Number of macrophages trended to be higher in the follicular than the luteal phase (p=0.07) and pre-pubertal gilts (p=0.11).
Infiltration of immune cells in the subepithelium: Immune cells in the subepithelium were demonstrated in Fig. 2a. Neutrophil was higher in the follicular than the other groups (p<0.05). Eosinophil in the luteal phase was higher than the follicular phase and pre-pubertal gilts (p<0.05).

Infiltration of immune cells in the glandular layer: Fig 2b demonstrated means number of immune cells in the glandular layer. Neutrophils and macrophage were rarely found in the glandular layer.

Correlation among E2, P4 and immune cells: Table 1 demonstrated means of serum E2 and P4 by groups. Lymphocytes in the epithelial, subepithelial and glandular layer correlated with P4 (r=-0.57, p<0.05; r=-0.44, p<0.05 and r=-0.58, p<0.05, resp.). In the subepithelial layers, number of eosinophils and neutrophils correlated with P4 (r=0.63, p<0.05; r=-0.43, p<0.05, resp.).

Table 1 Means±SD of serum E2 and P4 of anoestrous gilts

<table>
<thead>
<tr>
<th>Group of gilt</th>
<th>Oestradiol-17β (pmol/l)</th>
<th>Progesterone (nmol/l)</th>
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<tr>
<td>Follicular phase</td>
<td>134.7 ± 47.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.0 ± 1.6&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Luteal phase</td>
<td>111.2 ± 25.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.4 ± 40.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pre-puberty</td>
<td>140.4 ± 34.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.8 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
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The levels of E2 was lowest and P4 was highest during the luteal phase. This is in accordance with earlier studies (7, 8). However, the level of E2 was relatively high in pre-pubertal gilts. This might be due to that most of the gilts in the present study were nearly puberty.

In the present study, lymphocyte was frequently observed in all tissue layers of the endometrium, which is in accordance with previous findings (6-8). This indicates that lymphocytes is a dominant immune cell, and may play a major role in the endometrium. Neutrophils and macrophages were frequently observed in the endometrium during the follicular phase. The number of neutrophils and macrophages is in accordance with the previous study (7). Interestingly, plasma cells were also observed during follicular phase and in pre-pubertal gilts. These anoestrous gilts might have had a mild degree of chronic endometritis from the uterine infection in the pre-pubertal gilts and the previous oestrous cycle in the follicular gilts (4, 6). In the present study, eosinophils were also the dominant immune cell in anoestrous gilts. However, the number of eosinophils was about 20 times higher in pre-pubertal gilts compared to that report in normal sows (7), indicating that these may be a function to destroy foreign material in the uterus of the anoestrous gilts. Vascular endothelial growth factor (VEGF), could be produced by eosinophils (12), act as a stimulator of angiogenesis and vascular permeability (11, 13), which resulted in an increase of uterine weight and size during luteal phase.
This present study is in contrast to the study by Jiwakanon et al. (8) who found that lymphocytes and macrophages were the most common immune cells found in anoestrous gilts and sows. This might be due to that the gilts in the present study were delayed puberty indicated that antigen exposure into the uterus had been high and some gilts might have had a mild degree of endometritis. In conclusions, the infiltration of immune cells in the anoestrous gilts endometrium were affected by the levels of reproductive hormones, i.e., E\textsubscript{2} and P\textsubscript{4}. Infiltration of the immune cells, especially neutrophils, plasma cells and eosinophils could be used to indicate abnormal function of the endometrium of the anoestrous gilts.

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**References**

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