Vulva Discharge in Gilts: Distribution and Infiltration of Immune Cells in the Endometrium

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Introduction
It has been shown that reproductive failure account for 47% of the culled gilts (1,2). Vulva discharge (VD) is a common reproductive problem that has an incidence about 23-24% under field conditions (2,3,4,5). VD cause economic loss in the pig farm by decrease longevity of the female, reduce farrowing rate and litter size and increase infertility problems in the herd (6). Early diagnosis and well therapeutic plan may reduce these economic losses. VD may cause by endometritis, metritis, cervicitis, vaginitis and/or urinary tract infection. The amount of pus exudates usually indicated degrees of uterine infection. The difficulty for the early diagnosis of VD is that no typical clinical signs except vulva discharge (7). Kaeoket et al. (8) demonstrated that the distribution of the immune cells in the female reproductive tracts indicate the immune response of the sow endometrium and could be used as a diagnostic tool for VD. Studies on the infiltration of the immune cells in different parts of the genital tracts need to be investigated to understand the mechanism of uterine infection in the female pig (8,9,10,11). The present study was performed to evaluate the distribution and infiltration of immune cells in the endometrium of gilts culled due to VD.

Materials and Method
Animals and tissues: The study included genital organs from 24 crossbred Landrace×Yorkshire gilts aged 300±41 d with a body weight of 133±20 kg from two herds. All of the gilts were culled due to VD. After slaughter, the genital organs were collected and investigated for gross pathology. The numbers of follicles (F), corpora lutea (CL) and corpora albicantia (CA) in the ovaries were measured. The gilts were classified into 3 groups, i.e., follicular phase (n=10), luteal phase (n=10) and pre-puberty (n=4). Pus exudate in the uterine lumen was investigated. The gilts were classified into two groups, i.e., subclinical endometritis (no pus in the uterus) and clinical endometritis. The uterine samples were collected from each uterine horns and uterine bodies (7 sections per gilt).

Histological examination: Uterine samples in H&E stained were evaluated. Each uterine section was divided into three compartments, i.e., surface epithelium, subepithelial and glandular connective tissue layers. A light microscope with magnification of 400x and ocular micrometer corresponded to 15,625 μm² of real tissue area were used. Immune cells including lymphocyte, neutrophil, macrophage eosinophil and plasma cell in each layer were quantified. For each section and each layer, 20 microscopic fields were arbitrarily selected for investigation.

Statistical analyses: Data were analyzed using the SAS version 9.0 (SAS Inst Cary NC USA, 2002). The total number of immune cells per 100 squares (62,500 μm²) were analysed. Descriptive statistics were used to present the infiltration of immune cells data in each cycle. Proportions of various types of immune cells were used to present the distribution of the cells in the gilt’s endometrium. A natural logarithmic transformation was applied to achieve the assumption required for analysis of variance. Differences between groups were analyzed using MIXED procedure. The fixed effect of groups and the random effects of gilts and section nested within group were included in the statistical models Tukey-Kramer t-test was used to compare least squares mean among groups. \( p \leq 0.05 \) was considered to be statistically significant.

![Fig. 1 Distribution of immune cells (proportion) in the endometrium of gilts that have clinical (a) and subclinical (b) endometritis](image)
Results
All of gilts uteri in the present study showed histological evidence of endometritis. Clinical endometritis was observed in 9 gilts (37.5%) and subclinical endometritis was observed in 15 gilts (62.5%). Three gilts (12.5%) had cystic ovaries. The distribution of immune cells in the endometrium of clinical endometritis was similar to the subclinical endometritis (Fig. 1). Most of the immune cells including lymphocytes, neutrophils, eosinophils and plasma cells were mainly located in the subepithelial connective tissue layer. In contrast, most of macrophages were found in the surface epithelium (Fig 1).
The numbers of immune cells among follicular phase, luteal phase and pre-pubertal gilts that had endometritis are presented in Fig. 2. Neutrophils were the predominant immune cells found in all layers of endometrium. In sub-epithelium, plasma cells and lymphocytes were frequently observed during follicular phase. Lymphocytes in the follicular phase were higher than luteal phase ($p \leq 0.05$). Plasma cells were significantly higher in follicular phase than luteal phase and pre-pubertal gilts ($p \leq 0.05$) (Fig. 2). Eosinophils and macrophages were also observed in subepithelial and glandular layer. The predominant immune cells in glandular connective tissue of all gilts were lymphocytes and neutrophil (Fig 2).

Fig. 2 Numbers of immune cells (mean±SD) in the subepithelial (a) and glandular layers (b) of gilt culled due to vulva discharge syndrome

Discussion
In the present study, the proportion of subclinical endometritis was relatively high among VD gilts. This is accordance with Dalin et al. (12). In gilts with VD, three of them had cystic ovaries. Cystic ovaries have been reported as a co-factor that increases the severity of endometritis (13). Jana et al. (13) suggested that degree of endometritis depended on the number of bacterial infection. Lipopolysaccharide (LPS) from gram negative bacteria in an inflamed uterus plays a role in the rise of plasma prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) and act as vasodilator and myorelaxant. These causes an inflammatory process of the endometrium. Interestingly, the distribution patterns of immune cells in the clinical endometritis were similar to the subclinical endometritis. This indicated that the distribution of the immune cells might be influenced by the oestrous cycle, while the uterine infection increased the infiltration of immune cells in the endometrium of the gilts.
Neutrophil was the dominant immune cell in the subepithelial layer of VD gilts, while it was very low or absent in normal gilts (10). During the follicular phase of the VD gilts, the numbers of neutrophils in subepithelial and glandular connective tissue layers were 3-4 times higher than that reported in normal sows (8). During the luteal phase of the VD gilts, the number of neutrophils in subepithelial and glandular layer was 100 times and 40 times higher than that observed in normal sows, respectively (8). Additionally, the number of lymphocytes in subepithelial connective tissue of the VD gilts was 2 times higher than normal pre-pubertal gilts (10) and normal sows (8). During the follicular phase of the VD gilts, plasma cells in subepithelial connective tissue layer were 100 times higher than that observed in normal sows, respectively (8). Earlier study demonstrated that, a high number of lymphocytes and plasma cells were observed in sows that had chronic endometritis (7). In the present study, the number of eosinophils and macrophages was similar to that in normal sows (8), indicating that at this stage of endometritis, these immune cells may not alter.

In conclusion, our study revealed that 37.5% of the gilts culled due to VD could be identified by gross pathology, while the rest of the gilts had mild degree of endometritis. The infiltration and distribution of the immune cells, especially neutrophils, lymphocytes and plasma cells indicate the immune response of the endometrium and could be used as a diagnostic tool for the uterine malfunction in gilts.

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References
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