Expression of Cyclooxygenase-2 in the Endometrium of Gilts with Different Stages of Endometritis

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ABSTRACT. The present study determined the association among the expression of COX-2, stages of endometritis, and types and number of local immune cells infiltrating into the gilts’ endometrium. The uterine tissues from 24 Landrace x Yorkshire gilts identified as acute endometritis (n=7), chronic endometritis (n=7), and normal endometrium (n=10) were included. The tissues were prepared for both histological and immunohistochemical investigations. The immunoexpression of COX-2 in every layer of the gilts’ endometria was appraised by avidin-biotin-peroxidase complex method via image analysis; and was reported as percentage of positive area and staining index. The results revealed that the immunoexpression of COX-2 was found only in the surface epithelial layer. The gilts with acute endometritis possessed higher both percentage of positive area (68.99% versus 4.50% and 3.43%, appraised by avidin-biotin-peroxidase complex method via image analysis; and was reported as percentage of positive area and staining index. The results revealed that the immunoexpression of COX-2 was found only in the surface epithelial layer. The gilts with acute endometritis possessed higher both percentage of positive area (68.99% versus 4.50% and 3.43%, P<0.001) and staining index (1.13 versus 0.05 and 0.04, P<0.001) than those with chronic endometritis and normal endometrium, respectively. Positive correlations between the number of surface epithelial neutrophils and percentage of COX-2 positive area (r=0.47, P=0.022), as well as mean staining index (r=0.44, P=0.032) were observed. In conclusion, the immunoexpression of COX-2 was found strongest in the gilts with acute endometritis, meanwhile it was not different between those with chronic endometritis and normal endometrium. This suggested that the expression of COX-2 might be dependent not only on the infiltration of local immune cells in the endometrium, but also on the duration of exposure with inflammatory agents.

KEY WORDS: cyclooxygenase-2, endometritis, endometrium, gilts, local immune cells.

Swine production is one of the important livestock industries in Thailand, and the number of sows on production is, today, approximately 1 million. Generally, 40–60% of the sows are annually substituted by replacement gilts [9, 27]. As a result, reproductive performance of the gilts largely influences the total production of the pig farm. Under field conditions, certain gilts are culled for various reasons before the first litter can be successfully produced. Furthermore, during summertime, poor reproductive performance is more evident in the gilts than the sows [28]. In Thailand, reproductive disorders tenanted 47% of culled gilts. Based on gross morphological findings, 14% of the gilts culled due to reproductive disorders have endometritis [25]. In addition, endometritis has been observed in both virgin and mated gilts. The reproductive reasons for culling these endometritis gilts include anestrus, repeated breeding, repeated breeding, not being pregnant, and abnormal vaginal discharge [26].

The inflammation degree of the endometrium can be classified by type and number of local immune cells infiltrating into the endometrium [5, 26]. However, an elucidation of endometritis on the criterion of histological examination is relatively complicated since estrous cycle and/or insemination can be the factors predominiating on the infiltration of immune cells into the endometrium [5, 15, 24]. Earlier studies have demonstrated that an increase in serum progesterone (P\textsubscript{4}) concentration contributes to a decrease in the number of local immune cells infiltrating into the endometrium [24, 29]. Furthermore, most endometritis cases are clinically observed when the pigs are in luteal phase which the level of P\textsubscript{4} is very high. In addition, the preceding study indicates that the porcine female reproductive tract under luteal phase is very susceptible to an infection [29].

Endometritis is considered one of the general reproductive disorders in domestic animals. It has been well documented that metritis contributes to a number of alterations in synthesis rate of Prostaglandins (PGs), especially PGE\textsubscript{2a}, PGE\textsubscript{2}, and PGI\textsubscript{2}, in uterine tissues, together with lymph blood and lymph vessels. Furthermore, quantity of PGE\textsubscript{2a} and PGE\textsubscript{2} production in the inflamed porcine uterus is dependent on intensity of inflammatory process, time, and type of uterine tissues [14, 18]. PGs produced by endometrium are important to the regulation of estrous cycle and successful implantation in domestic animals, including pigs [19]. Cyclooxygenase (COX) is well acknowledged that it is the rate-limiting enzyme in the biosynthesis of PGs by catalyzing an alteration from arachidonic acid to PGH\textsubscript{2}. Subsequently, PGH\textsubscript{2} is converted to various eicosanoids, including PGE\textsubscript{2}, PGD\textsubscript{2}, PGF\textsubscript{2a}, PGI\textsubscript{2}, and thromboxane A\textsubscript{2} [7]. Currently, three isoforms of COX have been identified: COX-1 [11, 20], COX-2 [16, 30], and COX-3 [3]. COX-1 is a constitutive isoform and is localized in various cell types and tissues under basal condition [4]. COX-2 is a highly inducible isoform and can be expressed in assorted cell
obtained and stored at –20°C until assay. The blood samples were centrifuged at 3,000 rpm for 2 hr, and followed by adding 100 µl of P4-horseradish peroxidase (1:65,000). The plates were incubated at room temperature for 2 hr, and followed by adding 100 µl of ABTS substrate (prepared by mixing 40 µl, 0.5 M H2O2, 125 µl 40 mM ABTS in 12.5 µl 0.96% citric acid solution). An optical density (OD) of the content in the each well was determined at A450 nm (ELISA reader, TECAN SUNRISE, Austria). The OD for 0 well was >0.7 to <1.0 OD. The sensitivity of assay at 90% binding was 0.016 ng/well. The intra-assay coefficient variation (CV) for low and high controls was 6.15% and 9.05%, respectively.

Histological examination: Endometrial sections were classified as “acute endometritis,” “chronic endometritis,” and “normal endometrium” according to both gross morphological findings and histological examination from hematoxylin and eosin (H&E) stained sections [5, 26]. Moreover, immunocytes, including neutrophils, lymphocytes, eosinophils, macrophages, and plasma cells in different layers (surface epithelial, subepithelial connective tissue, and glandular connective tissue layers) were quantified under light microscope (400 ×) in 20 randomly microscopic fields per endometrial layer [15, 24]. The number of immunocytes counted was reported as the total number of cells per 20 microscopic fields (312,500 μm2, 400 ×). Acute endometritis would be defined by a number of neutrophil infiltration (>100 cells per 20 microscopic fields), as well as very scanty mononuclear cells. “Chronic endometritis” was classified by a plenty of lymphocytes (>40 cells per 20 microscopic fields), together with an existence of plasma cells (>10 cells per 20 microscopic fields), and a rather low number of neutrophils (<20 cells per 20 microscopic fields). “Normal endometrium” was considered when neutrophils were less than 40 cells, lymphocytes were less than 40 cells, and plasma cells were less than 10 cells per 20 microscopic fields [22].

Immunohistochemistry of Cyclooxygenase–2: Prior to performing immunohistochemistry, all the prepared specimens were deparaffinized in xylene and, afterwards, dehydrated in ascending concentrations of alcohol. Antigen retrieval was carried out by heating in a microwave oven at 800 watts with 0.01 M citrate buffer, pH 6.0, for 30 min. Thereafter, an endogenous peroxidase activity was blocked by 3% hydrogen peroxide in methanol for 10 min and all slides were rinsed with phosphate-buffered saline (PBS). After exceeding fluid was removed from the sections, they were incubated with normal goat serum for 30 min. Rabbit polyclonal anti-COX-2 primary antibody (Cayman chemi-

Tissue collection: The uterine tissue samples of Landrace x Yorkshire (LY) crossbred gilts from two swine commercial herds in Thailand were obtained at the slaughterhouses between March 2005 and September 2006. In total, reproductive tracts of 212 gilts were examined, and 24 gilts were finally chosen to be investigated. Historical data of individual, including date of birth, date at herd entry, date at culling, estrus symptoms, and mating were collected. Moreover, venipuncture was done from jugular vein individually prior to being slaughtered to evaluate the serum P4 concentration. The blood samples were centrifuged at 3,000 rpm (1,160 × g for 10 min). The sera, subsequently, were obtained and stored at –20°C until assay. Postmortem examination was performed grossly in all samples. Ovary and its structures: follicles, corpus luteum (CL), and corpus albican (CA), were carefully examined to identify the estrous phase and gross pathology. ‘Inactive phase’ was identified if only small follicles (<7 mm) were found, lacking CL or CA. ‘Follicular phase’ was characterized by the appearance of large follicles (7–12 mm), including CA. ‘Luteal phase’ was defined by an existence of CL [25]. Subsequently, the uterine horns were longitudinally opened to investigate the appearance of the endometrium. Gross morphological findings of these samples were reported [25]. The assessment of the endometrium was based on the appearance of uterine discharge (exudates or transudates), degree of edema, color, and the stage of estrous cycle. Endometritis was sentenced if signs of inflammation, i.e., severe edema and congestion, dark red color, and purulent exudates in the lumen were visible. All other endometria were defined as normal. The endometria were sampled, fixed in 10% neutral-buffered formalin for 24 hr, and paraffinized by an automatic tissue processor (Tissue-Tek TEC, Tokyo, Japan). The paraffinized embeddings were cut into 5 µm thick in order to further examine histologically and immunohistochemically. In total, the uterine tissues from 24 LY crossbred gilts Landrace x Yorkshire crossbred gilts identified as acute endometritis (n=7), chronic endometritis (n=7), and normal endometrium (n=10) were included in the present study.

Progesterone assay: Serum P4 was analyzed by enzyme-linked immunoassay as described by Munro and Stubenfeldt [21] using a P4 monoclonal antibody (CL 425). Briefly, plates (NUNC, Maxisorb, Rochester, NY, U.S.A.), except for nonspecific binding wells, were coated with 50 µl of antibody (1:7,500), and incubated at 4°C for 12 hr. The plates were washed for 5 times by washing buffer. Thereafter, 50 µl of P4 standards (0.78–200 pg/well), control, and serum samples were added. The plates were incubated and followed by adding 50 µl of P4-horseradish peroxidase (1:65,000). The plates were incubated at room temperature for 2 hr, and followed by adding 100 µl of ABTS substrate (prepared by mixing 40 µl, 0.5 M H2O2, 125 µl 40 mM ABTS in 12.5 µl 0.96% citric acid solution). An optical density (OD) of the content in the each well was determined at A450 nm (ELISA reader, TECAN SUNRISE, Austria). The OD for 0 well was >0.7 to <1.0 OD. The sensitivity of assay at 90% binding was 0.016 ng/well. The intra-assay coefficient variation (CV) for low and high controls was 6.15% and 9.05%, respectively.

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Microscopic image analysis of Cyclooxygenase–2: The immunoreaction of COX–2 in each section was separately evaluated in surface epithelium, subepithelial connective tissue, and glandular connective tissue layers. The immunostaining of COX–2 in each layer of endometrium was assessed on the criterion of staining intensity by using image analysis (Image-Pro® PLUS 6.0 Programming software, Media Cybernetics, Inc., MD, U.S.A.). Ten microscopic fields (200 ×) were randomly selected for investigating the immunoreaction of COX–2 in the endometrium. The staining intensity of COX–2 immunoreaction was displayed as percentage of positive area per entire selected area; and was calculated as staining index (0–3).

Percentage of positive area and staining index: The percentage of COX–2 positive area, measured by the image analysis program, represented the proportion of the tissues stained with moderate-to-very strong immunoreaction. The tissue areas without or with scant immunostaining were regarded as negative. In addition, staining index of COX–2 immunoreaction in each section was calculated from the summation of the percentage of positive area multiplied by the intensity scores (moderate=1, strong=2, and very strong=3) and divided by 100. After the calculation, both the percentage of positive area and the staining index were regarded as continuous variables.

Statistical analysis: Data were analyzed using SAS version 9.0 (SAS Institute Inc., Cary, NC, U.S.A.). Descriptive statistics, including number of non-missing values, means, SEMs, and ranges of the data were conducted for all reproductive data of the gilts included in the experiment (Table 1). The number of immune cells infiltrating into each layer of the endometrium was demonstrated as the mean number per 20 microscopic fields (312,500 μm²/400 ×). Pearson’s correlation was used to analyze an association among percentage of positive area, staining index, P4 level and the number of immune cells infiltrating into different layers of the endometrium. The percentage of COX–2 positive area, the mean staining index of COX–2 immunoeexpression and the number of immune cells infiltrating into each layer of the endometrium were analyzed by multiple ANOVA using general linear model procedure of SAS (PROC GLM). Stages of endometritis (acute, chronic, normal), mating history (mated versus virgin) and estrous cycle (follicular versus luteal phases) were regarded as independent variables. Normality of the residual distribution of both percentage of positive area and staining index was determined by Shapiro-Wilk statistics, skewness, and kurtosis. It was found that the distribution of both variables did not differ significantly from normal distribution (P>0.05). Therefore, both variables were assumed as normal distribution. All independent variables were included in the statistical models and were tested for significant levels (F-statistic). Since both mating history and estrous phases were not statistically significant in any model (P>0.1), both variables were omitted from the final model. Least-squares means were obtained from each class of the factors and were compared by least significant different test with Tukey-Kramer adjustment for multiple comparisons. Values with P<0.05 were considered statistically significant.

RESULTS

According to gross morphological examination of the reproductive tracts, it was found that the number of gilts in follicular and luteal phases was 6 and 18 respectively. Reproductive data of all gilts were presented in Table 1. Briefly, the gilts entered the breeding house at age 254.2 days and were removed at age 303.1 days. Eighteen out of 24 gilts showed first estrus signs at 243.5 days of age. Of these 24 gilts, 13 gilts were mated at age 274.2 days, while the number of virgin gilts was 3/7, 6/7, and 2/10 in those 24 gilts, respectively. Ages at first estrus and culling were not statistically different (F=0.05). Twelve gilts showed estrous cycles in follicular phases and 6 gilts showed estrous cycles in luteal phases.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number of gilts</th>
<th>Mean ± SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at entering the breeding house (day)</td>
<td>19</td>
<td>254.2 ± 3.7</td>
<td>214.0–289.0</td>
</tr>
<tr>
<td>Age at culling (day)</td>
<td>24</td>
<td>303.1 ± 6.5</td>
<td>260.0–371.0</td>
</tr>
<tr>
<td>Non-productive day (day)</td>
<td>19</td>
<td>55.9 ± 8.6</td>
<td>6.0–157.0</td>
</tr>
<tr>
<td>Age at first estrus (day)</td>
<td>18</td>
<td>243.5 ± 5.9</td>
<td>184.0–301.0</td>
</tr>
<tr>
<td>Age at first insemination (day)</td>
<td>13</td>
<td>274.2 ± 5.4</td>
<td>247.0–313.0</td>
</tr>
<tr>
<td>Number of ovulation</td>
<td>22</td>
<td>15.2 ± 0.6</td>
<td>8.0–20.0</td>
</tr>
</tbody>
</table>
highest percentage of positive area and staining index of COX-2 immunoreactivity, comparing with such of those having chronic endometritis ($P<0.001$; Fig. 1B) and normal endometrium ($P<0.001$; Fig. 1C). Neither the stage of estrous cycle nor the mating history influenced the percentage of positive area and the staining index of COX-2 immunoreactivity ($P>0.05$).

The correlation coefficients among local immune cells infiltrating into the gilts' endometrium, percentage of COX-2 positive area, and staining index were displayed in Table 3. It could be seen that positive correlations were observed between the percentage of positive area and the number of neutrophils in the surface epithelial layer ($r=0.47, P=0.022$), staining index and number of neutrophils in the surface epithelial layers ($r=0.44, P=0.032$). Moreover, the number of surface epithelial neutrophils correlated with the subepithelial neutrophils ($r=0.73, P<0.001$). However, no correlation between the number of lymphocytes and percentage of positive area, together with staining index; and the number of macrophages and percentage of positive area, as well as staining index was found ($P>0.05$).

The serum $P_4$ level, on average, of the gilts in the current study was $76.0 \pm 9.5$ nmol/l (range $1.0–143.4$ nmol/l). The serum $P_4$ of gilts during the luteal phase was significantly higher than the follicular phase ($95.2 \pm 8.5$ versus $18.4 \pm 7.4$ nmol/l, $P<0.001$). The serum $P_4$ did not differ significantly among gilts with different stages of endometritis ($P=0.78$). Moreover, it was found that $P_4$ was neither correlated with...
stimulated inflammatory processes occurred at the luminal face epithelial layer in responding to any agent which tion from the subepithelial connective tissue layer to the su-
epithelial layers signified the need of neutrophilic mobiliza-
tion. Furthermore, the positive correlation between the num-
ber of neutrophils in the subepithelial and in the surface 
epithelial layer of the endometrium was an important agent to stimulate COX-2 expression in the gilts’ endometrium. As seen in the endometria of the gilts with acute endometritis, these findings implied that the quantity of neutrophils in the glandular epithelium of normal uteri was faintly and moderately stained in that study. The endometria of the gilts with acute endometritis were strongly COX-2 stained, in the present study, might be caused by an induction of proinflammatory cytokines released from numerous polymorphonuclear cells in the endometrium [10]. Correspondingly, the positive correlation between the number of infiltrated neutrophils in the surface epithelial layer and percentage of positive area in the gilts’ endometria was noticed. Also, the number of neutrophils in the surface epithelial layer correlated with the staining index. These implied that the quantity of neutrophils in the surface epithelial layer of the endometrium was an important agent to stimulate COX-2 expression in the gilts’ endometria. As seen in the endometria of the gilts with chronic inflammation, a far smaller population of neutrophils and percentage of positive area was found in all layers than those of the endometria of the gilts with acute inflammation. Furthermore, the positive correlation between the number of neutrophils in the subepithelial and in the surface epithelial layers signified the need of neutrophilic mobilization from the subepithelial connective tissue layer to the surface epithelial layer in responding to any agent which stimulated inflammatory processes occurred at the luminal epithelium. In addition, the preceding in vitro studies demonstrated that proinflammatory cytokines, such as interleukin-1β and tumor necrotic factorα could activate COX-2 expression in human neutrophils and monocytes [17], human and rat myometrium [6, 10] and human fetal membranes [2]. Apart from the surface epithelial layer of the endometrium, other compartments, i.e., subepithelial connective tissue layer, glandular connective tissue layer, and endothelial and two layers of smooth muscle of blood vessels in the endometrium were COX-2 immunonegative in the current study. Nevertheless, Jana et al. [13] found moderate-to-strong immunostaining of COX-2 in glandular epithelium of severe acute endometritis gilts but faint staining was observed in such layer of normal uteri. Moreover, Jana et al. [13] found an undulating immunoreaction of COX-2 among endothelial, circular muscular, and longitudinal muscular layers of arteries in the gilts’ uteri. These findings might be due to markedly induced inflammation with injecting 50 ml of E. coli suspension containing 10⁶ colony-forming unit/ml into both uterine horns of the gilts in the third day of estrous cycle. From this day, the female pigs were in luteal phase which the reproductive system was under the predomination of P₄. The previous study revealed that the reproductive tract of the female pigs were the most susceptible to an infection by any pathogen in the luteal phase since the pattern of uterine resistance and susceptibility to infection was relevant to the alteration of sex steroid hormone during estrous cycle [29]. If the pigs were under the P₄ domination, immune function would be down-regu-
lated [24]. On the contrary, if the pigs were under an influence of estradiol, the function of immune cells would be up-regulated [29]. Specifically, the preceding studies revealed that the correlation between serum P₄ concentration and the number of eosinophils was found [5, 22]. Likewise, the tendency of positive correlation between serum P₄ concentration and the infiltration of eosinophils in the current study was observed. However, the association between serum P₄ and COX-2 immunoeexpression was not found in the current study. This implied that P₄ level might not be considerably related to the immunoexpression of COX-2 in natural-inflamed uteri. The gilts with chronic endometritis in the present study showed low immunoreaction of COX-2 in the endometrium. Duncan et al. [8] indicated that purulent and localized inflammations, such as pyometra and pyothorax, were good stimulators of neutrophilia of inflammation in reacting with local foreign materials. However, long-standing inflammation did not provoke the neutrophilia of inflammation. It meant various cytokines, growth factors, and mediators were less released from infected tissues than it was found in acute inflammation. On account of the low quantity of polymorphonuclear cells infiltrating into the endometrium in the

**DISCUSSION**

In the present study, the gilts having an acute inflamma-
tion of the endometrium showed the strongest immunoreac-
tion of COX-2, compared to such of those with chronic inflam-
mation of the endometrium and with normal endometrium. This was in accordance with the findings of Jana et al. [14] that COX-2 immunoreaction was very strong in the luminal epithelium of the severe acute endometritis gilts which were experimentally challenged by Escherichia coli (E. coli) inoculation. By contrast, such epithelium of normal uterine was faintly and moderately stained in that study. The endometria of the gilts with acute endometritis were strongly COX-2 stained, in the present study, might be caused by an induction of proinflammatory cytokines released from numerous polymorphonuclear cells in the endometrium [10]. Correspondingly, the positive correlation between the number of infiltrated neutrophils in the surface epithelial layer and percentage of positive area in the gilts’ endometria was noticed. Also, the number of neutrophils in the surface epithelial layer correlated with the staining index. These implied that the quantity of neutrophils in the surface epithelial layer of the endometrium was an important agent to stimulate COX-2 expression in the gilts’ endometria. As seen in the endometria of the gilts with chronic inflammation, a far smaller population of neutrophils and percentage of positive area was found in all layers than those of the endometria of the gilts with acute inflammation. Furthermore, the positive correlation between the number of neutrophils in the subepithelial and in the surface epithelial layers signified the need of neutrophilic mobilization from the subepithelial connective tissue layer to the surface epithelial layer in responding to any agent which stimulated inflammatory processes occurred at the luminal epithelium. In addition, the preceding in vitro studies demonstrated that proinflammatory cytokines, such as interleukin-1β and tumor necrotic factorα could activate COX-2 expression in human neutrophils and monocytes [17], human and rat myometrium [6, 10] and human fetal membranes [2]. Apart from the surface epithelial layer of the endometrium, other compartments, i.e., subepithelial connective tissue layer, glandular connective tissue layer, and endothelial and two layers of smooth muscle of blood vessels in the endometrium were COX-2 immunonegative in the current study. Nevertheless, Jana et al. [13] found moderate-to-strong immunostaining of COX-2 in glandular epithelium of severe acute endometritis gilts but faint staining was observed in such layer of normal uteri. Moreover, Jana et al. [13] found an undulating immunoreaction of COX-2 among endothelial, circular muscular, and longitudinal muscular layers of arteries in the gilts’ uteri. These findings might be due to markedly induced inflammation with injecting 50 ml of E. coli suspension containing 10⁶ colony-forming unit/ml into both uterine horns of the gilts in the third day of estrous cycle. From this day, the female pigs were in luteal phase which the reproductive system was under the predomination of P₄. The previous study revealed that the reproductive tract of the female pigs were the most susceptible to an infection by any pathogen in the luteal phase since the pattern of uterine resistance and susceptibility to infection was relevant to the alteration of sex steroid hormone during estrous cycle [29]. If the pigs were under the P₄ domination, immune function would be down-regulated [24]. On the contrary, if the pigs were under an influence of estradiol, the function of immune cells would be up-regulated [29]. Specifically, the preceding studies revealed that the correlation between serum P₄ concentration and the number of eosinophils was found [5, 22]. Likewise, the tendency of positive correlation between serum P₄ concentration and the infiltration of eosinophils in the current study was observed. However, the association between serum P₄ and COX-2 immunoeexpression was not found in the current study. This implied that P₄ level might not be considerably related to the immunoexpression of COX-2 in natural-inflamed uteri. The gilts with chronic endometritis in the present study showed low immunoreaction of COX-2 in the endometrium. Duncan et al. [8] indicated that purulent and localized inflammations, such as pyometra and pyothorax, were good stimulators of neutrophilia of inflammation in reacting with local foreign materials. However, long-standing inflammation did not provoke the neutrophilia of inflammation. It meant various cytokines, growth factors, and mediators were less released from infected tissues than it was found in acute inflammation. On account of the low quantity of polymorphonuclear cells infiltrating into the endometrium in the

**Table 3. Pearson’s correlation coefficient (r) and significant level (P value) among local immune cells infiltrating into the surface epithelial layer of the endometrium, percentage of Cyclooxygenase-2 (COX-2) positive area, and staining index**

<table>
<thead>
<tr>
<th>Local immune cells</th>
<th>Percentage of COX-2 positive area r</th>
<th>Staining index</th>
<th>r</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>0.47</td>
<td></td>
<td>0.44</td>
<td>0.032</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>–0.20</td>
<td>0.355</td>
<td>–0.14</td>
<td>0.522</td>
</tr>
<tr>
<td>Macrophages</td>
<td>0.31</td>
<td>0.146</td>
<td>0.13</td>
<td>0.536</td>
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</table>
chronic inflammatory process, the COX-2 immunoreactivity in the gilts with chronic endometritis in the present study was significantly lower than such in the gilts with acute endometritis. Moreover, the COX-2 immunoreaction in the gilts with chronic endometritis in the current study was not significantly different from those with normal endometrium. Ackermann [1] pointed out that once the acute inflammatory response failed; chronic inflammation would be ensued since the animal’s body attempted to surmount the provocative stimuli via the responsibility of macrophages and adaptive immune response. If this process failed, fibroblasts would produce collagen to encapsulate the stimulus and tried to place out of the body. This response could be beneficial and in time could contribute to a return to normal condition. This suggested that the gilts with chronic endometritis in the current study might be progressing to chronic endometritis gilts and those with normal endometrium, while such immunoexpression in those with acute endometritis, in the current study, and was not significantly different between chronic endometritis gilts and those with normal endometrium.

In summary, the current study showed the different association of COX-2 immunoexpression among different patterns of endometritis. The gilts with acute endometritis showed the strongest COX-2 immunoreaction in the endometrium, while such immunoreexpression in those with chronic endometritis and normal endometrium was not different. This suggested that the expression of COX-2 might be dependent on the infiltration of local polymorphonuclear cells which were the 1st-line defense mechanism in reacting against any immunogen; and the duration, as well as the concentration of inflammatory agonists. Furthermore, necropsy and local immunocyte quantification could reveal subjective degrees of endometrial inflammation. Nevertheless, immunohistochemistry of COX-2, the more objective method, in endometrium could enhance the accuracy of clinically diagnosing porcine endometritis.

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