Influence of Multiple Ovarian Cysts on the Immunohistochemical Localization of Progesterone Receptor in the Gilt Endometrium

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Introduction

In the swine farms, the reproductive disorders composed of repeat breeding, vaginal discharge, anestrus, not getting pregnant and abortion are the main problem resulting in culling lots of gilts (1). It is known that reproductive function of gilts is complicated to clinically explore under field surroundings, thus the reproductive organs from the slaughterhouses are the possible source for investigating the various reproductive problems (2). The pig uterus is regulated by the intricate actions of ovarian steroid hormones and the appropriate hormonal levels can build a suitable milieu within endometrium (3). Undeniably, the ovarian abnormalities must disrupt the uterine function leading to infertility (4). In Thailand, most ovarian disturbances found in gilts culled due to reproductive failure are the ovarian cysts (60%) and the multiple cysts were significantly detected among these gilts (5). Since the ovarian cysts are counted as the hormonal disease and the female steroid hormones accomplish their functions by binding through exact receptors (6). It is expected that the expression of these receptors in the target tissues could reveal the efficiency of female hormonal function. Therefore, the aim of this study was to determine the impact of the multiple ovarian cysts on the localization of progesterone receptor in the culling gilt endometrium.

Materials and Methods

The genital tracts of crossbred Landrace × York Shire culling gilts (n=10) were collected from the abattoirs. Both ovaries were categorized into the multiple ovarian cysts (n=5) and ovary at follicular phase as control group (n=5). Uterine horns were dissected out of the tract, fixed in 4% paraformaldehyde, embedded in paraffin and cut at 5 µm-thickness. The process of Avidin-Biotin Peroxidase immunohistochemical method (Vector Laboratories Inc.) was performed and the primary antibody applied was monoclonal mouse progesterone receptor (PR) antibody (PR-2C5, Invitrogen Ltd., Paisley, UK) at a dilution of 1:200. Positive labeling was perceived using 3,3'-diaminobenzidine (DAB, DAKO, Glostrup, Denmark) substrate and counterstained by hematoxylin. Negative control slides were carried out by replacing antibody with normal mouse IgG. The PR immunolocalization revealed in epithelium, subepithelial connective tissue (CNT) layer, and superficial glandular epithelium was assessed under a light microscope (BX50, Olympus, Tokyo, Japan). The intensity of positive immunostaining presented in each compartments was scored as weak (1), moderate (2) or strong (3) and all score data was statistically analyzed using the SAS statistical package (version 9.0).

Results and Discussion

The result depicted that positive PR immunolocalization was detected as an apparently brown nuclear staining in most of the uterine epithelial cells, stromal cells in the subepithelial CNT layer and also superficial glandular epithelial cells of the control group (Fig. 1A).

![Image](image1.png)

Figure 1 Immunohistochemical positive staining of PR (black arrows) in the nuclei of epithelial cells in luminal epithelium (LE), stromal cells in subepithelial CNT layer (STR) and epithelial cells in superficial glandular epithelium (SGE). Control group (A) compared to with group of multiple ovarian cysts (B). Negative control demonstrated in the inset of A.

The PR immunoreaction was found moderate to weak in all endometrial compartments of the gilts with multiple ovarian cysts (Fig. 1B). The intensity scores of PR positive staining were summarized in Table 1. The higher intensity score was significantly (p<0.05) revealed in the control group compared with multiple ovarian cysts.
Table 1 The PR immunohistochemical staining intensity scores presented in the endometrium of culled gilts.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Luminal epithelium</th>
<th>Stromal CNT layer</th>
<th>Glandular epithelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.60±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.00±0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.80±0.55&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Multiple cysts</td>
<td>1.10±1.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.20±0.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.50±1.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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<sup>*Different letters (a, b) within the same column represent significant differences (<p<0.05).</sup>

It is accepted that progesterone and its receptor were important for variety functions of the uterus, e.g. hypertrophy and secretory activity of uterine glands including the activities of luminal epithelium and stromal cells, to adjust proper micro-environment for development embryos (7). Besides, progesterone that mainly increased after ovulation affected the transportation of spermatozoa and embryos (8). The present results were corresponded with the earlier investigation which indicated that the changes of female steroid receptors as detected by immunohistochemical reaction in the uterus related in the pathological situations occurred in the gilts culled due to reproductive disturbances (9). Additionally, the multiple ovarian cysts showed exact endocrinological effect and caused the morphological deteriorations in the gilt endometrium (10). In this study, we conclude that the decrease of PR intensity levels in the target tissues involved in the multiple ovarian cysts and might directly lead to the endometrial abnormalities of these gilts.

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References