Original Article

Immunohistochemical Detection of Estrogen and Progesterone Receptors in the Uterine and Oviductal Tissues of Culling Gilts with Ovarian Cysts

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Abstract

The most important removal cause of replacement gilts that occurred in the swine herds is the reproductive failure. The widespread causes of reproductive problem are induced by various factors and ovarian cysts were frequently found among these. The objective of this study was to scrutinize the impact of ovarian cysts on the expression of female steroid receptors in the uterine horns and oviducts of culling gilts. The genital organs were collected from culled replacement gilts which were categorized into three groups: gilts with single large cyst, multiple large cysts and normal ovary at follicular phase (control). The historical data were gathered for analyzing the relationship between culling reasons and ovarian cystic types. Blood samples were collected for examining the progesterone level. The immunohistochemical study was performed to elucidate the localization of estrogen receptor alpha (ERα) and progesterone receptor (PR). Anestrus was the major culling reasons (50%) that found in the replacement gilts with multiple large ovarian cysts and progesterone level (45.8±21.2 nmol/l) was elevated in this group. The intensity and proportion of ERα and PR nuclear positive staining detected in uterine horns and oviducts were lower in the group of multiple large ovarian cysts compared with the other groups. The investigations indicated that the malfunctions in ovarian hormonal synthesis, particularly the abnormal level of progesterone, influence on the expression of female steroid receptors in the uterine horns and oviducts. This occurrence may describe that the multiple large ovarian cysts were the important reason to reduce the functional efficiency of reproductive tracts and may be the main reason of anestrus found in the replacement gilts.

Keywords: cystic ovary, estrogen receptor, gilt, progesterone receptor, reproductive organ

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Introduction

In the swine farms, the reproductive disorders composed of repeat breeding, abnormal vaginal expulsion, anestrus, not getting pregnant and abortion are the main problem for removing replacement gilts (Stein et al., 1990; Lucia et al., 2000). These problems have been documented as the cost-effective losses in the porcine industry (Dijkhuizen, 1989). The reproductive function is actually complicated to clinically detect under field environments; therefore the entire reproductive tracts of culling pigs from the slaughterhouses are the practical source for investigating the abnormalities (Fitko et al., 1995). In sows, numerous previous studies indicated that the pathological lesions of these reproductive organs could not be usually observed in Thailand or European countries (Kunavongkrit et al., 1986; Dalin et al., 1997; Heinonen et al., 1998). Likewise, Tummaruk et al. (2009) confirmed that more than 50% of the culling gilts displayed normal reproductive organs. For this reason, the microscopic investigations were necessary to scrutinize the reproductive tracts and the abnormalities of oviduct and uterine horn were apparently revealed in different manners in the gilts culled with anestrus or repeat breeding (Tienthai and Sajjarengpong, 2007a, 2007b; Teamsuwan et al., 2010). Definitely, the uterine horn and oviduct in domestic animals including pig are under control by the intricate mechanisms of ovarian steroid hormones and the appropriate hormonal levels affect the normal function in these organs to create a favorable micro-environment for gametes and embryo (Tsai and O'Malley, 1994; Hunter, 2005). In previous study, Karveliene et al. (2007) reported that the low level of estrogen and progesterone in anestrous pigs is correlated between ovarian dysfunction and the weight of reproduction organs. To ascertain the abnormalities occurred in reproductive tracts, the gross pathological and microscopic morphological examinations could be inadequate; as a result the additional study involved in steroid hormonal receptors is required for more understanding in the regulations of ovarian hormones and their receptors predominantly in the culling gilts found the abnormal ovaries.

The cystic ovarian disease is a varied endocrinal disorder that correlated to ovarian dysfunction causing the reproductive disturbance (Ogasa et al., 1983). Generally, the ovarian cysts were regularly categorized into single or multiple cysts and subdivided into small or large cysts (Miller, 1984). The incidence of ovarian cysts in pigs culled due to infertility fluctuates between 2% to 24% (Einarsson et al., 1974; Ryan and Raeside, 1991; Heinonen et al., 1998). Only in replacement gilts, the cystic ovaries were detected about 14% and more than 60% of these gilts were multiple ovarian cysts (Tummaruk et al., 2009). The changes of physiology and behavior in sows or gilts depend on the forms of ovarian cysts and most multiple cysts are suggested to be the serious cause for breeding because the gross pathological signs were not seen in reproductive tracts of these animals (Karveliene et al., 2007). Since the ovarian cysts are counted as the hormonal disease and the female steroid hormones accomplish their functions by binding through exact receptors at the target organs including uterus and oviduct (Yamashita, 1990). It is expected that the changes in the expression of female steroid receptors in the reproductive tracts resulting in the functional efficiency of ovary as well. Therefore, the principle objective of this study was to determine the influence of the ovarian cysts primarily the multiple large cysts on the immunostaining intensities of estrogen and progesterone receptors in the uterine horns and oviducts of gilts culled due to fertility problems.

Materials and Methods

Animal tissue collection: The reproductive organs of the crossbred Landrace × Yorkshire replacement gilts (n=40) were collected at the abattoirs. Their historical data were recorded particularly the culling reasons and blood samples were kept prior to slaughter for analyzing serum progesterone. The genital tracts were taken to the laboratory in a cool container after gathering approximately 6 to 8 hr for general macroscopic evaluation. The ovaries, uterine horns and oviducts were excised out of the whole genital tracts and culling gilt ovaries were categorized into 3 groups: normal ovaries at follicular stage (n=9), single large cyst on the ovaries (n=13) and multiple large cysts on the ovaries (n=18) as previously distinguished by Tummaruk et al. (2009). The uterine samples were cut off from the mid-portion of the uterine horns, whereas the oviductal tissues were separated into utero-tubal junction (UTJ), isthmus and ampulla. All samples were immersed in 4% paraformaldehyde for immunohistochemical procedure.

Progesterone hormone assay: Blood samples from culling gilts were centrifuged at 3,000 rpm for 10 min and the collected plasma was then frozen and kept at –20°C until analyzed. The progesterone level in plasma was examined by a solid-phase radioimmunoassay (Coat-A-Count®, Diagnostic Products Corporation, CA, USA) as previously described by Tummaruk et al. (2004) and the assay was carried out according to the manufacturer's instructions.

Immunohistochemistry: The tissue samples of uterine horns and oviducts from each group were randomly selected for immunohistochemical procedure composed of normal ovary (n=5), single large cyst (n=7) and multiple large cysts (n=10). The fixed samples were routinely prepared by histological techniques, embedded in paraffin blocks, cut in ~4 μm thick sections and mounted on the coated slides (Superfrost™ Plus, Menzel-Glaser, Freiburg, Germany). All tissue sections were heated in the incubator at 40°C for 4 hours, deparaffinized in xylene and rehydrated in decreasing concentration of alcohol prior to rinsing in phosphate buffered saline (PBS, pH 7.2). The procedure of standard Avidin-Biotin Peroxidase immunohistochemical technique (Vectastain ABC-Elite standard; Vector Laboratories Inc., Burlingame, CA, USA) to determine the estrogen alpha (ERα) and progesterone (PR) receptors as earlier performed by Tienthai et al. (2008, 2009). In brief, the antigen retrieval method was completed by boiling the

sections with 0.01 M citrate buffer (pH 6.0), 5×2 min in a microwave at 750 W. The endogenous peroxidase action was obstructed by immersing with 3% hydrogen peroxide in methanol and a non-specific background staining was reduced by incubation with normal horse serum (Vector Laboratories Inc.). Mouse monoclonal antibody to ERα (C-311, sc-787, Santa Cruz Biotechnology Inc., CA, USA) at a dilution of 1:50 and mouse monoclonal antibody to PR (PR-2C5, Invitrogen Ltd., Paisley, UK) at a dilution of 1:200 were used as primary antibodies. The incubation period for the primary antibody was 18-20 hr at 4°C. Negative controls were accomplished by substituting the primary antibodies with normal mouse IgG (sc-2025; Santa Cruz Biotechnology Inc.) of the same dilution of the primary antibodies. The sections were then applied with the secondary biotinylated horse anti-mouse antibody (Vector Laboratories Inc.) at a dilution of 1:500 followed by adding with ABC-mouse reagent (Vector Laboratories Inc.). The positive immunostaining was visualized using freshly prepared the 3, 3'-diaminobenzidine (DAB kit, Vector laboratories Inc.) in H_2O_2 and all tissue sections were counterstained with Mayer's hematoxylin and then mounted with glycerine-gelatin.

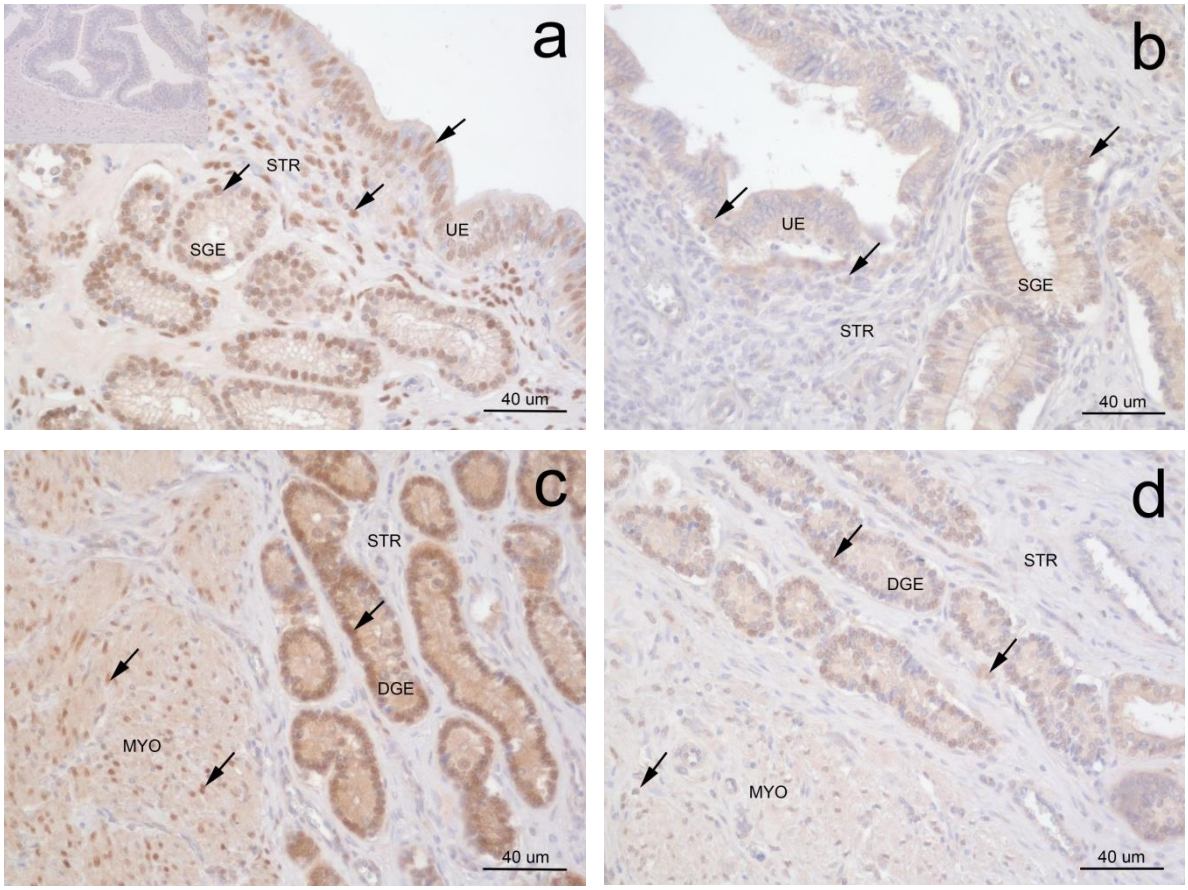

Figure 1 Immunohistochemical staining of ERα in different compartments of the selected gilt uterine tissues with normal ovaries at follicular stage (a, c) compared to culling gilts with multiple large ovarian cysts (b, d). The nuclear ERα positive cells were stained brown (arrows) in the uterine epithelium (UE), subepithelial stromal layer (STR), superficial uterine glandular epithelium (SGE), deep glandular epithelium (DGE) and smooth muscle layer or myometrium (MYO). Negative control was illustrated in the inset of picture a. Bar = 40 μm.

Categorization of positive immunohistochemical reaction: The uterine and oviductal sections were examined under light microscopy (BX50, Olympus, Tokyo, Japan) supplied with a digital camera Micropublisher 5.0 (Qimage, Surrey, Canada). The tissue micrographs were taken by selected program of Image Pro® Plus version 6 (Media Cybernatics Inc., MD, USA). The manual scoring of ERα and PR positive immunostaining cells was completed by the same person who was unaware of the independence of culling gilts. In uterine horns, five different compartments; luminal epithelium, superficial uterine glandular epithelium, deep uterine glandular epithelium, subepithelial connective tissue (CNT) layer and the smooth muscle layer were carried out, whereas only three partitions; luminal epithelium, subepithelial CNT layer and smooth muscle layer, in each parts of the oviducts were evaluated. Positive immunostaining intensity was classified into three different scores; weak (1), moderate (2) or strong (3) as previously performed by Tienthai et al., (2008, 2009). The proportions of the nuclei positive cells were estimated into four different scores (Karveliene et al., 2007); low proportion (<30% of positive cells, A), moderate proportion (31-50% of positive cells, B), high proportion (51-80% of positive cells, C) and almost all cells positive (>81%, D)

Statistical analyzes: Data was statistically scrutinized using the SAS statistical package (version 9.0, SAS

Institute Inc., Cary, NC, USA). The mean and standard deviation of all parameters were calculated and were used to demonstrate data. The intensity scores from each tissue compartment of uterine horn and oviduct were compared using of Wilcoxon Scores test and Kruskal-Wallis's test (NPAR1WAY procedure of SAS). A value of $p<0.05$ was considered statistically significant.

Results

Reproductive data: The historical data of culling replacement gilts and the incidence of ovarian cysts categorized by the culling reasons are displayed in Table 1 and 2, respectively. The gilts with multiple large cysts on the ovaries demonstrated a shorter ($p<0.05$) interval from entry-to-cull than the gilts with single large cyst on the ovary and normal ovary and the level of plasma progesterone in the culling gilts with multiple large cysts (45.8±21.2 nmol/l) was significantly higher ($p<0.05$) than the other groups (Table 1). All gilts (31 gilts) were sent to the abattoir with different culling reasons including anestrus, repeat breeding, unusual vaginal discharge, abortion and miscellaneous involved in reproductive tract. There were 50% of the culling gilts with multiple large cystic ovaries (18 gilts) were removed because of anestrus.

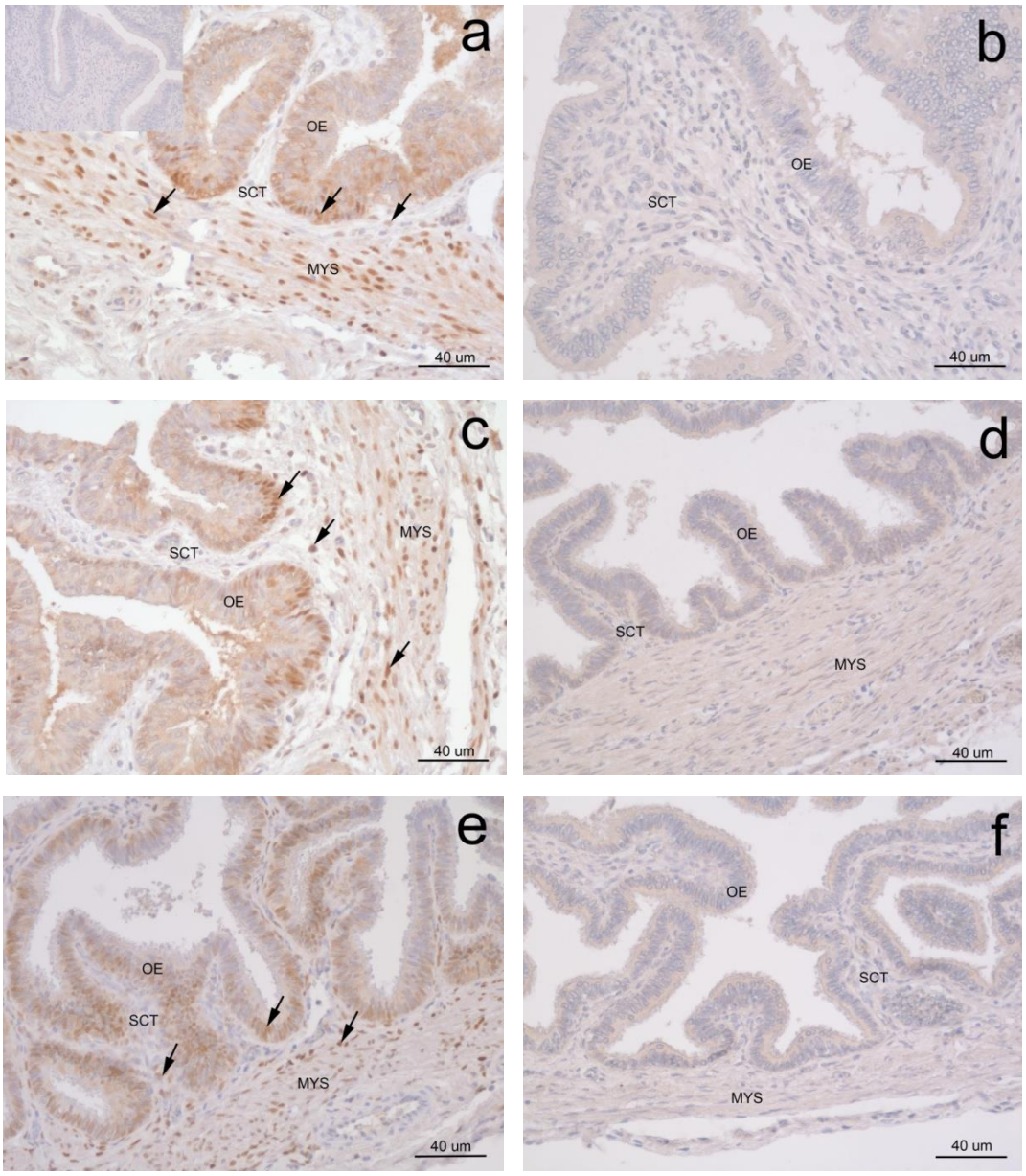

Figure 2 Immunohistochemical staining of ERα in different compartments of UTJ (a, b), isthmus (c, d) and ampulla (e, f) of the chosen gilt oviducts with normal ovaries at follicular stage (a, c, e) compared to culling gilts with multiple large ovarian cysts (b, d, f). The nuclear ERα positive cells were marked brown (arrows) in the oviductal epithelium (OE), subepithelial connective tissue layer (SCT), and smooth muscle layer or myosalpinx (MYS). Negative control was illustrated in the inset of picture a. Bar = 40 µm.

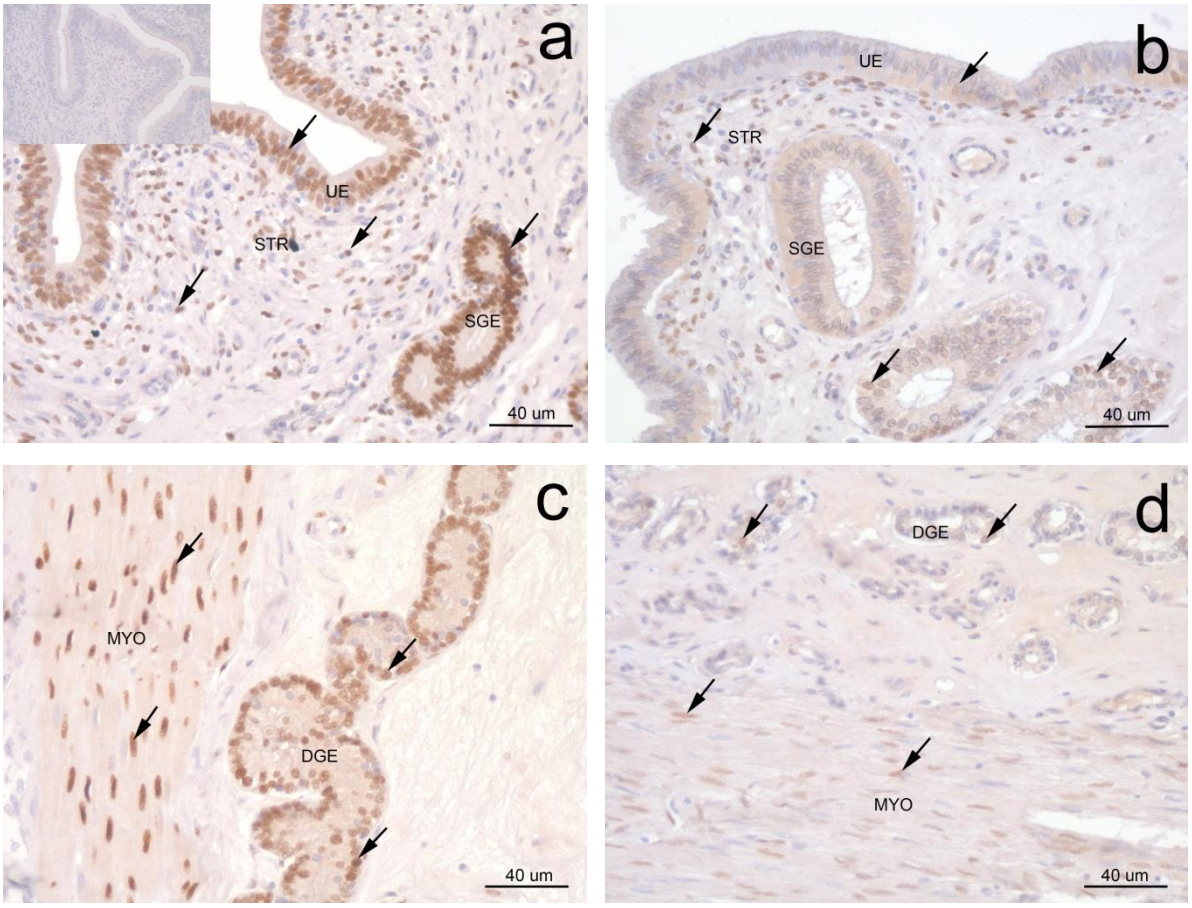

Figure 3 Immunohistochemical localization of PR in different compartments of the selected gilt uterine tissues with normal ovaries at follicular phase (a, c) compared to culling gilts with multiple large ovarian cysts (b, d). The nuclear ERα positive cells were stained brown (arrows) in the uterine epithelium (UE), subepithelial stromal layer (STR), superficial uterine glandular epithelium (SGE), deep glandular epithelium (DGE) and smooth muscle layer or myometrium (MYO). Negative control was illustrated in the inset of picture a. Bar = 40 μm.

Immunohistochemical staining of ERα in uterine horns and oviducts: As expected, the positive immunohistochemical labeling of ERα was clearly presented in the nucleus of different cells within all uterine and oviductal tissue compartments in the control gilts (Figs. 1a, c and 2a, c, e) compared to the alteration of ERα immunostaining that usually found in the gilts with multiple large ovarian cysts (Figs. 1b, d and 2b, d, f) whereas the negative controls displayed no staining (inset in Figs. 1a, 2a). The intensity (mean±SD) and proportion manual scoring of ERα positive nuclear staining in both uterine horns and oviducts of the control, single large cyst and multiple large cysts was showed in Table 3 and 4, respectively. Almost all cells (D) were positively stained in most uterine compartment except the subepithelial connective tissue layer (C) whereas the proportion of positive cells of oviductal compartment in the control gilts varied between score C and D. However, the proportion of the nuclei positive cells of both uterus and oviduct was decrease in the multiple large cysts. In uterine horns, the strongest intensities of ERα positive cells were observed in all tissue compartments in the control and the positive intensity staining was not significantly different from the single large cystic ovary. The intensity results from the multiple large cystic ovaries were obviously lower (p<0.05) than the control and single large cystic ovary. In oviducts, likewise the uterine tissues, the greatest intensities of the nuclear positive labeling were presented in all tissue compartments in the gilt with normal ovary. In the single cystic group, only the subepithelial CNT layer of UTJ, isthmus and ampulla demonstrated weak intensity immunostaining which was considerably different (p<0.05) from control group; meanwhile, the positive intensities observed in all oviductal tissue compartments of the multiple large cystic gilts were significantly lower (p<0.05) than both groups.

Immunohistochemical staining of PR in uterine horns and oviducts: Similar to ERα, the positive immunohistochemical expression of PR in the gilts with ovaries at follicular phase was conspicuously appeared in the nuclei of different uterine and oviductal cell types (Figs. 3a, c and 4a, c, e) while the negative controls exhibited no staining (inset in Figs. 3a, 4a). The intensity and proportion manual scoring data of PR immunostaining presented in different tissue compartments of both organs were described in Table 5 and 6. In all uterine tissue compositions, the greatest positive intensities of PR were observed in the control gilts and were not considerably different from the single large cystic gilts; whereas, the weakest intensity staining was seen in all tissue compartments of the multiple large cystic gilts compared with the other groups (p<0.05). In UTJ, isthmus and ampulla,

the strongest intensities of the positive labeling were presented in all tissue compartments in the gilt with follicular ovary. In the gilts with single large cyst, the subepithelial CNT layer showed very weak immunostaining similar to the multiple large cystic groups which were absolutely different (p<0.05) from the control group. In the multiple large cysts, the intensities of positive staining of the gilt oviduct were significantly lesser (p<0.05) in all tissue compartments than the control groups.

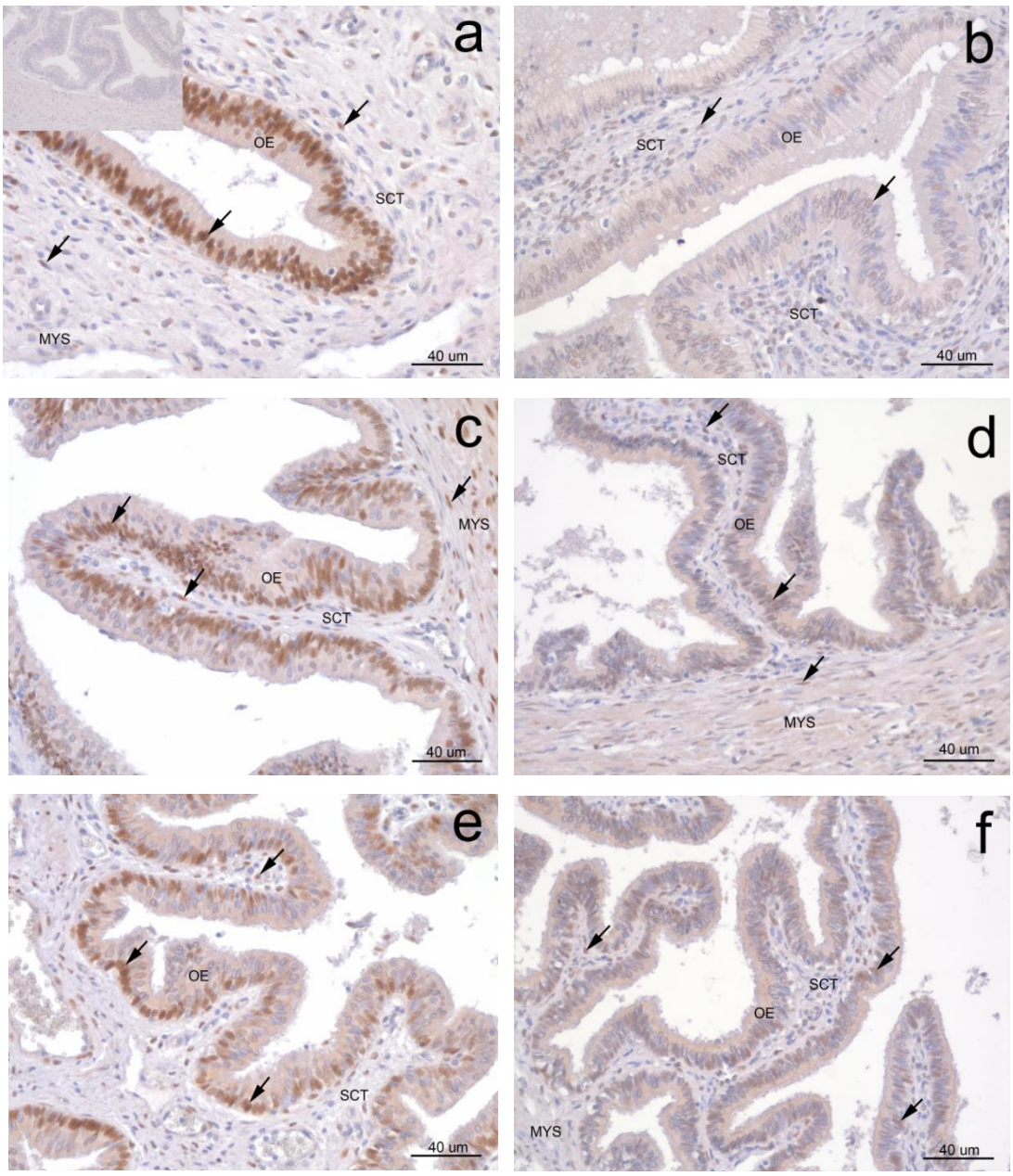

Figure 4 Immunohistochemical staining of PR in different compartments of UTJ (a, b), isthmus (c, d) and ampulla (e, f) of the chosen gilt oviducts with normal ovaries at follicular stage (a, c, e) compared to culling gilts with multiple large ovarian cysts (b, d, f). The nuclear PR positive cells were stained brown (arrows) in the oviductal epithelium (OE), subepithelial connective tissue (SCT), and smooth muscle layer or myosalpinx (MYS). Negative control was illustrated in the inset of picture a. Bar = 40 μm.

Discussion

In the present study, we found that more than half of the replacement gilts culled with multiple large cystic ovaries demonstrated anestrus. Tummaruk et al. (2009) suggested that the replacement gilts in Thailand were culled due to the main reproductive problems composed of anestrus (44%), abnormal vaginal discharge (20.5%), repeat breeding (15.5%) and not being pregnant and abortion (10%) and miscellaneous reasons (10%). It is interesting that the pathological signs in the reproductive tracts were not observed in these culling gilts (50.5%) whereas 20% of the replacement gilts culled due to reproductive disorders was seen accompanied with the ovarian cysts. Additionally, the high occurrence of return to estrus after artificial insemination and low incidence of farrowing rate were also observed in the sows with the ovarian cysts (Castagna et al., 2004). These findings confirmed the impact of ovarian cysts on the reproductive performance of female pigs and the cause of this problem was not thoroughly studied. Therefore,

we attempt to describe the association between the types of ovarian cysts and the culling reasons of replacement gilts. The present results indicated the gilts culled due to anestrus associated with the multiple large ovarian cysts correspond with the previous research reported that only multiple ovarian cysts affect the estrous cycle and conception because this cystic type mostly came from luteinized ovarian follicles and they synthesized progesterone in the adequate amount to interrupt the regular estrous cycle (Wrathal, 1980; Tummaruk and Kesdangsakonwut, 2012). This information was approved that the high plasma progesterone level (45.8±21.2 nmol/l) found in these culling gilts was present in the sows with multiple large cysts (Babolola and Shapiro, 1990; Szulanczyk-Menzel et al., 2010) and it appeared nearly the similar level of normal sows (Britt et al., 1999) and gilts (Teamsuwan et al., 2010) during diestrus. Therefore, the hormonal variation particularly the progesterone resulting in the multiple large ovarian cysts observed in the present study could be the key explanation for the gilts culled by the anestrus problem. So far, the etiology of cystic ovarian disease in pigs has not been completely elucidated but it was suggested that the actions of adrenergic nerves and noradrenaline induced the multiple cystic ovaries in rat and pig (Dorfman et al., 2003; Jana et al., 2005; Kozlowska et al., 2008). Moreover, different factors might be associated with the formation of ovarian cysts, for example blocking of ovulation by tissue-type plasminogen activator (Whisnant et al., 1998), unstable or insufficient in releasing of GnRH (Babolola and Shapiro, 1990) and LH-peak levels (Almond and Richards, 1991). Furthermore, the deficiency of LH and FSH receptors in the developing follicles (Miller, 1984), the increase of ACTH and cortisol because of diverse stressful stimuli were expected as the stimulus of ovarian cystic disease in gilts (Liptrap and McNally, 1977; Miller, 1984). In order to reduce the incidence of different kinds of cystic ovaries, the stressful stimuli, for example the concentrated acclimatization, overcrowding, and unsuitable climates must be diminished in the replacement gilts.

Table 1 Reproductive data of culling gilts with normal ovaries at follicular phase, single large cystic ovaries and multiple large cystic ovaries (mean±SD).

Variable factors	Normal (n=9)	Single (n=13)	Multiple (n=18)
Age at culling (day)	304.8±8.2[a]	309.7±11.6[a]	292.8±9.8[a]
Body weight (kg)	149.7±2.9[a]	153.4±4.0[a]	154.2±3.4[a]
Entry-to-cull (day)	76.4±9.1[a]	72.2±13.9[a]	49.1±11.2[b]
Average daily gain (g/day)	511.5±15.4[a]	492.0±21.4[a]	502.9±18.2[a]
Ovarian weight (g)	4.8±2.2[a]	8.9±3.7[ab]	17.5±3.2[b]
Uterine weight (g)	735.7±46.7[a]	695.8±66.1[a]	711.7±56.1[a]
Uterine length (cm)	232.6±12.3[a]	262.0±17.4[a]	277.3±14.8[a]
Progesterone (nmol/l)	3.1±0.7[a]	8.4±5.3[a]	45.8±21.2[b]

[a,b]Different superscript within column differed significantly (p<0.05)

Table 2 Culling reasons of reproductive disturbances associated with single large cystic ovaries and multiple large cystic ovaries in the replacement gilts.

Culling reasons	Single (n=13)	Multiple (n=18)
Anestrus	3 (23.1%)	9 (50.0%)
Repeat breeding	4 (30.8%)	3 (16.7%)
Abnormal vaginal discharge	3 (23.1%)	2 (11.1%)
Abortion	1 (7.7%)	3 (16.7%)
Miscellaneous	2 (15.4%)	1 (5.5%)

Table 3 Immunostaining intensity (mean±SD) and proportion of ERα as determined by manual scoring in assorted tissue compartments of culling gilt uterine horns.

Tissue compartments	Normal (n=5)	Single (n=7)	Multiple (n=10)
Uterine epithelium	2.60±0.55[a]/D	2.49±0.48[a]/C	0.80±1.03[b]/C
Subepithelial stromal layer	2.60±0.55[a]/C	2.43±0.53[a]/C	1.50±0.70[b]/B
Sup. glandular epithelium	2.60±0.55[a]/D	2.28±0.49[a]/C	1.00±1.15[b]/C
Deep glandular epithelium	3.00±0.00[a]/D	2.71±0.49[a]/C	2.00±0.66[b]/C
Smooth muscle layer	3.00±0.00[a]/D	2.71±0.49[a]/D	2.00±0.66[b]/D

Different superscript letters within the same row are significantly different (p<0.05)

Based on the earlier reports, the pathological lesions of reproductive organs could not be usually observed in pigs (Kunavongkrit et al., 1988; Dalin et al., 1997; Heinonen et al., 1998) and it was concerned that 50% of replacement gilts culled due to reproductive failure found normal reproductive organs by gross examination (Tummaruk et al, 2009). Definitely, we required the supplementary findings, such as the expression of female hormonal receptors, to determine the mechanisms of steroid hormones on their receptors. In the present results, the intensity scores of ERα and PR in the uterine horns and oviducts of these gilts were significantly lesser than single large cystic and the control gilts. As we have known that the female reproductive tracts are under dynamic changes during the estrous cycle, fertilization, conception and

pregnancy by the main regulation of estrogen and progesterone (Steffl et al., 2008). Estrogen was related to the cell proliferation, cell differentiation, and ciliogenesis whereas major function of progesterone was directly involved in the secretory cells, modifications of stromal cells and endometrial glands (Abe and Oikawa 1993; Graham and Clark, 1997; Molenda et al., 2003). These functions of female steroid hormones were completely carried out by binding via their specific receptors in the target tissues of reproductive organs (Tsai and O'Malley, 1994; Edwards, 2005). In cycling pigs, the immunolocalization pattern of ERα and PR was clearly appeared positive brown nuclear staining in different cell types of all tissue compositions in uterus (Geisert et al., 1993; 2004; Sukjumlong et al., 2003; 2005) and oviduct (Stanchev et al., 1985; Steffl et al., 2004) in which the variation of intensity staining of both receptors was depended on cellular types and estrous cycle phases. The positive intensity was conspicuously revealed in all tissue compartments of uterus and oviduct during proestrus, estrus and metestrus corresponding to the present results in the normal gilts at the follicular phase (control group). According to the description from several findings the high level of plasma estrogen promoted the expression of both ERα and PR in reproductive tracts of other species (Stanchev et al., 1990; Geisert et al., 1994). However, we found that the staining variation of ERα and PR was appeared in each tissue layer in these control gilts, and this condition was also reported in pigs (Geisert et al., 1993; Sukjumlong et al., 2003; 2005) and different domestic animals (Wang et al., 2000; Boos et al., 2006; Tienthai et al., 2008; 2009). The lower intensity appearance of ERα and PR in the uterine horns and oviducts of gilts with multiple large ovarian cysts was the interesting aspect in the present study and indicated the relationship between this disease and the reproductive failure, especially anestrus. Previous studies performed the expression of ER and PR the uterus of sows and prepubertal gilts culled due to various reasons of reproductive disorders and the varied intensity immunostaining was performed in these pigs (Karveliene et al., 2007; Srisuwatanasagul et al., 2010). On the contrary, the information of immunolocalization of these steroid receptors is still absent in the oviducts of culling pigs. However, the present results indicated the changes of steroid receptors could associate with the culling causes that found in these gilts. As previously explanation by Roberts et al. (1983), they suggested that the plasma ovarian hormones impact the expression levels of specific receptors in the uterus, i.e. the amount of estrogen improved the uterine proliferation by elevating tissue levels of ERα and PR while the progesterone opposed this activity. Additionally, the physiological modifications in the uterus and oviduct had a relationship not only the hormonal mechanism of receptor localization by both estrogen and progesterone but also their intensities of receptor proteins (Stanchev et al., 1990; Geisert et al., 1994). The present findings show the lowest intensities of both ERα and PR in all tissue compartments of uterine horns and oviducts of culling gilts with the multiple large ovarian cysts implying the influence of abnormal progesterone levels down-regulated the expression of ERα and PR. With this finding, it is possible that the insufficient functions of the reproductive organs might occur in these gilts.

Table 4 Immunostaining intensity (mean±SD) and proportion of ERα as determined by manual scoring in various tissue compartments of culling gilt oviducts.

Tissue compartments	Normal (n=5)	Single (n=7)	Multiple (n=10)
UTJ			
Luminal epithelium	2.80±0.45^{a}/C	2.43±0.53^{a}/C	1.30±0.94^{b}/A
Subepithelial stromal layer	2.80±0.45^{a}/D	1.86±0.69^{b}/C	1.20±0.63^{c}/A
Smooth muscle layer	2.80±0.45^{a}/C	2.43±0.53^{a}/C	1.70±0.67^{b}/A
Isthmus			
Luminal epithelium	2.80±0.45^{a}/C	2.43±0.53^{a}/C	1.30±0.95^{b}/A
Subepithelial stromal layer	2.60±0.55^{a}/D	1.71±0.49^{b}/C	1.20±0.63^{c}/A
Smooth muscle layer	2.80±0.45^{a}/C	2.43±0.53^{a}/C	1.70±0.67^{b}/A
Ampulla			
Luminal epithelium	2.80±0.45^{a}/C	2.43±0.53^{a}/C	1.30±0.95^{b}/A
Subepithelial stromal layer	2.40±0.55^{a}/D	1.71±0.49^{a}/C	1.20±0.63^{b}/A
Smooth muscle layer	2.80±0.45^{a}/C	2.43±0.53^{a}/C	1.70±0.67^{b}/A

Different superscript letters within the same row are significantly different (p<0.05)

Table 5 Immunostaining intensity (mean±SD) and proportion of PR as determined by manual scoring in assorted tissue compartments of culling gilt uterine horns.

Tissue compartments	Normal (n=5)	Single (n=7)	Multiple (n=10)
Luminal epithelium	2.80±0.45^{a}/D	2.28±0.75^{ab}/C	1.10±0.73^{b}/C
Subepithelial CNT layer	3.00±0.00^{a}/C	2.43±0.53^{ab}/C	2.20±1.03^{b}/B
Sup. glandular epithelium	2.80±0.45^{a}/D	2.28±0.75^{ab}/C	1.50±0.97^{b}/C
Deep glandular epithelium	3.00±0.00^{a}/D	2.71±0.49^{ab}/C	2.40±0.51^{b}/C
Smooth muscle layer	3.00±0.00^{a}/D	2.71±0.49^{ab}/C	2.40±0.51^{b}/C

Different superscript letters within the same row are significantly different (p<0.05)

Table 6 Immunostaining intensity (mean ± SD) and proportion of PR as determined by manual scoring in various tissue compartments of culling gilt oviducts.

Tissue compartments	Normal (n=5)	Single (n=7)	Multiple (n=10)
UTJ			
Luminal epithelium	2.80±0.45^{a}/C	2.43±0.53^{ab}/C	1.90±0.73^{b}/B
Subepithelial stromal layer	2.60±0.54^{a}/B	1.86±0.69^{ab}/B	1.90±0.73^{b}/B
Smooth muscle layer	2.80±0.45^{a}/C	2.43±0.53^{ab}/C	2.00±0.47^{b}/B
Isthmus			
Luminal epithelium	2.80±0.45^{a}/C	2.43±0.53^{ab}/C	1.90±0.74^{b}/B
Subepithelial stromal layer	2.60±0.54^{a}/B	1.86±0.69^{ab}/B	1.90±0.57^{b}/B
Smooth muscle layer	2.80±0.45^{a}/C	2.43±0.53^{ab}/C	2.00±0.47^{b}/B
Ampulla			
Luminal epithelium	2.80±0.00^{a}/C	2.43±0.53^{ab}/C	1.60±0.70^{b}/B
Subepithelial stromal layer	2.60±0.54^{a}/B	1.86±0.69^{ab}/B	1.70±0.67^{b}/B
Smooth muscle layer	2.80±0.45^{a}/C	2.43±0.53^{ab}/C	2.00±0.47^{b}/B

Different superscript letters within the same row are significantly different (p<0.05)

In conclusion, the information in our present study indicate that the gilts culled due to the reproductive failure with multiple large ovarian cysts frequently had anestrus and most of them revealed the lower proportion and intensity of ERα and PR immunolocalization in most compartments of uterine horns and oviducts. It is expected that the decrease expression of ERα and PR in the uterus and oviduct of these culling gilts with the multiple large ovarian cysts reflected the abnormal progesterone level could interrupt the physiological functions of porcine reproductive tracts. As the types of ovarian cysts are complicated, the impact of follicular and luteal ovarian cysts on the gilt reproductive tracts must be certainly investigated to understand the cause of reproductive failure.

Acknowledgements

The authors are grateful to Silpchai Pienchop, Witoon Mabutr and Jantima Intarapunya, Department of Anatomy, Faculty of Veterinary Science, Chulalongkorn University, for their extraordinary technical assistance. This study was financial sustained by Ratchadaphiseksomphot Endowment Fund, 2012, Chulalongkorn University.

References

Abe H and Oikawa T 1993. Effects of estradiol and progesterone on the cytodifferentiation of epithelial cells in the oviduct of the newborn golden hamster. Anat. Rec. 235(3): 390-398.

Almond G and Richards RG 1991. Endocrine changes associated with cystic ovarian degeneration in sows. J. Am. Vet. Med. Assoc. 199(7): 883-886.

Babalola GO and Shapiro BH 1990. Sex steroid changes in porcine cystic ovarian disease. Steroids 55(7): 319-324.

Boos A, Kohtes J, Janssen V, Mülling C, Stelljes A, Zerbe H, Hässig M and Thole HH 2006. Pregnancy effects on distribution of progesterone receptors, oestrogen receptor alpha, glucocorticoid receptors, Ki-67 antigen and apoptosis in the bovine interplacentomal uterine wall and foetal membranes. Anim. Reprod. Sci. 91(1-2): 55-76.

Britt JH, Almond GW and Flowers WL 1999. Diseases of the reproductive system. In: Straw, B.E., D'Allaire, S., Mengeling, W.L., Taylor, D.J. Diseases of Swine. Cap. 60, 8th ed. Iowa State University Press, Ames, Iowa, USA.

Castagna CD, Peixoto CH, Bortolozzo FP, Wentz I, Neto GB and Ruschel F 2004. Ovarian cysts and their consequences on the reproductive performance of swine herds. Anim. Reprod. Sci. 81(1-2): 115-123.

Dalin AM, Gidlund K and Eiliasson-Selling L 1997. Post-mortem examination of genital organs from sows with reproductive disturbances in a sow-pool. Acta. Vet. Scand. 38(3): 253-262.

Dijkhuizen AA 1989. Economic aspects of common health and fertility problems for the individual pig producer: an overview. Vet. Q. 11(2): 116-124.

Dorfman M, Arancibia S, Fiedler JL and Lara HE 2003. Chronic intermittent cold stress activates ovarian sympathetic nerves and modifies ovarian follicular development in the rat. Biol. Reprod. 68(6): 2038-2043.

Edwards DP 2005. Regulation of signal transduction pathways by estrogen and progesterone. Annu. Rev. Physiol. 67: 335-367.

Einarsson S, Linde C and Setergren I 1974. Studies of the genital organs of gilts culled for anoestrus. Theriogenology 2(5): 109-113.

Fitko R, Kucharski J, Szlezyngier B and Jana B 1996. The concentration of GnRH in hypothalamus, LH and FSH in pituitary, LH, PRL and sex steroids in peripheral and ovarian venous plasma of hypo- and hyperthyroid, cysts-bearing gilts. Anim. Reprod. Sci. 45(1-2): 123-138.

Geisert RD, Brenner RM, Moffatt RJ, Harney JP, Yellin T and Bazer FW 1993. Changes in oestrogen receptor protein, mRNA expression and localization in the endometrium of cyclic and pregnant gilts. Reprod. Fertil. Dev. 5(3): 247-260.

Geisert RD, Pratt TN, Bazer FW, Mayes JS and Watson GH 1994. Immunocytochemical localization and changes in endometrial progestin receptor protein during the porcine oestrous cycle and early pregnancy. Reprod. Fertil. Dev. 6(6): 749-760.

Graham JD and Clarke CL 1997. Physiological action of progesterone in target tissues. Endocr. Rev. 18: 502-519.

Heinonen M, Leppavuori A and Pyorala S 1998. Evaluation of reproductive failure of female pigs

based on slaughterhouse material and herd record survey. Anim. Reprod. Sci. 52(3): 235-44.

Hunter RH 2005. The Fallopian tubes in domestic mammals: how vital is their physiological activity? Reprod. Nutr. Dev. 45(3): 281-290.

Jana B, Dzienis A, Rogozińska A, Piskuła M., Jedlinska-Krakowska M, Wojtkiewicz J and Majewski M 2005. Dexamethasone-induced changes in sympathetic innervation of porcine ovaries and in their steroidogenic activity. J. Reprod. Dev. 51(6): 715-725.

Karveliene B, Zilinskas H and Riskevicience V 2007. Post-mortem examination of sow genital organs culled for reproductive disturbances and immunohistochemical studies on ER alpha and PR A receptors in the anoestral sows uterus. Repro. Domest. Anim. 42(3): 275-281.

Koziorowski M, Kotwica G, Stefanczyk S and Krzymowski T 1984. Estradiol, progesterone and testosterone receptors for pig endometrium and myometrium at various stages of the estrous cycle. Exp. Clin. Endocrinol. 84(3): 285-293.

Kozlowska A, Wojtkiewicz J, Majewski M and Jana B 2008. Cholinergic innervations of cystic porcine ovaries. Medyyna Wet. 64(4B): 565-570.

Kunavongkrit A, Chantaraprateep C, Prateep P and Poomsuwan P 1986. Ovarian activitiers and abnormalities in slaughtered gilts. Proc. 9th IPVS Congress, Barcelona, Spain, p. 34.

Liptrap RM and McNally PJ 1977. Effect of the uterus on induced cystic ovarian follicles in the sow. Res. Vet. Sci. 22(2): 181-189.

Lucia T, Dial GD and Marsh WE 2000. Lifetime reproductive performance in female pigs having distinct reasons for removal. Livest. Prod. Sci. 63(3): 213-222.

Miller DM 1984. Cystic ovaries in swine. Comp. Cont. Ed. 6: S31-S35.

Molenda HA, Kilts CP, Allen RL and Tetel MJ 2003. Nuclear receptor coactivator functions in reproductive physiology and behavior. *Biol. Reprod.* **69**(5): 1449-1457.

Ogasa A, Domeki I, Yokoki Y, Itoh S, Tsutsui T, Kawakami E 1988. The formation of cystic ovarian follicles in the sows after treatment with ACTH. Jap. J. Zoo-tech. Sci. 59(12): 1004-1012.

Roberts DK, Van Sickle M and Kelly RW 1983. The role of sex steroid receptors in obstetrics and gynecology. Obstet. Gynecol. Annu. 12: 61-78.

Ryan PL and Raeside JI 1991. Cystic ovarian degeneration in pigs: a review. Ir. Vet. 44: 22-36.

Srisuwatanasagul S, Tummaruk P and Kunavongkrit A 2010. Studies of oestrogen and progesterone receptors in reproductive organs of prepubertal gilts with reproductive disturbance. Thai J. Vet. Med. 40(1): 15-24.

Stanchev P, Rodriguez-Martinez H, Edqvist LE and Eriksson H 1985. Oestradiol and progesterone receptors in the pig oviduct during the oestrous cycle. J. Steroid Biochem. 22(1): 115-120.

Stanchev P, Rodriguez-Martinez H, Edqvist LE and Eriksson H 1990. Characterization of uterine sex steroid receptors in the pig and their variation during the oestrous cycle. J. Steroid Biochem. 35(6): 689-699.

Steffl M, Schweiger M and Amselgruber WM 2004. Immunophenotype of porcine oviduct epithelial cells during the oestrous cycle: a double-labelling immunohistochemical study. Histochem. Cell Biol. 121(3): 239-244.

Steffl M, Schweiger M, Sugiyama T and Amselgruberm WM 2008. Review of apoptotic and non-apoptotic events in non-ciliated cells of the mammalian oviduct. Ann. Anat. 190(1): 46-52.

Stein TE, Dijkhuizen AA, D'Allaire S and Morris RS 1990. Sow culling and mortality in commercial swine breeding herds. Prev. Vet. Med. 9(2): 85-94.

Sukjumlong S, Kaeoket K, Dalin AM and Persson A 2003. Immunohistochemical studies on oestrogen receptor alpha (ER alpha) and the proliferative marker Ki-67 in the sow uterus at different stages of the oestrous cycle. Reprod. Domest. Anim. 38(1): 5-12.

Sukjumlong S, Dalin AM, Sahlin L and Persson E 2005. Immunohistochemical studies on the progesterone receptor (PR) in the sow uterus during the oestrous cycle and in inseminated sows at oestrus and early pregnancy. Reproduction 129(3): 349-359.

Szulanczyk-Menzel K, Rzasa A and Bielas W 2010. Relationships between ovarian cysts and morphological and hormonal state of ovarian cortex in sows. Anim. Reprod. Sci. 121(3-4): 273-278.

Tsai MJ and O'Malley BW 1994. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu. Rev. Biochem. 63: 451-486.

Teamsuwan Y, Kaeoket K, Tienthai P and Tummaruk P. 2010. Morphological changes and infiltration of immune cells in the endometrium of anoestrus gilt in relation to the ovarian appearance and serum progesterone. Thai J. Vet. Med. 40(1): 31-40.

Tienthai P and Sajjarengpong K 2007a. A scanning electron microscopic study of oviduct epithelium in culling repeat breeder gilts. Thai J. Vet. Med. 37(3): 19-30.

Tienthai P and Sajjarengpong K 2007b. The study of oviductal epithelium of replacement gilts culled due to anestrus by scanning electron microscope. J. Thai Vet. Med. Assoc. 58(3): 14-25.

Tienthai P, Sajjarengpong K and Techakumphu M 2008. Estrogen receptor alpha localization in Thai swamp buffalo oviduct during the follicular and luteal phases. Thai J. Vet. Med. 38(4): 35-44.

Tienthai P, Sajjarengpong K and Techakumphu M 2009. Expression of the progesterone receptor in the Thai swamp buffalo oviduct at the follicular and luteal phases. Thai J. Vet. Med. 39(1): 17-29.

Tummaruk P, Suwimonteerabutr J, Singlor J, Tantasuparuk W, Techakumphu M and Kunavongkrit A 1994. The relationship between plasma and foecal progesterone in gilts. Thai J. Vet. Med. 34(3): 93-101.

Tummaruk P, Kesdangsakonwut S and Kunavongkrit A 2009. Relationship among specific reasons for culling, reproductive data, and gross morphology of the genital tracts in gilts culled due to reproductive failure in Thailand. Theriogenology 71(2): 369-375.

Tummaruk P and Kesdangsakonwut S 2012. Factors affecting the incidence of cystic ovaries in replacement gilts. Comp. Clin. Pathol. 21(1): 1-7.

Wang H, Eriksson H and Sahlin L 2000. Estrogen receptors alpha and beta in the female reproductive tract of the rat during the estrous cycle. Biol. Reprod. 63(5): 1331-1340.

Wang A, Ji, L, Shang W, Li M, Chen L, White RE and Han G 2011. Expression of GPR30, ER alpha and ER beta in endometrium during window of implantation in patients with polycystic ovary syndrome: a pilot study. Gynecol. Endocrinol. 27(4): 251-255.

Whisnant CS, Benoit AM and Dailey RA 1998. Concentrations of tissue-type plasminogen activator and relaxin in normal and induced-cystic follicles of gilts. Domest. Anim. Endocrinol. 15(3): 169-175.

Wrathal AE 1980. Ovarian disorders in the sow. Vet. Bull. 50: 253-272.

Yamashita S, Newbold RR, McLachlan JA and Korach KS 1990. The role of the estrogen receptor in uterine epithelial proliferation and cytodifferentiation in neonatal mice. Endocrinol. 127(5): 2456-2463.

บทคัดย่อ

การปรากฏของตัวรับฮอร์โมนเอสโตรเจนและโปรเจสเตอโรนภายในเนื้อเยื่อมดลูกและท่อนำไข่ของสุกรสาวคัดทิ้งที่พบภาวะถุงน้ำรังไข่

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สาเหตุที่สำคัญในการคัดทิ้งสุกรสาวทดแทนที่เกิดขึ้นกับฝูงสุกรในฟาร์มคือ ปัญหาความบกพร่องทางระบบสืบพันธุ์ ซึ่งเสาเหตุต่างๆ ของปัญหาความบกพร่องทางระบบสืบพันธุ์มาจากหลายปัจจัย โดยภาวะถุงน้ำรังไข่มีความเกี่ยวข้องกับปัญหาดังกล่าวอยู่เป็นประจำ การวิจัยในครั้งนี้ จึงมีวัตถุประสงค์หลักเพื่อศึกษาผลกระทบของภาวะถุงน้ำรังไข่ต่อการปรากฏของตัวรับสเตียรอยด์ฮอร์โมนในปีกมดลูกและท่อนำไข่ของสุกรสาวทดแทนที่ถูกคัดทิ้ง ด้วยสาเหตุความบกพร่องทางระบบสืบพันธุ์ซึ่งตรวจพบภาวะถุงน้ำรังไข่ เก็บอวัยวะสืบพันธุ์สุกรสาวจากโรงฆ่าสัตว์โดยแบ่งออกเป็น 3 กลุ่ม คือ สุกรสาวคัดทิ้งที่พบถุงน้ำรังไข่ขนาดใหญ่ชนิดใบเดียว สุกรสาวคัดทิ้งที่พบถุงน้ำรังไข่ขนาดใหญ่ชนิดหลายใบ และสุกรสาวคัดทิ้งที่มีรังไข่ปกติในระยะฟอลลิคูลาร์ (กลุ่มควบคุม) บันทึกข้อมูลประวัติของสุกรสาวทดแทนทุกตัว สำหรับการวิเคราะห์ความสัมพันธ์ระหว่างสาเหตุการคัดทิ้งและชนิดของถุงน้ำที่ตรวจพบ รวมทั้งเก็บตัวอย่างเลือดเพื่อตรวจหาระดับโปรเจสเตอโรน ศึกษาการปรากฏของตัวรับเอสโตรเจนอัลฟาและตัวรับโปรเจสเตอโรนในชั้นต่าง ๆ ของปีกมดลูกและท่อนำไข่ด้วยวิธีอิมมูนโนฮีสโตเคมี ผลการศึกษาครั้งนี้ พบว่า การคัดทิ้งสุกรสาวทดแทนที่พบภาวะถุงน้ำขนาดใหญ่ชนิดหลายใบมีสาเหตุมาจากการไม่เป็นสัดสูงถึงร้อยละ 50 และระดับโปรเจสเตอโรนในพลาสมา (45.8±21.2 nmol/l) สูงขึ้นในสุกรสาวที่พบภาวะถุงน้ำขนาดใหญ่ชนิดหลายใบ ผลของความเข้มและการกระจายตัวในการติดสีบวกของตัวรับเอสโตรเจนอัลฟา และตัวรับโปรเจสเตอโรนในเนื้อเยื่อชั้นต่างๆ ของปีกมดลูกและท่อนำไข่โดยส่วนใหญ่มีระดับความเข้มลดต่ำลงในกลุ่มสุกรสาวคัดทิ้งที่พบถุงน้ำรังไข่ขนาดใหญ่ชนิดหลายใบ เมื่อเปรียบเทียบกับสุกรสาวกลุ่มควบคุมและสุกรสาวที่พบถุงน้ำรังไข่ขนาดใหญ่ชนิดใบเดียว ผลการวิจัยบ่งชี้ถึงความผิดปกติบางอย่างในการผลิตฮอร์โมนจากรังไข่โดยเฉพาะอย่างยิ่งฮอร์โมนโปรเจสเตอโรนที่ผิดปกติ ซึ่งอาจส่งผลกระทบต่อการแสดงออกของตัวรับฮอร์โมนเพศเมียในปีกมดลูกและท่อนำไข่ อุบัติการณ์ในการศึกษาในครั้งนี้ อธิบายได้ว่าถุงน้ำรังไข่ขนาดใหญ่ชนิดหลายใบอาจเกี่ยวข้องหรือเหนี่ยวนำให้เกิดความบกพร่องในการทำหน้าที่ของทางเดินสืบพันธุ์เพศเมีย และอาจเป็นสาเหตุหลักที่ก่อให้เกิดภาวะการไม่เป็นสัดในสุกรสาวทดแทน

คำสำคัญ: ถุงน้ำรังไข่ ตัวรับเอสโตรเจน สุกรสาว ตัวรับโปรเจสเตอโรน อวัยวะสืบพันธุ์เพศเมีย

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