รายงานโครงการวิจัยเงินทุนคณะสัตวแพทยศาสตร์

การศึกษาเปรียบเทียบตัวรับเอสโตรเจนและโปรเจสเตอโรนในมดลูกของสุกรสาวในระยะต่าง ๆ ของวงรอบการเป็นสัด

Comparative study of oestrogen and progesterone receptors in the gilt uterus at different stages of the oestrous cycle

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Comparative study of oestrogen and progesterone receptors in the gilt uterus at different stages of the oestrous cycle

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Abstract

Ovarian steroid hormones are known to be the important modulator in regulation of reproductive functions in female. The levels of oestrogen and progesterone have been well documented of their interaction during the entire oestrous cycle but not their specific receptor in the target cells. Therefore, comparative study of oestrogen (ER) and progesterone receptors (PR) at a certain oestrous stage should be help in prediction of their interaction in specific uterine compartments.

The tissue samples were collected at different stages of the oestrous cycle: oestrus (n=3), early dioestrus (n=3) and late dioestrus (n=3). They were fixed in 10% formaldehyde and embedded in paraffin. Immunohistochemistry was done by using mouse monoclonal antibodies against oestrogen receptor (ER-6F11) and progesterone receptor (PGR-312).

In general, most of the uterine cells stained positive but with different intensity except for connective tissue stroma that the proportion of positive cells was different between ER and PR and/or at each stage of the oestrous cycle. At oestrus, both ER and PR were obviously strong in the epithelia and myometrium. For the superficial laying glandular epithelium (SGE), all SGE cells stained positive for ER whereas lower proportion of positive cells was observed for PR. At early dioestrus, it was interesting that cytoplamic staining was observed in the epithelia but this was not found for PR staining. When comparing between both receptors at early dioestrus, stronger intensity was observed in all compartments for PR. Moreover, in the stroma, higher proportion of PR positive stained cells was found. At late dioestrus ER and PR expression was not different that every compartment of the uterus stained weaker for both receptor proteins.

To summarize, the results from this study showed that both ER and PR may be regulated by the same mechanisms in some compartments and at specific stage of the oestrous cycle and that each compartment of the uterus had different expression of ER and PR which may according to its different role in reproductive physiology.

Keywords: immunohistochemistry, oestrogen receptor, progesterone receptor, gilt uterus

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บทคัดย่อ

ระดับของฮอร์โมนสเตียรอยด์เอสโตรเจน และโปรเจสเตอโรนเป็นตัวการสำคัญในการเปลี่ยนแปลงทางสรีรวิทยาในวงรอบการเป็นสัดของระบบสืบพันธุ์เพศเมีย ดังนั้นการศึกษาเปรียบเทียบตัวรับของเอสโตรเจนและโปรเจสเตอโรนในมดลูกจะช่วยในการทำความเข้าใจเกี่ยวกับกลไกการทำงานของฮอร์โมนสเตียรอยด์ในส่วนต่าง ๆ ของมดลูกได้ การศึกษานี้ใช้วิธีอิมมูนโนฮิสโตเคมีตรวจสอบตัวรับสเตียรอยด์จากตัวอย่างมดลูกสุกรสาวในระยะต่าง ๆ ของวงรอบการเป็นสัดได้แก่ oestrus, early dioestrusและ late dioestrus ผลการทดลองพบว่าเซลล์ที่ให้ผลบวกจะย้อมติดสีในนิวเคลียสและพบเซลล์ที่ให้ผลบวกได้ทุกส่วนของมดลูก ในระยะoestrus สามารถพบตัวรับเอสโตรเจนและโปรเจสเตอโรนได้สูงสุดในเซลล์เยื่อบุและเซลล์กล้ามเนื้อ ในระยะearly dioestrusสามารถพบเซลล์เยื่อบุที่ติดสีในไซโตพลาสซึมสำหรับการย้อมดูตัวรับเอสโตรเจน แต่ไม่พบลักษณะเช่นนี้ในการย้อมตัวรับโปรเจสเตอโรน เมื่อเปรียบเทียบกันระหว่างตัวรับเสตียรอยด์ทั้งสองชนิดในระยะ early dioestrusพบว่าตัวรับโปรเจสเตอโรนติดสีเข้มกว่าในทุกๆ ส่วนของมดลูกโดยเฉพาะอย่างยิ่งในชั้นกล้ามเนื้อของมดลูก ในระยะ late dioestrus พบว่าการย้อมติดสีของตัวรับเสตียรอยด์ทั้งสองชนิดมีจำนวนลดลงทั้งตัวรับเอสโตรเจนและโปรเจสเตอโรน จากการศึกษานี้สามารถสรุปได้ว่าตัวรับเอสโตรเจนและโปรเจสเตอโรนในบางส่วนของมดลูกสุกรสาวและในบางระยะของวงรอบการเป็นสัดถูกควบคุมด้วยกลไกชนิดเดียวกัน และจากการที่พบความแตกต่างของตัวรับเอสโตรเจนและโปรเจสเตอโรนในแต่ละส่วนของเนื้อเยื่อมดลูกอาจเป็นเพราะแต่ละส่วนมีหน้าที่ทางสรีรวิทยาในระบบสืบพันธุ์ที่ต่างกัน

คำสำคัญ: อิมมูนโนฮิสโตเคมี ตัวรับเอสโตรเจน ตัวรับโปรเจสเตอโรน มดลูกสุกรสาว

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Introduction

Steroid hormones are known to be the important substances in regulating reproductive functions in female. These hormones elicit their functions via their specific receptors in the target cells (DeMayo et al., 2002). In the oestrous cycle, the steroid hormones mainly oestrogen and progesterone work together by express their functions through specific receptor proteins named oestrogen receptor (ER) and progesterone receptor (PR) respectively.

The uterus, as one of the important target organs for oestrogen and progesterone changed remarkably during the oestrous cycle under the influence of these hormones(Kaeoket et al., 2001). Studies of the receptors for oestrogen and progesterone may explain some of the regulatory mechanisms which involved in the physiological changes of the uterus during the oestrous cycle and may also lead to a better understating in some reproductive pathology in the gilts.

Material and method

Animals

The animals used were the commercial gilts with normal reproductive performance sold to the slaughterhouse from Department of animal husbandry, Chulalongkorn University. They were checked for the oestrous cycle by inspection of standing reflex as well as inspection of vulva swelling and reddening.

Tissue collection

The tissue samples were collected from the gilts at different stages of the oestrous cycle: oestrous or d1 of standing reflex (n=3), early dioestrus or d 4 after standing reflex was observed (n=3); late dioestrus or d 17 after standing reflex was observed (n=3). Immediately after slaughter, the uteri were removed and macroscopic examination was done in order to check for normality of the uteri. The samples were collected from the middle part of the uterine horn at the mesometrial side of the horns. After that they were fixed in 10% formaldehyde for 24-36h and were embedded in paraffin block until immunohistochemistry was performed.

Immunohistochemistry

The immunohistochemistry of oestrogen receptor (ER) and progesterone receptor (PR) were studied at light microscopic level by using avidin biotin complex method (ABC method). In brief, 4 μm thick sections were cut and mounted on the silane coated slides in order to prevent the sections from falling during the procedure. To enhance the immunoreaction, the sections were boiled in citrate buffer using microwave oven for 5 min 2 times and the buffer was added in between to prevent the sections from drying. Then the sections were washed shortly in phosphate buffer saline (PBS, 0.1M, pH 7.4) and incubated with 3% H_2O_2 in methanol. After another washing in buffer, the sections were pretreated with normal horse serum before incubation with primary antibody for 90 min. The primary antibodies used were mouse monoclonal antibody to ER (NCL-ER-6F11) in a dilution of 1:25 and mouse monoclonal antibody to PR (NCL-PGR-312) in a dilution of 1:200. Negative controls were run by omission of primary antibody and by replacement of the primary antibody with PBS. After primary antibody incubation, the sections were washed in PBS and incubated with biotinylated secondary antibody for 30 min. Finally, after another washing in PBS, the sections were incubated with avidin biotin complex for 30 min. The immunoreaction was visualized by using 3-3'diaminobenaindine (DAB kit, Vector) and all sections were counterstained with Hematoxylin.

Evaluation of the results

Semiquantitative method was used to investigated the immunohistochemical reaction of ERα and PR in the gilt uteri. As the uterus composed of several different tissues, the evaluation of the results was done separately in each uterine compartment which were surface epithelium (SE), connective tissue stroma (STR), glandular epithelium (GE) and myometrium (M). All the semiquantitative results were shown in range of staining intensity as seen in the table.

Results

Table1 Staining intensity in different uterine compartments at different stages of the oestrous cycle comparing between oestrogen receptor (ER) and progesterone receptor (PR) immunostaining

Stages of the oestrous cycle	SE		GE		Stroma		Myometrium	
	ER	PR	ER	PR	ER	PR	ER	PR
Oestrus	+++	+++	+++	+++	+++	+++	+++	+++
Early dioestrus	++	+++	++	+++	++	+++	+	+++
Late dioestrus	+	_	++	+	++	++	+	+

- negative
\+ weak staining intensity
++ moderate staining intensity
+++ strong staining intensity

The semiquantitative results of staining intensity at different stages of the oestrous cycle were shown in Table1 with separated uterine compartment and the staining intensity was shown in Figure 1 and 2. In general, both ER and PR immunostainings were confined to nuclei of all uterine cell types. For the negative controls, no specific staining was observed in any uterine compartment.

At oestrus (Fig 1A, 1D, 2A and 2D) both receptors were found in every compartments of the uterus with strong intensity (Table 1) and high proportion (almost 100%) of positive cells. There was no difference between both receptor protein expressions.

At early dioestrus, strong intensity was observed in the epithelia (both surface epithelium and glandular epithelium)(Fig 1B, 1E 2B and 2E). However, cytoplasmic staining was observed only in the surface epithelium of ER staining (Fig 1B). When compared between both receptors stronger intensity was observed for PR staining in every uterine compartment especially in the myometrium (Fig 2B and 2E).

At late dioestrus, staining intensity was weaker in every compartment for both ER and PR (Fig 1C, 1F, 2C and 2F). However, in the SE, no positive cell was found for PR immunostaining (Fig 1F). Moreover, all GE cells stained positive for ER staining while negative cell could be observed for PR staining in the same compartment of the uterus (Fig 2C and 2F).

For the connective tissue stroma, more positive cells were observed in the subepithelial layer of the surface epithelium and moderate to strong intensity was found at all stages of the oestrous cycle (Fig 1A-1F).

Discussion

From the results of the present study, it was shown that at oestrus, both ER and PR may be up-regulated by the high level of oestrogen in the uterus. This was in agreement with many earlier studies that reported positive effect of oestrogen on the expression of steroid receptors (Wathes and Hamon, 1993; Dhaliwal et al., 1997; Vermeirsch et al., 2000; Kimmins and MacLaren, 2001; Robinson et al., 2001). However, the level of oestrogen may not be the only regulator to up-regulate steroid receptors. This was confirmed by ER and PR stainings in the epithelia which were still high during early dioestrus. Moreover, the stronger intensity in myometrium for PR at early dioestrus may be under the influence of progesterone since progesterone treatment could result in myometrium hypertrophy (De Bosscher et al., 2002; Kamernitskii et al., 2002) which may be mediated by progesterone receptors in the myocytes when the level of progesterone was high. At late dioestrus, lower expression of both steroid receptors observed in all compartments of the uterus was in accordance with the earlier study (Geisert et al., 1992). However, when compared between two receptor proteins, negative staining in the SE for PR may be explained that at late dioestrus, the SE was not the main target cells for progesterone. There are several studies showed that progesterone plays a major role in reproductive physiology associated with pregnancy via progesterone receptor (Spencer and Bazer, 2002), therefore withdrawal of PR in the SE should be observed at late dioestrus, when the gilt was not pregnant and going to start a new oestrous cycle. Moreover, there was a study indicated that down-regulation of progesterone receptors in the uterine epithelium may involved in the synthesis and release of prostaglandin F2 alpha (PGF2 alpha) for luteolysis (Geisert et al., 1992). For the stroma, most of the cells stained positive with medium to strong intensity throughout the oestrous cycle may indicated that steroid effects on the epithelia were mediated by stromal cells in paracrine manner as described by the other studies (Cooke et al., 1997; Buchanan et al., 1998; Cooke et al., 1998; Buchanan et al., 1999; Vermeirsch et al., 2000; Robinson et al., 2001; Bigsby, 2002; Spencer et al., 2002).

It is also interesting that cytoplasmic staining was observed in the SE for ER staining at early dioestrus. This finding supported the concept of receptor translocation between nucleus and cytoplasm in the target cell (Dauvois et al., 1993; Guiochon-Mantel and Milgrom, 1999). However, this finding should be more clearified whether it was the cytoplamic ER protein or it was only unspecific staining from the antibody used in the present study.

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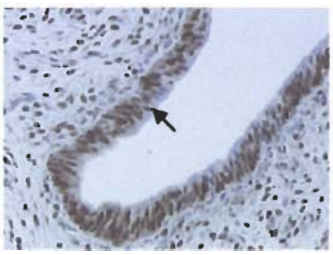
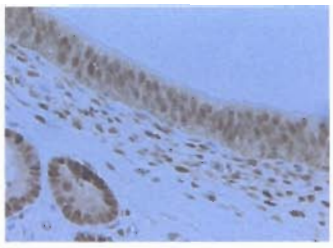
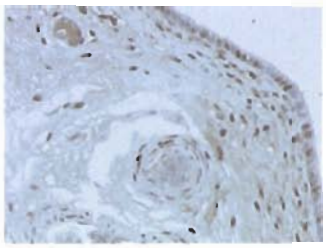


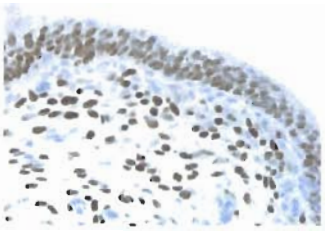
1A, ER at oestrus



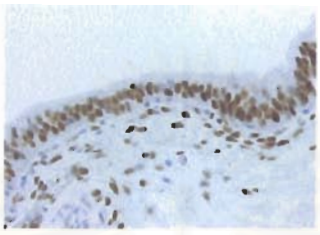
1B, ER at early dioestrus



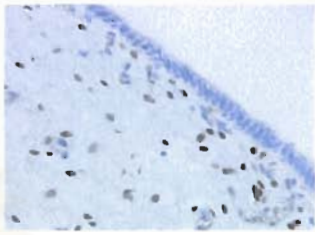
1C, ER at late dioestrus



1D, PR at oestrus

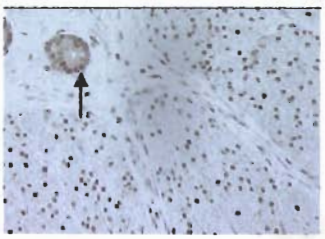


1E, PR at early dioestrus

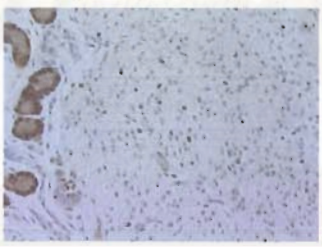


1F, PR at late dioestrus

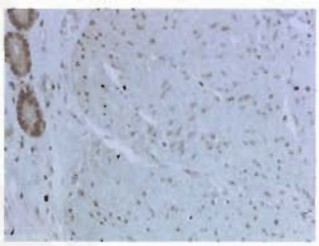
Fig. 1 Immunohistochemical staining of ER and PR in the surface epithelium at different stages of the oestrous cycle. At oestrus (1A and 1D), at early dioestrus (1B and 1E) and at late dioestrus (1C and 1F). Positive cell was stained with reddish brown color in the nucleus as shown by the arrow (x400).



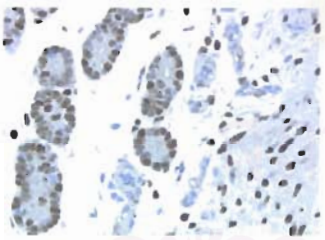
2A, ER at oestrus



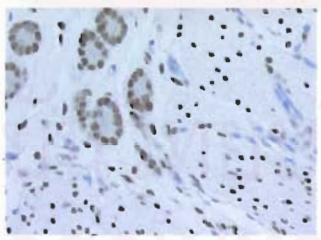
2B, ER at early dioestrus



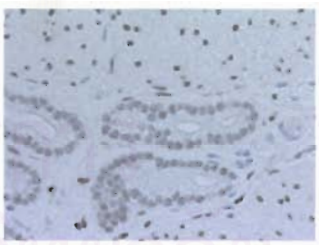
2C, ER at late dioestrus



2D, PR at oestrus



2E, PR at early dioestrus



2F, PR at late dioestrus

Fig 2 Immunohistochemical staining of ER and PR in the Glandular epithelium and Myometrium at diffent stages of the oestrous cycle. At oestrus (2A and 2D), at early dioestrus (2B and 2E) and at late dioestrus (2C and 2F). Positive cells was reddish brown in the nucleus as shown by the arrow. (x400).