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THE EXPRESSION OF OESTROGEN AND PROGESTERONE RECEPTORS IN THE GILT UTERUS AT DIFFERENT STAGES OF THE OESTROUS CYCLE

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Abstract

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THE EXPRESSION OF OESTROGEN AND PROGESTERONE RECEPTORS IN THE GILT UTERUS AT DIFFERENT STAGES OF THE OESTROUS CYCLE

Ovarian steroid hormones are known to be an important modulator in the regulation of reproductive function in the female. The levels of oestrogen and progesterone have been well documented whilst interacting during the entire oestrous cycle but their specific receptors in the target cells are less understood. Therefore, a comparative study of oestrogen (ER) and progesterone receptors (PR) at certain oestrous stages should be of help in the prediction of their interaction in specific uterine compartments.

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, using mouse monoclonal antibodies against the oestrogen receptor (ER-6F11) and the progesterone receptor (PGR-312).

In general, most of the uterine cells stained positive but with a different intensity. During oestrus, both ER and PR were obviously strong in the epithelia and the myometrium. For the glandular epithelium (GE), all GE cells stained positive for ER whereas a lower proportion of positive cells was observed for PR. During early dioestrus, it was interesting that cytoplamic staining was observed in the epithelia but not for PR staining. When comparing both receptors during early dioestrus, a stronger intensity was observed in all compartments for PR, especially in the myometrium. During late dioestrus ER and PR, expression showed no difference and 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 stages of the oestrous cycle and that each compartment of the uterus had a different expression of ER and PR which could accord with their different roles in reproductive physiology.

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

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***ผู้รับผิดชอบบทความ**

บทคัดย่อ

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การแสดงออกของตัวรับเอสโตรเจนและโปรเจสเตอโรนในมดลูกของสุกรสาวในระยะต่างๆ ของวงรอบการเป็นสัด

ระดับของฮอร์โมนสเตียรอยด์เอสโตรเจน และโปรเจสเตอโรนเป็นตัวการสำคัญในการเปลี่ยนแปลงทางสรีรวิทยาในวงรอบการเป็นสัดของระบบสืบพันธุ์เพศเมีย ดังนั้นการศึกษาเปรียบเทียบตัวรับของเอสโตรเจนและโปรเจสเตอโรนในมดลูกจะช่วยอธิบายกลไกการทำงานของฮอร์โมนสเตียรอยด์ในส่วนต่าง ๆ ของมดลูกได้ การศึกษานี้ใช้วิธีอิมมูนโนฮิสโตเคมีตรวจสอบตัวรับสเตียรอยด์จากตัวอย่างมดลูกสุกรสาวในระยะต่าง ๆ ของวงรอบการเป็นสัดได้แก่ oestrus, early dioestrusและ late dioestrus ผลการทดลองพบว่าเซลล์ที่ให้ผลบวกจะย้อมติดสีในนิวเคลียสและพบเซลล์ที่ให้ผลบวกได้ทุกส่วนของมดลูก ในระยะ oestrus สามารถพบตัวรับเอสโตรเจนและโปรเจสเตอโรนได้มากในเซลล์เยื่อบุและเซลล์กล้ามเนื้อ ในระยะ early dioestrus สามารถพบเซลล์เยื่อบุที่ติดสีในไซโตพลาสมสำหรับการย้อมดูตัวรับเอสโตรเจน แต่ไม่พบลักษณะเช่นนี้ในการย้อมตัวรับโปรเจสเตอโรน เมื่อเปรียบเทียบกันระหว่างตัวรับสเตียรอยด์ทั้งสองชนิดในระยะ early dioestrus พบว่าตัวรับโปรเจสเตอโรนติดสีเข้มกว่าในทุกๆ ส่วนของมดลูกโดยเฉพาะอย่างยิ่งในชั้นกล้ามเนื้อของมดลูก ในระยะ late dioestrus พบว่าการย้อมติดสีของตัวรับเสตียรอยด์ทั้งสองชนิดมีจำนวนลดลงทั้งตัวรับเอสโตรเจนและโปรเจสเตอโรน จากการศึกษานี้สามารถสรุปได้ว่าตัวรับเอสโตรเจนและโปรเจสเตอโรนในบางส่วนของมดลูกสุกรสาวและในบางระยะของวงรอบการเป็นสัดถูกควบคุมด้วยกลไกชนิดเดียวกัน และจากการที่พบความแตกต่างของตัวรับเอสโตรเจนและโปรเจสเตอโรนในแต่ละส่วนของเนื้อเยื่อมดลูกอาจเป็นเพราะแต่ละส่วนมีหน้าที่ทางสรีรวิทยาในระบบสืบพันธุ์ที่ต่างกัน

คำสำคัญ : อิมมูนโนฮิสโตเคมี ตัวรับเอสโตรเจน ตัวรับโปรเจสเตอโรน มดลูกสุกรสาว

Introduction

Steroid hormones are known to be important substances in regulating reproductive function in the female. These hormones elicit their functions via specific receptors in the target cells (Brannvall et al., 2002; DeMayo et al., 2002; Punyadeera et al., 2003). In the oestrous cycle, steroid hormones, mainly oestrogen and progesterone, work together by expressing their functions through specific receptor proteins named, oestrogen receptors (ER) and progesterone receptors (PR), respectively.

The uterus, as one of the important target organs for oestrogen and progesterone, changes remarkably during the oestrous cycle, under the influence of these hormones (Evans et al., 1990; Kaeoket et al., 2001). Studies of the receptors for oestrogen and progesterone may explain some of the regulatory mechanisms which are involved in the physiological changes of the uterus during the oestrous cycle and may also lead to a better understating of some reproductive pathology in gilts. The aim of this study was to investigate the expression of oestrogen and progesterone receptors in the gilt uterus at different oestrous stages and evaluate whether there is any relationship between the different compartments of the uterus.

Materials and methods

Animals

The animals used were commercial crossbred gilts (Landrace X Large white X Duroc), aged 4-6 months with a normal reproductive performance. Before being sold to the slaughterhouse by the Department of Animal Husbandry, Chulalongkorn University, they were checked for an oestrous cycle by attempting a standing reflex as well as inspection of any swelling and reddening of the vulva.

Tissue collection

Tissue samples were collected from the gilts at

different stages of the oestrous cycle: oestrous or d1 of the standing reflex (n=3), early dioestrus or d 4 after the standing reflex was observed (n=3); late dioestrus or d 17 after the 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. They were then fixed in 10% formaldehyde for 24-36 hrs and were embedded in a paraffin block until immunohistochemistry was performed.

Immunohistochemistry

Immunohistochemistry of the oestrogen receptors (ER) and progesterone receptors (PR) was studied at the light microscopic level by using the avidin biotin complex method (Vectastain Elite ABC kit, Vector Laboratories, Inc., USA). In brief, 4 μm thick sections were cut and mounted on silane coated slides in order to prevent the sections from falling during the procedure. To enhance the immunoreaction, the sections were boiled twice in citrate buffer, pH 6.0, using a microwave oven for 5 min. Buffer was added in between to prevent the sections from drying. The sections were then washed in phosphate buffer saline (PBS, 0.1M, pH 7.4) and incubated with 3% H_2O_2 in methanol for 10 min. All the procedures after heating were done at room temperature. After another washing in buffer, the sections were pretreated with normal horse serum for 30 min before incubation with primary antibody for 90 min. The primary antibodies used were mouse monoclonal antibody to ER (NCL-ER-6F11, Novocastra, UK), in a dilution of 1:25 and mouse monoclonal antibody to PR (NCL-PGR-312, Novocastra, UK), in a dilution of 1:200. Negative controls were run by the omission of primary antibody and by the 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 observed by using 3-3' diaminobezindine (DAB kit, Vector) and all sections were counterstained with Meyer's hematoxylin.

Evaluation of the results

A semiquantitative method was used to investigated the immunohistochemical reaction of ER and PR in the gilt uteri. As the uterus composed of several different tissues, the evaluation of the results was done separately for each uterine compartment namely surface epithelium (SE), connective tissue stroma (STR), glandular epithelium (GE) and myometrium (M). All the semiquantitative results are shown by their level of staining intensity as seen in the table 1.

Table 1 Staining intensity in different uterine compartments, at different stages of the oestrous cycle, for 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

Results

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

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

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

During late dioestrus, staining intensity was weaker in every compartment, for both ER and PR (Figs 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 cells could be observed for PR staining, in the same compartment of the uterus (Figs 2C and 2F).

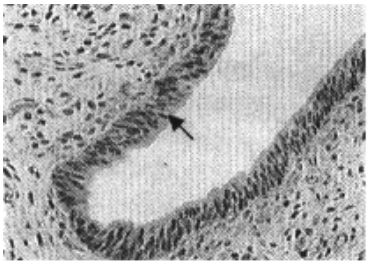
For the connective tissue stroma, more positive cells were observed in the subepithelial layer of the surface epithelium and moderate to strong intensity was found at all stages of the oestrous cycle (Figs 1A-1F).

Discussion

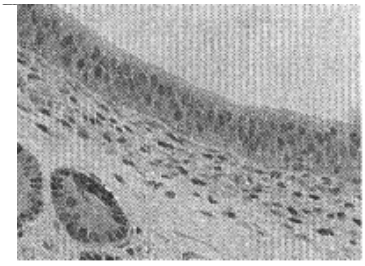
From the results of the present study, it was shown that both ER and PR may be up-regulated in the uterus by the high plasma level of oestrogen seen at oestrus. This was in agreement with many earlier studies that reported the positive effect of oestrogen on the expression of steroid receptors (Wathes 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 the ER and PR stainings in the epithelia which were still high during early dioestrus. Moreover, the stronger intensity in the myometrium for PR during 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). This may be mediated by progesterone receptors in the myocytes when the level of progesterone is high. During late dioestrus, a lower expression of both steroid receptors, observed in all compartments of the uterus, was in accordance with earlier studies (Geisert et al., 1992). However, when comparing the two receptor proteins, negative staining in the SE for PR may be that during late dioestrus, the SE was not the main target for progesterone. There are several studies that show that progesterone plays a major role in reproductive physiology associated with pregnancy via a progesterone receptor (Conneely et al., 2001; Conneely et al., 2002; Rider, 2002; Spencer and Bazer, 2002), therefore withdrawal of PR in the SE should be observed during late dioestrus, when the gilt is not pregnant and is about to start a new oestrous cycle. Moreover, there was a study indicating that down-regulation of progesterone receptors in the uterine epithelium may be involved in the synthesis and release of prostaglandin F2 alpha ($PGF_{2\alpha}$), for the purpose of luteolysis (Geisert et al., 1992).

For the stroma, most of the cells stained positive, with a medium to strong intensity, throughout the oestrous cycle which may indicate that steroid effects on the epithelia were mediated by stromal cells in a paracrine manner as described by 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 and Bazer, 2002).

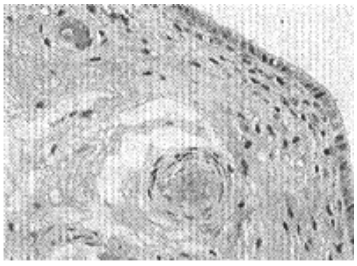
It is also interesting that ER cytoplasmic staining was observed in the SE during early dioestrus. This



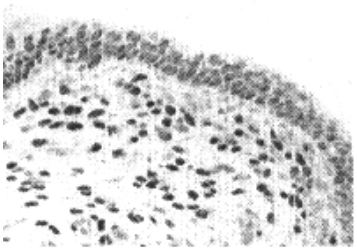
1A, ER at oestrus



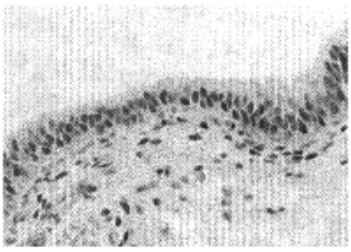
1B, ER at early dioestrus



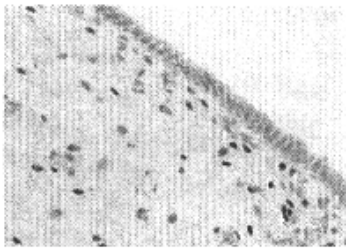
1C, ER at late dioestrus



1D, PR at oestrus

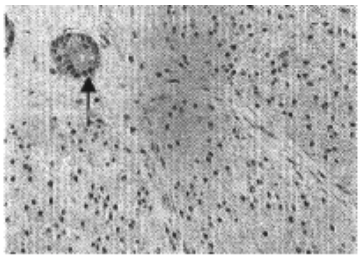


1E, PR at early dioestrus

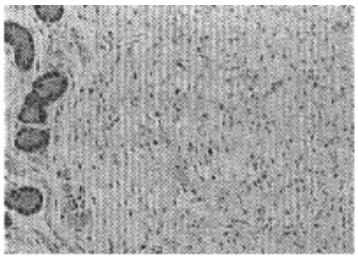


1F, PR at late dioestrus

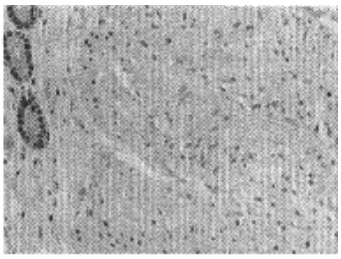
Fig. 1 Immunohistochemical staining of ER and PR in the surface epithelium, at different stages of the oestrous cycle. During oestrus (1A and 1D), during early dioestrus (1B and 1E) and during late dioestrus (1C and 1F). Positive cells were stained a reddish brown color in the nucleus, as shown by the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



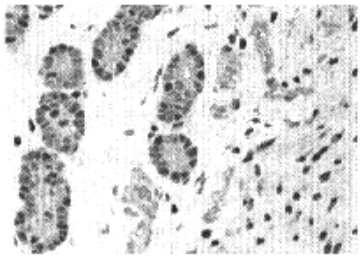
2A, ER at oestrus



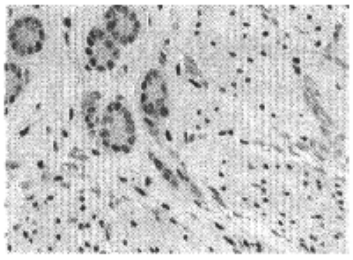
2B, ER at early dioestrus



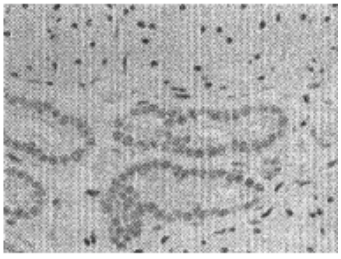
2C, ER at late dioestrus



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 the myometrium at different stages of the oestrous cycle. During oestrus (2A and 2D), during early dioestrus (2B and 2E) and during late dioestrus (2C and 2F). Positive cells were reddish brown in the nucleus, as shown by the arrow. (x400).

finding supports the concept of receptor translocation between the nucleus and the cytoplasm in the target cell (Dauvois et al., 1993; Guiochon-Mantel and Milgrom, 1999). However, this finding needs to be clarified as to whether it is positivity due to the cytoplamic ER protein or to unspecific staining from the antibody used in the present study.

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