

## Original Article

# The putative role of estrogen receptor and progesterone receptor on the pathogenesis of endometrial simple hyperplasia

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**Abstract:** The estrogen receptor (ER) and progesterone receptor (PR) play important roles in the normal cycling of endometrium; however their exact role in pathogenesis of endometrial simple hyperplasia (SH) is still not clear enough. In the present study, immunohistochemical analysis were performed for the expression of ER and PR in 77 archived formalin-fixed and paraffin embedded endometrial tissues, including 27 cases of normal proliferative endometrium (NPE) (childbearing age, n=10; perimenopausal period, n=17), and 50 cases of SH (childbearing age, n=30; perimenopausal period, n=20). The ER and PR expression was respectively evaluated in the stromal cells and glandular epithelial cells. In SH, the positive ER expression in the stromal cells was significantly higher than that of NPE (P=0.000). On the contrary, no differences in PR expression both in glandular epithelial and stromal cells were found between SH and NPE groups (stromal cells: P=0.457; glandular epithelium: P=0.706). Further analysis showed that the positive expression of stromal ER of SH in both childbearing age and perimenopausal age groups were all significantly higher than that in NPE (P=0.000; P=0.001), but no such difference was found in glandular epithelial cells (P=0.442; P=0.177). No differences in the expression of PR of both stromal and glandular cells between the two different age groups. The results indicate that the significance of ER expression in stromal and glandular epithelial cells of SH is different and higher stromal ER expression may play important role in the pathogenesis of endometrial simple hyperplasia.

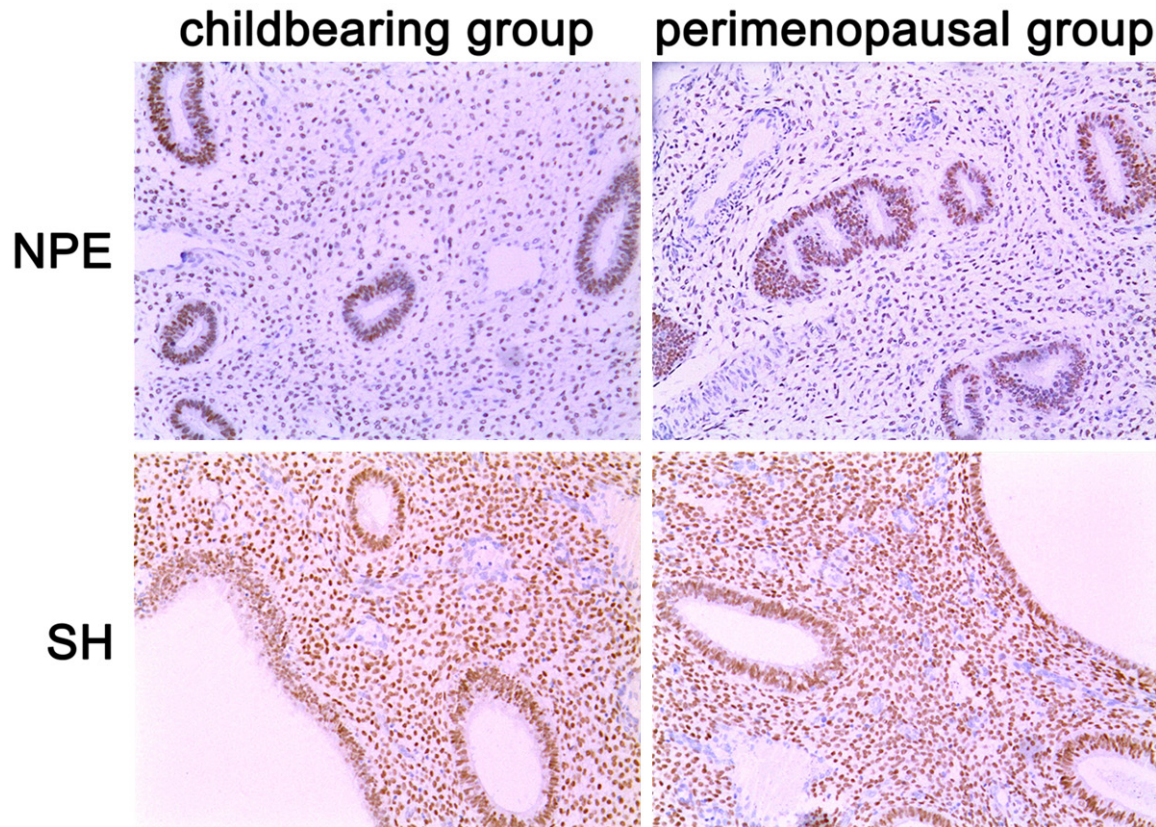
**Keywords:** Endometrial simple hyperplasia, estrogen receptor, progesterone receptor

## Introduction

Endometrial hyperplasia is a heterogeneous set of pathologic lesions that range from mild, reversible glandular proliferations to direct cancer precursors [1, 2]. There are four diagnostic categories of endometrial hyperplasia: simple hyperplasia (SH), complex hyperplasia (CH), simple atypical hyperplasia (SAH) and complex atypical hyperplasia (CAH) [3-5]. Simple hyperplasia is the most common gynecological diseases with clinical manifestations of irregular vaginal bleeding. The histological morphology of SH includes diffuse hyperplasia of endometrial glands and the stroma, local cystic dilatation, increased interstitial blood vessels, or thick-walled blood sinus [6].

The cyclic change of endometrium is regulated elaborately by hormone. Estrogen promoted

hyperplasia of gland and stroma; progesterone promoted gland transformation from proliferating phase to the secretory phase, with stromal decidual change. Estrogen and progesterone specific binding with their receptors are the most important step in their biological effects [7]. Therefore, the quantity and function of ER and PR were the basis to ensure the periodically changes of endometrium. Currently, there are several investigations on the expressions of ER and PR in endometrial lesions; however, the results are conflicting rather than conclusive. Sánchez et al found that there were no statistical significant differences on the cell density with ER between the normal endometrial and the simple and complex hyperplasia [8]. While in the study of Teleman, high level of both ER and PR in simple and complex hyperplasias and a significant decrease of these in atypical hyperplasia were demonstrated [9]. Therefore,



**Figure 1.** Immunohistochemical analysis of ER in endometrium.

further study on the expression pattern of ER and PR is imperative.

An interesting phenomenon attracted our attention was that different expression pattern and significance of ER and PR existed between glandular epithelial and stromal cells. Antunes et al found that only the final score for ER expression in the stroma of endometrial polyps was higher in the benign group than in the pre-malignant/malignant group, and this difference was significant. However, no difference was identified in glandular epithelial cells [10]. And differences in the stromal expression of PR were also found between endometrial polyps and normal endometrium in another study [11]. Whether the expression of ER and PR was different in SH between glandular epithelial and stromal cells need to be explored.

It is generally known that, along with the growth of age, the changes of endometrial ER and PR exist. Loss of PR in endometrial carcinoma cell nuclei correlated with an increased age in patients, whereas ER was not found to be cor-

related with age [12]. Gul et al also found differences in the expression of ER and PR in their study of solitary endometrial polyp between different age groups, both ER and PR expressions were higher in glandular epithelium compared with stroma in postmenopausal patients, however, this difference was not found in premenopausal patients [13]. Up to now, no study has been carried out to evaluate the age factors in the pathogenesis of SH. In the present study, by observing the expressions of ER and PR in different age groups, we aimed to analyze the similarities and differences of normal endometrium and endometrial simple hyperplasia between childbearing period and perimenopausal period, and to explore the pathogenesis of endometrial simple hyperplasia, providing theoretical basis for its clinical treatment.

#### **Materials and methods**

##### *Subjects*

Seventy-seven formalin-fixed and paraffin-embedded tissue specimen of SH were obtained

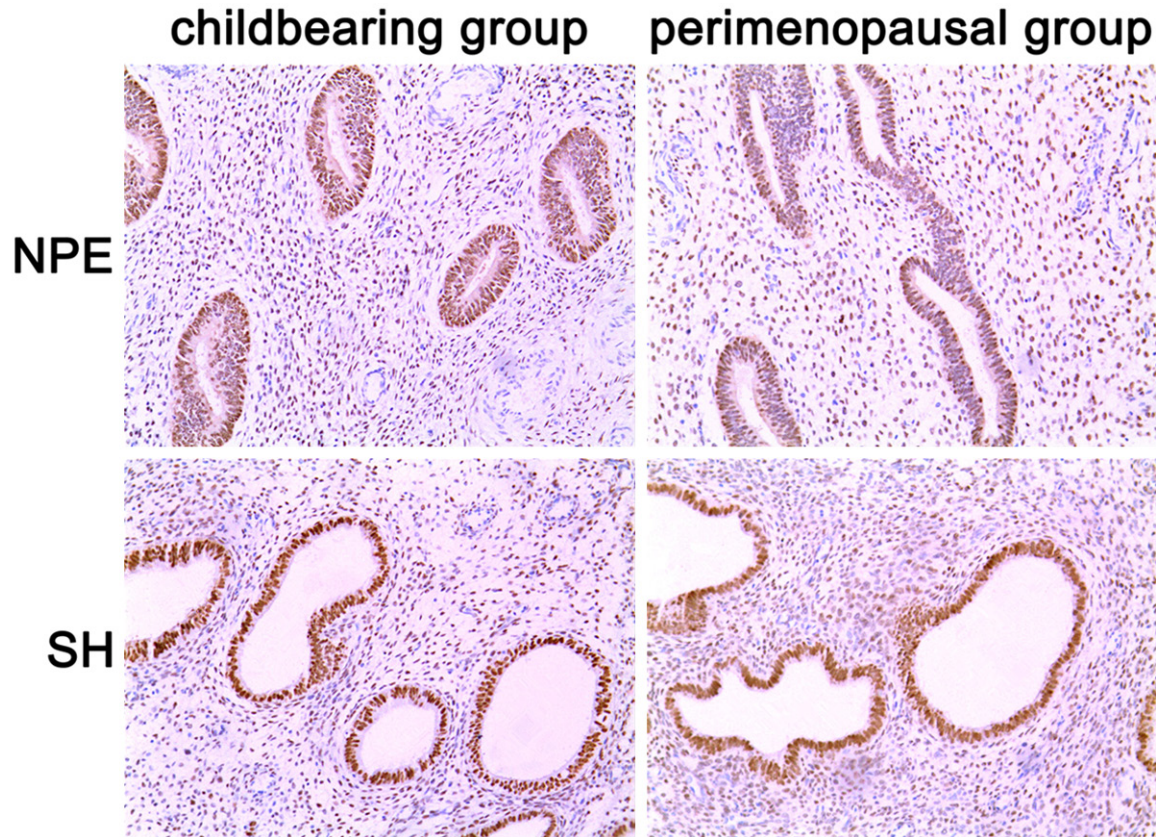


Figure 2. Immunohistochemical analysis of PR in endometrium (IHC 100 $\times$ ).

from the archives of the Department of Pathology, the Second Hospital of Hebei Medical University, during December 2009 and December 2012. Based on the age of the patients, the cases were divided to two groups: childbearing period (age: 22~39), 30 cases and perimenopausal period, 20 cases. The diagnosis was made according to the criteria of the Standard International Society of Gynecological Pathologists and World Health Organization (WHO). None of patients included in this study were nonsteroidal hormone usage within three months and without any other uterine lesions. Samples of control group were from patients with uterine leiomyoma. Of the 27 normal proliferative phase endometrium control cases, 10 were from childbearing period and 17 were from perimenopausal period.

#### Immunohistochemistry

Sections (4  $\mu$ m thick) were prepared from paraffin blocks. After deparaffinization, antigen retrieval was performed under citrate buffer for 15 minutes. Endogenous peroxidase activity

was blocked with 3% hydrogen peroxide in methanol for 10 minutes. Incubation with primary antibodies (ER and PR) and then conducted overnight at 4 $^{\circ}$ C in a humidified chamber. After washing with phosphate-buffered saline, the sections were stained according to their instructions of Elivision Plus Kit (Maixin-Bio). Co-nerstaining was performed with hematoxylin. Paralleled staining was performed in the absence of the primary antibody to serve as negative controls.

Positive control tissue sections of breast carcinoma (oestrogen and progesterone receptors) were used throughout. These tissues were known to contain the antigen and always stained positive, thus providing the means to monitor any loss of sensitivity in detection of the antibody. Negative controls were without the primary antibody (buffer only).

#### Immunohistochemical staining evaluation

Positive immunohistochemical staining of ER and PR was located in nucleus as brown gran-

## Expression of ER and PR in endometrial simple hyperplasia

**Table 1.** Positive expression of ER and PR in SH and NPE

	n	N (%)			
		ER		PR	
		Glandular epithelium	Stroma	Glandular epithelium	Stroma
SH	50	47 (94.0)	42 (84.0)	45 (90.0)	29 (58.0)
NPE	27	25 (92.6)	6 (22.2)	25 (92.6)	18 (66.7)
<i>P</i>		0.811	0.000	0.706	0.457

SH: endometrial simple hyperplasia; NPE: normal proliferative endometrium.

**Table 2.** Positive expression of ER in childbearing and perimenopausal patients

	n	Expression of ER			
		Glandular epithelium		Stroma	
		n (%)	<i>P</i>	n (%)	<i>P</i>
Childbearing patients					
SH	30	29 (96.7)	0.442	29 (96.7)	0.000
NPE	10	9 (90.0)		4 (40.0)	
Perimenopausal patients					
SH	20	19 (95.0)	0.177	13 (65.0)	0.001
NPE	17	16 (94.1)		2 (11.8)	

**Table 3.** Positive expression of PR in childbearing and perimenopausal patients

	n	Expression of PR			
		Glandular epithelium		Stroma	
		n (%)	<i>P</i>	n (%)	<i>P</i>
Childbearing patients					
SH	30	28 (93.3)	1.000	20 (66.7)	1.000
NPE	10	9 (90.0)		6 (60.0)	
Perimenopausal patients					
SH	20	17 (85.0)	0.609	9 (45.0)	0.185
NPE	17	16 (94.1)		12 (70.6)	

ule (illustrated in **Figures 1** and **2**). The glandular epithelium and stromal compartments were scored separately. Positive staining is considered when the percentage of positive cells was  $\geq 10\%$  for ER and PR.

### Statistical analysis

The experimental data was analyzed with normality test of Kolmogorov-Smirnov, homogeneity of variance test of Levene Statistic and Chi-square test, Mann-Whitney U of Nonparametric test with statistical software of SPSS 13.0 edition. There was statistically significant when  $P < 0.05$ .

## Results

### Expression of ER in endometrial simple hyperplasia and normal proliferative endometrium

Immunohistochemical results showed that the percentage of ER expression was 94.0% and 92.6% in the glandular epithelium among 50 cases of SH and 27 cases of normal proliferative endometrium, respectively, and no significant difference was found in glandular ER expression between the two groups. While in the stromal cells, the expression of ER was significantly higher in SH than that of normal proliferative endometrium ( $P=0.000$ ) (**Figure 1; Table 1**). The results demonstrated that up-regulation of stromal expression of ER might play an important role in the pathogenesis of SH.

### Expression of PR in endometrial simple hyperplasia and normal proliferative endometrium

The results showed that no significant difference was found in the expression of PR between SH and NPE both in glandular epithelial and stromal cells (glandular epithelial:  $P=0.706$ ; stroma:  $P=0.457$ ) (**Figure 1; Table 1**), suggesting that PR may not be the key factors in the pathogenesis of SH.

### Expression of ER in different age groups

Further analysis of ER expression in SH of different age groups showed that in childbearing age group, no significant differences were found in the expression of ER in glandular epithelium cells between SH and NPE ( $P=0.442$ ). Whereas in stromal cells, the positive expression of ER in SH was significantly higher than that in normal proliferative endometrium ( $P=0.000$ ). The similar results were observed in the perimenopausal age group, a higher positive expression of stromal ER was also found in SH ( $P=0.001$ ) (**Figure 1; Table 2**).

### Expression of PR in different age groups

In childbearing age group, no significant differences were found in the expression of PR in both glandular epithelial and stromal cells between SH and normal proliferative endometrium (glandular epithelium:  $P=1.000$ ; stroma:  $P=1.000$ ). Similar to childbearing group, no differences were found in the expression of PR in both glandular epithelial and stromal cells

## Expression of ER and PR in endometrial simple hyperplasia

between SH and NPE in the perimenopausal age group (**Figure 1; Table 3**).

Thus, the data indicated that no difference in ER and PR expression of SH was found between different age groups.

### Discussion

The present study was conducted to evaluate the possible roles of ER and PR in the pathogenesis of endometrial simple hyperplasia and at the same time, to explore the putative difference in the pathogenesis of SH for different age groups. The results indicated that SH exhibit a higher positive expression of stromal ER than normal proliferative endometrium, while PR expression was not found to correlate with SH. Further analysis demonstrated that no difference in ER and PR expression of SH was seen between different age groups.

The relationship between the expression of ER and cell proliferation has already been demonstrated in both normal and malignant endometrium. However, the role of ER and PR in SH hasn't yet been investigated. In contrast to the low ER expression observed in carcinomatous endometrium [14], our study has demonstrated that higher positive expression of ER is an important event in SH, and that SH exhibits a higher level of stromal ER than the normal endometrium. This appears to indicate that SH patients may respond to an increased number of receptors, as a consequence of low estrogen levels. In addition, high expression in the stroma indicates a higher sensitivity of these structures to steroid hormones, which may be responsible for the development of SH in the presence of low serum estrogen levels, while malignant polyps appear to be developed by a different etiology [10]. In the present study, we also found that this higher stromal expression of the ER could be found both in childbearing and perimenopausal age group, demonstrated that no difference in ER expression of SH was seen between different age groups.

No significant difference was found in the expression of PR in SH and NPE. This result indicated that the role of PR was less important in SH than ER. The activity of the ER is based on specific regions of the gene and furthermore, the formation and concentration of new receptors appear to be self-regulated and dependent

on hormonal factors. However, for progesterone, the tissue expression of the PR has not been found to correlate with the hormonal status found in postmenopausal patients, in which progesterational activity is not observed. In addition, the induction of PR formation in the endometrium is mainly a consequence of estrogen stimulation [15].

In conclusion, the observations of the present study indicated different significance of ER expression in stromal and glandular epithelial cells of SH and high stromal ER expression may play important role in the pathogenesis of endometrium simple hyperplasia. Further analysis showed that no difference in ER and PR expression of SH was seen between different age groups. Therefore, the antiestrogenic drugs may be used in the treatment of SH and alleviate the symptom of irregular vaginal bleeding.

### Disclosure of conflict of interest

None.

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