

Endometrial oestrogen and progesterone receptors and their relationship to sonographic appearance of the endometrium

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TABLE OF CONTENTS

Introduction	560	<p>somewhat less in echo pattern B (not triple line) endometrium compared with pattern A (triple line) endometrium. Oestrogen and progesterone receptor concentrations appeared stable on gonadotrophin induction, though fewer numbers were found during clomiphene cycles than in natural cycles. With regard to the distribution of receptor concentration between clomiphene and natural cycles, most women using clomiphene had very low oestrogen receptor populations. Pregnancy rates were low, in spite of high ovulatory rates during clomiphene treatment and were mainly related to low oestrogen receptor concentrations in preovulatory endometrium.</p>
Clinical evidence of the sonographic appearance of the endometrium	561	
Relationship between endometrial oestrogen and progesterone receptors and sonographic endometrial appearance	562	
Effect of clomiphene citrate stimulation on endometrial oestrogen and progesterone receptors	563	
References	564	

The rapid development of ultrasonographic equipment now permits instantaneous assessment of follicles and endometrium. The sonographic appearance of the endometrium has been discussed in relation to in-vitro fertilization (IVF) cycles. However, a generally agreed view of the relationship of the sonographic appearance to fecundity in IVF cycles has not emerged. We have studied the relationship between steroid receptors and the sonographic appearance of the preovulatory endometrium in natural cycles and ovulation induction cycles. Preovulatory endometrial thickness was not found to be indicative of fecundity, although a preovulatory endometrial thickness of <9 mm related to an elevated miscarriage rate. The preovulatory endometrial echo pattern did not predict fecundity. No relationships were found among endometrial appearance, endometrial steroid receptors and steroid hormone concentrations in serum. Oestrogen or progesterone receptor concentrations were not related to endometrial thickness or to concentrations of serum oestradiol, the only significant correlation being found between the endometrial concentrations of oestrogen and progesterone receptors. The ratio of progesterone:oestrogen receptor concentration was

Key words: clomiphene citrate/endometrium/oestrogen receptor/progesterone receptor/transvaginal ultrasound

Introduction

The use of modern ultrasonographic equipment permits easy assessment of endometrial condition during the menstrual cycle. Indeed, the sonographic appearance of the endometrium now offers—instantaneously—large amounts of information that allow the measurement of endometrial thickness and echo pattern. One major factor with regard to endometrial appearance, either when human chorionic gonadotrophin (HCG) is administered or at oocyte retrieval or embryo transfer, is the ability to predict pregnancy in an in-vitro fertilization (IVF) cycle. In some studies, endometrial thickness was shown to be significantly greater in conception than in non-conception cycles (Gonen *et al.*, 1989; Check *et al.*, 1991, 1993), while in others, a relationship between echo pattern and fecundity was reported (Serafini *et al.*, 1994). However, there is no regular viewpoint on the relationship between the appearance of the endometrium and fecundity. The present study was carried out to evaluate the relationship between oestrogen and

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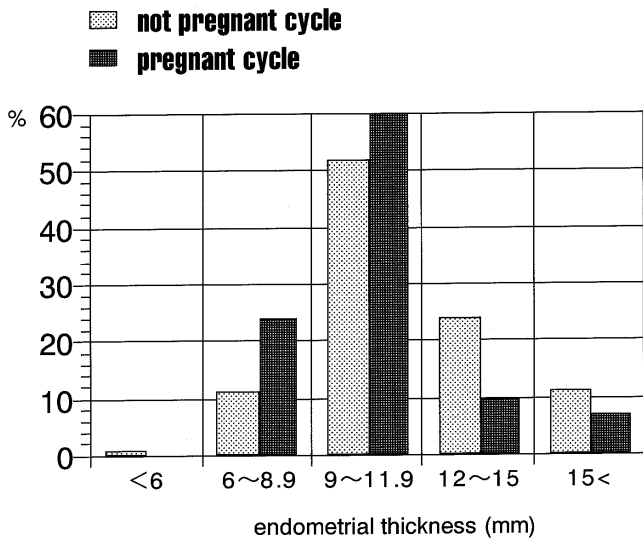


Figure 1. Percentage distribution of preovulatory endometrial thickness in 42 pregnancies and 63 non-pregnant cycles. All patients were confirmed to have ovulated within 2 days.

progesterone receptors, endometrial thickness and echo patterns as measured by vaginal ultrasonography on the day before ovulation. In addition, the effects of clomiphene citrate on oestrogen and progesterone receptor populations in the preovulatory endometrium were investigated.

Clinical evidence of the sonographic appearance of the endometrium

In 42 pregnant women, the preovulatory endometrial thickness and pattern was evaluated retrospectively for 105 cycles (39 without medication and 66 after ovulation induction with gonadotrophin). There were no differences in the distribution of preovulatory endometrial thickness in 42 pregnant cycles and 63 non-pregnant cycles (Figure 1). Similarly, recent reports have indicated that pregnancy can occur independently of endometrial thickness during IVF cycles (Strohmer *et al.*, 1994; Turnbull *et al.*, 1994).

Previous studies have shown that pregnancy did not occur when endometrial thickness was <6 mm on the day of HCG administration (Gonen *et al.*, 1989; Dickey *et al.*, 1993). In the present study, an endometrial thickness of <6 mm rarely occurred just before ovulation in both natural and stimulated cycles and thus we could not conclude that pregnancies would not arise when endometrial thickness was <6 mm. In our experience, most women with a preovulatory endometrial thickness of <6 mm previously had an

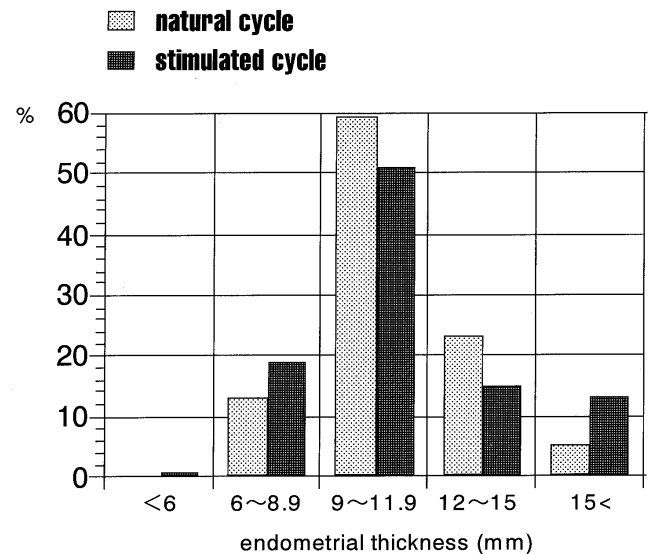


Figure 2. Percentage distribution of preovulatory endometrial thickness in 42 pregnancies in natural cycles and in gonadotrophin-stimulated cycles.

episode of endometrial curettage or had received long-term clomiphene citrate treatment.

The distribution of endometrial thickness during natural and stimulated cycles is shown in Figure 2. No relationship between ovulation induction and preovulatory endometrial thickness was found. These results agreed with those of previous investigations which suggested that monitoring of endometrial thickness was not useful during stimulated cycles (Strohmer *et al.*, 1994). However, data were obtained which showed an increase in oestrogen concentrations that correlated with follicular growth by monitoring of endometrial thickness in natural and stimulated cycles.

The relationship of preovulatory endometrial thickness to miscarriage is shown in Table I. When thickness was <9 mm, the miscarriage rate was 40%, but when thickness was >9 mm, the rate fell to only 6.3%. In conclusion, preovulatory endometrial thickness is not predictive of fecundity, though a thin preovulatory endometrial thickness in pregnant cycles indicates a higher risk of miscarriage.

Recently, higher predictive values of endometrial echo patterns were reported in IVF cycles (Serafini *et al.*, 1994; Turnbull *et al.*, 1994). In fact, abnormal echo patterns were observed in the endometrium with pathological abnormalities such as endometrial polyp, hyperplasia and cancer. In addition, uterine fibroids occasionally affected the endometrial echo pattern. These conditions may interfere with uterine receptivity and thus such cases were excluded from present study.

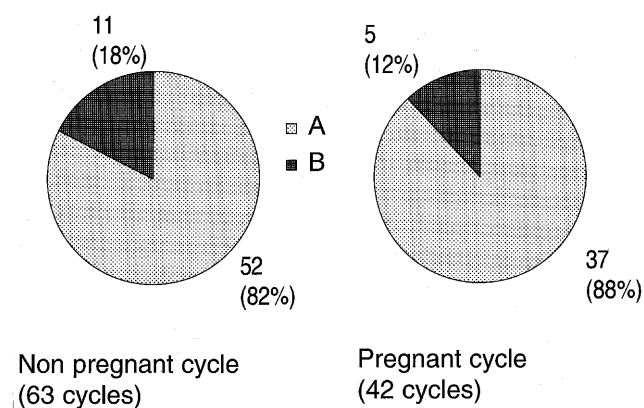


Figure 3. Percentage distribution of endometrial echo pattern among 42 pregnancies and 63 non-pregnant cycles.

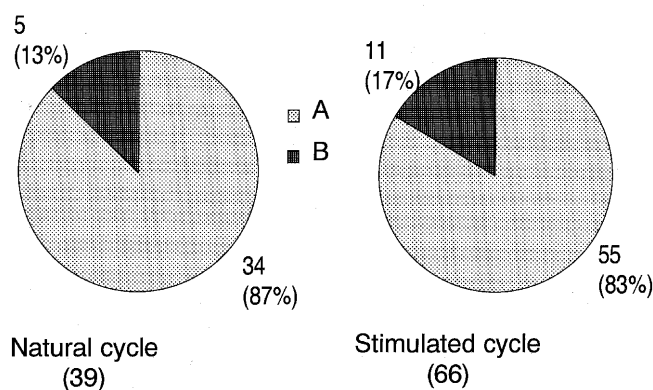


Figure 4. Percentage distribution of endometrial echo pattern in 42 pregnancies in natural cycles and gonadotrophin-stimulated cycles.

Table I. The relationship of preovulatory endometrial thickness to miscarriage

Endometrial thickness (mm)	No. of pregnancies	No. of miscarriages (%)
<6	0	–
6–8.9	10	4 (40)
9–11.9	25	2 (8)
12–15	4	0 (0)
>15	3	0 (0)
Total	42	6 (14)

The relationship between preovulatory endometrial pattern to per cycle fecundity is shown in Figure 3. The endometrial echo patterns visualized sonographically were divided two categories. Those in group A presented a triple-line pattern or multilayered endometrium in which hyperechogenic outer lines and a well-defined central echogenic line were visualized, with hypoechogenic or black areas seen between lines. Those in group B presented an echo pattern in which the endometrium had the same echogenicity as the myometrium, or it was homogenous and echo-dense in comparison with the myometrium. Echo pattern A occurred in 89 (85%) of cycles, while pattern B occurred 16 (15%) of cycles. Per cycle fecundity showed no significant differences between both echo patterns.

The relationship of ovulation induction to endometrial echo pattern is shown in Figure 4. Ovulation induction was unrelated to preovulatory endometrial echo pattern, though wide variations in the percentages of pattern B have been identified in previous reports. Such variations may have arisen because the echo pattern is influenced either by the performance of the machine, by the angle between endometrium and ultrasonic beam, and/or by a premature increase in progesterone.

Relationship between endometrial oestrogen and progesterone receptors and sonographic endometrial appearance

In a previous study (Ohno *et al.*, 1995), 67 infertile women with or without ovulation induction were carefully monitored by sonographic scanning. When the diameter of the leading follicle reached ≥ 15 mm, endometrial and blood samples were collected. Forty-eight endometrial samples associated with >100 pg/ml serum oestradiol and <2 ng/ml progesterone were used to measure oestrogen and progesterone receptors by enzyme immunoassay using monoclonal antibodies.

Generally, it is considered that serum oestradiol concentrations do not relate to endometrial thickness (Check *et al.*, 1991; Ueno *et al.*, 1991; Dickey *et al.*, 1993) and such lack of correlation was found in the present study. In addition, neither concentrations of endometrial oestrogen receptors nor progesterone receptors were found to be related to endometrial thickness or to serum concentrations of oestradiol. There were no differences in mean concentrations of oestrogen and progesterone receptors, despite differences in endometrial echo pattern and thickness.

The only significant correlation was found between concentrations of oestrogen and progesterone receptors, the progesterone:oestrogen receptor ratio being low in echo pattern B endometrium (1.6 ± 0.86) compared with pattern A endometrium (2.7 ± 1.2), though the difference was not significant. It is accepted that, in target tissue, the synthesis of progesterone receptors is regulated by oestrogen concentration; thus, the progesterone receptor can be used as a marker of the presence of functional oestrogen receptors (Janne *et al.*, 1975). These data suggest that high progesterone receptor expression during the preovulatory period is related to an adequate endometrial growth, and thereby

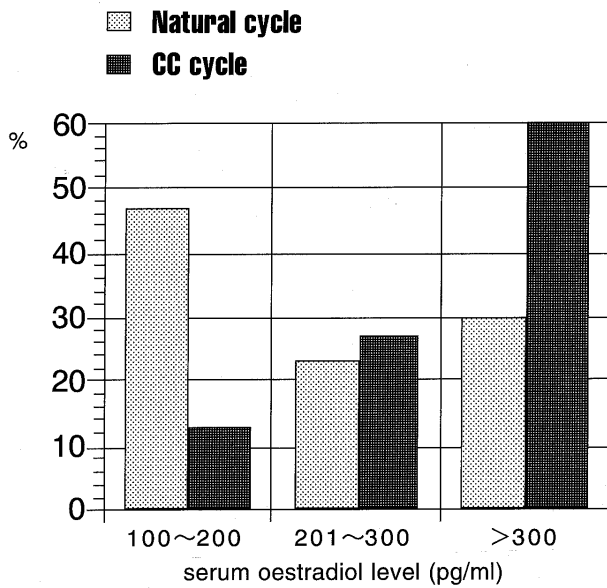


Figure 5. Percentage distribution of concentrations of serum oestradiol in 34 natural cycles and 15 clomiphene-treated cycles. Endometrial and blood samples were collected when the diameter of the leading follicle was ≥ 15 mm.

increases the responsiveness of the endometrium to progesterone stimulation after ovulation. However, clinical data do not correspond with this hypothesis.

One reason for these conclusions imputed the concentration of oestrogen and progesterone receptors, which displayed a very wide range (16–1500 and 40–1350 fmol/mg protein, respectively). This observation resembles that found in other studies using enzyme immunoassay on midluteal endometrium, which indicated a wide range of oestrogen and progesterone receptor concentrations (Balasch *et al.*, 1992). Recently, impedance to uterine blood flow in IVF cycles was found to be an important tool in assessing ovarian stimulation (Battaglia *et al.*, 1997). The differences of peripheral impedance in the uterine vascular beds suggested that serum oestradiol concentrations were unrelated to endometrial oestrogen receptor concentrations. We conclude that it was difficult to find statistical differences among steroid receptors, endocrinological data and sonographic appearance.

Effect of clomiphene citrate stimulation on endometrial oestrogen and progesterone receptors

Ovarian stimulation with gonadotrophin in infertile women results in an elevation in oestradiol concentrations. It should be noted that inadequate endometrial development, or a reduction in steroid receptor concentrations,

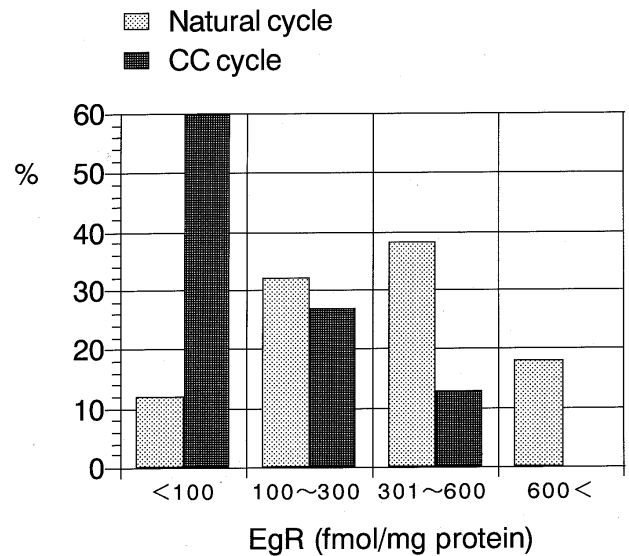


Figure 6. Percentage distribution of endometrial oestrogen receptor (EgR) concentrations in 34 natural cycles and 15 clomiphene-treated cycles.

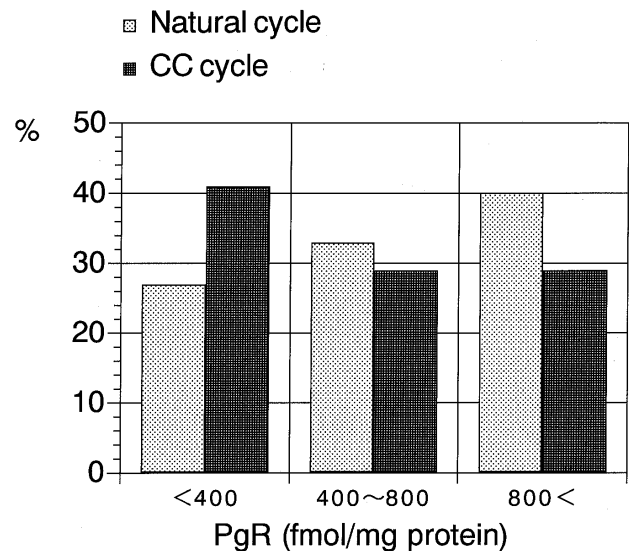


Figure 7. Percentage distribution of endometrial progesterone receptor (PgR) concentrations in 34 natural cycles and 15 clomiphene-treated cycles.

might have an unfavourable influence on the outcome of implantation (Bonhoff *et al.*, 1993; Hadi *et al.*, 1994). In our previous study (Ohno *et al.*, 1995), oestrogen and progesterone receptor concentrations appeared not to be altered following gonadotrophin induction, though amounts of these receptors were lower in clomiphene-stimulated cycles than in spontaneous cycles. However, the previous study included a very small group of women treated with clomiphene citrate. It is well known that clomiphene treatment reduces endometrial thickness (Randall and Temple-

ton, 1991; Yagel *et al.*, 1992) and that, in such patients, pregnancy rates are low in spite of high ovulatory rates.

The distribution of serum oestradiol concentrations in clomiphene and natural cycles is shown in Figure 5. As a result of the growth of two or more follicles, 60% of women with clomiphene cycles attained oestradiol concentrations >300 pg/ml. In contrast, 60% of women with clomiphene cycles had <100 fmol/mg protein in their endometrial oestrogen receptor concentration (Figure 6). However, women with high oestradiol populations did not always show individually low oestrogen receptor concentrations. Most women receiving clomiphene treatment had very low oestrogen receptor concentrations in the preovulatory endometrium, though these results were not based on the same number of treated or non-treated women, and it is thus uncertain whether clomiphene acts directly on the endometrium or whether it reduces the oestrogen receptor population. In a previous study, clomiphene citrate treatment did not affect the concentration of immunoassayable endometrial oestrogen and progesterone receptors during the mid-cycle or late luteal phase in isolated treatment cycle of normal ovulatory women (Fritz *et al.*, 1991). The clinical pregnancy rate during clomiphene treatment is highest in the first treatment cycle, and declines as treatment advances. In contrast, pregnancy rates in gonadotrophin-treated cycles gradually increased as treatment advanced. An adverse effect of long-term clomiphene administration on the endometrium may provoke an abnormally low oestrogen receptor population that predisposes to an inadequate endometrial appearance.

The difference in mean progesterone receptor concentrations during natural and clomiphene cycles was significant, as was that for oestrogen receptor concentrations (Figures 6 and 7). However, the distributions of progesterone receptor concentrations assumed a different aspect. There were no remarkable differences in the distribution of progesterone receptor concentrations between the natural and clomiphene-treated cycles. Previously, we supposed that there could be a difference due to an interference in the development of progesterone receptors induced when clomiphene acted as an anti-oestrogen, and so decreased the responsiveness of the endometrium to progesterone stimulation. Now, an additional study has revealed that the decline of oestrogen receptor concentrations in clomiphene-treated

preovulatory endometrium is more serious than a decline in the progesterone receptor population.

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