CLINICAL ASSISTED REPRODUCTION

Endometrial Evaluation Is Not Predictive for In Vitro Fertilization Treatment

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Purpose: The main purpose of this study was to evaluate ovarian function by clomiphene citrate (CC) challenge test in a group of tubal infertile women and to study endometrial morphological maturation in the early luteal phase of CC-stimulated cycles as compared to in vitro fertilization (IVF) treatment outcome.

Methods and Results: Four women presented with strongly retarded, proliferative endometrium in the luteal phase. Of these, three presented with impaired ovarian function, high basal follicle-stimulating hormone, and high follicle-stimulating hormone levels after clomiphene stimulation on cycle day 10. In the remaining 30 women, showing an in-phase endometrium after CC stimulation, a comparison of six morphological characteristics did not reveal any significant differences between the 14 women who did become pregnant and the 16 who did not. No significant differences in endometrial thickness were observed between the groups. Significant differences were found when comparing estradiol and progesterone area under the curve during the luteal phase ($P < 0.001$ and $P < 0.01$, respectively) between those who did and those who did not become pregnant.

Conclusions: Luteal endometrium morphology was not a sharp instrument to detect differences between women who did and women who did not become pregnant following IVF treatment, while ovarian function, as measured by hormonal markers, seemed to be a more reliable prognostic factor for IVF treatment outcome.

KEY WORDS: endometrium; clomiphene citrate; IVF outcome; menstrual cycle; ovarian function; tubal infertility.

INTRODUCTION

The major determinants for successful implantation after in vitro fertilization (IVF) seem to be preembryo quality, endometrial maturation, and the synchronization of these factors (1). The endometrial quality during the time of implantation is highly dependent on a relatively precise sequence of preovulatory estradiol (E_2) followed by E_2 and progesterone (P_4) priming during the luteal phase. Inadequate hormonal stimulation and/or reduced responsiveness of the endometrium may result in failure of implantation.

Since its introduction in 1960, clomiphene citrate (CC), a nonsteroidal synthetic compound with both estrogenic and antiestrogenic activity on estrogen-sensitive tissues, has been an important tool for ovulation induction. Several studies have demonstrated the usefulness of the drug, either alone or in combination with gonadotropins, for multiple follicular development aimed to facilitate IVF (2, 3). However, CC, being an antiestrogen, could also have a specific effect on the endometrium (4).

The main purpose of the present study was to evaluate ovarian function by the CC challenge test in a group of tubal infertile women and to study endometrial morphological maturation in the early luteal phase of the CC-stimulated cycles compared to IVF treatment outcome.

MATERIALS AND METHODS

Study Group

Thirty-four tubal infertile women waiting for IVF treatment who had failed to conceive for at least 2 years

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following tubal reconstructive surgery volunteered for the study. All women were regularly menstruating and underwent, prior to surgery, a complete investigation for infertility including basal body temperature, characterization of the menstrual cycle by repeated serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), E_2 , and P_4 in the luteal phase. All fulfilled our standard criteria for normal ovulatory cycles (5). All men had normal spermograms on at least two occasions according to the World Health Organization criteria (6).

Study Plan

CC, 100 mg, was administered daily for 5 days, beginning on cycle day 5. Blood samples were obtained from all subjects in the CC-treated cycle by venepuncture in the morning between 0730 and 0830 hr. Five milliliters of blood was drawn into a test tube each time and centrifuged within 30 min, and the serum was frozen and stored at -20°C until assayed. Blood was collected on days 3, 10–15, and 19–26. On days 10–15 urine samples were collected to estimate the day of the LH peak, using a self-test (OvuQuick; Monoclonal Antibodies Inc., Sunnyvale, CA). The LH peak was also determined in serum samples. E_2 and P_4 were measured during the luteal phase, on cycle days 19–26. Hormonal assays of E_2 , P_4 , FSH, and LH were performed with the Immulite system (DPC, LA, chemiluminescence). E_2 and P_4 were analyzed by competitive immunoassay. FSH and LH were analyzed by noncompetitive immunoassay. The limits of determination were as follows: E_2 , 73–7340 pM; P_4 , 0.64–127 nM; FSH, 0.1–170 IU/L; and LH, 0.7–200 IU/L. The precision (within-run) values were as follows: E_2 , 6.3–15%; P_4 , 6.9–13%; FSH, 5.4–7.7%; and LH, 4.8–6.5%.

In the luteal phase, P_4 and E_2 concentrations are expressed as areas under the curve (AUC), which were calculated as sums of partial areas obtained according to the following formula: $\text{area} = \text{time} \times (\text{level a} + \text{level b})/2 + \text{time} \times (\text{level b} + \text{level c})/2 + \text{time} \times (\text{level c} + \text{level d})/2 + \text{time} \times (\text{level d} + \text{level e})/2$.

Endometrial Assessment. In the same menstrual cycle endometrial biopsies were obtained from the anterior wall of the uterine cavity using a Randall curette (Stille Werner AB, Stockholm, Sweden) without cervical dilatation or anesthesia. The criteria of Noyes *et al.* (7) were used for the histological dating. The day of biopsy was grouped in relation to the LH peak and varied from LH+2 to LH+6. Endometrium out of phase was defined as histological dating differing more

than 2 days from that in relation to the LH peak. The specimens were fixed immediately in Bouin's solution for light microscopic examination after paraffin embedding and hematoxylin and eosin staining.

Morphometry. Ten arbitrarily chosen microscopic fields were analyzed in each biopsy specimen (Zeiss light microscope, $\times 10$, wide-angle eyepieces with micrometer disk, objective $\times 40$; Carl Zeiss, Germany). In each microscopic field the following six indices were assessed: (1) number of transversely sectioned glands (expressed as glands per mm^2), (2) number of glandular mitoses (expressed as mitoses per 1000 glandular cells), (3) number of stromal mitoses (expressed as mitoses per 1000 stromal cells), (4) diameter (μm) of the glandular lumen in the transversely sectioned glands, (5) height (μm) of the glandular epithelium, and (6) number of glandular cells with basal or subnuclear vacuolation (expressed as basal vacuolated cells per 1000 glandular cells). Morphological evaluation of the endometrial biopsies was performed blindly by one of the authors.

IVF Treatment Protocol. All patients followed our standard long gonadotropin-releasing hormone agonist (GnRHa)/human menopausal gonadotropin (hMG) protocol for stimulation. Gamete and preembryo handling as well as preembryo replacement has been described previously (2). During the study period treatment was restricted to three cycles (two women in the nonpregnant group had four cycles start but only three treatment cycles) and the number of replaced preembryos was limited to three.

Statistical Methods

The Mann–Whitney test and the Student *t* test for unpaired data were used to test for the significance of any differences ($P < 0.05$) using the Stat View statistical package (Abacus Concepts Inc., Berkeley, CA). The results are presented as means $\pm$ SD (Tables I–III).

Ethical Aspects

The study was approved by the local Ethics Committee of the Karolinska Hospital.

RESULTS

The clinical characteristics of the 34 infertile women with respect to the outcome of IVF treatment such as age, height, weight, body mass index, and cycle length

Table I. Certain Characteristics After CC Treatment in Four Women Showing an Out-of-Phase Endometrium

	Subject			
	I-1	I-2	I-3	I-4
FSH (IU/L) on cycle day 3	15.9	36.4	4.9	4.4
FSH (IU/L) on cycle day 10	26.3	45.3	13.9	4.3
E ₂ AUC (pM) × days	3131	3314	3564	3687
P ₄ AUC (nM) × days	85.3	255.0	255.0	469.2
P ₄ LH + 3–4 (nM)	9.8	25.8	25.8	44.4
P ₄ LH + 6–7 (nM)	18.6	30.9	30.9	62.6
P ₄ LH + 9–10 (nM)	12.8	25.4	25.4	38.1
Biopsy day related to LH peak (day 0)	+5	+6	+5	+5
Endometrial thickness just before endometrial biopsy	6.3	6.8	6.7	6.9
Endometrial dating (7)	Late prolif.	Mid prolif.	Late prolif.	Late prolif.

did not differ between women who did and those who did not become pregnant.

Four of twenty women who did not become pregnant had a strongly retarded, proliferative endometrium in the luteal phase (Table I). Three of these four women (I-1–I-3) presented with impaired ovarian function as indicated by high basal FSH and high FSH levels on cycle day 10 after CC treatment and low E₂ and P₄ AUC in the luteal phase (8), while one woman (I-4) presented with satisfactory ovarian function. These four women were excluded from further comparison.

Significant differences were found by comparing FSH on cycle days 3 and 10 and E₂ and P₄ AUC during the luteal phase between those women who did and those who did not become pregnant in the remaining 30 women (Table II). A comparison of six morphological characteristics in 14 infertile women who became pregnant and 16 who did not become pregnant following IVF treatment (Table III) did not reveal any differences. No significant differences in endometrial thickness were found between those two groups.

DISCUSSION

Three women presented with strongly retarded endometrial development during the luteal phase after

CC treatment, in combination with impaired ovarian function. Only one woman showed retarded endometrial development despite normal ovarian function. This woman presented with normal, in-phase endometrial characteristics during an untreated natural cycle. Studies have indicated that CC treatment fails to increase the uterine volume, and retarded endometrial thickening has been observed despite supraphysiological E₂ levels (9,10). This effect may be related to the antiestrogenic characteristics of CC, which competitively binds to the E₂ receptor at numerous sites throughout the reproductive system. Women receiving CC have an increased incidence of deficient luteal phase, characterized by an out-of-phase endometrium (11,12). This retardation in endometrial development has been linked to low conception and also to increased spontaneous abortion rates (13). Our findings appear to indicate that CC might have an antiestrogenic effect on the endometrium, measured by decreased mitotic counts and fewer secretory vacuoles. The prevalence of this problem seems to be relatively low—only 1 of 34 women.

In the remaining 30 women we found no differences in morphological qualities or in endometrial thickness in CC-treated screening cycles between women who did and those who did not become pregnant following

Table II. Certain Characteristics in 30 Infertile Women in Relation to the Outcome of IVF Treatment with In-Phase Endometrial Dating

	Pregnant (n = 14)	Not pregnant (n = 16)	Statistical analysis, pregnant vs. not pregnant ^a
FSH (IU/L) on cycle day 3	4.1 ± 1.5	7.1 ± 3.4	P < 0.05
FSH (IU/L) on cycle day 10	4.5 ± 1.2	12.8 ± 9.5	P < 0.01
E ₂ AUC (pM) × days in luteal phase	10,143 ± 3,290	4,739 ± 1,933	P < 0.001
P ₄ AUC (nM) × days in luteal phase	711 ± 285	364 ± 197	P < 0.01
Endometrial thickness just before endometrial biopsy (mm)	12.3 ± 1.1	11.8 ± 2.3	NS
Endometrial dating (7) LH + 4–6	In phase	In phase	NS

^a ANOVA.

Table III. Morphometric Analyses of Various Indices of Endometrium and Endometrial Thickness in 30 Infertile Women Related to the Outcome of IVF Treatment (Means $\pm$ SD)^a

Type of cycle	LH + 3 and LH + 4		LH + 5 and LH + 6	
	Pregnant	Not pregnant	Pregnant	Not pregnant
<i>n</i>	7	3	7	13
Number of glands (mm ²)	24.8 $\pm$ 2.3	22.2 $\pm$ 2.8	25.4 $\pm$ 4.5	22.7 $\pm$ 2.8
Glandular mitosis per 1000 glandular cells (<i>n</i>)	13.1 $\pm$ 1.7	14.6 $\pm$ 2.6	2.6 $\pm$ 0.3	3.0 $\pm$ 2.8
Stromal mitosis per 1000 stromal cells (<i>n</i>)	1.9 $\pm$ 0.5	2.2 $\pm$ 0.8	1.2 $\pm$ 0.5	1.3 $\pm$ 0.8
Glandular diameter (μ m)	49.4 $\pm$ 4.2	47.9 $\pm$ 3.6	59.9 $\pm$ 5.2	52.8 $\pm$ 3.7
Glandular epithelial height (μ m)	22.9 $\pm$ 0.6	23.3 $\pm$ 0.9	19.4 $\pm$ 3.2	21.6 $\pm$ 2.5
Vacuolated cells per 1000 glandular cells (<i>n</i>)	386.6 $\pm$ 43.6	367.7 $\pm$ 67.4	87.6 $\pm$ 5.8	98.6 $\pm$ 7.8
Endometrial thickness (mm)	10.8 $\pm$ 1.1	10.2 $\pm$ 0.8	11.8 $\pm$ 0.9	11.5 $\pm$ 1.4

^a ANOVA NS.

IVF treatment. However, E₂ and P₄ AUC during the luteal phase differed significantly between the two groups as reported previously (8). Hormonal markers of the luteal phase seem to be of certain prognostic value for the outcome of IVF treatment, while morphological examination of the luteal endometrium and ultrasound evaluation of endometrial thickness do not seem to be sharp enough instruments to detect prognostic differences, except in a few cases sensitive to the antiestrogenic effect of CC.

Molecular and biochemical markers of endometrial receptivity could have better prognostic values than morphologic and sonographic evaluation. Endometrial protein 14 or pregnancy-associated endometrial α 1-globulin, insulin-like growth factor binding protein, and integrins are some of the active proteins that may participate in the receptor-mediated interaction between maternal and embryonic cells. There are still many unanswered questions about the mechanisms of implantation.

Luteal endometrium morphology was not a sharp instrument to detect differences between women who became and those who did not become pregnant following IVF treatment, while ovarian function, as measured by hormonal markers, seemed to be a more reliable prognostic factor for IVF treatment outcome.

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