Endometrial Pattern on the Day of Oocyte Retrieval Is More Predictive of Implantation Success than the Pattern or Thickness on the Day of hCG Administration

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Purpose: Multiple studies have confirmed a lower implantation (IR) and pregnancy rate (PR) in women who exhibit a homogeneous pattern (pattern II) of the endometrium compared to a triple-line pattern (pattern I) on the day of hCG administration. However, no data are available to evaluate if patients alter their endometrial thickness and pattern between the day of hCG administration (D_{hCG}) and the day of oocyte retrieval (D_{RET}) and whether these changes adversely affect endometrial receptivity.

Methods: We prospectively evaluated 86 women (mean age, 32.9 ± 3.8 years; range, 24–40 years) undergoing 103 IVF/ET cycles.

Results: Pattern II was noted in 7 cycles (6.8%) on D_{hCG} , compared to 96 cycles with pattern I (93.2%). However, 20 cycles (19.4%) had pattern II on D_{RET} . The ongoing IR was 13.0% (3/23) in the pattern II group compared to 20.8% (76/365) in the pattern I group on D_{hCG} ($P = NS$). However, a significant decrease in the ongoing IR, to 9.9% (7/71), was noted in pattern II, compared to 23.3% (71/305) in pattern I, on D_{RET} ($P = 0.019$). There was no difference in age, basal FSH, peak E_2 , P_4 on the day of hCG, number of oocytes, number of ET, or endometrial thickness between pregnant and nonpregnant patients, or between patients with pattern I and those with pattern II. A trend toward higher progesterone levels on D_{hCG} was noted in women with pattern II ($P = 0.078$).

Conclusions: Endometrial pattern, rather than thickness, on the day of oocyte retrieval appears to be an important prognosticator of endometrial receptivity.

KEY WORDS: in vitro fertilization; ultrasound; endometrial pattern; endometrial thickness; pregnancy; implantation.

INTRODUCTION

Endometrial receptivity is essential for the occurrence of pregnancy. The ideal method for predicting this receptivity has yet to be established. Over the last decade, ultrasonographic examination of the endometrial thickness and pattern has been proposed as a noninvasive test of endometrial receptivity. Indeed, both endometrial thickness and endometrial pattern have been implicated as prognostic parameters for successful outcome in in vitro fertilization (IVF). However, endometrial thickness lacks specificity, and no consensus exists whether endometrial thickness is related to IVF outcome, with nearly as many studies showing a positive correlation with pregnancy rate (PR) (1–11), as showing no correlation (12–23). In contrast to the studies on endometrial thickness, nine studies have confirmed a lower PR in women who exhibit a homogeneous, hyperechogenic pattern (pattern II) of the endometrium on transvaginal ultrasound compared to a multilayered, hypoechoic, triple-line pattern (pattern I) on the day of human chorionic gonadotropin (hCG) administration, regardless of endometrial thickness (1,4–7,10,11,13,20,24). Conversely, six other studies failed to confirm these findings (15–18,21,22). In addition, only one study addressed whether implantation rates (IR) are correlated with endometrial thickness and pattern (9).

There is also no consensus on when the ultrasonographic examination is performed that best predicts the occurrence of pregnancy. Most of the studies evaluated endometrial thickness and patterns on the day of hCG administration (3,6,7,10,13,16–18,22,24), while others were assessed on the day following hCG administration (4,5,8,9,13), the day of oocyte retrieval (11),

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the day of embryo transfer (ET) (23), or on the days of both hCG administration and ET (15). However, no data are available to evaluate if patients alter their endometrial thickness and pattern between the day of hCG administration (D_{hCG}) and the day of oocyte retrieval (D_{RET}) and whether these changes adversely affect endometrial receptivity.

MATERIALS AND METHODS

Population

Exclusion criteria included an age >40 years, a basal follicle stimulating hormone (FSH) >11 IU/L, visible endometriomas on baseline ultrasound, the presence of uterine leiomyoma, the presence of endometrial polyps or submucosal and/or intramyometrial leiomyomata, and severely retroverted uteri (making an adequate assessment of the endometrial thickness and pattern difficult). Ninety-six women undergoing 116 IVF cycles who received an ET were thus prospectively evaluated between January 1997 and July 1998. Ten women (10.4%) undergoing 13 cycles were excluded for the absence of still photographs of the endometrium on either the day of hCG administration or the day of oocyte retrieval. Eighty-six women who completed a total of 103 IVF and ET cycles were included in the study.

Thirty-four patients had tubal disease (39.5%), 22 had male-factor infertility (25.6%), 13 had ovulatory dysfunction (15.1%), 10 had endometriosis (11.9%), and 7 had unexplained infertility (8.1%). Some patients had more than one infertility factor.

Because measurement of the endometrial thickness and pattern is an integral part of cycle monitoring in our program, institutional review board approval for the study protocol was not obtained.

Ultrasound Measurements

All measurements were performed using a 6.5-MHz vaginal probe (Performa; Acoustic Imaging, Dornier Medical Systems, Phoenix, AZ) by two of the authors (F.I.S. and H.D.M.). Endometrial thickness and pattern were recorded by obtaining permanent photographic images of the endometrium at its thickest measurement in the sagittal plane about 1 cm from the uterine fundus on the day of hCG administration and, again, on the day of oocyte retrieval. The widest endometrial thickness was measured as the distance between the two echogenic interfaces of the endometrial-myometrial

junction, with the calipers placed on the outer walls of the endometrium. The information obtained was not used for clinical decision making. The pictures were reviewed first independently by two of the authors (F.I.S. and J.L.) and then reviewed once again by both authors together. A trilaminar pattern identified as a hypoechoic layer with a central hyperechoic line or an isoechoic layer with a central echogenic line was classified as triple-line (pattern I). An endometrium which was homogeneously hyperechoic was considered to have a homogeneous pattern (pattern II).

The reported inter- and intraobserver coefficients of variation for endometrial thickness range from 1 to 9%, which are not considered statistically significant (13,17,25).

Stimulation Protocols

Women with a basal FSH <8.5 IU/L or younger than 38 years of age underwent a standard long luteal leuprolide acetate (LA) protocol (Lupron; TAP, North Chicago, IL) ($N = 61$). After pituitary desensitization, FSH and/or human menopausal gonadotropin (hMG) (Metrodin, Fertinex; and Gonal-F, Serono, Randolph, MA; Follistim and Humegon, Organon, West Orange, NJ) were started at 2–4 ampoules/day. For women older than 38 years and those with FSH ≥ 8.5 , a modified microdose LA flare protocol was used ($N = 42$). This consists of 50 μ g bid of LA along with 6 ampoules daily of FSH and/or hMG starting on day 3 of a withdrawal bleed following at least 3 weeks of oral contraceptives. When at least three follicles were ≥ 18 mm, 10,000 U hCG (Profasi; Serono) was administered. Oocyte retrieval was performed 34–36 hr later. ET was performed 72 hr later under ultrasound guidance using a Wallace catheter (Edwards–Wallace catheter; Marlow Technologies, Willoughby, OH). The luteal phase was supported using 50–100 mg progesterone in oil im daily. Biochemical pregnancies were considered as failure to conceive. An ultrasound to confirm the number of sacs and fetal viability was performed at 5–6 weeks of gestation. Ongoing PR implies delivered or ongoing pregnancies beyond 20 weeks. IR denotes the number of gestational sacs divided by the number of replaced embryos.

Laboratory Analysis

For serum FSH, a microparticle enzyme immunoassay was used (Abbott AxSYM system; Abbott Pharmaceuticals, Abbott Park, IL). The interassay and intraassay coefficients of variation were 3.48 and 4.

52%, respectively. The upper limit of normal for FSH in our laboratory is 10 IU/L (conversion factor to SI units, 1.0), which is equivalent to 18 IU/L by RIA Leeco assay (Leeco Diagnostics, Southfield, MI). For estradiol (E_2), a radioimmunoassay was used (Coat-a-count; Diagnostic Products Corporation, Los Angeles, CA). The interassay and intraassay coefficients of variation were 7.8 and 5.8%, respectively.

Statistical Analysis

Data are expressed as mean $\pm$ SD. Fisher's exact test, chi-square test, Mann-Whitney rank sum test, and Student *t* test analyses were used where appropriate. Pearson product-moment correlation was used to evaluate the relationship between age and endometrial thickness, and between peak E_2 and endometrial thickness. *P* was significant at <0.05 . Power calculations were performed where needed.

RESULTS

The mean age was 32.9 ± 3.8 years (range, 24–40 years). The mean basal FSH was 6.8 ± 1.8 IU/L (range, 1.4–10.4 IU/L). Basal E_2 was <50 pg/ml in all patients. The mean endometrial thickness was 10.2 ± 1.8 mm on D_{hCG} (range, 5.8–15 mm) and 9.8 ± 1.9 mm on D_{RET} (range, 5.3–15 mm). The mean E_2 on the day of hCG administration was 2548 ± 1028 pg/ml (range, 790–5389 pg/ml), with a mean number of retrieved oocytes of 15.5 ± 6.5 (range, 5–44). The mean progesterone (P_4) on the day of hCG administration was 1.5 ± 0.7 ng/dl (range, 0.3–3.7 ng/dl). The mean number of ET was 3.8 ± 1.0 (range, 1–6). There was a total of 47 pregnancies (45.6%), 44 of whom were delivered or ongoing (42.7%). The clinical and ongoing IR was 21.4 and 20.9%, respectively. We found a significant correlation between age and endometrial thickness on D_{hCG} ($P = 0.024$), but not between peak E_2 and endometrial thickness ($P = 0.40$; power, 62%).

The two authors reviewing the still pictures were in agreement 100% of the time on the thickness and pattern of the endometrium. Endometrial thickness was not different between D_{hCG} and D_{RET} (10.0 mm, 25–75% interquartile range, 8.3–11.0; and 10.0 mm, 25–75% interquartile range, 9.0–11.0; $P = 0.09$). A pattern II on D_{hCG} was noted in 7 cycles (6.8%), compared to 96 cycles with pattern I (93.2%). However, 13 additional cycles had pattern II on D_{RET} , for a total of 20 cycles (19.4%). No patients altered their pattern from II to I between D_{hCG} and D_{RET} . The clinical and

ongoing PR for women with pattern II on D_{hCG} was 42.8% (3/7), compared to 45.8% (44/96) and 43.8% (42/96) in women with pattern I ($P = 0.81$ and 0.72 , respectively) (Fig. 1). However, the clinical and ongoing PR for women with pattern II on D_{RET} declined to 30.0% (6/20) and 25.0% (5/20), compared to 49.4% (41/83) and 47.0% (39/83) in women with pattern I on D_{RET} ($P = 0.19$ and 0.12 , respectively). Even though a trend toward a lower clinical and ongoing PR is noted when pattern II is noted on the day of oocyte retrieval, the power of the study to detect a significant difference was low (31.8%).

The clinical and ongoing IR on D_{hCG} was 13.0% (3/23) in the pattern II group, compared to 21.4% (78/365) and 20.8% (76/365) in the pattern I group ($P = 0.49$ and 0.53 , respectively). However, a significant decrease in the clinical and ongoing IR, of 11.3% (8/71) and 9.9% (7/71), was noted in the pattern II group, compared to 23.9% (73/305) and 23.3% (71/305) in the pattern I group, on D_{RET} ($P = 0.029$ and 0.019 , respectively) (Fig. 1). There was no difference in age, basal FSH, peak E_2 , P_4 on the day of hCG, number of oocytes, number of ET, or endometrial thickness between pregnant and nonpregnant patients (Table I) or between patients with pattern I and those with pattern II on the day of hCG administration or the day of oocyte retrieval (Table II). The only exception was a trend toward a higher progesterone level on the day of hCG administration in the pattern II group (median, 1.80 ng/dl; 25–75% interquartile range, 1.5–2.8; and range, 0.8–3.7 ng/dl; compared to 1.45 ng/dl, 1.0–1.9, and 0.3–2.8 ng/dl in the pattern I group; $P = 0.078$; power, 62.9%). There was also no difference in endometrial thickness or pattern relative to the stimulation regimen.

Because we noted a significantly higher IR and a trend toward a higher PR in women with pattern I, we analyzed only those cycles where a pattern I was noted to determine the effect of endometrial thickness on PR, on both D_{hCG} and D_{RET} . There were no differences in endometrial thickness between pregnant and nonpregnant subjects on either day ($P = 0.21$ on D_{hCG} and $P = 0.20$ on D_{RET} , respectively). The lowest endometrial thickness noted in an ongoing pregnancy was 5.8 mm on D_{hCG} and 5.3 mm in the same patient on D_{RET} .

DISCUSSION

To our knowledge, this is the first study assessing prospectively the endometrial thickness and pattern on the days of both hCG administration and oocyte

Fig. 1. Ongoing PR and IR by pattern and day of observation. Pattern I, hypoechoic, triple-line; pattern II, hyperechoic, homogeneous. D_{hCG}, day of hCG administration; D_{RET}, day of oocyte retrieval.

Table I. Characteristics of the 103 IVF Cycles Grouped According to the Pregnancy Outcome

	Pregnant (N = 47)	Not pregnant (N = 56)	P
Age (years)	32.8 ± 3.4	33.0 ± 4.2	NS
FSH (IU/L)	6.8 ± 1.5	7.0 ± 1.5	NS
Peak E ₂ (pg/ml)	2,405 ± 1,029	2,668 ± 1,039	NS
P ₄ (ng/dl)	1.5 ± 0.7	1.6 ± 0.6	NS
Endometrial thickness (mm)	10.0 ± 2.1	9.6 ± 1.7	NS
No. of oocytes	15.1 ± 5.9	15.7 ± 7.0	NS
No. of ET	3.8 ± 1.0	3.7 ± 1.1	NS

Table II. Characteristics of the 103 IVF Cycles Grouped According to the Endometrial Pattern on the Day of Oocyte Retrieval

	Pattern I (N = 83)	Pattern II (N = 20)	P
Age (years)	33.0 ± 3.8	32.4 ± 3.9	NS
FSH (IU/L)	7.0 ± 1.5	6.5 ± 1.7	NS
Peak E ₂ (pg/ml)	2,539 ± 1,050	2,677 ± 815	NS
P ₄ (ng/dl)	1.5 ± 0.6	2.1 ± 0.9	0.078
Endometrial-thickness (mm)	9.8 ± 1.9	9.5 ± 1.6	NS
No. of oocytes	15.3 ± 6.1	16.1 ± 8.0	NS
No. of ET	3.8 ± 1.1	3.6 ± 0.8	NS

retrieval. The endometrial thickness was slightly thinner on the day of oocyte retrieval, but this difference was not significant. Confirming multiple studies, we did not find a difference in endometrial thickness between pregnant and nonpregnant women (12–23). In contrast to a previous study (17), we could not detect a difference in endometrial thickness between pregnant and nonpregnant subjects when only cycles with pattern I were analyzed. As noted in prior studies, the E₂ level on the day of hCG administration was not correlated with the endometrial thickness (2,4,13–15,19,26). However, we found a significantly negative correlation between age and endometrial thickness. Even though we evaluated only women 40 years old and younger, our results are in agreement with those of Sher *et al.* (7,24), who showed a significantly higher incidence of poor endometrial grade (endometrial thickness <9 mm with pattern I or II) in women aged 41–45 compared to those younger than 41.

In contrast to endometrial thickness, we found a significant decrease in IR and a trend toward a lower PR in women who have a homogeneous, hyperechoic pattern compared to those with a triple-line pattern on the day of oocyte retrieval. This is in agreement with prior studies in both IVF and ovulation induction/intra-uterine insemination cycles (1,4–7,9–11,13,20,24,27–29). Of the six studies that failed to show a significant reduction in PR when a homogeneous pattern was

noted on the day of hCG administration (15–18,21,22), two used a combination clomiphene citrate and hMG for ovarian stimulation (16,21), which has been shown to alter endometrial development compared to hMG alone (3,28,30), two had a small sample size but showed a trend toward a lower PR in the homogeneous group (15,22), and one found a lower PR only in donor oocyte recipients (18). None of the above studies evaluated IR. In a recent review, Friedler *et al.* evaluated 3258 IVF, cryopreserved, and oocyte donation cycles and found a high negative predictive value of 85.7% for conception when a homogeneous pattern was noted (31). Conversely, a triple-line pattern had a low positive predictive value of 33.1% and a low specificity of 13.7% to predict a clinical pregnancy (31).

Only one prior study evaluated the endometrium at two time points in the same treatment cycle (15). In this study, Khalifa and co-workers evaluated the endometrial thickness and pattern on the day of hCG administration and, again, on the day of ET. On the day of ET, 16 of 19 cases with a pattern II endometrium on the day of hCG administration remained pattern II (84%), while 3 cases (16%) became pattern I. However, of the 55 pattern I cases on the day of hCG administration, 26 (47%) changed to pattern II. The PR was not different between pattern I and pattern II on either day, but the sample size was relatively small, and IR were not assessed. In agreement with the results of Khalifa *et al.* (15), our results show that the endometrial pattern was altered to a less receptive endometrium in an additional 12.6% of the cycles between the day of hCG administration and the day of oocyte retrieval. Therefore, assessing the endometrium only on the day of hCG administration would have resulted in missing 13 additional women whose endometrium became less receptive. In our study, the P₄ level on the day of hCG administration was the only variable that nearly achieved statistical significance between those women who developed a homogeneous pattern and those who maintained a triple-line pattern. This is in agreement with Check *et al.* (24), who noted that the proportion of women with homogeneous, hyperechoic endometrial patterns increased from 6.8 to 15.6% once the progesterone level exceeded 1.5 ng/dl. Larger studies are needed to confirm this finding.

Unlike the results noted above by Khalifa and co-workers (15), none of our subjects experienced a change from a pattern II to a pattern I endometrium. We question whether a change from an early secretory (pattern II) to a late proliferative (pattern I) endometrium is physiologically possible.

In contrast to most studies that did not have a pregnancy when the endometrial thickness was less than 6 mm (4,5,16,18,27), a successful ongoing pregnancy was noted in one of our patients whose maximum thickness was 5.8 mm. This is in agreement with a recent report of a successful pregnancy with an endometrial thickness as low as 4 mm (32). Both our patient and the one described by Sundstrom (32) had a pattern I endometrium on both the day of hCG administration and the day of oocyte retrieval.

Despite a lower PR and IR when a homogeneous, hyperechoic pattern is noted, we disagree with some investigators who recommend embryo cryopreservation and subsequent ET in a frozen cycle (5,11,24,30). We agree with Serafini *et al.* (20) and Friedler *et al.* (31) that endometrial pattern offers important predictive information but should not be used as an absolute predictor of conception. Therefore, we believe that such patients should be adequately counseled and given the option to proceed with an ET or cryopreserve all resulting embryos for later transfer.

In conclusion, compared with the published literature that evaluated the pattern on the day of hCG, the endometrial pattern on the day of oocyte retrieval is more predictive of implantation success and, therefore, is of greater clinical significance. A triple-line pattern may indeed be the sonographic parameter that mostly reflects endometrial receptivity, while a homogeneous pattern is a bad prognostic factor, regardless of endometrial thickness. Assessing the endometrial pattern and thickness on the day of oocyte retrieval should be performed routinely, and patients counseled accordingly.

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