Endometrial thickness is related to miscarriage rate, but not to the estradiol concentration, in cycles down-regulated with gonadotropin-releasing hormone antagonist

In this retrospective cohort study of 102 ovarian stimulation cycles for IVF/intracytoplasmic sperm injection using GnRH antagonist and gonadotropins, we sought to assess the effect of high E_2 levels on endometrial stripe thickness and its association with pregnancy outcomes and serum E_2 levels. We found no significant correlation between serum E_2 levels (both peak and area under the curve E_2 concentration) and the endometrial thickness. However, there was a statistically significant inverse relationship with early pregnancy loss (31%) if the endometrial thickness was <9.8 mm (sensitivity 71%; specificity 76%). (Fertil Steril® 2008;89:998–1001. ©2008 by American Society for Reproductive Medicine.)

The endometrium is an important determinant for the success of artificial reproduction techniques. Endometrial development is related to estrogen stimulation (mostly E_2) during the follicular phase, which is manifested by the hyperplastic growth of endometrial glands and stroma. During controlled ovarian stimulation for assisted reproduction techniques (ART), E_2 reaches supraphysiologic levels that are 3 to 10 times the normal peak concentration reached in an unstimulated ovarian cycle. Even more important, the E_2 levels stay higher for longer than in natural cycles.

Ovulation induction cycles using GnRH antagonists down-regulation, although accomplishing similar E_2 levels, have been reported to have worse pregnancy outcomes when compared with GnRH agonists (GnRH-a), maybe because of a more substantial down-regulation of LH (1). These results prompted the development of numerous small nuances in the stimulation method, pertaining to the type of gonadotropin used, the addition of LH after initiation of GnRH, and the ovulation triggering with GnRH-a instead of hCG. Morever, GnRH antagonists for pituitary down-regulation during ovulation induction have also been shown to directly influence the development of extrapituitary tissues, including the endometrium (2). Conceptually, GnRH antagonists would decrease the effect of endometrial growth factors and thus decrease endometrial receptivity (2, 3).

Despite the abundance of studies that looked at endometrial thickness and implantation and pregnancy outcomes in IVF/ intracytoplasmic sperm injection cycles down-regulated with GnRH-a, there is a lack of studies on cycles down-regulated with GnRH antagonists. The purpose of this study was to assess the effect of endometrial stripe thickness on implantation and pregnancy outcome and its association with high E_2 levels during ovulation induction with GnRH antagonists for pituitary down-regulation.

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MATERIALS AND METHODS

In this retrospective study approved by the institutional review board at Wayne State University, we evaluated 88 women who underwent 102 consecutive conventional ART cycles for infertility from March 2003 to March 2005. We excluded donor cycles (in which the recipient did not undergo ovarian stimulation) and cycles in which an embryo transfer was not performed (because no pregnancy data would be available). All cycles underwent ovarian stimulation with GnRH antagonist (Antagon; Organon, West Orange, NJ) and gonadotropins (Gonal-F, Serono, Zurich, Switzerland, and Repronex; Ferring, Albuquerque, NM) after a step-up protocol. Patients were given oral contraceptives 1 month before the stimulation for pituitary down-regulation. Gonadotropins were administered from stimulation day 1 until the day of the hCG trigger, and GnRH antagonist (0.25 mg/day) was added from the day when at least one follicle reached 14 mm in diameter and continued daily until administration of the hCG ovulation-triggering dose. From the day of GnRH-antagonist administration on, all patients increased the dose of preparations containing an equal amount of FSH and LH by one ampule per day (75 IU FSH + 75 IU LH). Human chorionic gonadotropin was administered when at least two follicles were >18 mm in diameter. Estradiol peak and endometrial thickness were both measured on the day of hCG trigger. The thickest endometrial segment (between the two interfaces of the endometrial-myometrial junction) was measured transvaginally on a "frozen" midplane, longitudinal section of the uterus by two-dimensional ultrasonography.

For statistical analysis we used Pearson and partial correlations, *t*-test, McNemar change test, ANOVA, receiver operator characteristic curve (ROC), and χ^2 using SPSS statistical package for Windows, version 14.0 (SPSS Inc.,

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Chicago, IL). We also calculated the area under the curve (AUC) of E_2 concentration on the basis of the formula by Pruessner et al. (4) This calculates the AUC by summing the trapezoids under the E_2 measurements obtained for each patient (range, 5–9 measurements), with respect to the baseline E_2 measurements. The main outcome measures were serum E_2 levels, implantation rates, early pregnancy loss (miscarriage and biochemical pregnancy), and ongoing pregnancy rates in relation to the endometrial thickness. A P value $<.05$ was considered significant.

RESULTS

Demographics of the patient population are detailed in Table 1. The overall pregnancy rate was 57% (56/102), and the ongoing pregnancy rate was 37% (38/102): 28 were singleton, 5 twin, and 5 triplet pregnancies. Twelve of 56 pregnancies were biochemical, which was defined as a positive serum β-hCG with no gestational sac identified on ultrasound examination. Five more pregnancies ended in miscarriage in the first trimester after a gestational sac and/or an embryonal heart rate was detected by ultrasound examination. All miscarriages were of singleton pregnancies, and none of the patients requested a chromosomal analysis of the products of conception. One pregnancy that ended in elective abortion for mitochondrial disease found after chorionic villous sampling was counted in the pregnancy rate but was excluded from the early pregnancy loss patient group (defined as miscarriages plus biochemical pregnancies = 17/55 = 31%).

We found a statistically significant difference in mean endometrial thickness (P=.01) between the early pregnancy loss group (n = 17) (9.3 ± 2.4 mm; range, 6.5–15 mm) and the ongoing pregnancy group (n = 38) (11.0 ± 2.1 mm; range, 7–15 mm). No significant difference was found between the group of patients who did not achieve pregnancy (n = 46) (10.5 ± 2.3 mm; range, 5–17 mm) and the early pregnancy loss group, nor the ongoing pregnancy group.

The minimal endometrial thickness for a successful pregnancy was 7 mm. There was no significant difference in endometrial thickness between pregnant patients with singletons (n = 28) and those with multiples (n = 10; twins or triplets) (mean thickness was 11.0 ± 2.1 mm and 10.7 ± 2.2 mm in the two groups, respectively). Also, when the 14 patients who underwent more than one cycle were evaluated separately, there was no significant difference in endometrial thickness between the different cycles and no correlation with conception versus nonconception cycles.

There was no significant correlation between endometrial thickness and serum E_2 level on the day of hCG administration, the AUC of E_2 concentration constructed for each patient, implantation rate (22%), and ongoing pregnancy rate (37%). Similarly, no correlation was found between

TABLE 1

Characteristics of the study population (n = 102 patients).

Variable	Mean ± SD	Range
Age (y)	33.4 ± 4	25–45
Body mass index (kg/m^2)	25.7 ± 5	17.4–42.9
Total FSH dose (IU)	4,967 ± 3,920	938–17,025
No. of antagonist days	4.7 ± 1	3–8
Length of stimulation (d)	11.5 ± 2.0	8–14
Day 3 FSH (mIU/mL)	6 ± 4	1–13
E_2 on stimulation day 1 (pg/mL)	31 ± 13	<20–79
E_2 on hCG day (pg/mL)	2,453 ± 1,061	606–5,668
Endometrial stripe (mm)	10.5 ± 2.3	5–17
Total No. of follicles >14 mm	9 ± 5	3–25
No. of oocytes retrieved	15 ± 9	2–44
No. of embryos transferred	3 ± 1	1–5
Pregnancy rate, %[a] (56/102)	55	—
Implantation rate, %[b] (59/274)	22	—
Miscarriage rate, %[c] (5/55)	9	—
Biochemical pregnancy rate, %[c] (12/55)	22	—
Ongoing pregnancy rate, % (38/102)	37	—

[a] Includes one pregnancy ended in elective abortion.
[b] Number of gestational sacs/number of embryo transferred per cycle × 100.
[c] Number of losses/number of pregnancies (one pregnancy that ended in elective abortion was excluded from the total pregnancy count).

Detti. Early loss is correlated with a thin endometrium. Fertil Steril 2008.

endometrial thickness and body mass index (kilograms per square meter), E_2 level on day 1 of stimulation, length of stimulation (days), length of GnRH antagonist use (days), total FSH dose received, total number of follicles >14 mm diameter, and number of oocytes retrieved. When the cycles were stratified by patients' age (<30, 31–35, 36–40, and >40 years), no correlation with the same variables was found. There was, instead, a significant inverse relationship of endometrial thickness with early pregnancy loss ($r = -0.23$), with a P=.02. This relationship was not influenced by the age of the patients at the time of conception. On the basis of the ROC curve constructed with the variables "endometrial thickness" and "early pregnancy loss" (defined as miscarriages plus biochemical pregnancies), we identified the lowest value of endometrial thickness to exclude a pregnancy loss at 9.8 mm (sensitivity 71%; specificity 76%).

DISCUSSION

In our series, we found a statistically significant difference in endometrial thickness between cycles that ended in early pregnancy loss and those that ended in term pregnancies but no significant difference between cycles that failed to conceive and those with an early pregnancy loss or a term pregnancy. Pregnancy has been reported, in GnRH-a down-regulated cycles, when the endometrial thickness on the day of hCG administration was as thin as 4 mm or as thick as 14 mm, and most studies were not able to correlate the extent of endometrial thickness with the achievement of pregnancy (5, 6). When considering early pregnancy loss, it would be important to evaluate the products of conception for chromosomal abnormalities, because of the high incidence of genetic defects in these circumstances. The majority of our early losses derived from biochemical pregnancies, where no products were available for evaluation, and the event of a loss was independent from the patient's age at the time of conception. After we excluded all the studies with different endometrial thickness measurement techniques and different dates than the ones evaluated in our study, we found only one study that could, and four that could not, predict early pregnancy loss as a function of the endometrial thickness considered among other ultrasonographic parameters (7–11). Of note, all these studies used GnRH-a for pituitary down-regulation. Oliveira and colleagues attempted increasingly sophisticated combinations of ultrasonographic parameters over time but were not able to find a significant relationship between endometrial thickness and the event of a miscarriage (9–11). Miscarriages, but not biochemical pregnancies, were included in the numerator of the pregnancy loss to total pregnancy ratios of these authors' three studies (also, it is not clear whether biochemical pregnancies were included or excluded from the total number of pregnancies in their analyses). Serafini et al. evaluated endometrial thickness and pattern (different degrees of sonolucency) in 102 ART cycles and found the pattern to be the only parameter able to predict both pregnancy and miscarriage (8). However, in this study the biochemical pregnancies were not mentioned, as in the previous studies (9–11). Dickey et al. evaluated the endometrial thickness in 200 patients who underwent ART and were able to correlate it positively with the achievement of pregnancy (if thickness was >9 mm), as well as with the occurrence of a biochemical pregnancy (if thickness was <9 mm or >13 mm) (7). In our study, the occurrence of a miscarriage or a biochemical pregnancy was more likely when the thickness was <9.8 mm, even though a successful pregnancy was possible with a minimum endometrial thickness of 7 mm. Only three of 38 successful pregnancies occurred when the thickness was <9.8 mm.

Endometrial development had no direct correlation with the level of serum E_2 on the day of hCG administration. Our result is consistent with results of two previous studies by Ueno et al. and Czemiczky et al., where the investigators found no correlation between E_2 levels and endometrial thickness (12, 13).

In conclusion, we found it difficult to define the extent of the endometrial thickness related with a successful pregnancy, but the correlation with the probability of an early loss if the endometrial thickness is <9.8 mm could help in counseling patients undergoing ART using GnRH antagonist for pituitary down-regulation.

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