Ultrasound assessment of endometrial thickness: correlation with ovarian stimulation and pregnancy rates in IVF cycles

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Summary

Purpose: To study the correlation between endometrial thickness and IVF outcome and factors affecting this relation. *Methods:* Transvaginal ultrasound evaluation of endometrial thickness on hCG administration day in 112 IVF-ET cycles and comparison to indices of ovarian stimulation. Outcome was considered positive when fetal sac and fetal heart pulse were present at ultrasound. GnRH-agonist and antagonist protocols were also compared. Statistical analysis was performed by the SPSS system, chi-square and t-test. *Results:* 38 cycles displayed clinical pregnancy. In cases of higher endometrial thickness, pregnancy rates, mean serum estradiol levels, oocyte and mature oocyte numbers as well as mean large follicle numbers were higher, while the mean age was lower. *Conclusions:* In 38 cycles resulting in pregnancy, mean endometrial thickness was higher compared to cycles with negative outcomes. Higher serum estradiol is associated with higher endometrial thickness and pregnancy rates. Women achieving pregnancy and pregnant women with endometrium thicker than 9 mm were younger. Follicle stimulation was better with higher endometrial thickness. After adjustments for age, no statistical difference was found in endometrial thickness between agonist and antagonist protocols.

Key words: Endometrial thickness; Transvaginal ultrasound; IVF outcome; Ovarian stimulation.

Introduction

Endometrial receptivity and its relation to implantation of fertilized oocytes have been widely investigated by means of correlation with in vitro fertilization (IVF) outcome. Transvaginal ultrasound examination provides an easy and non-invasive way of assessing endometrial receptivity. Thickness, blood flow and pattern are related to endometrial receptivity and can be quite precisely determined by transvaginal ultrasound examination. Endometrial thickness is the most easily measured index.

Many studies have been conducted to compare endometrial thickness between successful and unsuccessful IVF cycles. Large studies (1,186 and 897 IVF cycles, respectively) have demonstrated that achieving pregnancy through IVF cycles is unlikely in cases of thin endometrium [1, 2]. Other investigators have not concluded to this relation, based either on small populations [3], or on stimulation protocols using clomiphene citrate combined with hMG (human menopausal gonadotrophin) [4].

The ideal endometrial thickness has also been investigated with most scientists suggesting that 7-14 mm is the best range [5], while others have not confirmed this limit, supporting that implantation and pregnancy rates do not significantly differ between endometrial thicknesses of more or less than 14 mm [6]. Factors affecting endometrial thickness have also been studied, such as elevated serum estradiol levels [7] and duration of ovarian stimulation [2].

The induction of ovarian stimulation in IVF, leading to the production of many follicles and as a result many oocytes, has increased IVF success rates [8]. Collection of many oocytes during oocyte retrieval is very much desirable. Speculation arises when follicles of different size and function are present, leading to oocytes of different maturation levels.

Ultrasound assessment of follicular size (diameter) can be used as an indicator of ovarian stimulation and oocyte maturity in IVF cycles. The size of large follicles after all is determinant for the time of hCG (human Chorionic Gonadotropin) administration, which promotes maturation [9]. It has been suggested in the literature that mature oocytes are correlated to large follicles, while immature oocytes are related to smaller follicles [10], though reverse opinions stand too [11], suggesting that there is no significant difference in fertilization between oocytes originating from follicles of different diameters.

It is widely accepted though that ultrasound assessment of follicular size and serum estradiol levels in women undergoing IVF is the most reliable indicator of oocyte maturity in IVF cycles.

Furthermore, studies have been conducted to investigate and compare the effectiveness of GnRH-agonist and GnRH-antagonist protocols in IVF. The use of GnRH-antagonists in IVF cycles prevents a premature rise in serum LH levels in most women and rapidly inhibits secretion of gonadotropin and steroid hormones [12]. This conveys a potential advantage over GnRH-agonists in the management of ovarian stimulation. Researchers have compared pregnancy rates, ovarian stimulation and factors affecting the cycle outcomes between the two protocols.

The purpose of this study was to investigate the correlation between endometrial thickness, ovarian stimulation and pregnancy rates and to study the factors affecting this correlation, along with comparing GnRH-agonists to GnRH-antagonists.

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Materials and Methods

In the Assisted Reproduction Unit of Athens University, 3rd Department of Obstetrics and Gynecology at "Attikon" Hospital, 112 IVF cycles were studied out of those performed in our Unit during the year 2005. Using transvaginal ultrasound examination on the day of hCG administration we measured endometrial thickness in all cases and divided our measurements in two groups. Group A consisted of endometrial thickness less than 9 mm, while group B consisted of cases with endometrial thickness more than 9 mm. During the same ultrasound scan we assessed the number of follicles with a diameter > 17 mm.

Pituitary suppression was achieved by GnRH-agonists in 50 cases and GnRH-antagonists in 62 cases. Ovarian stimulation was achieved by recombinant FSH, hCG was administered when at least three follicles had reached the mean diameter of 17 mm and oocyte retrieval was performed after 36 hours. The numbers of oocytes, as well as mature oocytes (Metaphase II) were marked. Embryo-transfer was performed on day 2 or 3 of the cycle. Each cycle used each woman's own oocytes.

To study the correlation between endometrial thickness and achieving pregnancy with IVF we considered the outcome positive when establishing by ultrasound the presence of a fetal sac and fetal heart pulse. Factors considered important in this study were stimulation duration, mean serum estradiol levels on the day of hCG administration, women's age, total FSH dosage and protocol stimulation.

Statistical analysis was performed by the SPSS system. Quantitative control was based on the t-test, quality control was based on the chi-square method, and results were considered statistically significant when $p < 0.05$.

Results

Out of 112 cycles included in our study, clinical pregnancy rate per embryo transfer was determined in 34%, which corresponds to 38 cycles. The most important results derived, are quoted in Table 1.

Table 1. — *IVF outcome.*

	Pregnancy /ET n (%)	Follicles d < 17 mm (mean ± SD)	Oocytes (mean ± SD)	Mature oocytes (mean ± SD)	Estradiol levels (pg/ml) (mean ± SD)	Age (years) (mean ± SD)	Stimulation duration (days) (mean ± SD)
Group A E < 9 mm n = 20	4 (5%)	2.1 ± 2.4	5.1 ± 3.1	4.6 ± 2	1074 ± 689	38.9 ± 3.9	10 ± 0.6
Group B E > 9 mm n = 92	34 (37%)	4.1 ± 2.7	8 ± 3.2	8 ± 3.2	1813 ± 1040	31.7±3.4	10.3 ± 2.4
p	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	NS

E: endometrial thickness, ET: embryo-transfer, d: diameter, SD: standard deviation, p: probability, NS: not significant.

In group B, where endometrium was thicker than 9 mm, we found a statistically significantly higher pregnancy rate per embryo-transfer (37%) compared to the same rate in group A (5%) ($p < 0.05$). Women in group B also displayed higher (mean ± SD) serum estradiol levels (1813 ± 1040 vs 1074 ± 689 pg/ml, $p < 0.05$) and were younger than women in group A (mean ± SD) (31.7 ± 3.4 vs 38.9 ± 3.9 years old, $p < 0.05$). However a factor not found to be related to endometrial thickness was the duration of stimulation. The differences between the two groups were not statistically significant, with a slightly longer duration in group B (mean ± SD) (10.3 ± 2.4 vs 10 ± days).

Ovarian stimulation was more satisfactory in Group A. The mean number of follicles with a diameter higher than 17 mm, as well as the mean number of oocytes and mature oocytes were statistically significantly higher in cases of endometrial thickness > 9 mm.

Moreover, we ascertained that endometrial thickness was significantly higher in cases of positive outcome (fetal sac and fetal heart pulse in ultrasound examination), with a mean thickness of 11.2 mm compared to negative outcomes, where mean endometrial thickness was 9.8 mm ($p < 0.05$).

Comparing the effectiveness of GnRH-agonist and GnRH-antagonist protocols in these cycles we concluded that pregnancy rates did not show any statistically significant difference between the two protocols. The mean ± SD age was significantly higher in the antagonist group as compared to that of the agonists. By means of endometrial thickness though, GnRH-agonists showed a stronger association with thicker endometrium (Table 2), but after adjustment for differences of age this difference was not statistically significant.

Table 2. — *GnRH-agonists vs GnRH-antagonists.*

	Pregnancy/ET n (%)	E > 9 mm n (%)	E < 9 mm n (%)	Age (mean ± SD)
GnRH-agonists n = 52 (46%)	35% (n = 18)	96% (n = 50)	4% (n = 2)	31.7 ± 3.4
GnRH-antagonists n = 60 (54%)	30% (n = 20)	70% (n = 42)	30% (n = 18)	38.9 ± 3.9
p	NS	< 0.05	< 0.05	< 0.05

E: endometrial thickness, ET: embryo-transfer, d: diameter, SD: standard deviation, p: probability, NS: not significant.

Discussion

According to our study results endometrial thickness is a factor very much related to the positive outcome of IVF cycles by means of achieving pregnancy. In 38 cycles resulting in pregnancy, mean endometrial thickness was statistically significantly higher compared to cycles with a negative outcome (11.2 mm vs 9.8 mm, respectively). Moreover, high serum estradiol levels have been found to be associated with higher endometrial thickness and consequently with higher pregnancy rates. Women having achieved pregnancy after IVF, as well as pregnant women whose endometrial thickness had been higher than 9 mm, were younger. Therefore, endometrial thickness is a factor determinant of the positive outcome in IVF cycles affected by serum estradiol levels, but according to our results not by the stimulation duration. We also investigated the potential link between the treatment protocol and endometrial thickness. We compared the agonist and antagonist protocols and after adjustment for differences in age, no statistical difference was found.

The role of endometrial receptivity in IVF-ET cycle outcomes has been widely investigated. Several studies are concordant to our findings, demonstrating that higher endometrial thickness is correlated to higher pregnancy rates. De Geyter *et al.* [1] suggested a fall in pregnancy rates when endometrium is thin, in a large series of 1,186

IVF cycles. In the study of Zhang *et al.* (897 IVF cycles) [2] the outcome was found superior in cases of higher endometrial thickness on the day of hCG administration. These investigators have also suggested that this seems to be affected by the duration of ovarian stimulation. The dependency of endometrial thickness on serum estradiol levels shown in our study has also been demonstrated by other investigators. They have all predicated that endometrial thickness is higher, when serum estradiol levels are higher.

Another trial has determined the particular endometrial thickness considered ideal for achieving pregnancy [5]. This retrospective study referred to 809 IVF-ET cycles in 623 women. The investigators divided their material in two groups based on endometrial thickness on the day of hCG administration. The first group consisted of cases with endometrial thickness of 7-14 mm and the second of cases with endometrial thickness higher than 14 mm. Successful implantation rates were found in 15% and 3%, respectively and pregnancy rates in 29.7% and 8.1%, respectively. Dietterich *et al.* [6] conducted a retrospective study of 570 women during which they evaluated endometrial thickness using transvaginal ultrasound on the day of hCG administration. Of those women 510 exhibited endometrial thickness lower than 14 mm, while the remaining 60 women displayed endometrial thickness higher than 14 mm. Successful implantation, pregnancy and spontaneous abortion rates were found to be similar between the two groups in that trial.

On the other hand some investigators have demonstrated that endometrial ultrasound parameters (thickness and pattern) do not differ between women achieving or not pregnancy after IVF cycles [13]. Some trials have not supported the correlation between endometrial thickness and IVF cycle success, referring though either to small populations [3] or to stimulation protocols using clomiphene and hMG (human menopausal gonadotrophin) [4].

Jarvela *et al.* [14] suggested that endometrial thickness is not a factor of great importance in predicting IVF outcome compared to endometrial pattern. The investigators established that the presence of triple-line endometrium after ovarian stimulation correlates to improved outcome. The same study demonstrated no differences between women achieving or not pregnancy by means of endometrial thickness, volume, and vascularization.

It has been endorsed that apart from thickness, endometrial receptivity is also designated by endometrial volume and vascularization. The role of endometrial volume and thickness in the prognosis of clinical pregnancy after assisted reproductive techniques (ART) has been explored in perspective. Higher endometrial volume (> 2 ml) and higher endometrial thickness have been shown to correlate to improved outcome. Investigators have suggested that endometrial volume constitutes a more significant factor in the prognosis of achieving pregnancy compared to endometrial thickness [15].

During recent years three-dimensional (3D) power Doppler ultrasound has been widely used to evaluate parameters determinant of endometrial receptivity apart from thickness, such as endometrial volume and endometrial-subendometrial vascularization. Studies conducted in order to display the role of endometrial-subendometrial vascularization in the prognosis of IVF outcome have demonstrated that vascularization is better in women achieving pregnancy by IVF, compared to negative IVF outcome [16].

Follicular size and endometrial thickness are definitely factors of great importance in the prognosis of IVF outcome and ovarian stimulation itself. Recently investigators have examined the role of follicular and ovarian blood flow ultrasound in the prognosis of IVF using Doppler. It seems that these parameters along with follicular size and endometrial thickness can be used for this purpose [17].

By means of achieving pregnancy our results suggest higher – but with no statistical significance – pregnancy rates for the cases of GnRH-antagonists. There has been wide investigation of this matter. Al Inany and Aboulghar found a clinical pregnancy rate of 5% in favor of GnRH-agonists, which is concordant with our findings [18]. Meta-analyses on studies comparing the two protocols have yielded conflicting results as regards the likelihood of achieving pregnancy, the most recent of which suggested higher efficacy by using antagonists [19, 20]. Research cannot overlook though the fact that GnRH-antagonist cycles are more often conducted in older patients, which has important implications for the interpretation of findings.

New potentiality in evaluating prognostic factors for ART outcome can be provided by 3D and 4D ultrasound. The quest of prognostic parameters for IVF effectiveness is a field of wide and constant investigation. The prognostic value of factors affecting endometrial receptivity, such as volume and vascularization, are under exploration.

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