

Article

Characterization of endometrial growth in proliferative and early luteal phase in IVF cycles



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Abstract

Human endometrium has a definite role in implantation, although knowledge about its modifications in the course of IVF cycles is still limited. This study was performed to characterize endometrial growth throughout stimulation treatment in women undergoing IVF cycles, regardless of clinical outcomes. Endometrial growth was longitudinally evaluated by ultrasonography in the first induced ovarian stimulation cycle (717 patients). Acceleration and length of significant growth were used to assess the slope of the growth curve mathematically. Endometrial growth showed a parabolic trend and final thickness was significantly affected by age ($P < 0.01$). Endometria that tended to overgrowth had a more rapid and longer growth during the whole phase. A similar stimulation treatment was repeated within 6 months of the first one and a second evaluation was carried out to verify whether similar growth occurred. Similar growth was observed in 76% of the patients, with an absolute difference between the two cycles of <4 mm in 84% of cases. The endometrium seems to have an individual intrinsic potential that can be expressed regardless of the stimulation protocol. This supports the hypothesis that individual factors (intrinsic properties of the endometrium) significantly affect endometrial growth.

Keywords: assisted reproductive technology procedures, endometrial thickness, endometrium, IVF, stimulation

Introduction

The ovarian response to stimulation protocols in IVF cycles has been extensively studied, whereas endometrial growth in the proliferative phase has not been properly characterized as yet. The importance of proper endometrial development in allowing implantation is widely accepted (Killick, 2007). The relative contribution of uterine receptivity to conception has been estimated by a mathematical model to account for up to 64% (Rogers *et al.*, 1986). The human endometrium is receptive to blastocyst implantation during a very short time known as the 'implantation window' (Navot *et al.*, 1991; Duc-Goiran *et al.*, 1999). Endometrial biopsy represents the sole method of accurately dating endometrial maturity (Frydman *et al.*, 1982), though its invasiveness is not acceptable in the clinical context of assisted reproduction treatment cycles.

The sonographic measurement of endometrial thickness at embryo transfer has been associated with outcomes in IVF

cycles (Friedler *et al.*, 1996; Dietterich *et al.*, 2002), but this information is of little benefit as it is made post-transfer. Conversely, the assessment of endometrial characteristics (thickness and pattern) on the day of human chorionic gonadotrophin (HCG) administration was found to be of poor predictive value (Friedler *et al.*, 1996). Nevertheless, a thin endometrium at embryo transfer was reported to be associated with low pregnancy rates (Rashidi *et al.*, 2005), with a gradual increase of favourable outcomes as the endometrium thickens (Richter *et al.*, 2007). Recently, a parabolic trend was reported for clinical pregnancy rates according to endometrial thickness, with lower rates at extremes (Lamanna *et al.*, 2007). Furthermore, the different development of the endometrium in the proliferative phase seemed to differ in cases that ended up with thick or thin endometrium at embryo transfer (defined as >14 and <8 mm respectively). Therefore, it was decided to carry out a larger

retrospective study to characterize endometrial growth throughout the stimulation treatment (proliferative and early luteal phases) in women undergoing assisted reproduction cycles. Patients undergoing ovarian stimulation protocols for IVF were chosen for this study in order to ensure a homogeneous data set with careful sequential clinical and laboratory assessment.

Materials and methods

Study subjects

This was a single-centre retrospective study of all women who underwent assisted reproduction treatments at the University Hospital of Bari between September 2002 and September 2007. Only the first cycle of each patient was considered for this study ($n = 754$), to avoid any possible interference from previous stimulation (Friedler *et al.*, 1996). According to an internal protocol, all women underwent office hysteroscopy to evaluate the uterine cavity, with endometrial sampling in the early follicular phase 2 or 3 months before treatment. All patients with evidence of endometrial anomalies (polyps, hyperplasia, endometritis, synechiae, septum or submucous/intramural fibroids encroaching the endometrial cavity) were excluded from the analysis ($n = 37$). The study population was then made up of 717 women who received the same ovarian stimulation protocol after suppression with long acting gonadotrophin-releasing hormone (GnRH) agonists as previously described in detail (Lorusso *et al.*, 2004, 2005). Briefly, patients were down-regulated with a GnRH agonist (GnRHa, Decapeptyl 0.1 mg; Ipsen, Italy), starting in the mid-luteal phase (day 21) of the cycle. After down-regulation was achieved (serum oestradiol ≤ 30 pg/ml), ovarian stimulation was carried out with a fixed daily dose of 225 IU of recombinant FSH (rFSH, Gonal F; Serono, Switzerland), starting from days 2–3 of the cycle for the first 5 days. Thereafter, the dose was adapted depending on the ovarian response to the treatment. GnRHa was continued up to and including the day of HCG administration. A dose of 10,000 IU HCG (Profasi; Serono, Switzerland) was given intramuscularly for ovulation induction when two or more growing follicles ≥ 18 mm in diameter were present. Oocyte retrieval was carried out 35–36 h later by ultrasound-guided transvaginal puncture. Embryos were replaced on day 2 or day 3 after oocyte retrieval.

Longitudinal assessment of the endometrial response to the stimulation was performed by one of four gynaecologists with considerable experience in gynaecological ultrasound by high-resolution transvaginal ultrasonography using a 4–7 MHz transvaginal transducer on Acuson 128/XP (Mountain View, California) or Aloka ProSound alpha 5 machines. Endometrial thickness was measured on days 0 (baseline), 4, 7, 10, on the day of HCG administration, egg retrieval, and embryo transfer, with a minimum variation of ± 1 day allowed. Endometrial thickness was defined as the maximal distance between the echogenic interfaces of the myometrium and the endometrium, measured in the plane through the central longitudinal axis of the uterus.

Modifications in endometrial growth were assessed longitudinally after categorization of all patients into three

groups according to endometrial thickness at embryo transfer: thin (< 8 mm), medium (8–14 mm) or thick (> 14 mm) endometrium. Endometrial thickness cut-off values were defined according to an initial report on clinical pregnancy rates (Lamanna *et al.*, 2007). The relationship between endometrial growth and patient characteristics (age and body mass index) and stimulation parameters (length of stimulation, total rFSH dose administered, and oestradiol serum concentrations on the day of HCG administration) were studied. Additionally, it was decided to evaluate endometrial thickness in the subsequent (second) cycle in those cases where the treatment was repeated at least 6 months later using the same stimulation protocol ($n = 194$). These women were selected among those who had no evidence of pregnancy (either biochemical or clinical) and did not receive any oestrogen or progestin therapy during the interval between the two cycles. Such a long interval was chosen to avoid any residual stimulation from the previous cycle (Friedler *et al.*, 1996). The amount of gonadotrophins administered in the second cycle was based on the clinical response to the first treatment (total number of follicles developed, oocytes retrieved and endometrial growth).

Clinical pregnancy rates were reported for all groups.

Mathematical and statistical analyses

Categorical variables were compared with two-tailed chi-squared test with Yates correction or Fisher's exact test, as appropriate. Continuous variables were assessed by one-way analysis of variance between groups (ANOVA), if a Gaussian distribution was confirmed by the Kolmogorov–Smirnov test, or using the Mann–Whitney *U*-test. Differences among subgroups were evaluated with Tukey–Kramer multiple comparisons test.

Endometrial growth was assessed by studying the slope of each curve and the time at which the response (endometrial growth) reached 90% of the thickness at embryo transfer (settling time at 90%; ST_{90}) (Hanggi and Moschytz, 2000). The slope was evaluated as the difference between two consecutive measurements of endometrial thickness ($En_2 - En_1$; mm) divided by the temporal interval ($t_2 - t_1$; days) (this is known as difference quotient or Newton's quotient). The difference quotient (DQ) represents the acceleration (variation per unit of time interval) and the settling time shows how long the endometrium maintains significant growth.

The relationship between endometrial modifications and patient characteristics and stimulation parameters was assessed by linear regression. Data were analysed using GraphPad InStat (version 3.00, GraphPad Software Inc., San Diego, California, USA) and significance set at a *P*-value of < 0.05 .

Results

Demographic characteristics and baseline clinical data of patients are shown in **Table 1**. The groups were homogeneous for all considered parameters except age. Women who presented a thinner endometrium at embryo transfer were

older than those in the other groups ($P < 0.01$). Ovarian response to stimulation with gonadotrophins was similar in the three groups (**Table 1**). A parabolic trend in clinical pregnancy rates was confirmed as reported in **Table 1**.

Endometrial thickness was found to be significantly thinner ($P < 0.01$) in the group of women who ended up with a thin endometrium (<8 mm) compared with that of the other two groups in all days but day 0. All groups showed a parabolic trend of endometrial growth with a slow increase in the first third of the stimulation period, then an acceleration phase with a subsequent flattening of the slope (**Figure 1**). Differences between two subsequent measurements were more clearly visualized by means of DQ calculations (**Figure 2**). DQ represents the modification per day, thus the upward line indicates acceleration, whereas a downward tendency means slackening of growth. Cases that ended up with a thin endometrium (<8 mm) showed a positive trend until day 7, while the other two groups kept growing until day 10 and day 12 (for group 8–14 and >14 mm respectively). Moreover, substantial differences were found for maximum acceleration between the three groups ($P < 0.05$) being 0.53 mm/day on day 7, 0.85 mm/day on day 10, and 1.2 mm/day on day 12 in the thin, medium and thick groups respectively. Ninety per cent of the final potential of the endometrium (ST_{90}) was reached on average in 10.4 ± 2.3 , 11.3 ± 2.0 and 12.6 ± 1.2 days in the three groups ($P < 0.001$; **Figure 3**). These data indicate that the endometrium continued to grow for longer in those cases that ended up with a thick endometrium at embryo transfer.

Age was the only parameter studied that was related to endometrial growth ($P < 0.01$; **Table 1**), showing an inverse correlation with endometrial thickness at embryo transfer ($r = -0.16$, 95% confidence interval -0.49 to -0.18 ; $P < 0.001$).

Further analyses were carried out in the subgroup of patients ($n = 194$) that underwent a second stimulation protocol after at least 6 months (range 6–12 months). In all, 147 women (75.8%) showed a similar endometrial thickness at embryo transfer in the second cycle, remaining in the same thickness class (**Figure 4**). None of the patients who had a thin (<8 mm) endometrium in the first cycle showed a thick (>14 mm) endometrium in the second treatment (**Figure 4**), and *vice versa*. The amount of rFSH dose administered in the second cycle to women who had a poor endometrial response in the first stimulation (thickness at embryo transfer <8 mm) was significantly increased (3402 ± 1518 IU versus 3136 ± 1557 IU; $P < 0.05$). This allowed two-thirds (66.7%) of women to obtain a medium endometrial thickness in the second cycle (**Figure 4**). Only 18.4% of women with a medium (8–14 mm) endometrium in the first treatment presented a different thickness in the subsequent cycle, either thinner or thicker. It is interesting to note that patients with a thick endometrium in the first cycle showed a 56.3% probability of remaining in the same class, regardless of the total rFSH dose administered in the two cycles (2757 ± 1103 versus 2602 ± 938 IU in the first and second cycles, respectively; not significantly different). Nevertheless, differences found in endometrial thickness between the two consecutive cycles were within 3 mm in 72.1% of cases (**Figure 5**) in the whole study population.

Table 1. Demographic characteristics and clinical data of patients.

	Endometrial thickness (mm)		
	<8 (n = 59)	8–14 (n = 604)	>14 (n = 54)
Age (years)	37.2 ± 5.2	34.6 ± 4.8	34.2 ± 5.1
Body mass index (kg/m ²)	24.2 ± 3.0	24.5 ± 3.1	24.5 ± 3.9
Primary infertility	37 (62.7)	408 (67.5)	35 (64.8)
Duration of infertility (years)	4.6 ± 2.0	4.9 ± 2.7	4.4 ± 3.3
Aetiology			
Male factor	21 (35.6)	255 (42.2)	18 (33.3)
Tubal factor	17 (28.8)	154 (25.5)	14 (25.9)
Endometriosis	8 (13.6)	93 (15.4)	11 (20.4)
Anovulatory	8 (13.6)	59 (9.8)	8 (14.8)
Unexplained	5 (8.5)	43 (7.1)	3 (5.6)
Length of stimulation (days)	11.7 ± 1.8	11.6 ± 1.9	11.9 ± 1.4
Amount of recombinant FSH (IU)	2949 ± 1586	2719 ± 1019	2770 ± 846
Total number of follicles on HCG day (n)	10.9 ± 6.9	12.1 ± 6.3	11.0 ± 7.1
Number of follicles ≥ 18 mm on HCG day (n)	5.0 ± 3.9	6.3 ± 4.6	5.7 ± 2.8
Oestradiol on HCG day (pg/ml)	1374 ± 1001	1453 ± 973	1449 ± 808
Clinical pregnancy rate	12 (20.3)	167 (27.6)	10 (18.5)

Values are mean \pm SD or n (%).

Categorical and continuous variables were compared using the chi-squared or Fisher's tests and one-way analysis of variance or Mann–Whitney U-test, respectively.

*Women with the thinnest endometrium were significantly older than women in the other two groups ($P < 0.01$).

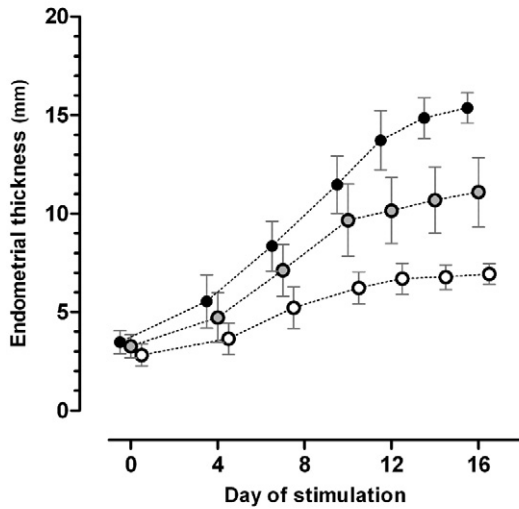


Figure 1. Longitudinal modifications of endometrial thickness throughout the stimulation treatment in the three groups: thin (<8 mm at embryo transfer, white circles), medium (8–14 mm at embryo transfer, grey circles) and thick (>14 mm at embryo transfer, black circles) endometrium. Significant differences ($P < 0.01$) at all days but day 0.

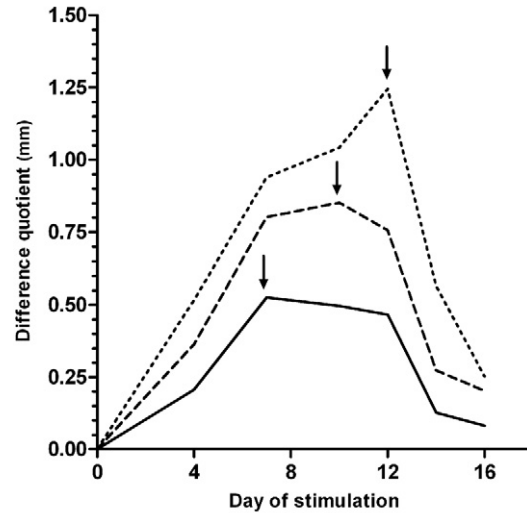


Figure 2. Difference quotient calculated for all groups according to the day of stimulation. Thin (unbroken line), medium (thick dotted line) and thick (thin dotted line) endometrium. Arrows indicate maximal activity.

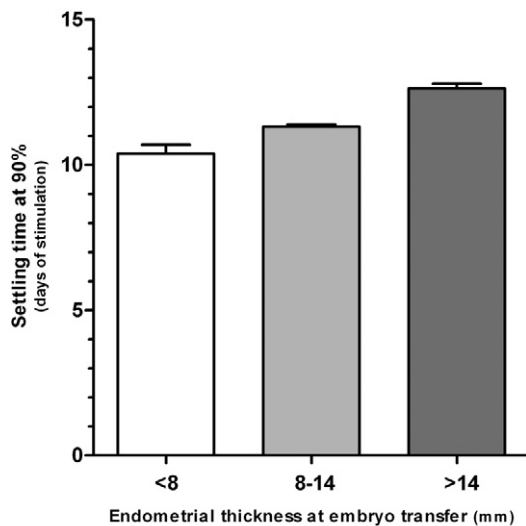


Figure 3. Settling time at 90% of the thickness at embryo transfer in the three groups.

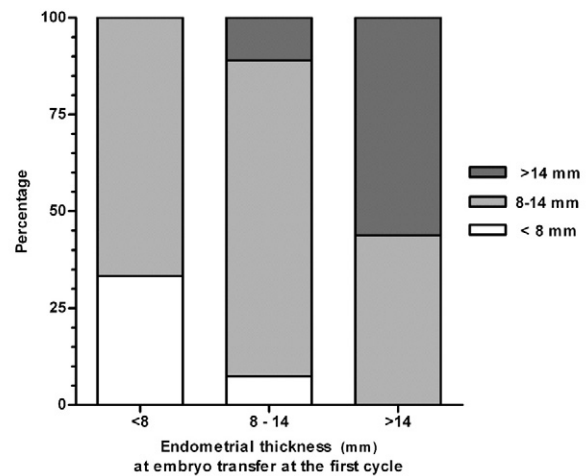


Figure 4. Class of endometrial thickness at embryo transfer in the second cycle expressed as percentages (y-axis).

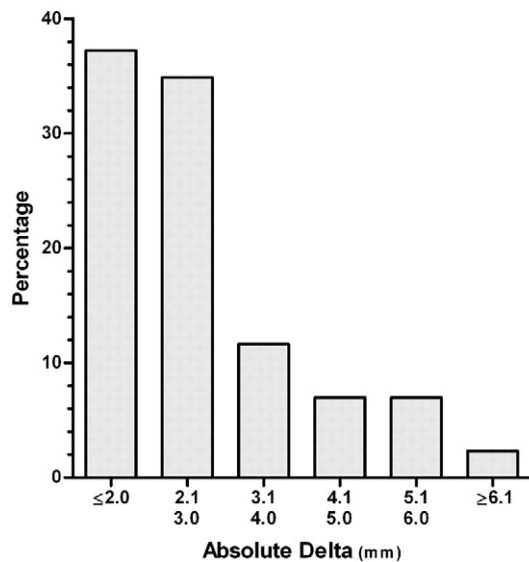


Figure 5. Differences in endometrial thickness between two consecutive cycles expressed as absolute value (delta).

Discussion

The findings seem to suggest that the endometrium has an individual intrinsic potential that can be expressed regardless of the stimulation protocol. In fact, different responses can be seen in women using the same protocol, with a significant probability for the endometrial thickness to be similar at embryo transfer in the same patient in the next cycle. Therefore, the data presented here support the hypothesis that individual factors (intrinsic properties of the endometrium and sub-endometrial vascularization) significantly affect endometrial growth.

This study follows up some observations reported in a recent article (Lamanna *et al.*, 2007) on the likelihood of a clinical pregnancy in assisted reproduction cycles according to endometrial thickness at embryo transfer. Since pregnancy rates had already been reported, the primary aim of this study was not to assess the prognostic value of endometrial thickness on clinical outcomes in IVF treatments, but to understand the longitudinal modifications that occur during endometrial growth. Several studies have attempted to identify sonographically the ideal endometrial thickness to predict implantation, resulting in small improvements for clinical practice (Friedler *et al.*, 1996; Rinaldi *et al.*, 1996). Most studies focused their attention on endometrial evaluation on the day of HCG administration to possibly optimize the timing of transfer by making modification to the stimulation protocol, oocyte retrieval scheduling and, in some cases, to delay embryo transfer (Friedler *et al.*, 1996). The findings show that the potential of the endometrium on the day of HCG administration is expressed by then, so little improvements can be achieved through protocol modifications later on. In a previous study, no endometrial thickness value with a good discriminatory ability to predict a clinical pregnancy was identified (Lamanna *et al.*, 2007). Nevertheless, a particularly thin endometrium at around the time of embryo transfer has been known for some time to have a strong negative predictive value for the subsequent occurrence of pregnancy (Gonen *et al.*, 1989; Shapiro *et al.*, 1993). Rogers and co-workers (1991) reported a correlation between histological aspects of the endometrium in early luteal phase and ultrasonography patterns in IVF cycles.

No relationship was found between the hormonal status and the stimulation treatment and endometrial growth. Notwithstanding this, the findings suggest that a small improvement can be obtained in women who showed a thin endometrium at embryo transfer in the first cycle by increasing the rFSH dose.

The biological basis of the decline in fecundity as female age increases has been associated with several factors such as reduced number and quality of oocytes (the ageing ovary), increased incidence of aneuploidy, and reduced intercourse frequency (Baird *et al.*, 2005). The findings highlight the importance of the endometrium in this situation, as poor growth may certainly impair fecundity (Gonen *et al.*, 1989; Shapiro *et al.*, 1993; Lamanna *et al.*, 2007).

Indeed, endometrial receptivity holds a key role for the achievement of a successful pregnancy, and several factors are involved in the endometrial maturational development and implantation (Cavagna and Mantese, 2003; Nardo, 2005). Abnormal or suboptimal endometrial development can be linked to ovarian stimulation treatment, as demonstrated by clinical and functional genomic studies (Martinez-Conejero *et al.*, 2007). The endometrium from ovarian stimulation cycles shows a shift in time in the differentiation, associated with a delay in the regulation of gene expression necessary for the formation of a receptive endometrium. Furthermore, a complex series of molecular and cellular interactions are involved in the implantation process that has to happen within a definite time frame (Guzeloglu-Kayisli *et al.*, 2007). The identification of this 'optimal' interval cannot be made unless through an invasive approach (biopsies or endometrial fluid sampling). In contrast, ultrasound evaluations of the endometrial aspect (thickness, pattern and vascularity) are not invasive and can be performed repeatedly, giving an idea of function as well as structural development (Killick *et al.*, 2007).

The application of mathematical analyses (DQ and ST₉₀) allowed understanding of the dynamic patterns of endometrial growth throughout the proliferative and early luteal phases. For instance, it was demonstrated that 'thick' endometria kept growing faster (DQ) and longer (ST₉₀) than other types during

the stimulation. Endometrial evaluation by ultrasonography appears to be a non-specific parameter for the prediction of conception, although it certainly is a useful clinical tool to follow up stimulation protocols.

The strength of the present study is that: (i) all patients received the same ovarian stimulation protocol; (ii) only the first cycle was considered for longitudinal modifications of the endometrium, as previous stimulations may interfere with endometrial growth (Friedler *et al.*, 1996); and (iii) the actual number of observations entered into the analysis was 6377 (5019 and 1358 for the first and second cycles respectively), as seven scans were considered for all patients ($n = 717$ first cycles; $n = 194$ second cycles). The longitudinal evaluation of endometrial growth presented in this study offers more information than a single observation on the day of HCG administration (Richter *et al.*, 2007) or at embryo transfer (Rashidi *et al.*, 2005; Esmailzadeh and Faramarzi, 2007). Indeed, this a retrospective study, and therefore has design limitations, such as the impossibility of prospectively modifying the stimulation protocol to assess potential modifications in endometrial growth.

In this study, the endometrial growth was extensively studied in the proliferative and early luteal phases in assisted reproduction cycles. It seems that influencing the endometrial growth is possible, even if by little (usually within 3 mm), as demonstrated in the second cycle of women with a thin endometrium at the first cycle. Further studies are needed to clarify the biological modifications that underlie endometrial growth.

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