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Endometrial ultrasonography as a predictor of pregnancy in an in-vitro fertilization programme

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The endometrial pattern and thickness were analysed by ultrasonography in 139 cycles stimulated for in-vitro fertilization (IVF) on the day of administration of human chorionic gonadotrophin (HCG). A semi-programmed schedule based on the pill + clomiphene citrate + human menopausal gonadotrophin (HMG) was used in all cycles. On the day of HCG administration, endometrial pattern and thickness were assessed with an Ultramark 4 (ATL) ultrasound equipped with a 5 MHz vaginal probe. Endometrial pattern I (a 'triple-line' multilayer) was observed in a total of 105 cycles (76%), and pattern II (fully homogeneous and hyperechogenic in relation to myometrial tissue) in 34 (24%). The incidence of clinical pregnancy did not differ ($P = 0.52$) between the groups with endometrial patterns I (23.8%) and II (29.4%). Endometrial thickness on the day of HCG administration in the group with pattern I (8.4 ± 1.9 mm) was similar ($P = 0.96$) to that observed in the group with pattern II (8.4 ± 2.0 mm). In addition, the endometrial thickness of the patients who became pregnant (8.0 ± 1.7 mm) did not differ ($P = 0.15$) from that of women who did not achieve pregnancy (8.6 ± 2.0 mm). The conclusion from the present data is that ultrasonographic analysis of endometrial thickness and refringency on the day of HCG administration had no predictive value for conception in IVF cycles.

Key words: endometrium/in-vitro fertilization/predictor/pregnancy

Introduction

Despite the advances in the understanding and manipulation of human reproductive processes, the biological mechanisms which regulate nidation are still obscure. This is particularly important for those who work in the area of assisted reproduction, especially with in-vitro fertilization (IVF), which does not always lead to conception despite recent progress in ovarian stimulation and egg collection techniques, laboratory embryo culture, and embryo transfer.

Echography has been recently utilized for better development of this field, especially after the advent of the vaginal transducer. Vaginal ultrasound has permitted earlier visualization of pregnancy and of fetal heartbeats, as well as more accurate monitoring of follicular growth and ovulation. In addition to these applications, the vaginal transducer provides a non-aggressive visualization of endometrial characteristics, expressed in terms of patterns of sound reflection (refringency) and of thickness. However, the real value of this examination in terms of clinical practice continues to be uncertain despite several reports which have related ultrasonographic images of the endometrium to pregnancy (Rabinowitz *et al.*, 1986; Gonen and Casper, 1990; Ueno *et al.*, 1991; Dickey *et al.*, 1992; Khalifa *et al.*, 1992).

Cohen *et al.* (1984) have demonstrated a lack of correlation between oestradiol and progesterone concentrations in the luteal phase and endometrial development, emphasizing the limited value of hormonal measurements in the assessment of the endometrial response.

The object of the present study was to assess by ultrasonography the different endometrial patterns and thicknesses on the day of human chorionic gonadotrophin (HCG) administration and to determine whether any correlation exists between these parameters and the later occurrence of clinical pregnancy during IVF cycles.

Materials and methods

The endometrial pattern and thickness on the day of HCG administration were analysed in 139 stimulated cycles of 121 patients included in an IVF–embryo transfer programme. Most of the couples (78%) presented a tubal–peritoneal factor as the major cause of infertility, followed by idiopathic aetiologies (10%), endometriosis (9%), and male factor (3%).

A semi-programmed schedule was used for ovarian stimulation as follows: a low-dose oral contraceptive (30 μg ethynyl-oestradiol and 75 μg gestodene; Gynera, Berlimed–Shering, São Paulo, Brazil) was used on the first day of the menstrual cycle preceding the ovulatory stimulus and continued for a minimum of 21 days and maximum of 28 days. Ovarian stimulation was obligatorily started on the fifth day after discontinuation of the contraceptive pill, with clomiphene citrate given at a dose of 100 mg/day for 5 consecutive days. Also on the fifth day, human menopausal gonadotrophin [HMG: follicle stimulating hormone (FSH) = 150 IU and luteinizing hormone (LH) = 150 IU per day] was administered on alternate days (minimum 4 ampoules), and dexamethasone was also started at a dose of 0.5 mg/day and maintained until embryo transfer.

The assessment of ovarian follicular development was started

Fig. 1. Endometrial pattern I: endometrium with layers, consisting of a middle line and of two clearly visible external lines (arrow), with a black hypo-echogenic region between lines.

Fig. 2. Endometrial pattern II: fully homogeneous endometrium (arrow), hyperechogenic compared to myometrial tissue.

on the eighth day of stimulation using an Ultramark 4-ATL ultrasound equipped with a 5 MHz vaginal transducer (Advanced Technology Laboratories, Bothell, WA, USA). When two or more follicles of ≥ 17 mm diameter were identified, in the absence of endogenous LH release, HCG was administered i.m. at a dose of 10 000 IU.

The endometrial pattern was documented with photographs taken with a type FTI-210 Fuji video processor (Fuji, Japan) and stored for later analysis. Two ultrasonographers were involved and agreement over the first observation was 95%. Endometrial thickness was also measured by two ultrasonographers, with an inter-observer coefficient of variation of 3.7%. The intra-observer coefficient of variation was 1.2% for observer 1 and 1% for observer 2.

On the day of HCG administration, maximal endometrial thickness was measured on a plane centrally located in relation to the longitudinal axis of the uterine body. The endometrjal pattern was also analysed and classified into two types: pattern I, a multiple layer presenting 'three defined lines' consisting of external hyperechogenic lines at the limit between endometrium and myometrium, and a central line of identical characteristics separated by a hypo-echogenic central region corresponding to endometrial tissue (Figure 1); pattern II, a fully homogeneous endometrium, hyperechogenic in relation to the myometrial tissue (Figure 2). Follicular puncture and aspiration was performed on patients sedated with ethomidate (Hyponomidate, São Paulo, Brazil) 34–36 h after HCG using a 17G needle guided by vaginal transducer. Embryo transfer was performed 40–44 h after the puncture. Maintenance of the luteal phase occurred in all cases when HCG or progesterone was used in the injectable form.

Serum β-HCG measurement was performed on the 14th day after embryo transfer to diagnose initial gestation. In addition, a vaginal ultrasonography examination was programmed for the sixth week of pregnancy to detect the presence of fetal heartbeats (clinical gestation).

Data are reported as means ± SD and were analysed statistically by the χ^2 and Student's t-tests, with the level of significance set at $P < 0.05$.

Table I. Distribution of age, number of oocytes collected and number of embryos transferred for subjects presenting endometrial patterns I and II[a]

Population variables	Endometrial pattern		Significance (Student's t-test)
	I[b]	II[b]	
Age (years)	31.4 ± 4.0	32.2 ± 4.7	$P = 0.36$
Oocytes retrieved	5.6 ± 3.0	6.0 ± 3.6	$P = 0.56$
Embryos transferred	3.0 ± 1.7	3.2 ± 1.4	$P = 0.79$

[a]See legends to Figures 1 and 2.
[b]Figures are mean ± SD.

Table II. Incidence of clinical pregnancies in in-vitro fertilization (IVF) cycles in relation to endometrial pattern[a]

Pattern	Pregnancy	
	Absent	Present
I	80	25
II	24	10
χ^2-test: $P = 0.52$		

[a]See legends to Figures 1 and 2.

Results

Patient age, number of oocytes collected and number of embryos transferred were analysed and compared with the endometrial pattern. Patients showing ultrasonographic pattern I had a mean age of 31.4 years (range 23–44 years) versus 32.2 years (range 24–40 years) for patients with pattern II. The mean number of oocytes collected was similar for both groups (5.6 for pattern I and 6.0 for pattern II), with 1–18 oocytes obtained per puncture for pattern I and 1–14 oocytes for pattern II. The number of embryos transferred was the same for the two groups, with a pooled mean of 3.1 embryos and a range of 1–5. Statistical

analysis did not show significant differences between groups for the above parameters (Table I).

Endometrial pattern I was observed in a total of 105 cycles (76%), and pattern II in 34 (24%). A total of 25 clinical pregnancies (23.8%) was obtained among the subjects with endometrial pattern I, and a total of 10 (29.4%) was obtained among the subjects with endometrial pattern II (Table II). The difference in pregnancy rate was not statistically significant ($P = 0.52$).

The endometrial thickness observed on the day of HCG administration was 8.4 ± 1.9 mm for the group with pattern I, a value that did not differ significantly ($P = 0.96$) from that observed for the group with pattern II (8.4 ± 2.0 mm). Furthermore, the endometrial thickness of the patients who became pregnant (8.0 ± 1.7 mm) did not differ ($P = 0.15$) from that of the patients who did not become pregnant (8.6 ± 2.0 mm).

Considering the data as a whole for both groups, endometrial thickness varied widely from 5 to 15 mm. However, pregnancy only occurred when thickness on the day of HCG administration was within the 6−12 mm range.

Discussion

Although recent advances in the treatment of infertility by assisted reproduction techniques have made it easy to obtain human embryos in the laboratory, this procdure has not been accompanied by comparable improvements in pregnancy rates. In general, several factors may be directly related to successful achievement of pregnancy in IVF, including the quality and number of transferred embryos and patient age. On the other hand, it is accepted by many investigators that the endometrial environment may also be of fundamental importance for the occurrence of normal nidation, i.e. its quality and state of preparation may affect the successful achievement of pregnancy after IVF.

Ultrasound is a non-invasive tool used to obtain repeated information. The ultrasonographic aspect varies throughout natural or stimulated ovulatory cycles. During the proliferative phase, the endometrium presents a strong hyperechogenic pattern as compared to myometrial tissue due to the presence of more developed vessels and glands, and thickness increases progressively during this phase. When ovulation is near, with a consequent increase in oestradiol concentration, the endometrium tends to change in appearance, presenting external refringent lines which surround a hypo-echogenic core, where a central line also occurs which is hyperechogenic like the external ones. However, it should be pointed out that the persistence of the high-refringency pattern or the presence of a mixed pattern is not uncommon. Soon after ovulation, the 'three-line' pattern is still present but changes progressively, acquiring greater refringency and thickness associated with an increase in the interfaces, which is evidence of the secretory activity of the endometrium (Smith *et al.*, 1984; Glissant *et al.*, 1985; Bonilla-Musoles *et al.*, 1988; Gonen *et al.*, 1989; Hackeloer, 1991).

The predictive value of the endometrial aspect during the process of ovarian stimulation could be established on any day of the cycle but the day of HCG administration is of greatest clinical importance. If an ideal endometrial pattern which will facilitate the occurrence of pregnancy is identified at that time, some action could be taken to optimize the chance of success, such as postponing the use of HCG and continuing stimulation. Recognition of a predictive value of endometrial refringency and thickness on the days following HCG administration would only be of theoretical relevance, since the entire process of oocyte maturation by then has been triggered and the only alternative outcome would be cancellation of the cycle.

In the present study, no significant differences were observed in patient age, number of oocytes collected or number of transferred embryos between the two endometrial patterns. Thus, these parameters did not affect pregnancy rates in either group.

On the other hand, the absence of hormonal measurements during the control of the ovulatory process prevented a comparison between circulating steroids and endometrial pattern or thickness. This deficiency, however, does not affect the conclusions of the present study, since other investigators have detected no significant differences in hormonal concentrations between different endometrial patterns and thicknesses (Rabinowitz *et al.*, 1986; Gonen and Casper, 1990; Ueno *et al.*, 1991; Khalifa *et al.*, 1992).

The present data did not indicate a positive or negative relation for any of the endometrial patterns analysed, in contrast to data reported by Welker *et al.* (1989), Gonen and Casper (1990), Ueno *et al.* (1991) and Dickey *et al.* (1992). In the first three of these reports, the endometrium was examined on the day of, or the day before, oocyte collection, possibly explaining the discrepancy in the results. However, Dickey *et al.* (1992) analysed the endometrium on the day of HCG administration and observed a significant correlation between pregnancy and endometrial pattern. The authors stated that this relationship could be used to predict the final outcome, but only for the techniques of gamete-intra-Fallopian transfer and tubal embryo transfer. In the case of IVF, these authors did not observe a significant difference in pregnancy rates between three identified endometrial patterns.

On the other hand, our data agree with those of Khalifa *et al.* (1992) who reported the absence of significant differences ($P > 0.05$) in IVF pregnancy rates between patients showing endometrial pattern B (equivalent to pattern I in the present study) or pattern A (equivalent to pattern II in the present study) on the day of HCG administration.

The classification used in the present study and in the study by Khalifa *et al.* (1992) differed from that used by others, who also considered a pattern III, or intermediate pattern represented by a hyperechogenic central line accompanied by a texture almost iso-echogenic with the myometrium. However, as also performed by Welker *et al.* (1989) and Gonen and Casper (1990), in the final analysis this pattern was associated with the fully hyperechogenic one (pattern II of our study) and no individual value was attributed to it.

As to endometrial thickness, our findings indicate the existence of a minimum thickness limit of 6 mm on the day of HCG administration for pregnancy to occur with which Welker *et al.* (1989), Gonen and Casper (1990) and Dickey *et al.* (1991) also agree. In contrast, Glissant *et al.* (1985), Fleischer *et al.* (1986), Rabinowitz *et al.* (1986) and Ueno *et al.* (1991) did not observe any correlation between endometrial thickness and pregnancy rates.

In conclusion, on the basis of the present data, ultrasonographic analysis of endometrial thickness and refringency on the day of HCG administration had no predictive value for conception in IVF cycles.

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