Human Reproduction vol.12 no.11 pp.2515–2518, 1997

Endometrial ultrasonography as a predictor of pregnancy in an in-vitro fertilization programme after ovarian stimulation and gonadotrophin-releasing hormone and gonadotrophins

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A total of 150 patients were submitted to in-vitro fertilization (IVF) and endometrial pattern and thickness were assessed on the day of administration of human chorionic gonadotrophin (HCG). Ovarian stimulation was performed with gonadotrophins [human menopausal (HMG) or follicle stimulating hormone (FSH)] after down-regulation with leuprolide acetate. The endometrium was evaluated by vaginal ultrasound and classified into two groups: pattern I (a 'triple line' multilayer) and pattern II (fully homogeneous and hyperechogenic in relation to myometrial tissue). Pattern I was detected in 129 cycles (86%) and pattern II in 21 cycles (14%). The clinical pregnancy rates per cycle were similar (P = 0.79) for pattern I (29.4%) and pattern II (33.3%). There was no significant difference (P = 0.40) in the number of miscarriages between patients with pattern I and those with pattern II. Endometrial thicknesses were similar (10.3 ± 2.0 mm and 11.2 ± 3.1 mm) (P = 0.25). The thicknesses were similar (P = 0.14) for pregnant (10.8 ± 2.1 mm) and non-pregnant (10.2 ± 2.2 mm) women, but no pregnancies occurred when thickness was <7.0 mm. However, there was a significant difference (P = 0.04) between pregnant (10.8 ± 1.9 mm) and non-pregnant (10.0 ± 1.9 mm) women who showed pattern I. The conclusions from these data are that endometrial ultrasound in terms of pattern and thickness is of no prognostic value in IVF cycles on the day of administration of HCG. However, a minimum thickness has to be achieved for pregnancy to occur (7.0 mm). The presence of pattern I appears more likely to favour pregnancy.

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

Introduction

Ultrasonography is a non-invasive method that can provide serial information about the characteristics of the endometrium. In general, the pattern of sound reflection (refringency) and the thickness of the endometrial line are analysed. These parameters are believed to be valid for the evaluation of endometrial receptivity in programmes of in-vitro fertilization (IVF).

Several investigators have postulated the existence of a correlation between the 'degree of endometrial development' evaluated by ultrasound and the probability of embryo implantation (Sher *et al.*, 1993; Coulam *et al.*, 1994; Serafini *et al.*, 1994; Bustillo *et al.*, 1995; Noyes *et al.*, 1995). However, the correlation proposed in these studies is not universally accepted. Other authors did not consider the examination of the endometrial echogenic pattern to be useful as a predictive factor for embryo implantation (Khalifa *et al.*, 1992; Eichler *et al.*, 1993; Mardesic *et al.*, 1995).

Oliveira *et al.* (1993) analysed the endometrial pattern in 130 IVF cycles on the day of administration of human chorionic gonadotrophin (HCG). Ovarian stimulation was performed using a semi-programmed scheme (an oral hormonal contraceptive, clomiphene citrate and human menopausal gonadotrophin). No significant differences in endometrial pattern or thickness were detected between the patients who became pregnant and those who did not.

The objective of the present study was to assess the prognostic value of endometrial ultrasonography in IVF cycles when ovarian stimulation was performed using a gonadotrophin-releasing hormone (GnRH) analogue in combination with gonadotrophins.

Materials and methods

A total of 115 patients underwent 150 IVF cycles and endometrial thickness and pattern were assessed on the day of HCG administration. The major indications for the IVF programme in this population were tubal factor in 42.6% of cases, unexplained infertility in 26%, endometriosis in 12.2%, male factor according to the World Health Organization (WHO) criteria in 9.6%, and immunological factors in 2.6%. Two or more associated factors were present in 7% of cases.

For the protocol of ovarian stimulation, leuprolide acetate was administered at a dose of 1.0 mg/day from the mid-luteal phase of the preceding cycle. Administration of gonadotrophins [human menopausal gonadotrophin (HMG) and/or pure follicle stimulating hormone (FSH)] commenced 14 days later if menstruation had occurred. The gonadotrophin dose varied according to the ovarian response of the patients.

Evaluation of ovarian follicle development was started on day 8 of stimulation using ultrasound with a 5 MHz vaginal transducer (Ultramark 4: ATL Advanced Technology Laboratories Inc., Bothell, WA, USA) or a 7 MHz vaginal transducer (RT 3200 Advantage II: GE Medical Systems, Milwaukee, WI, USA). When at least two follicles measuring 17 mm in widest diameter were identified, 10 000 IU HCG was administered i.m.

Endometrial thickness was measured on the central plane of the longitudinal axis of the uterine body starting from the interface between endometrium and myometrium. The total thickness from one interface to the other was considered. The ultrasonographers

Table I. Patient age distribution, number of oocytes collected and embryos transferred in patterns I and II

Variables	Endometrial pattern		Significance
	I	II	
Age (years)	34.6 ± 4.7	35.8 ± 3.7	$P = 0.11$*
Oocytes	8.0 ± 5.5	8.4 ± 5.0	$P = 0.47$**
Embryos	3.0 ± 1.2	3.4 ± 0.9	$P = 0.18$**

*Student's *t*-test.
**Mann–Whitney test.

Table II. Clinical pregnancy rate and subsequent abortion rate in the IVF cycles in relation to endometrial pattern

Pattern	Clinical pregnancy		Abortions
	n	% per cycle	*n*
I	38	29.4	13
II	7	33.3	1

Fisher test, $P = 0.79$ for clinical pregnancy rate, $P = 0.40$ for abortion rate.

involved in the process of endometrial evaluation presented an inter-observer coefficient of variation that did not exceed 3.7%. The intra-observer coefficient of variation ranged from 1.0 to 1.2%.

The endometrial pattern was classified into two types (Oliveira *et al.*, 1993): pattern I corresponded to multilayers presenting 'three marked lines' consisting of hyperechogenic external lines in the limit between endometrium and myometrium, and of a central line of identical characteristics separated by a central hypoechogenic region corresponding to endometrial tissue. Pattern II was fully homogeneous and hyperchogenic in relation to the adjacent myometrial tissue, also included the isoechogenic pattern.

Follicular punctures were performed 34–36 h after HCG administration using a 17G needle guided by a vaginal transducer, with the patient under sedation. The technical procedures used to handle the gametes and embryos followed the basic norms of our service (Franco Jr *et al.*, 1995).

Data are reported as means ± SD and were analysed statistically by the Fisher test, Student's *t*-test and Mann–Whitney test, with the level of significance set at $P < 0.05$.

Results

Endometrial pattern I was observed in a total of 129 cycles (86%) and pattern II in a total of 21 cycles (14%). There was no significant difference between age of patient, number of oocytes collected or number of embryos transferred between patients with pattern I and those with pattern II (Table I).

The overall pregnancy rate per oocyte collection was 30%. There was no significant difference in pregnancy rate between patients with pattern I and pattern II (Table II). Fourteen pregnancies (31.1%) terminated in abortion before week 12, with no significant difference ($P = 0.40$) between the two endometrial patterns (Table II). The endometrial thickness of patients who aborted (10.7 ± 2.0 mm) was similar ($P = 0.46$) to that of patients with normal pregnancy outcomes (10.8 ± 2.1 mm).

Endometrial thickness on the day of HCG administration ranged from 6.0 to 17 mm, and pregnancy was obtained within the 7.0–16 mm range. The endometrial thickness of patients who became pregnant (10.8 ± 2.1 mm) did not differ significantly from that of patients who did not become pregnant (10.2 ± 2.2 mm). The endometrial thickness of patients with endometrial pattern I (10.3 ± 2.0 mm) did not differ significantly ($P = 0.25$) from that of patients with endometrial pattern II (11.2 ± 3.1 mm).

Table III. Endometrial thickness in patterns I and II of pregnant and non-pregnant women

Pattern	Pregnancy		Significance*
	Absent	Present	
I	10.0 ± 1.9 mm	10.8 ± 1.9 mm	$P = 0.04$
II	11.5 ± 3.2 mm	10.7 ± 3.0 mm	$P = 0.62$

*Mann–Whitney test.

The endometrial thickness of pattern I was greater ($P = 0.04$) in the patients who became pregnant (10.8 ± 1.9 mm) than in the patients who did not (10.0 ± 1.9 mm). In pattern II, there was no significant difference in endometrial thickness between the patients who became pregnant and those who did not (Table III).

Discussion

The ultrasonographic aspect of the endometrium varies in terms of thickness and echogenicity throughout the natural or stimulated ovulatory cycle. During the proliferative phase the endometrium is initially thin and presents a strongly hyperechogenic pattern in relation to myometrial tissue. As follicular development progresses, endometrial thickness increases and tends to change in aspect with the onset of ovulation and the consequent increase in oestradiol concentrations. The endometrium then starts to exhibit refringent external lines surrounding a hypoechogenic core in which a central hyperchogenic line is present (multilayer or three-line endometrium). The hypoechogenic pattern can still be observed soon after ovulation but changes progressively, acquiring greater refraction (hyperechogenicity) and increased thickness typical of secretory activity of the endometrium (Sakamoto, 1985; Rabinowitz *et al.*, 1986). However, it is important to point out that a mixed pattern is not uncommon. Some authors have also reported the presence of fluid material inside the endometrial cavity during the immediate post-ovulatory period (Ueno *et al.*, 1991).

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 (isoechogenic), or intermediate pattern represented by a hyperechogenic central line accompanied by a texture almost isoechogenic with the myometrium. However, similar to the results of 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.

The predictive value of the endometrial aspect during the process of ovarian stimulation can be established on any day of the cycle. However, the day of HCG administration is of

greater clinical importance because, if an ideal endometrial pattern is not observed on this date, some measure can be taken, such as postponement of HCG administration and continued ovarian stimulation or freezing the embryos obtained for future transfer under better endometrial conditions. In the present study, analysis of the endometrium was performed on the day of HCG administration, as has also been done by others (Check *et al.*, 1991; Dickey *et al.*, 1992; Khalifa *et al.*, 1992; Eichler *et al.*, 1993; Sher *et al.*, 1993; Coulam *et al.*, 1994).

The presence of different ultrasonographers may interfere with data analysis, but the inter- and intra-observer evaluations performed here did not reveal variations that would impair the results.

The absence of hormonal measurements during the control of the ovulatory process prevents a comparison between the level of circulating steroids and the pattern and thickness of the endometrium. However, this deficiency does not affect the conclusions, considering that other investigators did not detect a significant difference in hormonal concentration between the various endometrial patterns and thicknesses (Gonen and Casper, 1990; Ueno *et al.*, 1991; Khalifa *et al.*, 1992).

On the other hand, the data obtained did not indicate a positive or negative correlation between endometrial pattern and clinical pregnancy rates, in contrast to the data reported by Welker *et al.* (1989), Gonen and Casper (1990), Check *et al.* (1991, 1993b), Ueno *et al.* (1991), Dickey *et al.* (1992, 1993a,b), Sher *et al.* (1993), Coulam *et al.* (1994), Serafini *et al.* (1994), Bustillo *et al.* (1995), Noyes *et al.* (1995) and Bohrer *et al.* (1996). However, the present data agree with those reported by Check *et al.* (1993a) in a study of cycles with oocyte donation, and with those reported by Khalifa *et al.* (1992), Eichler *et al.* (1993) and Mardesic *et al.* (1995) in a study of IVF cycles. Similarly, Oliveira *et al.* (1993), using clomiphene for ovarian stimulation in IVF cycles, did not detect a significant difference between endometrial patterns in terms of pregnancy rate.

In the present study, the abortion rate did not differ significantly between the two echogenic patterns. In contrast, Serafini *et al.* (1994) observed a lower abortion rate among patients with a hypoechogenic pattern compared to patients with hyperechogenic and intermediate patterns.

With respect to thickness, the present findings indicate that a minimum thickness of 7.0 mm on the day of HCG administration is necessary for pregnancy to occur. However, no difference in endometrial pattern was observed between the patients who became pregnant and those who did not. No maximum value that might be considered limiting for the occurrence of pregnancy was observed, in agreement with other investigators.

Our results are closely similar to those reported by Isaacs Jr *et al.* (1996) who, in a study of ovarian stimulation with human menopausal gonadotrophin (HMG) for IVF, identified a minimum value of 7.0 mm for the occurrence of pregnancy, with no difference in endometrial thickness between the patients who became pregnant and those who did not. Several authors also reported no relationship between endometrial thickness and pregnancy rates (Welker *et al.*, 1989; Eichler *et al.*, 1993; Coulam *et al.*, 1994; Serafini *et al.*, 1994; Bustillo *et al.*, 1995; Mardesic *et al.*, 1995).

Some investigators, however, disagree and attribute a prognostic value for the occurrence of pregnancy to endometrial thickness (Gonen and Casper, 1990; Check *et al.*, 1991; Dickey, 1992; Abdalla *et al.*, 1994; Rinaldi *et al.*, 1996).

Abortion rate and endometrial thickness also showed no correlation, in agreement with data reported by Serafini *et al.* (1994). However, when we analysed endometrial thickness separately in patients with endometrial pattern I (hypoechogenic), we observed greater thickness ($P = 0.04$) in patients who became pregnant compared to those who did not. These results are similar to those reported by Sher *et al.* (1993), who defined a hypoechogenic endometrium with a thickness ≥9.0 mm as the ideal response, with a greater probability of pregnancy.

In conclusion, separate ultrasonographic analysis of endometrial thickness and echogenic pattern on the day of HCG administration were of no prognostic value in terms of the occurrence of pregnancy or of abortion. However, a minimal endometrial thickness of 7.0 mm must be reached on this day for pregnancy to occur. Apparently, the thicker the hypoechogenic pattern, the more favourable it is to the occurrence of pregnancy.

Acknowledgements

The authors wish to thank Mrs Elettra Greene for revising the English text.

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Received on April 2, 1997; accepted on August 14, 1997