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Correlation of ultrasound assessment of endometrial growth and plasma steroid concentrations during superovulation for *in vitro* fertilization

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Summary

Endometrial and follicular development were investigated by ultrasound in 25 normally cycling women who received clomiphene citrate and human menopausal gonadotrophin to induce multiple follicular development for *in vitro* fertilization. Ultrasonic determination of endometrial thickness and reflectivity grading and follicular number were correlated with daily concentrations of estradiol (E_2) and progesterone (p) in peripheral serum. Serum E_2 showed a better positive correlation with endometrial thickness than with total number of developing follicles. There was a significant inverse correlation between endometrial thickness and plasma p concentration. The E_2 value per follicle and E_2/p ratio were both weakly correlated respectively with endometrial thickness. These data indicate that ultrasound determination of endometrial thickness is a useful ultrasonic parameter for monitoring ovarian function for *in vitro* fertilization. However, endometrial thickness should always be combined with total number of developing follicles, in order to reach decisions concerning timing of oocyte recovery.

Résumé

Nous avons etudie, au moyen de l'Ultrason, le developpement de l'endometre et des follicules chez 25 femmes a cycle regulier auxquelles ont ete administre de la citrate de clomiphene et de la gonadotrophine afin de provoquer une action folliculaire intense en vue de la fecondation in vitro. L'epaisseur de l'endometre, l'indice de reflectivite ainsi que le nombre de follicules ont ete mis en correlation avec les concentrations quotidiennes d'estradiol (E_2) et de progesterone (p) dans le serum. En utilisant le serum E_2, la variable epaisseur de l'endometre a revele une correlation plus grande que la variable total des follicules en developpement. Nous avons constate une correlation inverse significative entre l'epaisseur endometriale et la concentration de la plasma p. La valeur E_2 par follicule et la rapport E_2/p indiquent une faible epaisseur endometriale. Ces donnees montrent que la determination par l'Ultrason de l'eppaisseur endometriale est un parametre ultrasonique pour l'observation de la fonction ovarienne lors de la fecondation in vitro. Toutefois, l'epaisseur endometriale devrait normalement etre combinee avec le total des follicules en developpement afin d'aboutir a des reactions aptes a declencher la reprise des oocytes.

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Introduction

The monitoring of ovarian function is an essential step during superovulation for *in vitro* fertilization. It allows effective evaluation of ovarian follicular development for the optimal timing of human chronic gonadotrophin (HCG) administration. The method initially used for monitoring ovarian follicular development was the rapid measurement of estrogen in blood or urine [1–4]. Ultrasound determination of follicular diameter [5] and volume [4] have also been used. Close correlations have been reported to exist between follicular diameter and plasma estradiol concentrations [6]. An association has also been shown between plasma estradiol levels and total number of developing follicles [7]. Recently, we reported the use of ultrasound assessment of qualitative and quantitative endometrial changes

to evaluate ovarian function and to optimize HCG administration in our IVF program [8]. The purpose of this study was to evaluate the correlation between these ultrasonic endometrial changes and peripheral steroid concentrations during stimulated cycles in ovulatory patients who received clomiphene citrate and human menopausal gonadotrophin to induce multiple follicular development for *in vitro* fertilization.

Patients and methods

Patients

Twenty-five cycles in 25 women undergoing superovulation for *in vitro* fertilization (IVF) at the Wellington Hospital, London were used for the study. The women were aged between 24 and 42 years (mean 28.5 years) and had regular ovulatory menstrual cycles. The median length of their menstrual cycles was 28 days with a range of 26–29 days. The indications for IVF treatment included irreversible tubal damage in 23 patients and unexplained infertility in two.

Methods

All patients received clomiphene citrate and human menopausal gonadotrophin in a sequential regime. Clomiphene citrate (Clomid, Merrell Pharmaceuticals, Slough, UK) was given daily in a dose of 100 mg from days 3 to 7 of the cycle and 150 IU of human menopausal gonadotrophin (Pergonal, Serone Laboratories Ltd., Welwyn Garden City, UK) was given daily from day 7. Ultrasound examination and blood sampling were commenced on the 10th menstrual day and repeated every day until 5,000 IU of HCG was administered.

Ultrasound examination was performed with a Diasonics Mechanical Sector Scanner (Model DRFL, Diasonics Inc., Sunnyvale, C.A.) and by a single sonologist. Both ovaries were visualised as semi-solid ovoid structures in the pelvis; the number and diameter of developing Graafian follicles within the ovaries were determined. The uterus and endometrium were then scanned. The grey scale reflectivity of the endometrium was used to grade the degree of its development as previously described [11]. Grade D was the least mature endometrium and was characterised ultrasonically by a black (anechoic) region in the presence of a midline echo. Grade C endometrium showed a solid area of reduced reflectivity and was darker than the surrounding myometrium. Grade B endometrium had comparable reflectivity to that of the myometrium while Grade A endometrium (the most mature) showed an increased reflectivity pattern when compared with that of the myometrium.

At the same time, the maximum thickness of the endometrium on both sides of the midline was measured in the plane through the central longitudinal axis of the uterine body. Endometrial thickness and follicular diameter were measured the same day using omnidirectional calipers (velocity, 1540 m/sec) superimposed on the "frozen" ultrasound image.

Blood samples were taken in plain tubes soon after ultrasound examination, allowed to clot, centrifuged and separated into sera. Samples were stored at –20°C until assayed in a single batch for estradiol (E_2) and progesterone (p).

Steroid RIA

Standard radioimmunoassay techniques were used to determine serum concentrations of E_2 and p. Progesterone levels were determined with reagents supplied through the North East Thames Region Immunoassay Unit. Estradiol was estimated using a direct assay kit supplied by Steranti Laboratories.

Statistical analysis

Mann-Whitney's non-parametric rank sum test was used for unpaired comparison between the groups. The relationships between ultrasonic determination of endometrial quality and follicular number were determined using multiple regression analysis.

Results

As shown in Table 1, the median E_2 concentration after clomiphene/HMG administration increased in a linear fashion with increase in endometrial thickness as determined by ultrasound. When the endometrium was 0–2 mm thick, the median E_2 concentration was 777 pmol/litre (range 102–1465); this increased to 3600 pmol/litre (range 3335–3600) at 5–6 mm endometrial thickness. After an endometrial thickness of 6mm, there was no further appreciable increase in plasma E_2 concentrations. Two patients had low E_2 levels

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(1125 and 1205 pmol/litre respectively) despite good endometrial development (7 and 8mm, respectively); these patients had correspondingly low serum progesterone levels. As shown in Table 1, there was a progressive but less consistent fall in serum progesterone concentration with increase in endometrial thickness.

Table 2 demonstrates the relationship between serum E_2 levels and ultrasound grading of the endometrium. Grades A-C are associated with optimal serum E_2 concentration; with grade D endometrium, there was a significantly decreased E_2 concentration when compared with any of the rest ($P < 0.01$).

Serum E_2 and p concentration were significantly greater in patients with higher number of developing follicles in both ovaries (Table 3).

Table 1: Relationship between endometrial thickness and plasma steroid concen- tration

Endometrial Thickness (mm)	Plasma Estradiol Level pmol/l (Median and Range)	Plasma Progesterone Level (nmol/l) (Median and Range)
0–2	777 (102–1465)	31 (11–80)
3–4	1541 (242–3400)	40 (9–50)
5–6	3600 (3335–3600)	23 (8–27)
7–8	3490 (1125–4200)	19 (6–23)

Table 2: The relationship between endometrial grading and plasma E_2 concentration

Endometrial Grading	Serum E_2 Level pmol/l (Median and Range)
A	3600 (3500–4200)
B	3490 (3300–3600)
C	3690 (3515–3315)
D	1242 (242–3200)

Table 3: The relationship between total number of developing follicles and plasma steroid concentration

Total Number of Developing Follicles	Serum Estradiol Level (pmol/l) Median and Range	Serum Progesterone Level (nmol/ll) (Median and Range)
0–5	1205 (102–1465)	11 (9–23)
6–8	3503 (892–3600)	28 (9–80)
9–12	3445 (1465–4200)	29 (9–80)

When the total number of developing follicles was correlated with E_2 levels, a significant but weak positive correlation was demonstrated ($r = 0.36$, $P < 0.05$).

Ultrasound determination of endometrial thickness showed a better positive correlation with serum E_2 levels ($r = 0.62$, $P < 0.001$). However, there was a significant inverse correlation between endometrial thickness and plasma progesterone concentration ($r = 0.51$, $P < 0.02$), when the serum E_2/p ratio per patient correlation was shown, this was not better than with E_2 alone ($r = 0.5$, $P < 0.05$). The mean E_2 value per follicle also correlated weakly with endometrial thickness ($r = 0.45$, $P < 0.02$) indicating that E_2 secreted from all follicles contribute to endometrial development.

Discussion

This study demonstrates a weak correlation between real-time ultrasound imaging of total number of developing follicles and serum E_2 concentration. This finding agrees with previous reports [9, 10, 11] and suggests that ultrasound determination of total number of developing follicles cannot be relied upon as the sole index of ovarian function for successful *in vitro* fertilization.

However, the better positive correlation between serum E_2 and endometrial thickness is of interest. Since the endometrium is the end-organ for steroid hormone action, its development should constitute a good index of ovarian function. In the same vein, the ovarian Graafian follicle is the end-tissue for gonadotrophin action;

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therefore ovarian follicular number, diameter of volume should be good measures of gonadotrophin activity.

Our findings appear to suggest that ultrasound assessment of endometrial development is a better indicator of ovarian function than ultrasound determination of total number of ovarian follicles. However, the less than perfect correlation of endometrial thickness with E_2 implies that both parameters must be used together in monitoring ovarian function. From this study, the endometrium that is associated with optimal serum E_2 level is one with a thickness of at least 5 mm and a grading of C.

There was a significant inverse correlation between endometrial development and serum p concentrations. This finding is not surprising since progesterone is known to curtail endometrial development. It is known that circulating levels of progesterone during the follicular phase of the menstrual cycle may serve to prevent excessive endometrial development, a phenomenon that may compromise implantation of transferred embryos [12]. On the other hand, premature elevation of serum progesterone during the follicular phase as occurs in polycystic ovarian disease [13], may restrict endometrial development and interfere with implantation. The failure of the ratio of E_2 to p to show an improved correlation with endometrial thickness is an indication that factors other than E_2 and p are important in endometrial development. One of such factors may be the intrinsic capacity of the endometrium to respond to circulating steroids and this may possibly be mediated by endometrial steroid receptors.

Ultrasound assessment of endometrial development may be useful in one other area of *in vitro* fertilization: that of predicting the optimum endometrium for successful implantation of transferred embryos. It is our hope that further research and improvement in ultrasound imaging may reveal the best type of endometrium in which to attempt the transfer of cleaving embryos.

In conclusion, ultrasound is an extremely useful parameter in ovulation timing for oocyte recovery in *in vitro* fertilization programs. The best parameter seems to be endometrial thickness since this correlated best with plasma estradiol concentrations. However, endometrial thickness should always be combined with total number of developing follicles in order to reach decisions concerning timing of oocyte recovery.

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