Effect of ovulation induction on uterine blood flow and oestradiol and progesterone concentrations in early pregnancy

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To determine if oestradiol and progesterone concentrations are related to uterine blood flow in early pregnancy, we measured these hormones at the time of vaginal Doppler ultrasound before and after the beginning of intervillous circulation in spontaneous pregnancy (group I), after clomiphene citrate administration (group II), and after clomiphene citrate plus human menopausal gonadotrophin (HMG) administration (group III). Despite large increases of oestradiol concentration in groups II (60%) and III (300%) and of progesterone in groups II (100%) and III (300%), compared with group I, increases in blood flow were modest during the first 9 weeks of gestation. Uterine artery flow volume increased by 20% in group II and 33% in group III (P < 0.02); average velocity increased by 37% in group III (P < 0.003) compared with groups I and II; vessel diameter increased by 15% in group II (P < 0.025) and III (P < 0.001) compared with group I; and the uterine artery resistance index decreased by 3 to 5% in group III (P = 0.004) compared with groups I and II. Serum oestradiol and progesterone concentrations were unrelated to the uterine artery resistance index or volume by an analysis of covariance. We conclude that uterine artery blood flow is significantly increased during early pregnancy following HMG administration, and that the increase is unrelated to increases in oestradiol and progesterone concentrations.

Key words: oestradiol/ovulation induction/pregnancy/progesterone/uterine blood flow

Introduction

Two studies have suggested that increases in oestradiol and progesterone concentrations are responsible for increased uterine blood flow during the first 4 months of pregnancy (Jauniaux et al., 1992, 1994). If oestradiol and progesterone are the cause of increased uterine blood flow, then the increased concentrations of these hormones in cycles of ovulation induction (Robertson et al., 1971; Dickey et al., 1991, 1992, 1993; Ghosh et al., 1994) should be accompanied by a significant increase in uterine blood flow during early pregnancy. Uterine blood flow has been measured previously during ovulation induction with human menopausal gonadotrophin (HMG; Weiner et al., 1993) and at the time of embryo transfer (Sterzik et al., 1989; Steer et al., 1992), but such studies have not been continued into pregnancy. Serum oestradiol concentrations per follicle and implantation rates per preovulatory follicle are increased by clomiphene citrate plus HMG compared with clomiphene citrate alone (Dickey et al., 1993). We postulate that the 2-fold increase in pregnancy rate following clomiphene citrate plus HMG administration, compared with clomiphene citrate alone, may be caused by increased uterine blood flow as a result of increased oestradiol concentrations. The difference in uterine blood flow during early pregnancy may also be responsible for the marked variability in the growth rate of embryos of identical size (Dickey and Gasser, 1993). The deficiency of uterine blood flow might be responsible for the supposed increase in the spontaneous abortion rate following the use of clomiphene citrate.

In this study, we have analysed the relationships between serum oestradiol/progesterone concentration and uterine blood flow following spontaneous ovulation and in pregnancies initiated by ovulation induction with clomiphene citrate alone or clomiphene citrate plus HMG, to determine if increases in oestradiol and progesterone concentrations induced by ovulation induction cause similar increases in uterine blood flow. Uterine blood flow was measured by vaginal Doppler ultrasound before and after the beginning of intervillous circulation during week 10 of gestation (Jauniaux et al., 1994).

Materials and methods

Subjects

The study group consisted of 84 unselected patients who conceived singleton pregnancies after treatment for primary or secondary infertility. Only in-vitro fertilization and gamete intra-Fallopian transfer patients were excluded from our study. Patients who received clomiphene citrate or clomiphene citrate plus HMG had mild to moderate ovulatory dysfunction, and most also had mild to moderate endometriosis. Those who did not receive clomiphene citrate or HMG were pregnant following the use of surgical laser surgery or microsurgery, etc., to correct endometriosis or tubal adhesions. The presence of endometriosis did not affect uterine blood flow in this study. Patients were examined initially using colour Doppler ultrasound as soon as a positive urine pregnancy test was observed, at between 29 and 34 days post-menstruation (gestational week 4), and thereafter at 1–3 week intervals, until 15 weeks. The ovulation induction drugs used during the conception cycle were as follows: group I, no drugs, 38 patients, 136 ultrasound measurements; group II, 50–150 mg clomiphene citrate only from cycle days 3 to 9, 20 patients, 68 ultrasound measurements; and group III, 50–100 mg clomiphene citrate plus human menopausal gonadotrophin (HMG; Weiner et al., 1993) and at the time of embryo transfer (Sterzik et al., 1989; Steer et al., 1992), but such studies have not been continued into pregnancy. Serum oestradiol concentrations per follicle and implantation rates per preovulatory follicle are increased by clomiphene citrate plus HMG compared with clomiphene citrate alone (Dickey et al., 1993). We postulate that the 2-fold increase in pregnancy rate following clomiphene citrate plus HMG administration, compared with clomiphene citrate alone, may be caused by increased uterine blood flow as a result of increased oestradiol concentrations. The difference in uterine blood flow during early pregnancy may also be responsible for the marked variability in the growth rate of embryos of identical size (Dickey and Gasser, 1993). The deficiency of uterine blood flow might be responsible for the supposed increase in the spontaneous abortion rate following the use of clomiphene citrate.

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clomiphene citrate from cycle days 3 to 9, followed by 75–150 mIU HMG for 3–5 days. 26 patients, 100 ultrasound measurements. Patients received no progesterone or oestrogen supplementation. All patients had delivered or successfully completed 26 weeks of pregnancy at the time of analysis.

Ultrasound studies

Blood flow was measured with a 5.0 MHz transvaginal probe with real-time and pulsed Doppler capabilities (Ultramark 9, Advanced Technologies Laboratory, Bothell, WA, USA), with the high pass filter set at 100 MHz, the pulse frequency set at 2–12 kHz for Doppler spectral analysis, and a spatial peak time average intensity (Ispet) of 45 mW/cm². The ascending branch of the uterine arteries was identified at the level of the internal cervical os by its diameter and direction of blood flow. The minute blood flow volume of the ascending uterine arteries was determined from the product of the cross-sectional vessel, calculated from the mean of four measurements of diameter on a frozen two-dimensional scan, and the average mean velocity of three waveforms, with the ultrasound probe held at an angle of between 10 and 50° which resulted in the highest pulsatility. A built-in software package was used which adjusted for the angle of incidence. The resistance index [RI: Gosling et al., 1971; (peak systolic velocity – end diastolic velocity)/peak systolic velocity] was measured using an on-screen cursor from the best of five waveforms. Measurements were made with subjects in the recumbent position with knees bent following 10 min of equilibration. Intra- and interobserver coefficients of variation for recumbent uterine blood flow ultrasound measurements in our clinic have been reported previously (Dickey et al., 1994). The average time required for the completion of Doppler blood flow measurements in the recumbent position was 4 min.

Hormone assays

Serum was collected for oestradiol and progesterone determinations between 8:30 and 12:00 h on the day of ultrasound examination, and assayed daily. Serum 17β-oestradiol concentrations were determined by coated tube radioimmunoassay (Diagnostic Products Inc., Los Angeles, CA, USA). Intra- and interassay coefficients of variation were 2.6 and 8.4% respectively. Progesterone concentrations were determined by a competitive radioimmunoassay (Diagnostic Systems Laboratories Inc., Webster, TX, USA). Intra- and interassay coefficients of variation were 7.8 and 10.3% respectively.

Statistics

Graphs of mean hormone and uterine blood flow measurements were plotted by gestational week and treatment group. Differences between the groups, for weeks 4–9 and 10–15, before and after the onset of intermittent circulation (Jauniaux et al., 1994), were examined for significance using Student’s t-test. A multiple linear regression analysis was used initially to examine the relationships between variables for all weeks and separately for weeks 4–9 and 10–15. Differences found with the t-test and regression analysis were re-examined using analysis of covariance, with gestational age, subject and progesterone or oestradiol concentrations included as covariants, to remove the confounding effect of sex hormones on each other and the effect of the repetitive examination of subjects (Bland and Altman, 1995). Statistical analysis was performed using a computerized statistical program (SPSS/PC+, SPSS Inc., Chicago, IL, USA). P values >0.05 were considered statistically significant.

Results

The serum oestradiol concentrations in spontaneous and ovulation induction pregnancies in the three groups are shown in Figure 1A. Oestradiol concentrations in groups I and II rose continuously; they were initially 60% higher in group II than in group I (t = 4.48, P < 0.001) and remained higher for 15 weeks. Concentrations of oestradiol were initially 300% higher in group III than in group I (t = 7.10, P < 0.001), but decreased after week 10, until they were no different from group I and were lower than group II. By an analysis of covariance, oestradiol concentrations were found to be higher during weeks 4–9 in groups II (F = 53.8, P < 0.001) and III (F = 28.2, P < 0.001) compared with group I.

Serum progesterone concentrations in the three groups are shown in Figure 1B. Until week 9, progesterone concentrations were initially 100% higher in group II (t = -4.17, P < 0.001) and 300% higher in group III (t = -6.68, P < 0.001) than in group I. After week 9, there was no difference between groups I and II, but progesterone concentrations continued to be higher in group III until after week 11. Progesterone concentrations analysed by an analysis of covariance were significantly higher in group II pregnancies (F = 19.6, P < 0.003) during weeks 4–9 and in group III pregnancies (F = 74.9, P < 0.001) during all weeks compared with group I.

The effects of ovulation induction drugs on uterine artery Doppler measurements are shown in Figure 2. During weeks 4–9, uterine artery blood flow volume was higher by ~20% in group II and by ~33% in group III (t = -2.37, P = 0.019) compared with group I (Figure 2A). No effect of ovulation induction drugs on blood flow volume was found during weeks 10–15. Uterine artery average velocity was increased by ~37% in group III (t = 3.20, P = 0.002) compared with groups I and II (t = 3.05, P = 0.003) during weeks 4–9 (Figure 2C). Following an analysis of covariance, only the differences in flow volume and velocity, which occurred during weeks 4–9 in group III, were significantly different from group I (P = 0.01).

Uterine artery diameter was ~15% greater for groups II (t = 2.27, P = 0.025) and III (t = 3.39, P = 0.001) compared with group I during weeks 4–9 (Figure 2B). No difference in diameter was apparent during weeks 10–15. Following an analysis of covariance, only the difference between groups III and I during weeks 4–9 was significant (P = 0.001).

The average uterine artery resistance was ~3–5% lower in group III (t = 2.94, P = 0.004) than in groups I and II (Figure 2D) during weeks 4–9. After week 10, the effect of group III treatment on the uterine artery RI was reversed compared with groups I (t = 2.16, P = 0.032) and II (t = -2.06, P = 0.042). Following an analysis of covariance, only the difference between groups III and I during weeks 4–9 was significant (P = 0.004).

Oestradiol concentrations analysed by multiple linear regression and analysis of covariance were negatively related to the average vessel diameter for group I during weeks 10–15 (F = -1.89, P < 0.05), but were unrelated to the average diameter for groups II and III, and to flow volume, average velocity and uterine artery RI for any group. Serum progesterone concentrations analysed by multiple linear regression and analysis of covariance were positively related to uterine artery average velocity (F = 2.45, P < 0.001) for group I during all weeks.
Figure 1. (A) Serum oestradiol and (B) serum progesterone concentrations during the first 15 weeks of pregnancy, following spontaneous ovulation (group I, ×), clomiphene citrate (CC) without human menopausal gonadotrophin (HMG; group II, △), or clomiphene citrate followed by HMG (group III, ◊). Values shown are mean ± SE.

Figure 2. (A) Uterine artery total (right plus left) blood flow volume, (B) average diameter, (C) average velocity and (D) resistance index (RI) during the first 15 weeks of pregnancy, following spontaneous ovulation (group I, ×), clomiphene citrate (CC) without human menopausal gonadotrophin (HMG; group II, △), or clomiphene citrate followed by HMG (group III, ◊).
weeks, but were unrelated to average velocity for groups II and III, and to other blood flow variables for any group.

Discussion
The findings from our study, that uterine blood flow and serum oestradiol concentrations are increased compared with spontaneous ovulation during early pregnancy following ovulation induction with clomiphene citrate plus HMG, have not been reported previously. The finding that serum progesterone concentrations are elevated for the first 9 weeks of gestation following clomiphene citrate administration compared with spontaneous ovulation has been reported previously (Robertson et al., 1971; Dickey, 1984). However, the change in uterine blood flow in cycles where clomiphene citrate plus HMG were used compared with spontaneous cycles, during gestational weeks 4–9 before the beginning of intervillous circulation (Jauniaux et al., 1994), were relatively small (flow volume, +33%; velocity, +37%; diameter, +15%; and RI, –5%) when compared with the increases in serum oestradiol and progesterone (+300%) concentrations. The difference in magnitude of the increase in sex hormones compared with blood flow, and an examination of the relationship between oestradiol/progesterone concentrations and uterine blood flow by analysis of covariance (with gestational age, subject and the other sex hormone as covariants in ovulation induction cycles), suggest that there is no direct relationship between either oestradiol or progesterone concentration and uterine blood flow during early pregnancy. Therefore it can be assumed that oestradiol and progesterone are not responsible for the increased uterine blood flow in early pregnancy.

Our findings do not confirm those of previous authors (Jauniaux et al., 1992, 1994), that increased oestradiol and progesterone concentrations are responsible for the increase in uterine blood flow which occurs during the first 4 months of pregnancy. In these previous studies, a negative relationship was found between oestradiol (Jauniaux et al., 1992, 1994) and progesterone concentrations (Jauniaux et al., 1994) and the uterine RI, indicating to the authors that the decreased downstream resistance was caused by increases in those hormones. However, they found no association between oestradiol or progesterone concentration and the pulsatility index, which would be expected to parallel changes in the RI, and they did not measure, as we did, actual blood flow volume in the uterine arteries. The relationships between oestradiol or progesterone concentration and the uterine artery diameter in spontaneous cycles found by ourselves in this study, and the uterine artery RI found by Jauniaux et al. (1992, 1994), may be coincidental or a result of colinearity, because both sex hormones and blood flow increase during pregnancy. Duvetak et al. (1993), who measured maternal cardiovascular function and oestradiol, progesterone and plasma renin concentrations in early pregnancy, concluded that changes in cardiac output were triggered by a fall in systemic vascular tone, which was not related to oestradiol, progesterone or renin. The trophoblastic invasion of the myometrium, which begins by week 5 of gestation (Jaffe and Woods, 1993), is responsible for early changes in the spiral artery resistance, and may be the indirect cause of apparent changes in uterine artery blood flow during early pregnancy. The negative association between oestradiol concentration and uterine artery diameter which we found in spontaneous cycles could be explained by the finding that oestrogens can potentiate α-adrenergic vasoconstriction in response to prostaglandins (Colucci et al., 1982; Miller and Vanhoucke, 1990).

Rising oestrogen concentrations in early pregnancy might be expected to be the cause of the increased uterine blood flow, because oestradiol has been noted to cause decreased resistance in the uterine arteries (de Ziegler et al., 1991; Hillard et al., 1992) and systemic circulation (Penotti et al., 1993) when administered to post-menopausal women, and has been reported to cause dilation of the uterine arteries in sheep (Anderson et al., 1977). Progesterone opposes the effect of oestradiol in some studies (Hillard et al., 1992; Marsh et al., 1994) but not in others (de Ziegler et al., 1991). These findings may not be transferable to human pregnancy, where adequate blood flow is necessary for implantation (Goswamy et al., 1988; Sterzik et al., 1989; Steer et al., 1992) and where, therefore, the uterine vascular system may already be responding maximally to oestrogen and progesterone as a prerequisite for implantation.

Recumbent blood flow studies such as ours may overlook the effects of oestrogen on standing uterine blood flow. Recently, we have shown that uterine blood flow volume is decreased in 70% of non-pregnant women when going from a recumbent to a standing position (Dickey et al., 1994). As our study has demonstrated, the relationships between sex hormone concentration and uterine blood flow during early pregnancy are complex. Both blood flow volume and resistance indices need to be measured to allow us to fully appreciate this relationship. The finding that HMG increases uterine blood flow during early pregnancy may explain the higher implantation rate per pre-ovulatory follicle when HMG is used alone or in combination with clomiphene citrate, when compared with clomiphene citrate alone, which we reported in a previous study (Dickey et al., 1993). The possibility that HMG and follicle-stimulating hormone may stimulate embryo development during the first 9 or 10 weeks of pregnancy by increasing uterine blood flow deserves further investigation.

The effects of Doppler ultrasound on embryo and fetal development are not known with any degree of certainty. The bioeffects of Doppler ultrasound are related to the spatial peak time average intensity (I_{SPTA}) and duration of exposure. Doppler ultrasound during early pregnancy should be performed only by sonographers with experience in the technique of uterine blood flow examination, and should be limited to 500 s (8.3 min) at an I_{SPTA} <94 mW/cm² (American Institute of Ultrasound in Medicine, 1993).

References
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