Colour Doppler analysis of peri-implantation utero-ovarian haemodynamics in women with excessively high oestradiol concentrations after ovarian stimulation

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BACKGROUND: Gonadotrophins are used in many assisted reproduction units to achieve a better success rate by increasing the number of replaced embryos. However, high oestradiol concentrations are associated with altered physiological functions and its complications. We investigated whether high oestradiol concentrations (≥20 000 pmol/l) after ovarian stimulation in infertile women would affect the uterine haemodynamics at the time of embryo transfer. METHODS: Colour Doppler indices of utero-ovarian arteries and endometrial colour signals were measured. Fifty-eight women undergoing ovarian stimulation for IVF were classified according to serum oestradiol concentrations on the day of human chorionic gonadotrophin injection into moderate responders (oestradiol <20 000 pmol/l; n = 39) and high responders (oestradiol ≥20 000 pmol/l; n = 19). RESULTS: Haemodynamic parameters were significantly lower in high responders; the uterine arterial pulsatility index (PI) and resistance index (RI) were (median; range) 1.87 (0.84–2.82) and 0.79 (0.57–0.90) respectively; ovarian artery PI was 0.57 (0.40–1.12) and RI was 0.43 (0.33–0.64). In moderate responders the uterine PI and RI were 2.63 (1.46–5.92) and 0.88 (0.77–1.10) respectively. Ovarian PI was 0.81 (0.32–3.72) and RI was 0.55 (0.23–0.97). The number of women showing endometrial colour signals was significantly lower in high responders (63%) than in moderate responders (92%) (P < 0.05). A further increase in oestradiol (≥25 000 pmol/l; n = 8) showed significantly (P = 0.03) fewer endometrial colour signals [1.5 (0–8)] compared with moderate responders [4 (0–14)]. CONCLUSION: Despite low uterine PI and RI, the endometrial blood flow in high responders appears to be impaired. This may contribute to the decline in implantation efficiency noted in high responders.

Key words: Doppler haemodynamics/endometrial perfusion/high responders/luteal phase/ovarian stimulation syndrome

Introduction

Gonadotrophins are used in many assisted reproduction programmes to recruit a greater number of follicles. An increase in the number of embryos transferred results in higher conception rates. However, with gonadotrophin stimulation there is also an increased risk of an exaggerated ovarian response and its associated complications (MacDuggall et al., 1993). Furthermore, supraphysiological hormone concentrations in ovarian stimulation cycles have been associated with reduced implantation rates (Simón et al., 1995). Previous studies have examined changes in the Doppler indices of uterine and ovarian vessels during ovarian stimulation and spontaneous cycles (Baber et al., 1988; Scholtes et al., 1989; Steer, 1992). In a recent study (Ng et al., 2000) we have shown a significant reduction in the implantation and pregnancy rates in women with very high concentrations of oestradiol (≥20 000 pmol/l). In the literature there is a paucity of data that correlate excessively high concentrations of oestradiol with the blood flow measurements of the uterine and ovarian arteries. The present study was undertaken to compare the haemodynamic parameters of the utero-ovarian vasculature and the endometrial spiral arteries of women who showed a moderate response with women whose oestradiol concentrations were in excess of 20 000 pmol/l after ovarian stimulation.

Materials and methods

Fifty-eight cycles of women undergoing assisted reproduction treatment were studied prospectively. The indications for IVF treatment included tubal, male and unexplained factors of infertility. These women had regular menstrual cycles and there was no significant intra-uterine or ovarian abnormality detected in these women during
pretreatment evaluation. Ethical approval for the study was obtained from the Ethics Committee of the Faculty of Medicine, The University of Hong Kong.

All women were stimulated with the departmental standard protocol of ovarian stimulation (Basir et al., 2001) for IVF and embryo transfer. These women were pre-treated with a gonadotrophin-releasing hormone (GnRH) analogue, buserelin (Suprecur®; Hoechst, Frankfurt, Germany) nasal spray 150 µg four times a day from the mid-luteal phase of the cycle preceding the treatment cycle. Baseline transvaginal ultrasound scanning was performed on the second day of the cycle to assess uterine and ovarian morphology and to confirm pituitary down-regulation. Assay of serum oestradiol was also performed on the second day of the cycle. Human menopausal gonadotrophin (HMG, 75 IU FSH and LH; Pergonal®; Serono, Switzerland) injections were then started. The women underwent monitoring of ovarian response by assay of serum oestradiol concentrations and serial transvaginal ultrasonographic scans during the follicular phase. Human chorionic gonadotrophin (HCG, Pregnyl®; Organon, Oss, The Netherlands) 10 000 IU was given i.m. when the mean diameter of the leading follicle was >18 mm and there were at least three follicles with a mean diameter of ≥16 mm. The day of HCG administration (day 0) was used as the reference for determining the cycle day. Doppler evaluation was done on the day of embryo transfer.

Women recruited in the study were scanned transvaginally with colour blood flow imaging ~1 h before embryo transfer. Doppler ultrasonic measurements were carried out using a Doppler US (Acumen XP128/10; Acuson, Mountain View, CA, USA) machine equipped with a transvaginal transducer of 7 MHz. All scans were done by one operator (T.P.W.L.) experienced in the procedure. The women were studied between 0800 and 0900 h to exclude the effects of circadian rhythmicity on blood flow (Zaidi et al., 1995). Flow velocity waveforms were obtained from the ascending main branch of the uterine artery on the right and left side of the cervix in a longitudinal plane before it entered the uterus. Ovarian flow signals were obtained from within the ovarian parenchyma. The ovarian vessels studied were those detected in close proximity to the follicles. The ‘gate’ of the Doppler was positioned when the vessel with good colour signals was identified on the screen. A visual classification was made using three similar, consecutive waveforms of good quality on the right and left side; median values (range) were used. The pulsatility index (PI) and resistance index (RI) of the uterine and ovarian arteries were calculated electronically. As no significant differences in the Doppler velocimetry indices between the left and right side of the uterine and intra-ovarian arteries were obtained, the data were combined and the average value of both arteries was used. Furthermore, endometrial perfusion was also assessed by colour Doppler flow imaging. The presence or absence of colour and the number of colour signals obtained from the pulsatile vessels in the endometrium, which represented the spiral arteries, was then recorded. Women demonstrating ≥2 colour signals were considered to have absent or minimal flow. The velocity scale was set to detect low flow signals in the range of 0–0.23 m/s, while the colour gain was adjusted to minimize motion artifacts. The region of interest in the colour Doppler examination was adjusted to a minimum area with inclusion of the whole endometrium in mid-sagittal plane. The image was frozen on the screen and the number of pulsatile dots (representing spiral arteries) were counted at the upper two-thirds of the endometrium. However, the PI and RI of the spiral arteries were not recorded because the marked tortuosity of arteries made optimal placement of the ‘gain’ difficult.

Statistical analysis
Statistical analysis was performed using Statistical Package for Social Science (SPSS for Windows package release 10.0; SPSS Inc., Chicago, IL, USA). The non-parametric Mann–Whitney U-test was used in the evaluation of the skewed data. As the data were not normally distributed, continuous data are expressed as median and range. A P value of < 0.05 was considered significant. The χ² test or the Fisher’s exact test was used to estimate the significance of difference between discontinuous variables.

Results
To study the effect of high oestradiol concentrations, the study subjects were classified into moderate responders and high responders. We have previously shown (Ng et al., 2000) that the implantation and pregnancy rates were significantly lower in women with serum oestradiol concentrations ≥20 000 pmol/l. Therefore, women with serum oestradiol <20 000 pmol/l (range: 1800–17000 pmol/l) on the day of HCG administration were classified as moderate responders (n = 39) while those with serum oestradiol concentrations ≥20 000 pmol/l (range: 20000–44000 pmol/l) were classified as high responders (n = 19). All high responders were also found to have developed 15 or more follicles. There was no significant difference between the median age of moderate responders (33 years range; 30–37) and high responders (32 years range; 29–39). Similarly, the various factors of infertility and the duration of infertility did not differ significantly. The Doppler haemodynamic and ovarian response of women in the moderate and high response groups are shown in Table I. The uterine and ovarian arterial PI and RI showed a significant difference between the two groups. The uterine artery PI and RI for high responders were significantly lower than for moderate responders (P < 0.001) Similarly, the indices of ovarian arteries demonstrated significant differences between the two (P < 0.05) groups. Presence of colour signals in the endometrium was demonstrated in 92% of women in the moderate response group. In high responders, colour signals could be detected in only 63% of women. This difference between moderate and high responders was found to be statistically significant (P < 0.05).

We further analysed the effect of oestradiol induced blood flow changes in the endometrium, based on the number of colour signals detected. High responders were sub-classified into group A (oestradiol concentrations between 20 000–25 000 pmol/l, n = 11) and group B (oestradiol concentrations ≥25 000 pmol/l; range 25 000–44 400; n = 8). In moderate responders the median number of endometrial signals observed were 4 (0–14). Among high responders, those in group A had 3 (0–13) endometrial signals, and those in group B had 1.5 (0–8) signals. No difference in colour signals was demonstrated between the two subgroups. Although high responders in group A showed fewer number of signals than moderate responders, the difference was not statistically significant. In group B of high responders the number of endometrial spiral arterial signals was significantly lower (P = 0.03) than that of moderate responders. Similarly, 75% women in group B showed absent or minimal endometrial flow signals on Doppler examination. This was significantly higher (P < 0.05) when compared with women in the moderate response group (7%) and those in group A (9%) of the high response.
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Table I. Comparison between haemodynamic and ovarian response of moderate and high responders after ovarian stimulation. Values are in median and ranges.

<table>
<thead>
<tr>
<th></th>
<th>Moderate responders</th>
<th>High responders</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 39)</td>
<td>(n = 19)</td>
<td></td>
</tr>
<tr>
<td>Uterine PI</td>
<td>2.63 (1.46–5.92)</td>
<td>1.87 (0.84–2.82)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Uterine RI</td>
<td>0.88 (0.77–1.10)</td>
<td>0.79 (0.57–0.90)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Ovarian PI</td>
<td>0.81 (0.32–3.72)</td>
<td>0.57 (0.40–1.12)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Ovarian RI</td>
<td>0.55 (0.23–0.97)</td>
<td>0.43 (0.33–0.64)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Number of colour signals in the endometrium</td>
<td>4 (0–14)</td>
<td>2 (0–13)</td>
<td>NS</td>
</tr>
<tr>
<td>Number of women with endometrial colour signals</td>
<td>92%</td>
<td>63%</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Oestradiol (pmol/l) on day of ovulatory dose of HCG</td>
<td>8161 (1817–16701)</td>
<td>21408 (20413–44408)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Number of follicles ≥14mm</td>
<td>12 (3–38)</td>
<td>18 (7–33)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Number of oocytes obtained</td>
<td>7 (0–20)</td>
<td>25 (12–59)</td>
<td>&lt; 0.001</td>
</tr>
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</table>

*P value calculated by Fisher’s exact test.
All P values are calculated by the Mann–Whitney U-test.
PI = pulsatility index; RI = resistance index.

Discussion

Ovarian stimulation is commonly used nowadays in assisted reproduction to obtain a larger number of oocytes and to improve the pregnancy rate. However, some studies have shown that hyperstimulation impairs the chance of successful implantation (Forman et al., 1988; Simón et al., 1995) whereas others (Chenette et al., 1990; Sharara and McClamrock, 1999) have found no adverse effects. In literature there is controversy regarding the effect of high oestradiol concentrations on subsequent implantation and pregnancy rates in IVF. Recent clinical data (Ng et al., 2000) from our IVF program showed that implantation and pregnancy rates were significantly lower in high responders with high concentrations of serum oestradiol. In literature there is a paucity of data that correlates the probability of conception to haemodynamic changes associated with excessively high oestradiol concentrations. Oyesanya et al. (1996) found that intrafollicular haemodynamics before HCG administration did not predict the development of ovarian hyperstimulation syndrome but they did not measure the uterine PI and RI in patients with high oestradiol concentrations. Therefore, we conducted the present study to investigate whether the high oestradiol concentrations would affect the uterine haemodynamics at the time of embryo transfer. A significant decline in uterine and ovarian pulsatility and resistance indices was seen in women with high serum oestradiol concentrations. A lower uterine and intra-ovarian PI and RI imply reduced downstream impedance and increased blood flow in these women. In a recent study Yuval et al. (1999) concluded that lower resistance and a higher oestradiol concentration are interrelated, but do not predict implantation. Steer et al. (1992) reported that maximum pregnancies were achieved when uterine arterial PI was in the range of 2.00 to 2.99 and that lower arterial impedance indices on the day of embryo transfer did not lead to higher pregnancy rates.

Tekay et al. (1995) found no difference in mean uterine artery PI between asymptomatic and ovarian hyperstimulation cycles (Tekay et al., 1995). However, serum oestradiol concentrations in patients of ovarian hyperstimulation syndrome in Tekay’s study population were similar to those in asymptomatic controls. Moreover, the Doppler evaluation was done in the late luteal phase of the cycle, and this may explain the difference from our results.

Perhaps an important observation from this study is the increase in the number of subjects demonstrating absent or minimal endometrial colour signals at high concentrations of oestradiol. As a possible explanation of these findings we further investigated oestradiol induced endometrial haemodynamic changes. The endometrial haemodynamic analysis of women who developed an excessive response (group B) revealed that 75% were associated with absent or poor endometrial colour signals. Additionally, significantly fewer numbers of signals were observed in this group of women compared with moderate responders. There appears to be a trend towards inadequate endometrial flow at very high oestradiol concentrations. If indeed there was absence or inadequate endometrial perfusion while blood flow in the uterine arteries increased it would suggest that the blood flow was shunted away from the uterine endometrium. The low PI and RI of the ovarian arteries indicate neovascularization and increased capillary permeability in the ovarian tissue of high responders. Therefore, the blood flow might be directed through the utero–ovarian collaterals to the ovaries. Moreover, the increase in hormonal concentrations in the peripheral plasma leads to a decrease in peripheral vascular resistance (Goswamy et al., 1988; Goswamy and Steptoe, 1988; Long et al., 1989) and a decreased contractility of the uterine muscles. This results in relaxation and opening up of the small uterine vascular channels which may also cause an increase in the capillary permeability. In a recent study (Basir et al., 2001) on the endometrial morphological changes at high concentrations of oestradiol a significantly greater number of vessels and endometrial oedema in women who responded excessively to ovarian stimulation was demonstrated. Therefore, it was postulated that the blood flow through these minute endometrial vessels may be very slow and the weak Doppler flow signals arising from them could not be picked up by the colour Doppler despite low uterine PI and RI. The increase in capillary permeability and dilatation leads to extravasation of fluid from the intercellular to extracellular compartments and hence endometrial oedema. In another study (Gannon et al., 1997) the investigators suggested that the
blood flow per capillary might actually be reduced during oedema. Successful implantation and continuing development of implanted embryo depends on a complex series of cellular and molecular events (Finn, 1977; Cross et al., 1994) between the blastocyst and the endometrium. The decline in blood flow could therefore impede the exchange of essential nutrients, bioactive molecules and reactive compounds that are vital for implantation. Zaidi et al. (1996) concluded that absent endometrial and intra-endometrial vascularization appeared to be a useful predictor of failure of implantation in IVF cycles (Zaidi et al., 1996).

In this study our sample size was small and the spiral artery blood flow indices were not measured. Further larger prospective studies are required to confirm the effect of excessively high concentrations of serum oestradiol on endometrial blood flow. Power Doppler has a three-fold greater sensitivity to flow than colour Doppler, and is particularly useful for minute vessels with low-velocity flow (Schilds et al., 2000). This can serve to determine the haemodynamic environment of the endometrium under the influence of very high oestradiol. In addition to the utero-ovarian blood flow indices, subjective observation of spiral arteries may be a useful starting point to convert a semi-quantitative assessment into a quantitative value at least until more sophisticated techniques are available for routine monitoring of infertile women undergoing IVF. Present data provide substantial evidence to suggest that the circulatory adequacy of the endometrium, which is vital for embryo implantation, is compromised in high responders. Excessive concentrations of oestriadiol may result in suboptimal blood flow to the endometrium. Therefore, very low uterine PI and RI may not be associated with better endometrial perfusion. The altered endometrial perfusion in high responders may contribute to a decline in endometrial receptivity. This may explain and substantiate our clinical data, which showed markedly diminished implantation and pregnancy rates in high responders in our IVF programme.

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References


Uterine haemodynamic changes in high responders

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