Ultrasound evaluation of the uterine zonal anatomy during in-vitro fertilization and embryo transfer

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This study was designed to establish if ultrasound could detect differences in uterine zonal anatomy between conception and non-conception in-vitro fertilization (IVF)/embryo transfer cycles. A transvaginal ultrasound scan was performed on the day of downregulation (D0), on day 8 of ovulation induction (D8), on the day of human chorionic gonadotrophin (HCG) injection, at the time of oocyte retrieval, and at embryo transfer. Thicknesses of endometrium, junctional zone, myometrium and full thickness of the uterus were recorded for every patient and comparisons made at all the assessment points. Differences between measurements on D0 and all other measurements (temporal changes) and between every subsequent measurement (dynamic changes) were also compared. There were no statistically significant differences in endometrial thickness between pregnant and non-pregnant groups at any time. The diameter of the uterus increased during therapy and its measurements at the time of downregulation was significantly greater in the pregnant subset at the time of HCG injection, oocyte retrieval and embryo transfer ($P < 0.02$, 0.03 and 0.02 respectively). The myometrium was significantly thicker in the pregnant group on D0, on D8 and at HCG administration ($P < 0.03$, 0.004 and 0.02). There was a decrease in junctional zone thickness in both groups during the first week of ovulation induction, and on D8 the junctional zone in pregnant patients was significantly thinner ($P < 0.04$). The junctional zone became significantly thicker at embryo transfer in the pregnant group ($P < 0.01$). This was confirmed by significant temporal and dynamic changes at the time of oocyte retrieval and embryo transfer ($P < 0.01$, 0.0001 and $P < 0.05$, 0.01 respectively).

The purpose of this study was to determine if there are any differences in the changes observed in uterine zonal anatomy between conception and non-conception in in-vitro fertilization (IVF) and embryo transfer cycles.

Materials and methods

Patients

This is a retrospective group comparison study of 30 consecutive patients who conceived after assisted conception treatment, and 30 consecutive patients treated during the same period of time who failed to conceive. All women had a history of infertility due to tubal, male and unexplained factors. Patient selection criteria also included having three good embryos (grade 3/5–5/5) transferred and an easy embryo transfer (smooth introduction of the catheter, no tenaculum/dilators used). Embryo scoring was carried out as described previously (Puissant et al., 1987).

Medication

All patients were downregulated with a standard regimen of luteinizing hormone releasing hormone superagonist (Nafarelin;...
Dynamic changes (differences between each subsequent measurement: D8–D0; oocyte retrieval–D0 and embryo transfer–D0) were also assessed within all layers.

The thickness of the myometrium (Table IV) was significantly greater in the pregnant group than the non-pregnant group at the time of HCG injection. There were no statistical differences between non-pregnant and pregnant groups when temporal and dynamic changes within endometrial, myometrial and uterine thickness were maximal at the time of HCG injection. There were no significant differences in age, incidence of primary or secondary infertility, period of ovulation induction, numbers of ampoules used, numbers of aspirated follicles, numbers of retrieved oocytes or grade of embryos between pregnant and non-pregnant groups (Table I). Values of endometrial thickness and thickness of the uterus were maximal at the day of oocyte retrieval.

Imaging techniques

Transvaginal ultrasound scans (ATL Ultramark 4, 5 MHz transducer; Advanced Technology Laboratories, Seattle, USA) were performed on the day of downregulation (D0), on day 8 of ovulation induction (D8), and on the days of HCG injection, oocyte retrieval and embryo transfer. We routinely used the ABC Method (Figure 1; Tetlow et al., 1996) for measuring zonal anatomy in all scanned patients. Measurements of the endometrium, junctional zone, myometrium and full thickness of the uterus were recorded for every patient, and a comparison made between the pregnant and non-pregnant groups at all assessment points. To evaluate the temporal changes in a given layer, the differences between all measurements and D0 (D8–D0; HCG–D0; oocyte retrieval–D0 and embryo transfer–D0) were obtained and non-pregnant and pregnant subsets compared again. Dynamic changes (differences between each subsequent measurement: D8–D0; HCG–D8; oocyte retrieval–HCG; embryo transfer–oocyte retrieval) were also assessed within all layers.

These observations were evaluated on SPSS for Windows (SPSS UK Ltd, St Andrews House, Woking, UK) using a Mann–Whitney test for two independent samples following Lilliefors test for normality of distribution. A P value < 0.05 was regarded as statistically significant.

Results

There were no significant differences in age, incidence of primary or secondary infertility, period of ovulation induction, numbers of ampoules used, numbers of aspirated follicles, numbers of retrieved oocytes or grade of embryos between pregnant and non-pregnant groups (Table I). Values of endometrial thickness and thickness of the uterus were maximal at the time of HCG injection. There were no statistical differences found between the endometrial thickness values of pregnant and non-pregnant subsets at any point of the treatment cycle (Table II). The thickness of the uterus in pregnant patients was significantly greater than in non-pregnant patients at the time of HCG administration, oocyte retrieval and embryo transfer (P < 0.02, 0.03 and 0.02 respectively) (Table III). The thickness of the myometrium (Table IV) was significantly greater in the pregnant group than the non-pregnant group at D0, D8 and at HCG administration (P < 0.03, 0.004 and 0.02). There were no significant differences between non-pregnant and pregnant groups when temporal and dynamic changes within endometrial, myometrial and uterine thickness were evaluated.

Measurement of the junctional zone revealed two different profiles (Figure 2). There was a decrease in the thickness during the first week of ovulation induction in both groups, but at D8 the junctional zone in the pregnant group was

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-pregnant</th>
<th>Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Age (years)</td>
<td>30.6 ± 3.8</td>
<td>30.4 ± 3.5</td>
</tr>
<tr>
<td>Cause of infertility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubal factor</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Male factor</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Unexplained</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Primary infertility</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Embryo quality</td>
<td>3.6 ± 0.5</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>(3.4, 3.8)</td>
<td>(3.3, 3.7)</td>
<td></td>
</tr>
<tr>
<td>FSH ampoules used (n)</td>
<td>(27.1, 36.9)</td>
<td></td>
</tr>
<tr>
<td>Follicles aspirated (n)</td>
<td>(14.3 ± 5.9)</td>
<td></td>
</tr>
<tr>
<td>(12.1, 16.6)</td>
<td>(15.0, 19.8)</td>
<td></td>
</tr>
<tr>
<td>Oocytes retrieved (n)</td>
<td>(10.5 ± 5.6)</td>
<td></td>
</tr>
<tr>
<td>(8.4, 12.6)</td>
<td>(10.9, 14.1)</td>
<td></td>
</tr>
<tr>
<td>Ovulation induction (days)</td>
<td>12.3 ± 2.0</td>
<td>12.6 ± 2.8</td>
</tr>
<tr>
<td>(11.05, 13.02)</td>
<td>(11.55, 13.64)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD; values in parentheses are 95% confidence intervals.

The groups were not significantly different for any of the variables measured.

FSH = follicle stimulating hormone.

Table I. Characteristics of non-pregnant and pregnant patient groups
and pregnant subsets were those noted within the junctional zone (thickness, \( P < 0.05 \); temporal changes, \( P < 0.0004 \); dynamic changes, \( P < 0.04 \)) at the time of embryo transfer.

**Discussion**

High-resolution ultrasound has long been used as a non-invasive method of assessing uterine receptivity. In this study, we investigated the concept of ultrasound measurements of zonal anatomy as a research tool in patients who were infertile. This technique has been described previously (Tetlow et al., 1996) and found to be of value when comparing serial transvaginal scan measurements and evaluating research documentation in the form of videotaped images from multicentre trials.

Ultrasound assessment of endometrial receptivity has been performed most frequently as a single measurement at the time of HCG injection (Coulam et al., 1994), on the day before oocyte retrieval (Ueno et al., 1991), at the time of oocyte retrieval (Welker et al., 1989) or at embryo transfer (Strohmer et al., 1994). In our study, by performing several consecutive measurements during each IVF cycle, we observed changes within a given layer and defined profiles for pregnant and non-pregnant groups.

Endometrial thickness appeared to increase rapidly during the first week of ovulation induction, becoming maximal at the time of HCG injection. The subsequent assessments at the time of oocyte retrieval and embryo transfer revealed a small decrease or no increase in endometrial thickness values. These findings differ from other observations (Rabinowitz et al., 1986) which described an increase of 0.5 mm/day from day –3 to +2 (oocyte retrieval = day 0) and a slower linear increase (0.1 mm/day) during the luteal phase until day +11. In this study, conception cycles were characterized by an accelerated growth. A positive correlation between endometrial thickness in the luteal phase and IVF/embryo transfer outcome has also been reported (Imoedemhe et al., 1987). We did not continue

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**Table II.** Double thickness of endometrium (mm) during IVF cycle in non-pregnant and pregnant patients

<table>
<thead>
<tr>
<th>Time</th>
<th>Non-pregnant</th>
<th>Pregnant</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0</td>
<td>2.3 ± 1.2</td>
<td>2.2 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>D8</td>
<td>10.5 ± 2.9</td>
<td>10.3 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>HCG</td>
<td>12.4 ± 2.7</td>
<td>12.9 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Oocyte retrieval</td>
<td>11.1 ± 2.5</td>
<td>12.2 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>Embryo transfer</td>
<td>11.4 ± 2.8</td>
<td>11.9 ± 2.9</td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD.
HCG = human chorionic gonadotrophin.
The groups were not significantly different at any of the measurement times.

**Figure 2.** Line drop plot of changes in junctional zone thickness during IVF cycle. The solid line represents the pregnant group; the dotted line represents the non-pregnant group. D0 = day of downregulation; D8 = day of ovulation induction; ET = day of embryo transfer; HCG = day of human chorionic gonadotrophin injection; OR = oocyte retrieval.

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**Table IV.** Double thickness of the myometrium (mm) during IVF/embryo transfer cycle in non-pregnant and pregnant patients

<table>
<thead>
<tr>
<th>Time</th>
<th>Non-pregnant</th>
<th>Pregnant</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0</td>
<td>31.4 ± 3.4</td>
<td>33.5 ± 5.5</td>
<td>NS</td>
</tr>
<tr>
<td>D8</td>
<td>34.7 ± 4.3</td>
<td>37.0 ± 5.2</td>
<td>NS</td>
</tr>
<tr>
<td>HCG</td>
<td>36.7 ± 4.9</td>
<td>39.4 ± 5.3</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>Oocyte retrieval</td>
<td>36.3 ± 5.8</td>
<td>39.0 ± 5.6</td>
<td>&lt; 0.03</td>
</tr>
<tr>
<td>Embryo transfer</td>
<td>36.2 ± 4.1</td>
<td>38.9 ± 4.9</td>
<td>&lt; 0.02</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD.
HCG = human chorionic gonadotrophin; NS = not significant.

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**Table V.** Double thickness of junctional zone (mm) during IVF/embryo transfer cycle in non-pregnant and pregnant patients

<table>
<thead>
<tr>
<th>Time</th>
<th>Non-pregnant</th>
<th>Pregnant</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0</td>
<td>5.5 ± 1.7</td>
<td>4.8 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>D8</td>
<td>4.4 ± 1.5</td>
<td>3.6 ± 1.2</td>
<td>&lt; 0.04</td>
</tr>
<tr>
<td>HCG</td>
<td>4.1 ± 1.5</td>
<td>3.7 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Oocyte retrieval</td>
<td>4.2 ± 1.5</td>
<td>4.4 ± 1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Embryo transfer</td>
<td>4.2 ± 1.5</td>
<td>5.1 ± 1.1</td>
<td>&lt; 0.01^</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD.
HCG = human chorionic gonadotrophin; NS = not significant.
^Significant after Bonferroni correction.

significant thinner and then became significantly thicker at the time of embryo transfer (Table V). This was confirmed by significant temporal and dynamic changes at the time of oocyte retrieval and embryo transfer (\( P < 0.05 \), 0.0001 and \( P < 0.05 \), 0.01 respectively). In patients who failed to become pregnant, changes in junctional zone were less pronounced and a return to the initial thickness was less likely to occur.

When the Bonferroni correction was applied to all the results, the only significant differences between non-pregnant
with our measurements beyond the time of embryo transfer, but observations suggest that a marked increase in the width of endometrial thickness layer is unlikely unless conception has already occurred.

In this study there were no statistical differences in the mean endometrial thickness between pregnant and non-pregnant groups at any point of the IVF cycle. The same conclusion was reached (Khalifa et al., 1992) after performing transvaginal sonography (TVS) on the day of HCG administration, the day of oocyte retrieval and before embryo transfer. Lack of any correlation between mean endometrial thickness and treatment outcome has also been described (Ueno et al., 1991; Couplam et al., 1994; Serafini et al., 1994; Oliveira et al., 1997). Although other workers (Glissant et al., 1985; Gonen and Casper, 1990; Sher et al., 1991; Noyes et al., 1995; Rinaldi et al., 1996) have reported otherwise, their observations were based on a single measurement. There is a possibility that studies which described a positive correlation between endometrial thickness and IVF treatment outcome might have included the junctional zone measurement within that of endometrial thickness. Alternatively, a positive correlation might have resulted from the inclusion of some patients with very poor endometrial stimulation.

It has been reported (Gonen and Casper, 1990; Couplam et al., 1994; Noyes et al., 1995; Rinaldi et al., 1996) that a threshold value of $<6$ mm has a high negative predictive value for subsequent pregnancy. As all our patients (pregnant and non-pregnant) had an endometrial thickness $>7$ mm at the time of HCG injection, oocyte retrieval and embryo transfer, we were unable to comment on this point.

Our study found no significant endometrial changes between compared groups, despite obtaining several subsequent measurements and calculating temporal and dynamic changes. Our findings are confirmed by others (Strohmer et al., 1994), who concluded that endometrial thickness was related to the individual size of the uterus and therefore had no predictive value for implantation.

The junctional zone may play an important role in implantation. MR images obtained in a conception cycle at 7 days post ovulation showed loss of integrity or disruption of the junctional zone and a reduction in signal intensity of the overlying myometrium (Turnbull et al., 1995a). Similar findings (Barton et al., 1993) suggested involvement of the junctional zone in cases of incomplete abortions, but not in the case of ectopic pregnancy. An association between the relative magnetic resonance signal intensities of the uterine layers and successful implantation has been described (Turnbull et al., 1994). Women with reduced contrast between the myometrium and junctional zone were more likely to conceive, but the signal intensity of the myometrium appeared to be the most important factor related to outcome.

This study has demonstrated changes within junctional zone thickness during an IVF/embryo transfer cycle. Magnetic resonance imaging (MRI) studies (Zawin et al., 1990) have described the junctional zone as the most stable layer which remains unchanged during 6 months of gonadotrophin releasing hormone analogue (GnRHa) therapy for endometriosis, in contrast to extensive changes in the endometrium and myometrium. No difference was found between follicular and luteal phase junctional zone thickness measured by MRI (McCarthy et al., 1986), but the subsets were small (nine women using oral contraception and 12 in the natural cycle) and measurements were taken only once within the 10 days of the follicular phase or 15 days of the luteal phase. Although MRI provides precise measurements of uterine layers, its costs and complexity mean that investigated groups are usually small and observations are scattered in time and do not provide comparable information about menstrual cycles.

We observed a decrease in junctional zone thickness during ovulation induction, and this was reversed during the early luteal phase, suggesting either mechanical compression by the rapidly growing endometrium or the negative effect of increasing oestradiol concentrations. This was more prevalent among patients who would later conceive. The endometrial stromal cell nuclei have been shown to increase in size and become more round during the early luteal phase (Dockery et al., 1990). This observation appeared to be related to increased transcriptional activity. As the junctional zone is characterized by a three-fold increase in nuclei per unit area compared with the outer myometrium (Scoutt et al., 1991), it may react differently to changing hormone concentrations, possibly in a similar way to endometrium. The junctional zone has been recognized only recently as a separate structure (McCarthy et al., 1989; Brown et al., 1991; Scoutt et al., 1991) and lack of direct access to samples of this tissue makes collection for scientific studies much more difficult. However, digitized images of real-time ultrasound indicate that this myometrial layer may be responsible for subtle contractile activity, reflected by endometrial wave-like movements (Birnholz, 1984). Junctional zone contractions also contribute to sperm transport (Kunz et al., 1996), and their pattern changes in the presence of endometriosis (Leyendecker et al., 1996). Moreover, the junctional zone contractions are affected by the method of embryo transfer (Lesny et al., 1999b). Less active junctional zone contractility has recently been associated with higher pregnancy rate in both natural (Ijland et al., 1997) and assisted (Fanchin et al., 1998) reproduction cycles. The mechanism by which these contractions are generated is unknown. It has been suggested recently that local synthesis of nitric oxide (NO) within the uterus can regulate uterine contractility (Rosselli, 1997), and its synergistic action with progesterone may relax uterine contractions in a paracrine fashion (Telfer et al., 1995). More exaggerated changes in the thickness of the junctional zone in the group who became pregnant may reflect a better responsiveness of the junctional zone to successful implantation, but to what extent junctional zone thickness is related to its function is yet to be determined.

At D0, the myometrial layer was significantly thicker in the pregnant group and remained thus throughout ovulation induction (on D8 and at HCG). This was not due to previous pregnancies because the incidence of primary and secondary infertility in both subsets was similar. In this study there was no difference in myometrial measurements during the early luteal phase between the two outcome groups. Although our results are not directly comparable, a MRI study (Turnbull et al., 1994) also failed to yield any differences in myometrial...
volumes between pregnant and non-pregnant groups measured one day before embryo transfer. However, MRI showed differences in relative signal intensity ratios between analysed layers, suggesting a higher water content in the myometrium of fertile cycles, which in turn could reflect differences in metabolism.

The pronounced changes within the junctional zone were confirmed when the Bonferroni correction was applied. The highly significant differences between the junctional zone values at downregulation and embryo transfer (temporal change) between non-pregnant and pregnant groups \( (P < 0.0004) \) suggest that this parameter may be important with regard to uterine receptivity.

In summary, our results suggest that it is not possible to predict the likelihood of pregnancy from endometrial thickness at any time of the IVF/embryo transfer cycle. In contrast, changes in other uterine layers were more exaggerated in conception cycles than in non-conception cycles. The responsiveness of the junctional zone may be associated with implantation, but its precise role is not clear.

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References


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