Uterine junctional zone contractions during assisted reproduction cycles

VIDEO

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This study was designed to assess junctional zone contractions (JZ) during cycles of in-vitro fertilization (IVF) and embryo transfer in oocyte donors exposed to a long protocol regime for ovarian stimulation. Real-time transvaginal ultrasound and advanced audio-visual and computer technology were used to evaluate the contraction pattern, frequency and velocity. At the time of down-regulation JZ contractions were not observed. After 7 days of superovulation all patients displayed cervico-fundal, fundo-cervical and random contractions. Cervico-fundal waves dominated the picture at the time of human chorionic gonadotrophin injection. However, the activity was strongest on the day of oocyte retrieval. At that time the percentage of opposing waves increased and fundo-cervical waves disappeared. The highest wave frequency and velocity (4.29 ± 0.68 waves/min and 2.73 ± 0.54 mm/s respectively) were observed at the time of oocyte retrieval. All patients had some JZ activity on days 2, 3 and 4 after oocyte retrieval but regular wave-like contractility gradually decreased and only single random movements were seen on day 4 after oocyte retrieval. In conclusion, JZ activity throughout the IVF cycle is more exaggerated when compared to the results reported from observations of the natural cycle but follows a similar pattern. This fact can probably be explained by the vastly different hormone levels. Higher JZ activity and correspondingly increased mobility of the endometrium may impair its receptivity and affect implantation.

Key words: in-vitro fertilization and embryo transfer/transvaginal ultrasound/uterine junctional zone contractions

Introduction

Contractility of the non-pregnant uterus throughout the ovarian cycle has been described by Martinez et al. (1973), who used three rubber balloon catheters to record intrauterine pressure. He distinguished between propagated and non-propagated contractions and noted that the frequency of contractions is highest in the periovulatory phase of the ovarian cycle. The rapid development of real-time ultrasonography allowed non-invasive observation of endometrial movements transabdominally (Birnholz 1984) and transvaginally (Oike et al., 1988; de Vries et al., 1989; Abramowicz and Archer, 1990; Lyons et al., 1991; Fukuda and Fukuda, 1994; Kunz et al., 1996; Kunz and Leyendecker, 1996). Later, advanced audio-visual and computer technology was utilized to evaluate these particular kind of contractions in a more objective way (Ijland et al., 1996, 1997a,b; Fanchin et al., 1997; Lesny et al., 1998). The first study directly related to fertility in the natural cycle has revealed that lower endometrial activity throughout the entire cycle is associated with a higher pregnancy rate. It has also shown that fertile cycles have different contraction characteristics (Ijland et al., 1997a). Subendometrial contractions have been described in in-vitro fertilization (IVF)–embryo transfer cycles (Abramowicz and Archer, 1991; Fukuda and Fukuda, 1994) but these groups used different superovulation regimes and simpler imaging techniques. Recently increased endometrial mobility has

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been associated with embryo transfers which were traumatic (Lesny et al., 1998) and, if seen before transfer, with a lower pregnancy rate (Fanchin et al., 1997).

The aim of this study was to evaluate endometrial activity during a cycle of IVF–embryo transfer in patients exposed to a long protocol regimen with down-regulation, superovulation and luteal phase support. We have used the term ‘junctional zone (JZ) contractions’ as there is some evidence that this particular layer of myometrium, which consists of a discrete compartment of more compacted myocytes, may be responsible for the wave-like movements of the adjacent endometrium (Scoutt et al., 1991; Turnbull et al., 1994; Brosens et al., 1995; Tetlow et al., 1997).

Materials and methods

This research project was approved by Hull and East Riding Research Ethics Committee. All patients were counselled and participated on a voluntary basis after giving written consent. We asked 18 egg donors (multiparous women, age 23–34 years, mean 28.2 years) to act as model IVF–embryo transfer patients. None of them had any medical or gynaecological problems including a previous history of a gynaecological operation or Caesarean section. None were using medication other than the following. Superovulation prior to IVF was achieved with a standard regimen of pituitary down-regulation with a luteinizing hormone releasing hormone superagonist (Nafarelin; Searle Pharmaceuticals, High Wycombe, Bucks, UK) 800 µg daily administered intranasally from the mid-luteal phase, followed by appropriate doses of urofollitrophin (Metrodin High Purity; Serono Laboratories UK Ltd, Welwyn Garden City, Herts, UK). When the lead follicle reached a diameter of 20 mm, human chorionic gonadotrophin (HCG, Profasi; Serono) 10 000 IU, was given as an ovulatory trigger. Luteal support was provided by vaginal micronized progesterone (Utrogestan; Besins Iscovesco Laboratories, Paris, France) in a dose of 600 mg nocte from the day of oocyte retrieval until day 2, 3 or 4 after oocyte retrieval. All patients received 600 mg of ibuprofen (Brufen; Knoll Ltd, Nottingham, UK) 2 h before oocyte retrieval. Midazolam (Hypnovel; Roche Products, Welwyn Garden City, Herts, UK) was used for sedation and alfentanil (Rapifen; Janssen-Cilag Ltd, High Wycombe, Bucks, UK) was given for analgesia during the procedure.

A vaginal ultrasound scan (ATL Ultramark 4, 5 MHz transducer; Advanced Technology Laboratories, Seattle, Washington, USA) was used to record JZ activity after down-regulation (day 0), on day 8, day 10, on the day of HCG injection, during oocyte retrieval (just before and after), and 2, 3 and 4 days after oocyte retrieval. At each examination 3–5 min of video images of the uterus in both longitudinal and transverse planes were recorded (VHS P 4341; Goldstar, South Korea). The uterine zonal anatomy (Tetlow et al., 1996) and the length of cervix and uterine cavity were measured. A total of 97 recordings were made. After recording, the images were digitized using a computer equipped with a Perception Video Recorder 3500, PAL (Digital Processing System Inc. 1996, Scarborough, Canada) and converted to five times normal speed using Speed Razor Mach III (In:Synch Corporation, 1993, Bethesda, MD, USA). A frame time-coding system (25 frames/s) allowed us to evaluate timing of events with an accuracy of ±0.04 s. To describe wave patterns we used the classification system introduced by Ijland et al. (1996) which distinguished between waves from cervix to fundus; waves from fundus to cervix; opposing waves originating simultaneously at cervix and fundus; and random waves starting at various foci. Contraction pattern and frequency (35 episodes lasting 2 min) and velocity (25 randomly chosen waves) were rated by two independent observers. These observations were evaluated on SPSS for Windows (SPSS UK Ltd, St Andrew’s House, Woking, Surrey, UK) using Wilcoxon’s test for two paired samples which showed no differences between intra- and interobserver measurements.

Results

At the time of down-regulation JZ and midline cavity echo or thin endometrium were clearly seen but contractions were never observed (Video, Example 1: see CD ROM). After 8 days of superovulation JZ activity appeared in all cases (Figure 1). The most accentuated were contractions from cervix to fundus. Waves from fundus to cervix and random waves were also observed (Video, Example 2). At the time of HCG injection cervico-fundal waves dominated the picture, opposing waves appeared, and percentage of fundo-cervical and random waves decreased (Video, Example 3). Contractions recorded in the transverse plane appeared as a contraction ring which involved the full circumference of marginal endometrium (Video, Example 4). Assessment just prior to oocyte retrieval revealed an increasing number of opposing waves. Fundo-cervical waves were no longer seen and the share of cervico-fundal waves was reduced (Video, Example 5).

The cervico-fundal waves were initially slow and with low amplitude but on the day of HCG injection and at the time of oocyte retrieval they involved the whole length of endometrium and increased in frequency and velocity (Table 1). The strongest JZ activity reflected by higher frequency and velocity was immediately after oocyte retrieval with the wave-like patterns well pronounced but disrupted by rapid random movements not seen prior to this stage (Video, Example 6).
Table I. Frequency and velocity of junctional zone contractions from cervix to fundus during ovulation induction for an in-vitro fertilization cycle

<table>
<thead>
<tr>
<th>Day of cycle</th>
<th>HCG injection</th>
<th>Oocyte retrieval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>2.87 ± 0.78</td>
<td>3.26 ± 0.82</td>
</tr>
<tr>
<td>Velocity</td>
<td>1.42 ± 0.26</td>
<td>1.68 ± 0.28</td>
</tr>
</tbody>
</table>

HCG = human chorionic gonadotrophin.

Table II. Junctional zone contractions waves observed 2, 3 and 4 days after oocyte retrieval (early luteal phase)

<table>
<thead>
<tr>
<th>Type of waves</th>
<th>Random</th>
<th>Opposing</th>
<th>Cervico-fundal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2 (8 patients)</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Day 3 (6 patients)</td>
<td>6</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Day 4 (4 patients)</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

Table II shows characteristics of wave directions 2, 3 and 4 days after oocyte retrieval. When recording was performed 2 days after oocyte retrieval, cervico-fundal contractions were present in three out of eight patients, but they were short and never involved the whole endometrium. Endometrial activity on day 3 was less dynamic than on day 2, the waves being shorter and with limited spread and random waves prevailed. On day 4 the endometrium was very 'quiet', only focal random movements were observed (Video, Example 7A,B,C).

Discussion

Evaluation of JZ contractions presents several challenges. Simple factors, such as the scanning technique, can profoundly affect results. When one tries to obtain a perfect image of the endometrium and press too hard with a transvaginal transducer, even strong contractions during the periovulatory phase may become disrupted. We have demonstrated that contractions seen in the transverse plane involve simultaneously the whole circumference of marginal endometrium, giving the impression of a regular pulsation. However, the same contractions in the longitudinal plane were perceived as cervico-fundal waves. Any oblique image is a mixture of activity in transverse and longitudinal plane and will create a false picture of opposing or fundo-cervical waves. The above findings may be responsible for lack of consistency in the reported classification of waves (Abramowicz and Archer, 1991; Lyons et al., 1991). This has been even more confused by using the fast-forward facility of a video tape machine to do on-line analysis. With this method fine random waves or more complicated wave patterns cannot be visualized. We agree that the wave classification introduced by Ijland et al. (1996) is the most convenient, but even with digitization we found it difficult to quantify very minute movements or activity without an associated wave pattern. When we experimented with a
higher speed (20 and 40 times acceleration of recorded picture), it became evident that an apparently static image had low amplitude activity.

We have not observed any JZ contractility in down-regulated patients. This is similar to the referred lack of contractions of the inner third of the myometrium in postmenopausal women (de Vries et al., 1989). It has been documented that the JZ is the only uterine layer which is not significantly affected by prolonged treatment with lutetinizing hormone-releasing hormone superagonists (Demas et al., 1986; Zawin et al., 1990) or the menopause (Brown et al., 1991; Scoult et al., 1991). As in both situations the anatomical structure which may be capable of generating contractility is present, the lack of activity may be the result of low oestrogen level. During the early follicular phase of the natural cycle when oestriadiol level is low, some minimal contractility has been described (Lyons et al., 1991; Kunz et al., 1996; Lyendererde et al., 1996; Ijland et al., 1996, 1997a).

The timing of our second scan assessment (7 days of gonadotrophin injections) corresponds to the mid-follicular phase of the ovarian cycle. At that time all our patients displayed JZ contractility which differs from findings in the natural cycle. Lyons et al. (1991) have described endometrial mobility in 20–48% of observed subjects. Fukuda and Fukuda (1994) in 74% and Ijland et al. (1996) in 86% of their patients respectively. When the ultrasound scan images were viewed with the tape in fast-forward mode Fukuda reported the presence of endometrial waves in 75% of patients treated with clomiphene citrate and in 74% of women given gonadotrophins for both ovulation induction and superovulation. The only other published data using digitization is presented by Ijland’s group (1996, 1997a,b) to which we relate all of our comments concerning wave patterns during the natural cycle.

During the mid-follicular phase of an IVF cycle, cervico-fundal contractions prevailed (62%) compared with 28% in the natural cycle where random waves dominated (36%). The frequency and velocity of cervico-fundal waves was similar in IVF and natural cycle groups.

The injection of HCG 36 h before oocyte retrieval was used to define the late follicular phase. The dominant pattern at this time was cervico-fundal waves (75%), opposing waves appeared (13%) and the number of fundo-cervical waves declined from 13% to 6%. This varies from the findings of Ijland et al. (1996) who did not see any activity in 4% of patients during a natural cycle. They reported different wave patterns: opposing waves comprising 30%, cervico-fundal waves 36% and fundo-cervical waves 19% of all observations. The frequency of cervico-fundal waves was still within the same range in both groups but their velocity in an IVF cycle was higher (1.96 ± 0.33 compared to 1.53 ± 0.27 mm/s respectively). The above results may suggest that baseline JZ contractility is lower during the follicular phase of the natural cycle and not every patient is able to initiate contractions. Cervico-fundal waves seem to dominate the picture earlier in an IVF cycle.

Observation of the JZ contractions just before and after oocyte retrieval provides the closest possible assessment of the periovulatory phase. In previous studies related to the natural cycle, ovulation has been determined retrospectively by the disappearance of the leading follicle (Lyons et al., 1991; Ijland et al., 1996). We have performed a mock assessment of the periovulatory phase by comparing JZ contractions at the time of HCG injection (late follicular phase) to activity before and after oocyte retrieval. Before oocyte retrieval the percentage of opposing waves increased to 19% while fundo-cervical waves disappeared. The number of cervico-fundal waves was slightly reduced to 68%. The frequency and velocity of cervico-fundal waves was highest with velocity 2.73 ± 0.54 mm/s being nearly twice the value of natural cycle (1.50 ± 0.27 mm/s). These effects were observed in spite of 600 mg of ibuprofen given prior to oocyte retrieval when one might expect some muscle relaxant action induced by its anti-prostaglandin activity. Very strong but irregular contractions after oocyte retrieval may suggest that ovarian trauma or multiple ‘ovulation’ caused an excessive release of prostaglandins and others factors, which in turn affected JZ contractility. The presence of prostaglandins in the ovarian vein (Wallach and Dhamurajan, 1992), mediators of the inflammatory reaction (Espey, 1992) and neuropeptides (Kannisto et al., 1992) have been described in animal models and during physiological ovulation. JZ hyperactivity after oocyte retrieval has been observed despite drugs used for sedation and analgesia.

During the early luteal phase all our patients demonstrated some JZ contractility. This was not the case in 10% of women assessed during the natural cycle (Ijland et al., 1996). This may be the effect of oocyte retrieval and/or hyperactivity caused by higher than physiological hormone levels in an IVF cycle. It is also agreed that luteal phase support is required for down-regulated patients to prevent premature luteolysis (Smith et al., 1989; McClure et al., 1992), and, if it is still happens in spite of treatment, this may also have some implication for observed differences. However, it must be emphasized that our donors received progesterone for the luteal phase support vaginally, which is a well accepted and effective method (Edwards and Brody, 1995). We observed subtle differences such as the disappearance of cervico-fundal waves, shortening of opposing waves and reduction in JZ contractility in general.
(only single random wave present) at 96 h compared to 48 and 72 h after oocyte retrieval respectively. Diminishing contractility from early to late luteal phase of the natural cycle has been described before (Lyons et al., 1991; Ijland et al., 1996) and less observed endometrial mobility has been associated with a higher pregnancy rate (Ijland et al., 1997a). It has also been suggested that endometrial hyperperistalsis and dysperistalsis are possible mechanisms for subfertility in patients with endometriosis (Leyendecker et al., 1996).

Taking into consideration that JZ contractility is higher during the early luteal phase of the IVF cycle than the natural cycle, the mobility of the endometrium may affect the implantation process in a strictly mechanical way. In addition, embryos are transferred earlier to the uterine cavity than would have been the case if they came from the Fallopian tube (Croxatto et al., 1978; Buster et al., 1985) and therefore are exposed to a more ‘unsettled’ endometrium. The presence of JZ activity may alter implantation rates although there are some conflicting data in the published literature on whether this is a decrease (Fanchin et al., 1997) or increase (Woolcott and Stanger, 1997).

Some studies have demonstrated that the pregnancy rate is higher after embryo transfer on the third day as embryo quality can be better assessed (Huisman et al., 1994; Dawson et al., 1995). We suggest that one reason for an apparently better outcome is less active JZ contractility. If the JZ contractions express one aspect of the ‘implantation window’, endometrial receptivity may not be at its best during the early luteal phase of the IVF cycle.

In conclusion, JZ contractility throughout the IVF cycle is more exaggerated than during the natural cycle but follows a similar pattern. This could be explained by the vastly different hormone levels. Higher JZ activity and correspondingly increased mobility of the endometrium may impair its receptivity and affect early phases of implantation, but at present our data do not allow extrapolation to pregnancy rates.

**Legends to video example (see video on CD-ROM)**

**Example 1.** Junctional zone contractions are not present in down-regulated patients.

**Example 2.** Junctional zone contractions after 7 days of superovulation (early follicular phase, ELP). The most accentuated are contractions from cervix to fundus. Contractions from fundus to cervix and random contractions are also observed.

**Example 3.** Junctional zone contractions at the time of human chorionic gonadotrophin injection (late follicular phase, LFP). Cervico-fundal contractions dominate the picture, opposing contractions appear, percentage of fundo-cervical and random contractions decrease.

**Example 4.** Junctional zone contractions at the time of human chorionic gonadotrophin injection (LFP). Transverse plane. Contractions appear as a regular pulsation or contraction ring.

**Example 5.** Junctional zone contractions just before oocyte retrieval (periovulatory phase). Regular cervicofundal contractions are still present but number of opposing contractions increases.

**Example 6.** Junctional zone contractions just after oocyte retrieval. Strong contractility. Distinctive random and opposing waves appear. Wave pattern is less regular.

**Example 7A.** Junctional zone contractions 2 days after oocyte retrieval (ELP). Contractions from cervix to fundus can be still observed but have lesser frequency and velocity.

**Example 7B.** Junctional zone contractions 3 days after oocyte retrieval (ELP). Random contractions prevail, opposing contractions present. Waves hardly able to spread.

**Example 7C.** Junctional zone contractions 4 days after oocyte retrieval (ELP). Only single random movement can be noted.

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