Influence of controlled ovarian hyperstimulation on uterine peristalsis in infertile women

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STUDY QUESTION: Is there a difference in the characteristics of uterine peristalsis in natural and controlled ovarian hyperstimulation (COH) cycles?

SUMMARY ANSWER: COH significantly changed the uterine peristaltic pattern.

WHAT IS KNOWN ALREADY: In natural menstrual cycles, the periodic changes of uterine peristalsis are closely related to the reproductive process.

STUDY DESIGN, SIZE, DURATION: This is a prospective cohort study with a total of 64 subjects involved. The study was performed between May 2011 and August 2011.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Sixty-four infertile women with regular, ovulatory menstrual cycles underwent follicular tracking in one natural cycle and after ovarian stimulation (GnRH-agonist down-regulation) in the subsequent cycle (COH). Three time points were studied in both cycles: at LH surge/HCG plus 1 day, ovulation/oocyte retrieval and 2 days after ovulation/retrieval. The study was performed in an IVF center of the university-affiliated Xiangya hospital.

MAIN RESULTS AND THE ROLE OF CHANCE: Uterine peristaltic wave frequency was 1.31 times higher in the COH than in the natural cycle ($P < 0.01$). At all three time points in the COH cycle, waves moving from the cervix to fundus dominated, comprising 80–90% of the wave types observed, while ‘no activity’ was more frequently observed in the natural cycle. The wave frequency was positively correlated with the level of serum estradiol ($E_2$) ($r = 0.30; P < 0.01$) and negatively correlated with the progesterone level ($r = -0.48; P < 0.01$) for the physiological range of steroid levels. No correlation was found between the wave frequency and supraphysiological concentrations of $E_2$ or progesterone.

LIMITATIONS, REASONS FOR CAUTION: The two observers were not independent and this was a limitation of the study. Quantitative measurements of wave amplitude in the different cycles should be compared in future research.

WIDER IMPLICATIONS OF THE FINDINGS: Uterine peristalsis was much higher in the COH cycle than in the natural cycle. The endometrial movements did not weaken to the natural level before embryo transfer, even with high levels of progesterone. The wave frequency was positively correlated with serum $E_2$ level and negatively correlated with that of progesterone within the physiological range. No correlation was found between the wave frequency and supraphysiological concentrations of $E_2$ and progesterone.

STUDY FUNDING/COMPETING INTEREST(S): The authors declare that they have no study funding or competing interests in this study.

Key words: controlled ovarian hyperstimulation / uterine peristalsis / endometrium / wave frequency / IVF

Introduction

Uterine peristalsis is involved in the whole process of human reproduction. It originates in the subendothelial myometrial tissues and occurs with periodic changes during the natural menstrual cycle (Lyons et al., 1991; Chalubinski et al., 1993; Ijland et al., 1996; Bulletti et al., 2000). As the follicle develops, uterine peristalsis increases and reaches a peak in the pre-ovulatory phase. The active cervicofundal movements transport sperm from the vaginal fornix through the uterus into the oviduct ipsilateral to the dominant follicle (Kunz...
et al., 1996). After ovulation, the endometrial wave-like activity decreases significantly to provide a quiet environment for embryo implantation (Ijland et al., 1997a,b). When the uterine peristaltic wave amplitude and frequency are too high in the luteal phase, the embryo may not reach the optimal implantation site, contributing to the low embryo implantation rate or ectopic pregnancy (Ijland et al., 1997a; Lesny et al., 1998a,b; Eytan and Elad, 1999). Only a few cervical movements were observed in the luteal phase and these may prevent the embryo from implanting in the lower part of the uterus, which would result in cervical pregnancy or placenta praevia (Ijland et al., 1996; Kunz et al., 2006).

Controlled ovarian hyperstimulation (COH) increases the number of retrieved oocytes and subsequently the number of embryos available for transfer. However, the potential adverse effects on uterine peristalsis of stimulation regimens remain almost unknown. The present study was designed to describe, for the first time, the characteristics of endometrial wave-like activity in the natural and COH cycles in the same subjects as well as their relation to serum estradiol (E₂) and progesterone concentrations.

Materials and Methods

Patients

Between May 2011 and August 2011, we prospectively recruited 64 consecutive normo-ovulatory women who came for IVF or ICSI treatment at the Department of Reproductive Medicine at Xiangya Hospital of Central-south University. Women with anatomic disorders of the female reproductive system, leiomyoma, endometriosis, intrauterine adhesion, endometrial polypl, ovarian cyst, benign ovarian neoplasms and anovulation were excluded.

Study design

All subjects underwent follicular tracking in a natural cycle and after a GnRH-agonist down-regulation ovarian stimulation protocol in the subsequent (COH) cycle.

In the natural cycle, follicular tracking started on Day 10 by transvaginal ultrasonography (TVS). If the average diameter of the dominant follicle was <14 mm, the patient was asked to return every other day. Otherwise, a urine LH test (Sterility test strip, Kunming Yunda Biotechnology Co., Ltd.) was performed and the patient was asked to return the next day. When the diameter of the dominant follicle was >18 mm or the urine LH test was >45 mIU/ml, uterine peristalsis was monitored and a blood sample for the measurement of serum LH, E₂ and progesterone was collected every day until ovulation.

Pituitary down-regulation was achieved with a GnRH agonist (Triptorelin, Ferring GmbH) of 0.05–0.1 mg daily administered at 7 days after ovulation (mid-luteal phase) and continued for 14 days. In the COH cycle, gonadotrophin stimulation was initiated with recombinant FSH (Gonal-F, Merck Serono) or urinary FSH (Urofolitropin, Lixion, China) at a dose of 112.5–300 IU/day depending on the ovarian response, as determined by serum E₂ levels and a transvaginal ultrasound scan performed every 1–3 days. When there were at least three leading follicles measuring ≥18 mm in diameter, HCG (Lixion, China) 5000 IU–10,000 IU was given as an ovulatory trigger. Oocytes were retrieved 34–36 h after HCG injection under transvaginal ultrasound guidance. Luteal phase support was provided by progesterone injection in a dose of 60 mg/day and progesterone soft capsules in a dose of 200 mg/night from the day of oocyte retrieval. Embryo transfer was performed 2 or 3 days after oocyte retrieval.

Uterine peristalsis was monitored by an ultrasound scan performed at three standardized defined periods in each of the cycles, namely on the day of the LH surge, at ovulation and 2 days after ovulation (ovulation + 2) in natural cycles, and on Day 1 after HCG administration (HCG + 1), at oocyte retrieval and 2 days after oocyte retrieval (oocyte retrieval + 2) in COH cycles. Blood samples for the measurement by electrochemiluminescence immunoassay of serum E₂ and progesterone were collected on the days of LH surge, ovulation + 2, HCG + 1 and oocyte retrieval + 2.

Transvaginal ultrasonography of uterine peristalsis

Transvaginal ultrasonography (Mindray DC-6 Expert, 5–8 MHz transducer) was performed for 5 min while the patient was lying relaxed in a lithotomy position. At each examination, images of the mid-sagittal plane of the uterus were recorded (DSC-T100, Sony). The records were replayed at four times regular speed using a VLC media player 1.1.11 (VideoLAN team). Uterine peristaltic wave frequency and wave type were analyzed and agreed by two observers who had accumulated experience in identifying the peristaltic wave patterns during preliminary experiments performed in the month preceding the main research. Both observers reviewed each video at the same time and were blind to the timing of the measurements and the treatment condition (natural/stimulated cycle), both for reviewing the ultrasound videos and assessing outcomes. If disputes over the wave frequency or type arose, the ultrasound video was replayed and reanalyzed several times until agreement was reached. Most of the discrepancies were easily resolved. Only four cases of sharply differing opinion occurred and these were excluded from the analysis.

Wave types were described according to the classification system of Ijland et al. (1996), including five types of endometrial movement: no activity (N), waves from cervix to fundus (CF), waves from fundus to cervix (FC), opposing waves starting simultaneously at cervix and fundus (OP) and random waves starting at various foci (R). This study was approved by the Institutional Review Board. Informed consent was obtained from all patients.

Statistical analysis

The statistical analysis was carried out using the STATA (v10.0), SPSS (v19) software. Repeated measures data of uterine peristaltic wave frequency were analyzed with generalized estimating equation methods, paired Student’s t-test and presented as mean ± SD. Categorical variables were compared by the χ² test. Correlations between the uterine peristaltic wave frequency and respective serum concentrations of steroids were performed using linear correlation analysis (two-tailed). P < 0.05 was considered statistically significant.

Results

In the present study, the age distribution of the women ranged from 22 to 42 years (mean: 31.38), and the duration of infertility ranged from 1 to 18 years (mean: 5.16). Fifteen patients had primary infertility and 45 had secondary infertility.

Table 1 shows the uterine peristaltic wave frequency at different times in the natural and COH cycles. The uterine peristaltic wave frequency on the day of HCG + 1 was lower than that of LH surge, while it was higher at oocyte retrieval and oocyte retrieval + 2 than at ovulation and ovulation + 2. After controlling for relevant confounding factors, the wave frequency was 1.31 times higher in the COH cycle than that in the natural cycle (P < 0.01).
The relative distribution of uterine peristaltic wave types in the cycles studied is shown in Table II. The differences in proportion of uterine peristaltic wave types were compared between corresponding times in the natural and COH cycles, i.e., LH surge versus HCG + 1, ovulation versus oocyte retrieval and ovulation + 2 versus oocyte retrieval + 2. All comparisons were statistically significant. Compared with natural cycles, the percentage of CF waves was greater and the percentage of FC waves was lower with COH. In natural cycles, uterine endometrium was most dynamic on the days of LH surge and ovulation, with CF waves representing 74.88% and 79.83% of the total. Two days after ovulation, uterine peristasis decreased, with the CF waves reducing to 68.41%. Meanwhile, R waves increased compared with the LH surge and four cases of ‘no activity’ were observed in this phase. In the COH cycle, despite the lower wave frequency on HCG + 1 day compared with the LH surge, a higher percentage of CF waves was present on HCG + 1 day versus day of the LH surge. Both the frequency and percentage of CF waves reached a peak on the day of oocyte retrieval, while R waves disappeared at that point. Two days later, the percentage of CF waves dropped to 79.78%, and other types of uterine peristaltic waves rose slightly. In addition, during the observation times tested, the uterine peristaltic waves seemed to be more obvious and complex in the COH cycle than in the natural cycle (Fig. 1).

The correlations between the wave frequency and progesterone and E2 were analyzed in two separate parts: physiological and supra-physiological levels. A positive correlation between E2 and the wave frequency (r = 0.30; P < 0.01) and a significant negative correlation between progesterone and the wave frequency (r = −0.48; P < 0.01) were observed within the physiological range of steroid levels. However, no correlations were found between the uterine peristaltic wave frequency and supraphysiological concentrations of E2 (HCG + 1 day and oocyte retrieval + 2) and progesterone (oocyte retrieval + 2).

### Discussion

In the present study, for the first time we comprehensively analyzed the characteristics of uterine peristalsis in a natural cycle and a COH cycle in the same woman. Although previous studies have reported that uterine peristalsis was more pronounced in induced cycles than in natural cycles, the comparison was performed between different subjects, and even in separate studies (Ijland et al., 1998; Eytan et al., 2001). All comparisons in our study were matched, in that the different cycles were analyzed in the same woman, effectively enabling women to be their own control and thereby reducing bias. The rationale for the study was that understanding the changes in uterine peristalsis could influence further research into the mechanisms that regulate success rates of IVF following embryo transfer.

The uterine peristaltic wave frequency in a COH cycle was greater than that of a natural cycle, which is similar to the findings in previous research (Ijland et al., 1998; Eytan et al., 2001). However, the wave frequency on HCG + 1 day was lower than that at the LH surge, which differed from our expectation. It was reported that GnRH could significantly decrease the contraction intensity of uterine muscle strips, following the action of oxytocin and acetylcholine (Gohar et al., 1996). In addition, the treatment of pregnant rats with pharmacological doses of GnRH delayed parturition (Gohar et al., 1996). GnRH agonists may have a similar effect. The strongest uterine peristalsis in a COH cycle was observed at oocyte retrieval both in our study and that of Lesny et al. (1998a,b), suggesting that ovarian trauma or multiple “ovulation” may release excessive prostaglandins and other uterotonic agents which enhanced uterine contractility.

Although changes in the percentage of each wave type in a COH cycle followed a similar pattern when compared with the natural cycle, the relative distribution of uterine peristaltic wave types was different significantly in the natural and COH cycles. CF waves dominated

### Table I: Uterine peristaltic wave frequency (waves/min) in natural and COH cycles in infertile women.

<table>
<thead>
<tr>
<th>Cycle</th>
<th>N</th>
<th>LH surge/ HCG + 1</th>
<th>Ovulation/ oocyte retrieval</th>
<th>Ovulation + 2/oocyte retrieval + 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural cycle</td>
<td>60</td>
<td>2.9 ± 0.6</td>
<td>2.7 ± 0.7</td>
<td>2.0 ± 0.9</td>
</tr>
<tr>
<td>COH cycle</td>
<td>60</td>
<td>2.4 ± 0.7</td>
<td>3.6 ± 0.8</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td><em>P</em></td>
<td></td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Note: the data are mean ± SD, and the comparison was performed using paired Student’s t test. For natural cycles: ovulation + 2: 2 days after ovulation. For COH cycles: HCG + 1: Day 1 after HCG administration, oocyte retrieval + 2: 2 days after oocyte retrieval.

### Table II: The percentage distribution of uterine peristaltic wave types in natural and COH cycles in infertile women.

<table>
<thead>
<tr>
<th>Observation time</th>
<th>N</th>
<th>CF</th>
<th>FC</th>
<th>OP</th>
<th>R</th>
<th>n</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH surge</td>
<td>0 (0%)</td>
<td>647 (74.9%)</td>
<td>170 (19.7%)</td>
<td>46 (5.3%)</td>
<td>1 (0.1%)</td>
<td>1596</td>
<td>31.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HCG + 1</td>
<td>1 (1.7%)</td>
<td>621 (83.5%)</td>
<td>77 (10.3%)</td>
<td>31 (4.2%)</td>
<td>2 (0.3%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovulation</td>
<td>0 (0%)</td>
<td>649 (79.8%)</td>
<td>143 (17.6%)</td>
<td>16 (2.0%)</td>
<td>5 (0.6%)</td>
<td>1885</td>
<td>84.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Oocyte retrieval</td>
<td>0 (0%)</td>
<td>1004 (93.7%)</td>
<td>55 (5.1%)</td>
<td>13 (1.2%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovulation + 2</td>
<td>4 (6.7%)</td>
<td>442 (68.4%)</td>
<td>124 (19.2%)</td>
<td>12 (1.8%)</td>
<td>25 (3.9%)</td>
<td>1329</td>
<td>29.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Oocyte retrieval + 2</td>
<td>1 (1.7%)</td>
<td>585 (79.8%)</td>
<td>87 (11.9%)</td>
<td>28 (3.8%)</td>
<td>21 (2.8%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: wave type distributions were compared by the χ² test in the natural (top set of data at each time point) versus COH cycle (a lower set of data). N, no activity; CF, waves from cervix to fundus; FC, waves from fundus to cervix; OP, opposing waves starting simultaneously at cervix; R, fundus and random waves starting at various foci.
at all times in the COH cycle versus the natural cycle, while the proportion of FC waves was lower in the COH cycle than in the natural cycle. Previous studies (Ijland et al., 1996; Kunz et al., 2006) had observed a few CF waves during the luteal phase, perhaps protecting the embryo from implantation within the lower uterine regions. However, whether the increased CF waves and decreased FC waves in COH cycles could push embryos into Fallopian tubes remains unclear. It is worthwhile noting that four cases of 'no activity' were observed at ovulation + 2, whereas there was only one case at oocyte retrieval + 2. Uterine peristalsis decreased after oocyte retrieval but was still greater than in the natural cycle.

The uterine peristalsis before embryo transfer in COH cycles was more active than in the natural level, either in terms of wave frequency or the distribution of wave type. In addition, uterine peristalsis in the natural cycle mainly comprised transverse waves but both transverse and longitudinal waves occurred in COH cycles, not only undulating but also rolling forward (Fig. 1 and see videos in Supplementary data). These complex waves may move the embryo from side to side, resulting in implantation failure.

According to our research, uterine peristaltic wave frequency positively correlated with physiological levels of serum E2, which paralleled the conclusion of Oike et al. (1990). In the COH cycle, serum E2 levels
were 10 times that of the natural cycle. However, the uterine peristaltic wave frequency was slightly higher instead of increasing linearly, also confirming the results of Kunz et al. (1998) who found that supraphysiological serum concentrations of E2 or an oxytocin injection in the early and mid-follicular phases significantly increased the uterine peristaltic frequency but the increase was not significant in the late follicular phase. They speculated that the natural uterine peristaltic frequency had reached a level at mid-cycle which cannot be surpassed significantly by non-physiological stimulation, probably because the system has become refractory. A similar phenomenon was observed in recent research on oxytocin, various prostaglandins and a muscarinic-receptor agonist that induced the characteristic uterine contractility patterns in an extracorporeal perfusion model of the swine uterus (Dittrich et al., 2009). A dose-dependent increase in intrauterine pressure (IUP) was observed after administration of different uterotonic agents; however, no relevant changes in IUP were measured when a plateau was reached after administration of oxytocin or prostaglandin F2α.

In our study, progesterone correlated negatively with uterine peristalsis at physiological levels, once again providing evidence of the role of progesterone in inhibiting myometrium contractions, and ‘quieting’ the uterus. Nevertheless, there was no correlation between the uterine peristaltic wave frequency and supraphysiological concentrations of progesterone at oocyte retrieval + 2. On the one hand, the progesterone level of nearly all patients was >60 ng/ml but the actual level could not be determined as this was the upper detection limit of the assay. On the other hand, although there was abundant progesterone in the serum before embryo transfer, the uterine peristalsis at oocyte retrieval + 2 could not reduce to the natural level at ovulation + 2. This may suggest that active uterine peristalsis cannot be effectively suppressed by the administration of progesterone alone. Studies have shown that anticholinergic agents and non-steroidal anti-inflammatory drugs could suppress uterine peristalsis in infertile women and increase the probability of successful pregnancy (Moon et al., 2004; Nakai et al., 2008; Kido et al., 2009).

Such data suggest the possibility that the administration of other agents to modulate uterine peristalsis may contribute to an improvement in endometrial receptivity and thereby increase embryo implantation rate.

Uterine activity was first detected by measuring IUP in non-pregnant women following the development of IUP transducers (Hendricks, 1964, 1966; Cibils, 1967; Martinez-Gaudio et al., 1973). However, this invasive procedure may alter normal uterine activity and induce contractions. In our study, TVS was used, owing to its high rate of detection of uterine peristalsis, direction identification (Nakai et al., 2004), convenience and cost-effectiveness. Although magnetic resonance imaging (MRI) could offer superior image quality, especially when it is impossible for TVS to display a good sagittal view if the uterus is in a retroverted position, even ultrafast MRI requires time intervals between scans (Nakai et al., 2001).

One limitation of our study was the lack of quantitative measurement of wave amplitude because of the performance restriction of the transvaginal ultrasound machine. Recently, Meirzon et al. (2011) brought forward a new, computerized method to objectively and qualitatively calculate the uterine peristaltic wave frequency and amplitude using transvaginal ultrasound imaging. However, the complicated algorithm and professional technology hamper its popularization and application. Fully automatic computation of this new approach is of great interest for further study.

In conclusion, COH significantly changed the pattern of uterine peristalsis in infertile women. In particular, endometrial movements could not reduce to the natural level before embryo transfer, even with abundant progesterone. Further research is required to investigate the possible negative effect of active uterine peristalsis on embryo implantation and maintenance of gestation.

**Supplementary data**

Supplementary data are available at http://humrep.oxfordjournals.org/.

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**Authors’ roles**

Z.L. participated in following parts of the study: study design, acquisition of data, analysis and interpretation of data, drafting the article and critical discussion. L.Y. is the corresponding author of this paper and participated in the study conception and design, as well as revising it critically for important intellectual content. X.A. participated in the acquisition and analysis of data, critical discussion in the study. All authors approve the version to be published.

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**Conflict of interest**

None declared.

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