Uterine peristalsis exerts control over fluid migration after mock embryo transfer

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STUDY QUESTION: What is the effect of uterine peristalsis on fluid migration after mock embryo transfer?

SUMMARY ANSWER: Uterine peristaltic wave frequency was positively correlated with the distance that fluid moved after it was deposited in the uterine cavity.

WHAT IS KNOWN ALREADY: Embryos have been found outside the uterine cavity after embryo transfer. It has been suggested that uterine contractions expelled these embryos.

STUDY DESIGN, SIZE, DURATION: A prospective cohort study of a total of 112 infertile women was conducted between March 2013 and May 2013.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Uterine peristaltic activity was assessed before and after a mock embryo transfer, in which 20 μl of ultrasound contrast agent was placed in the uterine lumen 3 days after ovulation in a natural cycle. The movement of this fluid was measured by ultrasound at 0, 15 and 30 min after placement.

MAIN RESULTS AND THE ROLE OF CHANCE: The uterine peristaltic wave frequency was significantly higher after than before mock transfer (3.06 ± 0.99 versus 2.24 ± 0.74, P < 0.01). At the conclusion of the 30-min monitoring period, the fluid had remained in place (N = 94), leaked into the cervix (N = 5), or moved into the Fallopian tubes or the cornua of the uterus (N = 11). The fluid movement was positively correlated with uterine peristaltic wave frequencies before (r = 0.518, P < 0.01) and after embryo transfer (r = 0.371, P < 0.01) and uterine peristaltic wave frequency was significantly higher both before and after embryo transfer in cases where the fluid was extruded.

LIMITATIONS, REASONS FOR CAUTION: Mock embryo transfer was performed in the luteal phase of a natural cycle instead of a controlled ovarian stimulation cycle. The endometrial environment and uterine peristalsis may be different in a stimulated cycle.

WIDER IMPLICATIONS OF THE FINDINGS: Uterine peristalsis exerts control over embryo migration and could adversely affect the chances of pregnancy if the wave frequency is too high. It could be used as a predictor of uterine irritability before embryo transfer.

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Key words: uterine peristalsis / fluid migration / uterine receptivity / assisted reproductive technology

Introduction

In a spontaneous cycle, embryo implantation occurs in the luteal phase when the uterus is ready for reception with few uterine peristaltic movements (IJland et al., 1997a,b; Bulletti et al., 2000; Fanchin and Ayoubi, 2009). The quiet local milieu is essential to maintain the embryo drifting in the uterine cavity, facilitating the maternal–fetal dialogue and recognition (Bulletti and de Ziegler, 2006; Fanchin and Ayoubi, 2009). In assisted reproduction, embryo transfer is the final, crucial step, but the invasive insertion of the catheter may disturb the physiological processes of embryo implantation. Although the combination of a soft catheter and ultrasound guidance permit placement with high precision, intrauterine deposition of embryos does not always mean that they remain in situ (Poindexter et al., 1986; Knutzen et al., 1992; Mansour...
of mock transfers, respectively (Knutzen et al., 1992). Similarly, methylene blue dye was also visualized at the external cervical os after mock embryo transfer (Mansour et al., 1994). Lesny observed that touching the uterine fundus with the catheter, to mimic difficult embryo transfer, generated strong uterine contractions, which expelled the fluid into the cervix and Fallopian tubes (Lesny et al., 1998). In Woolcott and Stanger’s study, endometrial wave-like movements appeared to move the embryo-associated air bubble back and forth around a pivotal point. Standing up shortly after embryo transfer did not play a significant role in the final position of the embryo-associated air bubble. In one case with very active movement, the air bubble migrated to the cervix and then returned after the patient stood up from a supine position (Woolcott and Stanger, 1997, 1998). Thus previous studies show that transferred embryos may be expelled from the uterine cavity due to active uterine peristalsis, but how and to what extent uterine peristalsis will affect embryo migration after embryo transfer has been less investigated. In the present study, ‘embryo’ drifting was directly monitored after mock embryo transfer with ultrasound contrast agent in place of embryo-associated air bubble. Uterine peristalsis was quantitatively compared with the movement of this fluid.

Materials and Methods

Patients

In this prospective study, patients admitted to the Department of Reproductive Medicine at Xiangya Hospital of Central-south University were recruited between March and May 2013. A total of 112 consecutive patients, seeking IVF/ICSI-embryo transfer treatment and with normal ovulation, were analysed. Exclusion criteria were anatomic disorders of the female reproductive system, leiomyoma, endometriosis, intrauterine adhesion, endometrial polyp, hydrohystera and any medical illness requiring pharmacologic treatment. This study was approved by the institutional review board of Xiangya Hospital of Central-south University. All patients participating in the study gave their informed consent.

Study design

All subjects underwent mock embryo transfer in the early luteal phase of the natural menstrual cycle preceding their IVF treatment cycle. Serial ultrasound examinations for follicular tracking started on Day 10 in the natural cycle. If the average diameter of the dominant follicle was <14 mm, the patient was asked to come every other day. Otherwise a urine LH test was performed and the patient was asked to come next day. A urine LH test above 45 IU/l and the disappearance of the pre-ovulatory follicle were used to identify ovulation. Three days after ovulation, uterine peristalsis assessment and a mock embryo transfer were performed by a single operator (L.Z.). Just before the mock embryo transfer, a transvaginal ultrasound scan (Mindray DC-6 Expert, 5–8 MHz transducer, China) was performed to assess the length of the uterine cavity and the cervix, as well as the position and length of the uterus. As described in our previous study (Zhu et al., 2012), a 5-min transvaginal ultrasonographic observation of uterine peristalsis at the mid-sagittal plane of the uterus was recorded for each patient. All patients were asked to empty their bladder before the first ultrasound assessment and stay in a supine position till the last measurement was completed. No tranquilizers or medication was given to the patients throughout the whole procedure.

A mock embryo transfer guided by abdominal ultrasonography was performed using a catheter (CCD131230301, Ultrasonic Frydman Set with Guide, Laboratoire C.C.D., France) to deliver 20 µl of fluid SonoVue (Bracco Imaging S.p.A., Italy) into the uterine cavity. No cervix tenaculum was utilized. Immediately after the mock embryo transfer, transvaginal ultrasonography was performed to locate the position of the fluid in the uterine cavity. If the tracking medium divided into two or more parts, then the largest one was tracked. The distance from the fluid to the fundus of the uterine cavity in the mid-sagittal plane and to the bilateral endometrium in transverse plane was measured three times as soon as possible. The average of repeated measurements were expressed as db1 (distance between the fluid and the fundus of the uterine cavity), dl1 (distance between the fluid and the patient’s left endometrium) and dr1 (distance between the fluid and the patient’s right endometrium) (Fig. 1). Within 1 min after the mock transfer, uterine peristalsis was recorded for a further 5 min using a vaginal US probe (Mindray DC-6 Expert, 5–8 MHz transducer, China). At 15 and 30 min after embryo transfer, the position of the fluid was remeasured and recorded as db2, d2, dr2 and db3, dl3, dr3, respectively.

Uterine peristalsis assessment and fluid movement distance measurement

Images of uterine peristalsis were converted to four times normal speed using a VLC media player 1.1.11 (VideoLAN team). Uterine peristaltic wave frequency was analysed independently by two observers (L.Z. and L.X.). The final result of wave frequency was the mean of two numbers from different observers. The displacement of the fluid between two time points was calculated based on the movement in three planes over the three observation time points (Fig. 1).

Statistical analysis

The SPSS software version 19.0 was used for statistical analysis. Continuous data were presented as mean and standard deviation. Inter-observer variation of uterine peristaltic wave frequency was analysed using Wilcoxon signed-rank test. Changes in uterine peristaltic wave frequency before and after embryo transfer were assessed using paired Student’s t-test. Correlations between the uterine peristaltic wave frequency and the distance of fluid movements were performed using linear correlation analysis. P < 0.05 was considered statistically significant.

Results

A total of 112 subjects commenced this study. Two patients were excluded due to difficulties in visualizing the endometrium. Before the placement of the fluid, the endometrial contractility was 2.24 ± 0.74 waves/min. After the placement, the endometrial contractility increased to 3.06 ± 0.99 waves/min (P < 0.01) (Table I). The uterine peristaltic wave frequency, both before embryo transfer and after embryo transfer, was significantly higher in cases where the fluid was extruded (Table II). Following embryo transfer, fluid moved into the cervix (5 cases), or into the Fallopian tubes or the cornua of the uterus (11 cases). In one case, the fluid moved down into the inner os of the cervix, but after several cervicofundal uterine peristaltic waves, the fluid moved back into the uterine cavity (Fig. 2 and Supplementary data, Video S1).
The fluid was deposited into the uterine cavity at a distance of 9.56 ± 5.20 mm from the fundus. On average, the fluid moved 10.34 ± 7.46 mm within 30 min of embryo transfer. In the cases where the fluid was extruded, it moved much further. The placement of the fluid within the uterine cavity was not significantly correlated with the distance the fluid moved (P = 0.176). Uterine peristaltic wave frequencies before and after embryo transfer were both significantly correlated with the distance the fluid moved, with correlation coefficients of 0.518 (P < 0.01) and 0.371 (P < 0.01), respectively (Fig. 3).

A follow-up survey showed that for 21 patients embryo transfer was cancelled in the subsequent stimulated cycle. Of the 89 transferred cases, 40 patients were confirmed to have a clinical pregnancy by ultrasonographic visualization of gestational sacs. However, statistical analysis shows that there was no difference in uterine peristaltic wave frequency in the preceding cycle between the pregnant group and non-pregnant group (before mock transfer: 2.21 versus 2.12, P = 0.903; after mock transfer: 3.22 versus 3.04, P = 0.402).

**Discussion**

The current study indicated that uterine peristalsis was quantitatively correlated with the distance of fluid movement after embryo transfer. Our approach allowed the transferred embryo analogue to be directly
tracked by ultrasonography and demonstrated that uterine peristalsis did correlate with fluid migration. It could therefore potentially influence pregnancy outcomes.

The uterine peristaltic wave frequency after embryo transfer was significantly higher than that before embryo transfer, suggesting that an embryo transfer procedure may enhance uterine peristalsis. However, our result was in contrast to Torre’s study (Torre et al., 2010), which did not find any influence of mock embryo transfer on uterine contractility. Differences in insertion procedures of the catheter may contribute to the inconsistency of the results. Torre et al. introduced a catheter into the cervix up to the inner cervical os and immediately withdrew it, while we completely mimicked a real embryo transfer including the insertion of the soft inner catheter into the upper half of the uterine cavity and the ejection of medium. Uterine contractility occurs in vivo with specific patterns mainly related to the ovarian steroid levels. Estrogens promote and progesterone relaxes uterine contractility (Kunz et al., 1998; Zhu et al., 2012). Other factors that are strongly involved include prostaglandins from semen or from the myometrium and endometrium (Dittrich et al., 2009). Prostaglandins are likely to be released after catheter stimulation of the uterine lumen. This may indicate that catheter insertion and fluid ejection procedures should be as gentle as possible to avoid triggering an increase in uterine peristalsis.

The placement of the fluids in our study seemed to be a little closer to the uterine fundus than the recommended distance in previous studies (9.56 versus 10–20 mm, respectively) (Coroleu et al., 2002; Pacchiarotti et al., 2007; Mains and Van Voorhis, 2010; Tiras et al., 2010). However, these studies all measured the distance between the tip of the catheter and the uterine fundus. The ejection of transfer medium may push the embryo a little further forward and closer to the fundus. Lambers et al. (2007) and Friedman et al. (2011) monitored the location of the air bubble relative to the fundus after embryo transfer and found that the pregnancy rate was significantly higher when the air bubbles ended up closer to the fundus (<10 mm). Thus, the deposition of our fluids could be considered appropriate. Furthermore, a statistical analysis showed that fluid placement was not significantly correlated with the distance the fluid moved in our study, suggesting a negligible effect of proper deposition on fluid migration.

The movement of gametes and zygotes within the uterine lumen is influenced by ovarian steroid levels, myometrial activity and any myometrial or endometrial pathologies. In 14.5% of our transfers the fluids were observed to leave the uterine cavity, which was similar to the previous finding (Pointdexter et al., 1986). This cohort of patients had more active uterine movements than the others, both before embryo transfer and after embryo transfer. During the 30-min observation period, the fluid did not move uniformly forward in one direction; instead it was influenced by the uterine peristalsis. The cervicofundal uterine peristaltic waves tended to spread the fluid, pushing it forward in the direction of the wave. When the wave reached the fundus, the fluid would then

### Table II  Uterine peristaltic wave frequency and distance of fluid movement in patients with different outcome of transferred fluids.

<table>
<thead>
<tr>
<th>Fluids</th>
<th>N</th>
<th>Uterine peristaltic wave frequency (waves/min)</th>
<th>The rate of increase of uterine peristalsis</th>
<th>db l (mm)</th>
<th>Distance of fluid movement (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before embryo transfer</td>
<td>After embryo transfer</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inside the uterine cavity</td>
<td>94</td>
<td>2.15 ± 0.71</td>
<td>2.94 ± 0.91</td>
<td>36.7%</td>
<td>9.58 ± 5.14</td>
</tr>
<tr>
<td>Extruded outside</td>
<td>16</td>
<td>2.75 ± 0.67</td>
<td>3.72 ± 1.21</td>
<td>35.3%</td>
<td>9.42 ± 5.71</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>–</td>
<td>0.914</td>
</tr>
</tbody>
</table>

db l: distance between the fluid and the fundus of the uterine cavity after fluid deposition.

Figure 2 A sequence showing that fluid could move from the uterine lumen, down into the inner os of the cervix and back to the uterine cavity in response to cervicofundal uterine peristaltic waves. The red arrow points to the ultrasound contrast agent. SonoVue is made up of sulphur hexafluoride microbubbles. The images show 3 time points: 0, 15 and 30 min after placement.
move backwards to varying degrees. Within the cohort that extruded fluids, the uterine peristaltic wave frequency was significantly increased and the waves overlapped each other, so that there was no time for the fluid to move back to counteract the effect of the preceding waves. The cumulative effect of this to-and-fro motion caused the fluid migration and may contribute to intrauterine/ectopic pregnancies.

It was worthwhile to note that cervicofundal uterine peristalsis could return the fluid to the uterine cavity, even once it had reached the inner os of the cervix (Fig. 2 and Supplementary data, Video S1). This, for the first time, demonstrates the previous assumption (Kunz et al., 2006) that the cervicofundal uterine peristalsis in the luteal phase may also prevent the embryo from implanting in the lower part of the uterus, avoiding cervical pregnancy or placenta praevia. Interestingly fluid that moved into the Fallopian tubes (N = 9) did not come back into the uterine cavity.

Uterine peristalsis wave frequencies in the natural cycle between the pregnancy group and non-pregnancy group were not significantly different in our study. It may be because controlled ovarian stimulation changed the uterine peristaltic pattern in the subsequent cycle, as was observed in our previous study (Zhu et al., 2012). Additionally, there were many confounding factors such as the patients’ age, peak E2 concentration, number of oocytes retrieved, number of embryos transferred, embryo quality, sample size, etc. Fanchin et al. (1998) evaluated the uterine contractions just before embryo transfer and divided the patients into four groups according to uterine contraction frequency: ≤3.0, 3.1–4.0, 4.1–5.0, >5.0. A stepwise decrease in clinical pregnancy rates occurred from the lowest to the highest uterine contraction frequency groups (53, 46, 23 and 14%, respectively). If we grouped our patients in the same way, there would have been 80 patients with a uterine peristalsis wave frequency of no more than 3.0 before mock transfer, and only 7, 2, 0 patients in the 3.1–4.0, 4.1–5.0, >5.0 groups, respectively. A group of 21 patients whose embryo transfer was cancelled in the stimulated cycle were excluded. The clinical pregnancy rates would have been 45, 42.9, and 50% in the first three groups, but the wide variation in subgroup sample size and other confounding factors made the correlation analysis invalid. But if inter-subject,
observer and cycle differences are put aside, their results resemble our results in that the uterine peristalsis of those who expelled their embryos was \( \geq 3 \text{ waves/min} \). In the present study, there was an approximate 36% increase in uterine peristalsis after embryo transfer. Therefore, the post-transfer uterine peristaltic wave frequency might be roughly calculated by measuring the pre-transfer frequency, both of which might be taken as a noninvasive predictor of the uterine receptivity and pregnancy outcome. However, it is worthwhile to note that some other elements may also play a comprehensive role in controlling the luminal content migration, such as the thickness of the myometrium, the length of the uterine cavity, the endometrial thickness, the volume and viscosity of fluid in the endometrium, the amplitude and velocity of uterine peristaltic waves, the basal tone of the uterine contractility and others (IJland et al., 1997b, 1998; Eytan et al., 1999, 2001). Thus, the effectiveness of this new index remains to be seen.

One limitation of our study was that the mock embryo transfer was performed in a natural cycle instead of a stimulated cycle. Thus the endometrial environment and uterine peristalsis may be different from a real embryo transfer (Eytan et al., 2001; Zhu et al., 2012). Another point that is worthy of attention is that even though our mock embryo transfer using ultrasound contrast medium was as close as possible to a real embryo transfer, it was still different from an actual embryo because the contrast medium is not the same as the media used in embryo transfers.

In conclusion, uterine peristalsis was shown to play an essential part in fluid migration and this indicates that it could influence the outcomes of embryo transfers. We believe that further investigations are necessary to clarify the mechanism behind the regulation of uterine peristalsis and to apply interventions to reduce the adverse effect of active uterine peristalsis.

**Supplementary data**

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

**Authors’ roles**

L.Z. participated in the study design, transvaginal ultrasonographic scan, mock embryo transfer, analysis and interpretation of data. L.X. participated in the acquisition and analysis of data. H.S.C. took part in the transabdominal ultrasound guided embryo transfer, and acquisition of data. Y.P.L. participated in the study conception and design. J.T.L. provided the ultrasound contrast agent and critical advice in study design. All authors contributed to the production of the manuscript.

**Conflict of interest**

None declared.

**References**


