Uterine peristaltis before embryo transfer affects the chance of clinical pregnancy in fresh and frozen-thawed embryo transfer cycles

L. Zhu, H.S. Che, L. Xiao, and Y.P. Li*

Department of Reproductive Medicine, Xiangya Hospital, Central-south University, Changsha City, Hunan Province, P.R. China

*Correspondence address. Department of Reproductive Medicine, Xiangya Hospital, 87 Xiangya Road, Changsha City, Hunan Province, P.R. China. E-mail: lisayanping@sina.com

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STUDY QUESTION: Does uterine peristalsis influence the chance of clinical pregnancy in an embryo transfer cycle?

SUMMARY ANSWER: The uterine peristaltic wave frequency before embryo transfer is inversely related to the clinical pregnancy rates in fresh and frozen-thawed embryo transfer cycles.

WHAT IS KNOWN ALREADY: Uterine peristalsis participates in regulating fluid migration after mock embryo transfer, but whether it could potentially influence pregnancy outcomes had remained unclear.

STUDY DESIGN, SIZE, DURATION: This prospective cohort study included a total of 292 infertile women and was conducted between March 2013 and August 2013.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Patients underwent fresh embryo transfer in a fresh stimulation cycle with a long down-regulation protocol, a natural frozen-thawed embryo transfer cycle or an artificial frozen-thawed embryo transfer cycle. Uterine peristaltic activity was assessed before embryo transfer by transvaginal ultrasonography.

MAIN RESULTS AND THE ROLE OF CHANCE: The uterine peristaltic wave frequencies of most patients were between 1.1 and 3.0 waves/min before embryo transfer (ET). The clinical pregnancy rate was the highest when <2.0 waves/min was observed and it decreased with an increasing wave frequency thereafter, with an especially dramatic decrease with >3.0 waves/min. Uterine peristaltic wave frequencies of the non-pregnant patient group were higher than that of the clinically-pregnant patient group in all types of transfer, fresh embryo transfer, natural FET or artificial FET cycle. Binary logistic regression analysis demonstrated that the association between uterine peristaltic wave frequency before embryo transfer and clinical pregnancy was independently significant (odds ratio: 0.49; 95% confidence interval: 0.34–0.70, P < 0.001).

LIMITATIONS, REASONS FOR CAUTION: Uterine peristalsis after embryo transfer was not observed in case any possible negative effect of the observation disturbed embryo implantation or caused psychological stress. Uterine peristalsis after embryo transfer may differ from that before embryo transfer. Another limitation of the present study was the lack of uterine peristaltic wave type analysis which is also an important parameter to assess uterine activity.

WIDER IMPLICATIONS OF THE FINDINGS: Patients with uterine peristalsis of <3.0 waves/min before embryo transfer had a higher chance of pregnancy compared with those with higher frequencies. This could be a promising quantitative marker of uterine receptivity and pregnancy outcome in an embryo transfer cycle. The predictive validity of the cut-off value needs to be tested in further study.

STUDY FUNDING/COMPETING INTEREST(S): The study is supported by Hunan Provincial Innovation Foundation for Postgraduates. The authors declare that they have no competing interests in this study.

Key words: uterine peristalsis / uterine receptivity / clinical pregnancy / assisted reproductive technology
Introduction

Controlled ovarian stimulation improvements and technological advancements in the laboratory have provided adequate oocytes and high-quality embryos to infertile couples, leading to steadily increased success rates for IVF over the years. However comparatively speaking, considerably fewer efforts have been devoted to improving uterine receptivity, especially in clinical practice.

In contrast with an invasive endometrium biopsy, uterine peristalsis can be directly visualized by ultrasonography and has therefore broadened the horizon for exploring uterine receptivity in human reproduction. During the luteal phase in a natural cycle, uterine activity reaches an absolute minimum and the reduced uterine peristalsis is believed to facilitate embryo implantation ([Ijland et al., 1997a; Bulletti et al., 2000; Bulletti and de Ziegler, 2006]. Studies of mock embryo transfer (ET) have also testified to the important role of uterine peristalsis in controlling the migration of embryo analogues. Lesny et al. (1998) observed that the transferred fluid could be expelled out of the uterus by strong uterine contractions after touching the uterine fundus with the catheter. Our previously published study also found that uterine peristaltic wave frequency was positively correlated with the distance that fluid moved after it was deposited in the uterine cavity (Zhu et al., 2014). Since uterine peristalsis can exert an influence on the drift of an embryo analogue during the implantation window, we hypothesized that it may also have an effect on the subsequent clinical pregnancy outcome in a real embryo transfer cycle. This inspired us to comprehensively analyse the relationship between uterine peristalsis just before embryo transfer and the clinical pregnancy rates under different conditions, including fresh embryo transfer, natural frozen-thawed embryo transfer (FET) and artificial FET cycles.

Materials and Methods

Patients

From March 2013 to August 2013, all patients undergoing IVF/ICSI-ET and FET treatment at the Department of Reproductive Medicine at Xiangua Hospital of Central-south University were offered participation in the study. To limit the interference of confounding variables affecting embryo quality and/or uterine receptivity, patients were only selected if they (i) were 20–35 years of age; (ii) had a normal uterus; (iii) had two good-quality day-3 cleavage stage embryos available for transfer (good-quality day-3 embryos were defined by at least seven cells, <20% fragmentation, and no apparent morphological abnormalities); and (iv) underwent easy embryo transfer (i.e. the catheter was smoothly inserted without touching the fundus, no cervix tenacity was used and the catheter was clean of blood). After excluding 13 patients due to cancellation of embryo transfer (because of ovarian hyperstimulation syndrome or hydrolystera), a change to blastocyst transfer, or the use of another drug intervention, a total of 166 patients underwent fresh embryo transfer and 126 patients received either natural FET (n = 69) or artificial FET (n = 57) were analysed. Written informed consent was obtained from each patient before inclusion. The study was approved by the institutional review board (Clinical Trial Registration Number: ChiCTR-0CH-13003105).

Fresh cycles

Patients underwent ovarian stimulation in a long down-regulation protocol using urinary FSH (Urofollitropin, Livzon, China), recombinant follitropin alfa (Gonal-F, Merck Serono) or recombinant follitropin beta (Puregon, Merck Sharp & Dohme). FSH doses were adjusted individually according to the patient’s ovarian response as assessed by ultrasound and hormonal measurements performed every 1–3 days. A dose of 5000–10 000 IU of human chorionic gonadotrophin (HCG, Livzon, China) was administered when at least three follicles were ≥ 18 mm in diameter. Oocyte retrieval was conducted transvaginally 34–36 h after the HCG trigger. The luteal phase was supported with progesterone injections at a dose of 60 mg/day and progesterone soft capsules at a dose of 200 mg/night from the day of oocyte retrieval.

Cryopreservation cycles

In the natural FET cycles, the development of the dominant follicle and endometrium was monitored from Day 10 by regular vaginal ultrasound, urine LH tests and serum LH, E2 and progesterone measurements until ovulation. If the diameter of the dominant follicle was < 14 mm, the patient was asked to return every second day. If the dominant follicle was ≥ 14 mm, a urine LH test 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, serum LH, E2 and progesterone concentrations were measured to help estimate the maturation of the dominant follicle, predict ovulation and find out whether the follicle was luteinized. If the serum progesterone concentration was ≥ 1.0 ng/ml and the follicle was still unruptured, which was usually accompanied by heterogeneous echo of the follicular fluid, luteinized unruptured follicle was identified and that day was taken as the ovulation day. To preclude luteal phase defect, luteal phase support was provided from the day of ovulation day progesterone injection 40 mg/day and added progesterone soft capsules at a dose of 200 mg/night from the day of embryo transfer.

In the artificial FET cycles, patients received increasing doses (6 mg for days 3–7, 8 mg for 2 days, and 10 mg for 2 days thereafter) of estradiol valerate (Progonova, Bayer) in order to prepare the endometrium for embryo transfer. Endometrial thickness was monitored by transvaginal ultrasound every time before adjusting the doses of estradiol valerate, and when the endometrium reached or exceeded 7 mm, increasing doses (20 mg for 1 day, 40 mg for 1 day and 60 mg for 1 day thereafter) of progesterone were given intramuscularly once per day. Progesterone soft capsules at a dose of 200 mg/night were administered orally from the day of embryo transfer.

Embryo transfer

Two good-quality embryos were transferred under abdominal ultrasound guidance 3 days after oocyte retrieval in a fresh cycle, 2 days after ovulation in a natural FET cycle and 3 days after progesterone administration in an artificial FET cycle, respectively. All of the embryo transfers were performed by an experienced doctor (Y.P.L.), who was blind to the frequency of uterine peristalsis, using the same type of catheter (CCD131230301, Ultrasoft Frydman Set with Guide, Laboratoire C.C.D., France). Blood samples were collected on the day of embryo transfer to determine the serum E2 and P using electrochemiluminescence immunoassays. Approximately 80% of all fresh cycles were conducted as IVF and 20% were conducted as ICSI/half-ICSI. Patients were confirmed to be clinically pregnant by ultrasonographic visualization of a gestational sac 2–3 weeks after a positive pregnancy test. Luteal support was continued either until 1 month after confirmation of intrauterine pregnancy or until pregnancy was ruled out by a negative serum HCG measurement, in all types of treatment cycles.

Observation and assessment of uterine peristalsis

Transvaginal ultrasonography scans (Mindray DC-6 Expert, 5–8 MHz transducer) of uterine peristalsis were performed by a single examiner (L.Z.) about 1 h before embryo transfer while the patient was lying relaxed in a
lithotomy position. The probe was introduced into the vagina as gently as possible to avoid stimulating the cervix. After scanning the mid-sagittal plane of the uterus, the probe was fixed as steady as possible while a 5 min video of uterine peristalsis was recorded simultaneously. The records were analysed at 4 × regular speed using a VLC media player 1.1.11 (Video-LAN team) by two independent experienced observers (L.Z. and L.X.). The uterine peristaltic wave frequency was the mean of the results from each observer. Both observers were blind to the treatment condition (fresh cycle, natural FET cycle or artificial FET cycle) of all patients.

Statistical analysis

Statistical analyses were carried out using SPSS version 19.0. Continuous data were presented as mean and standard deviation (SD). A two-sample t-test was used to determine whether the uterine peristaltic wave frequency on embryo transfer day differs in the pregnancy and non-pregnancy patients. Student–Newman–Keuls (SNK) test was used in comparison of every combination of group pairs (fresh versus natural FET cycle, fresh versus artificial FET cycle and natural FET versus artificial FET cycle) for uterine peristaltic wave frequency as well as serum progesterone. Inter-observer agreement of uterine peristaltic wave frequency, meaning that the agreement between the two readers was very good.

The demographic characteristics of the study subjects according to clinical pregnancy outcomes are detailed in Table I. No difference was found between the two groups, except for the serum progesterone concentration on the day of embryo transfer.

All of the patients were subjectively divided into five groups according to uterine peristaltic wave frequency on embryo transfer day: ≤1.0, 1.1–2.0, 2.1–3.0, 3.1–4.0 and >4.0 waves/min. Most patients were distributed into group 1.1–2.0 and 2.1–3.0. The clinical pregnancy rate was the highest when no more than 2.0 waves/min were observed before embryo transfer and it decreased with increasing wave frequency thereafter, with a dramatic decrease when >3.0 waves/min were observed (Table II).

As shown in Table III, uterine peristaltic wave frequencies of non-pregnancy patients were significantly higher than that of clinical pregnancy patients no matter whether they underwent fresh embryo transfer, natural FET or artificial FET cycles, respectively. Additionally, the uterine peristaltic wave frequency before embryo transfer seemed to be a little lower in the fresh cycles (1.80 ± 0.77 waves/min) than in FET cycles (natural FET 2.05 ± 0.80 waves/min and artificial FET 2.15 ± 0.81 waves/min). But the differences in the comparison of every combination of group pairs were not significantly different. Eight patients were diagnosed with ectopic pregnancy in our study, and the uterine peristaltic wave frequency of those patients showed a higher trend compared with those with intrauterine pregnancy (2.21 ± 1.20 versus 1.73 ± 0.65 waves/min), although the difference did not reach statistical significance (P = 0.29).

Binary logistic regression analysis revealed that the uterine peristaltic wave frequency on the day of embryo transfer was inversely related to the clinical pregnancy rate. Women with high uterine peristaltic wave frequency were less likely to get pregnant than those with a relatively low

### Table I: Demographic comparison of pregnancy group versus non-pregnancy group.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Clinical pregnancy (n = 156)</th>
<th>Non-pregnancy (n = 136)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>29.1 ± 3.4</td>
<td>29.0 ± 3.7</td>
<td>0.720*</td>
</tr>
<tr>
<td>Duration of infertility (year)</td>
<td>4.2 ± 2.9</td>
<td>4.8 ± 3.0</td>
<td>0.102*</td>
</tr>
<tr>
<td>Treatment cycle (%)</td>
<td>Fresh cycle (60.9)</td>
<td>71 (52.2)</td>
<td>0.084a</td>
</tr>
<tr>
<td></td>
<td>Natural FET cycle (24.4)</td>
<td>31 (22.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Artificial FET cycle (14.7)</td>
<td>34 (25.0)</td>
<td></td>
</tr>
<tr>
<td>Basal serum FSH (IU/l)</td>
<td>6.4 ± 2.2</td>
<td>6.3 ± 2.0</td>
<td>0.790*</td>
</tr>
<tr>
<td>Basal serum LH (IU/l)</td>
<td>5.2 ± 3.2</td>
<td>5.5 ± 4.0</td>
<td>0.473*</td>
</tr>
<tr>
<td>Basal serum E2 (pg/ml)</td>
<td>41.2 ± 29.7</td>
<td>40.5 ± 38.1</td>
<td>0.854*</td>
</tr>
<tr>
<td>Serum E2 on embryo transfer day (pg/ml)</td>
<td>1068.3 ± 973.0</td>
<td>947.1 ± 903.6</td>
<td>0.273*</td>
</tr>
<tr>
<td>Serum P on embryo transfer day (ng/ml)</td>
<td>56.3 ± 11.5</td>
<td>52.8 ± 15.2</td>
<td>0.030*</td>
</tr>
<tr>
<td>Fresh cycle</td>
<td>58.3 ± 9.3</td>
<td>59.0 ± 7.2</td>
<td>0.582*</td>
</tr>
<tr>
<td>Natural FET cycle</td>
<td>53.2 ± 13.6</td>
<td>48.4 ± 16.4</td>
<td>0.188*</td>
</tr>
<tr>
<td>Artificial FET cycle</td>
<td>53.0 ± 14.5</td>
<td>43.8 ± 20.0</td>
<td>0.049*</td>
</tr>
<tr>
<td>Endometrium thickness on embryo transfer day (mm)</td>
<td>11.3 ± 2.7</td>
<td>11.2 ± 3.0</td>
<td>0.761a</td>
</tr>
<tr>
<td>Good-quality embryo rate (%)</td>
<td>68.6 ± 22.9</td>
<td>72.9 ± 21.9</td>
<td>0.104a</td>
</tr>
</tbody>
</table>

*Two-sample t-test.

Pearson χ².
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fresh cycles, natural FET cycles and artificial FET cycles to further
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embryo transfer and clinical pregnancy outcomes. To the best of our
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Discussion

In this prospective cohort study, we found a significant, independent as-
association between uterine peristaltic wave frequency on the day of
embryo transfer and clinical pregnancy outcomes. To the best of our
knowledge, this is the first comprehensive study which included both
fresh cycles, natural FET cycles and artificial FET cycles to further
explore the possible role of uterine peristalsis in embryo implantation.

The demographic characteristics of the study subjects were compar-
able (P > 0.05) between the pregnancy and non-pregnancy groups,
except for serum progesterone levels on the day of embryo transfer
(P = 0.030) (Table I). Although the serum progesterone level was signifi-
cantly different between the groups, they were both >40 ng/ml which is
much higher than the physiological level of mid-luteal phase (5.97–
14.15 ng/ml) in a natural cycle (John et al., 2008). Furthermore, logistic
regression analysis showed that the serum progesterone level on the day
of embryo transfer did not influence the clinical pregnancy rate in our
study, implying that the luteal support was adequate for pregnancy occu-
rence (Table IV).

Before embryo transfer, uterine peristalsis of most patients was main-
tained at a low level of <3.0 waves/min, similar to the phenomenon
observed in a natural cycle (Jliland et al., 1997a,b; Bulletti et al., 2000). In
line with this, the clinical pregnancy rate was much higher in these patients
compared with those with a frequency of >3.0 waves/min, indicating a
beneficial effect of low frequency uterine peristalsis on pregnancy
(Table II). Our result is in accordance with the study of Fanchin et al.
(1998), who also found a stepwise decrease in clinical pregnancy rates
with increasing uterine contractions before embryo transfer (53, 46, 23
and 14% for group ≤3.0, 3.1–4.0, 4.1–5.0 and >5.0, respectively).
The discrepancy in grouping may be attributed to inter-subject, observer
and cycle differences. It is noteworthy that in our previous study (Zhu et al.,
2014), the uterine peristalsis of those who expelled the transferred fluid
was also >3.0 waves/min. Thus, the result obtained in this study, together
with previously published studies, suggested that patients with the uterine

<table>
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<tr>
<th>Table II</th>
<th>Clinical pregnancy outcomes of different uterine peristaltic wave frequency groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine peristaltic wave frequency (waves/min)</td>
<td>n</td>
</tr>
<tr>
<td>≤1.0</td>
<td>39</td>
</tr>
<tr>
<td>1.1–2.0</td>
<td>118</td>
</tr>
<tr>
<td>2.1–3.0</td>
<td>117</td>
</tr>
<tr>
<td>3.1–4.0</td>
<td>16</td>
</tr>
<tr>
<td>&gt;4.0</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table III</th>
<th>Comparison of uterine peristaltic wave frequencies between clinical pregnancy and non-pregnancy women in different cycles.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles</td>
<td>Uterine peristaltic wave frequency</td>
</tr>
<tr>
<td></td>
<td>Clinical pregnancy (n = 156)</td>
</tr>
<tr>
<td>Fresh embryo transfer</td>
<td>1.69 ± 0.69</td>
</tr>
<tr>
<td>Natural FET</td>
<td>1.82 ± 0.66</td>
</tr>
<tr>
<td>Artificial FET</td>
<td>1.82 ± 0.59</td>
</tr>
<tr>
<td>Total</td>
<td>1.74 ± 0.67</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Table IV</th>
<th>Binary logistic regression analysis of factors potentially associated with the occurrence of clinical pregnancy.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.033</td>
</tr>
<tr>
<td>Duration of infertility</td>
<td>−0.072</td>
</tr>
<tr>
<td>Basal serum FSH</td>
<td>−0.005</td>
</tr>
<tr>
<td>Basal serum LH</td>
<td>−0.046</td>
</tr>
<tr>
<td>Basal serum E2</td>
<td>0.002</td>
</tr>
<tr>
<td>Serum E2 on embryo transfer day</td>
<td>0.000</td>
</tr>
<tr>
<td>Serum P on embryo transfer day</td>
<td>0.016</td>
</tr>
<tr>
<td>Endometrium thickness on embryo transfer day</td>
<td>0.033</td>
</tr>
<tr>
<td>Good-quality embryo rate</td>
<td>−0.006</td>
</tr>
<tr>
<td>Types of ART cycles</td>
<td>−0.251</td>
</tr>
<tr>
<td>Uterine peristaltic wave frequency on embryo transfer day</td>
<td>−0.714</td>
</tr>
</tbody>
</table>

Clinical pregnancy outcome was the dependent binary variable with clinical pregnancy coded as 1 (positive) and non-pregnancy coded as 0 (negative). β < 0 and OR > 1 means clinical pregnancy outcome is inclined to be negative.
peristalsis of <3.0 waves/min before embryo transfer have a better chance of becoming pregnant.

Our current findings also confirmed that embryo transfer cycles which ended with successful pregnancy showed less uterine peristalsis before embryo transfer than non-pregnant cycles (Table III), consistent previous research (Ijland et al., 1997a). It may be because the intense uterine peristalsis adversely affects embryo migration after embryo transfer and extrudes it out of the uterine cavity, resulting in implantation failure or ectopic pregnancy. Recently, the application of oxytocin antagonists to selectively decrease the uterine activity was applied with success (Pierzynski et al., 2007; Moraloglu et al., 2010; Pierzynski, 2011). Other research has also tried to improve pregnancy rates by suppressing uterine peristalsis with various agents (Moon et al., 2004; Nakai et al., 2008; Kido et al., 2009; Xu et al., 2013). However molecular mechanisms modulating uterine peristalsis around the implantation window are awaiting further study which may contribute to an improvement in endometrial receptivity and pregnancy rates. The incidence of ectopic pregnancy in this population was 2.7%, similar to the reported ectopic rate following assisted reproductive technologies (Schippert et al., 2012). Although the uterine peristaltic wave frequency was a little higher in the ectopic pregnancy group than those with intrauterine pregnancy, the difference was not significant. This may be due to the uneven distribution of sample size in the ectopic pregnancy group (8 cases) and intrauterine pregnancy group (148 cases).

However, it was contrary to our expectation that the uterine peristaltic wave frequency on the day of embryo transfer showed an uptrend in fresh, natural FET and artificial FET cycles, although the differences for comparison of each combination of group pairs did not reach statistical significance. Previous investigators have found that progesterone had an inhibitory action on mediating uterine peristalsis (Fanchin et al., 1998; Mueller et al., 2006; Zhu et al., 2012). In the present study, serum progesterone levels on the day of embryo transfer were 58.6 ± 8.5, 51.0 ± 15.0 and 47.5 ± 18.4 ng/ml for fresh, natural FET and artificial FET cycles. Statistical analysis showed that the serum progesterone levels in the fresh cycles were significantly different from that in the natural FET and artificial FET cycles, while the difference between the natural FET and artificial FET cycles was not significant. The decreasing supraphysiological serum progesterone may only partly account for the uptrend of uterine peristalsis, since we found that uterine peristalsis and pregnancy rates. The incidence of ectopic pregnancy in this population was 2.7%, similar to the reported ectopic rate following assisted reproductive technologies (Schippert et al., 2012). Although the uterine peristaltic wave frequency was a little higher in the ectopic pregnancy group than those with intrauterine pregnancy, the difference was not significant. This may be due to the uneven distribution of sample size in the ectopic pregnancy group (8 cases) and intrauterine pregnancy group (148 cases).

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The uterine peristaltic wave frequency before embryo transfer in the non-pregnant group was 2.14 ± 0.88 waves/min, which seemed to be moderate for embryo implantation. Nevertheless, if the embryo transfer procedure is taken into consideration, uterine peristalsis might be enhanced thereafter (Lesny et al., 1998; Zhu et al., 2014). In the present study, we did not observe uterine peristalsis after embryo transfer in case any possible negative effect disturbed embryo implantation or caused psychological stress for the patients. In our previous study, an approximate 36% increase in uterine peristalsis was observed after mock embryo transfer, no matter whether the fluid was kept inside the uterine cavity or extruded outside (Zhu et al., 2014). When we take a rough estimate of the uterine peristalsis after embryo transfer in the present study accordingly, it would be 2.91 waves/min. ROC curve analysis showed that the cut-off value of uterine peristalsis was 2.45 waves/min in predicting clinical pregnancy outcome, which was a medium between 2.14 and 2.91 waves/min. This deduction sheds light on the predictive validity of the cut-off value and places it as a promising quantitative marker of uterine receptivity and pregnancy outcome in an embryo transfer cycle. Moreover, binary logistic regression analysis demonstrated that the association between uterine peristaltic wave frequency before embryo transfer and clinical pregnancy was independently significant (odds ratio: 0.49; 95% confidence interval: 0.34–0.70, P < 0.001). We believe that further investigations are necessary to testify to the effectiveness of uterine peristalsis in predicting clinical pregnancy before definitive conclusions can be drawn.

Subjective and objective methods were both applied in uterine peristaltic wave counting. Although several studies (Fanchin et al., 1998; Oczeretko et al., 2006; Meirzon et al., 2011) have explored objective methods to reduce the influence of the subjective factor, the complicated approach and professional technology requirement prohibits their widespread use and application. It is also noteworthy that computer analysed results do not deserve complete trust since a subtle movement of the ultrasound probe may induce a false wave for analysis which could be avoided by dynamic analysis of uterine peristalsis video. Furthermore, comparisons of counting uterine peristaltic wave frequencies of accelerated images and other objective methods have shown that they are equivalent (Ayoubi et al., 2001; Nakai et al., 2004). Thus, with the rich experience accumulated in our preliminary experiments and previous studies, we chose the classic and convenient method to assess uterine peristalsis.

One limitation of the present study was the lack of uterine peristaltic wave type analysis which is also an important parameter to assess uterine activity. Previous studies (Kunz et al., 1996; Leyendecker et al., 1996; Ijland et al., 1997a; Fanchin et al., 1998; Bulletti et al., 2000; Nakai et al., 2008; Zhu et al., 2012) have reached a consensus on the percentage distribution of uterine peristalsis wave types in menstrual cycle, i.e. cervicofundal and fundocervical waves took a predominant and second position, respectively, with other types showing lower proportions. Nevertheless, it was too complicated to tell the definite or even possible function of each type. We speculate that the cervicofundal waves may counteract the effect of waves of other types and function predominantly. Thus, we focused on the frequency of uterine peristalsis in the present...
study and did not analyse the uterine peristalsis wave types. In order to clarify the function of uterine peristalsis of different wave types, we think it would be better to design a more rigorous study in the future.

In conclusion, the uterine peristaltic wave frequency before embryo transfer was inversely related to the clinical pregnancy in fresh and frozen-thawed embryo transfer cycles. Uterine peristalsis of <3.0 waves/min before embryo transfer indicates a favourable pregnancy outcome. This may represent a convenient, although invasive, indicator of uterine receptivity and pregnancy outcome in an embryo transfer cycle.

Authors’ roles

L.Z. contributed to the study design, transvaginal ultrasonographic scans, data analysis and drafting of the manuscript. H.S.C. performed the follow-up studies and statistical analysis of the data. L.X. participated in the acquisition and analysis of data. Y.P.L. participated in the study conception and design as well as the embryo transfer procedure. All authors contributed to the production of the manuscript.

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Conflict of interest

The authors declare that they have no competing interests in this study.

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