ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Clinical validation of pinopode as a marker of endometrial receptivity: a randomized controlled trial

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Objective: To determine the clinical value of pinopodes in the human.
Design: Randomized controlled trial.
Setting: Academic fertility center.
Patient(s): From December 2014 to December 2016, in a phase I trial, 363 patients were recruited in their first IVF cycles. In a phase II trial, 136 patients with at least two previous embryo implantation failures were recruited.
Intervention(s): In the phase I trial, in the midluteal phase, endometrial tissues were obtained for scanning electron microscopy, and their IVF treatment outcomes were followed up. In the phase II trial, participants were allocated into two groups by random number table, and the experimental group had twice the number of the control group. Individual ETs (iETs) were performed in the experimental group, while frozen-thawed embryos were routinely transferred in the control group.
Main Outcome Measure(s): Clinical pregnancy rate (CPR) and ongoing pregnancy rate (OPR).
Result(s): In the phase I trial, through receiver operation characteristic curve analysis, the optimal pinopode scoring for successful clinical pregnancy was determined to be 85. The CPR (74.29% vs. 19.77%) and OPR (62.86% vs 11.86%) were significantly higher in the patients whose pinopode scoring was >85. In the phase II trial, the CPR was dramatically higher in the iET group (33.82% vs. 8.11%).
Conclusion(s): Pinopode scoring is a reliable marker of endometrial receptivity, and iET based on pinopode scoring is a promising solution for recurrent implantation failure.
Trial Registration Numbers: ChiCTR-OOC-14005617; ChiCTR-OOC-15005882. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Pinopode, window of implantation, individual embryo transfer

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In the human endometrium, during the receptive state, the surface of the endometrial epithelium undergoes striking modifications: the hair-like cell microvilli fuse to a mushroom-like membrane projection called the "pinopode (protrusion)" (1). This highly dynamic microstructure was preserved through evolution. It has already been identified in a wide variety of animals, but the biologic function of pinopodes is still unclear. The pinopode is named for the function of pinocytosis, but this process is not observed in humans (2). Whether pinopodes are a reliable marker for the window of implantation in the human remains controversial (1–6). To determine the clinical value of this highly dynamic microstructure, we monitored pinopodes expression, analyzed the relationship between pinopodes expression and IVF pregnancy outcomes, and performed individual ET (iET) based on pinopode scoring. Through the 2-year clinical trials, pinopodes proved to be a reliable morphologic marker of endometrial receptivity.

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MATERIALS AND METHODS

Study Setting and Patients

This parallel study was conducted at the Center for Reproductive Medicine at Xiang-Ya Hospital in Changsha, Hunan, People's Republic of China, was approved by the Reproductive Medicine Ethics Committee of Xiang-Ya Hospital, and was registered in the Chinese Clinical Trial Registry (registration nos.: ChiCTR-OOC-14005617; ChiCTR-OOC-1500588).

Inclusion Criteria

Inclusion criteria included age < 40, normal ovarian response (FSH < 10 IU/L, antimüllerian hormone > 1.5 ng/mL,

antral follicle count > 5), and body mass index (BMI) ranged from 18 to 25. In the phase I trial, participants were also required to be on their first IVF cycle with tubal factor as the main indication for treatment; in the phase II trial, participants were required to have at least two previous implantation failures.

Exclusion Criteria

Participants were excluded for the following reasons: severe hydrosalpinx, advanced endometriosis (stage III–IV), uterine adhesion, or myoma or adenomyosis adjacent to uterine cavity.

Ovulation Monitor and Scanning Electron Microscopy (SEM)

During the menstrual cycle previous to IVF treatment, urine LH peaks were detected through daily urine LH test (urine LH test kit, YunDa) starting from day 10 of the menstrual cycle. On the seventh and ninth day post-LH peak (LH+7, LH+9), endometrial tissues were obtained via catheter suction (endometrial sampler, AiMu), avoiding repeated sampling from the same uterine wall. These specimens were first rinsed in saline and then were kept in a 2.5% glutaraldehyde solution for more than 48 hours before SEM.

Two independent observers who were blinded to the identity of the slides counted the number of mature pinopodes at magnification ×2,000. Twenty fields of endometrial epithelium were examined (the apical surface of ~150 cells can be visualized per field: 150 × 20 = 3,000 cells visualized per sample). The total number of pinopodes seen in all 20 fields was added up, and the mean value was used as the final sample scoring. The number of the fields full of cluster pinopodes was also counted. The diameter of a nascent blastocyst hatching from zona pellucida was 100 ~ 300 μm (7). The field at magnification ×2,000 was approximately 1/3 ~ 1/2 of a nascent embryo in size, enough for a hatched blastocyst adhesion.

Ovarian Stimulation, Luteal Phase Support, and ET

In the phase I trial (ChiCTR-OOC-14005617), induction of multiple follicular growth was accomplished by the administration of exogenous highly purified FSH (HP-FSH; Lishenbao, LiZhu) after a long desensitization protocol with short-acting GnRH agonist (Triptorelin, Ferring AG). After hCG (Rongcuxinsuzhen, LiZhu) administration, oocytes were collected transvaginally. From the day of oocyte retrieval, the luteal phase was supported with vaginal micronized P capsule (Utrogestan, Besins Healthcare) 200 mg twice a day.

Cleavage-stage embryos were evaluated according to La Asociación para el Estudio de la Biología de la Reproducción embryo assessment criteria (8). On day 3 after oocyte retrieval, up to two cleavage-stage embryos were transferred under ultrasound guidance.

Embryo selection for transfer and/or cryopreservation (viable embryo) was development to at least six blastomeres, <25% fragmentation, and absence of multinucleation. Clinical pregnancy was defined as the presence of a gestational sac with or without fetal heart activity as evaluated by ultrasound 4–5 weeks after ET. Ongoing pregnancy was defined as an intact pregnancy without termination before the 20th week of gestation.

Individual ET

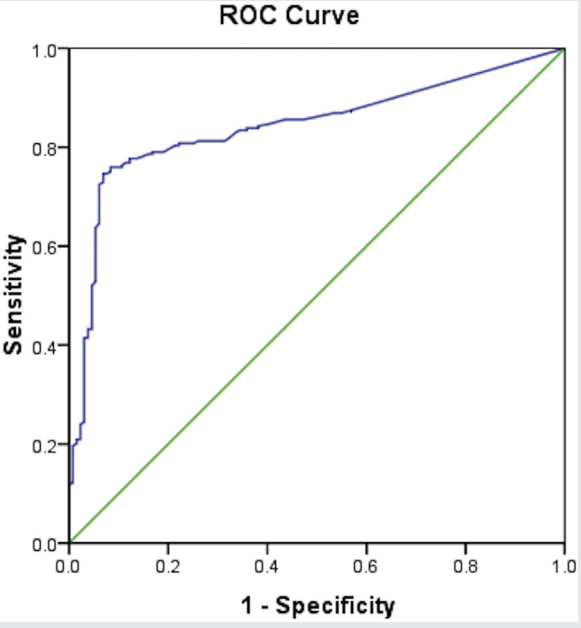
In the phase II trial (ChiCTR-OOC-1500588), sample size was calculated with the formula $N = Z^2 * (Prop * [1 - Prop])/E^2$, estimated design effect ($Z = 1.64$), desired level of absolute precision ($E = 10\%$), and estimate of the expected proportion (Prop = 40%). Using the formula, the sample size for the experimental group was calculated to be N = 65. After signing written informed consent forms, the patients' endometrial tissues were obtained and pinopode scores were calculated. Those participants with abnormal pinopode score (<85) were recruited and allocated into two groups by a random number table, and the experimental group had twice the number of the control group. Individual ET was carried out in the experimental group, where synchronization with the embryo was arranged so that the predicted most receptive day (determined by pinopode scoring) coincided with embryonic age day 6. In the control group, frozen-thawed embryos were transferred as a routine.

Statistical Analysis

The main outcome measures were the clinical pregnancy rate (CPR) and ongoing pregnancy rate (OPR). Statistical analysis was performed using SPSS version 16.0 software (SPSS Inc.).

A receiver operating characteristic (ROC) curve was drawn to determine the optimum cutoff value of pinopode score on day LH+7 for accumulated clinical pregnancy (Fig. 1). Based on this cutoff value, patients were divided into two groups. The pregnancy outcomes were compared

FIGURE 1



ROC curve of pinopode score for successful clinical pregnancy. The area under the ROC curve is 0.842 (95% confidence interval, 0.799~0.884), and the cutoff value is 85. The sensitivity is 74.7%, and the specificity is 93.1%.

Qiong. Optimal pinopode scoring. Fertil Steril 2017.