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● *Original Contribution*

COMPARISON OF UTERINE RECEPTIVITY BETWEEN FERTILE AND UNEXPLAINED INFERTILE WOMEN BY ASSESSMENT OF ENDOMETRIAL AND SUBENDOMETRIAL PERFUSION USING CONTRAST-ENHANCED ULTRASOUND: WHICH INDEX IS BETTER—PEAK INTENSITY OR AREA UNDER THE CURVE?

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Abstract—The goal of this study was to compare uterine receptivity between women with normal fertility and those with unexplained infertility during natural cycles by assessment of endometrial and subendometrial perfusion using contrast-enhanced ultrasound (CEUS). We wanted to determine the better index: peak intensity (PI) or area under the curve (AUC). Thirty women with unexplained infertility were recruited into the study group, and 30 women with normal fertility were recruited into the control group. All women underwent CEUS during the late proliferative phase, ovulation phase, and implantation window of a menstrual cycle. Endometrial PI, endometrial AUC, subendometrial PI and subendometrial AUC were analyzed. In the late proliferative phase, the control group had a significantly higher endometrial PI ($p < 0.001$) as well as subendometrial PI ($p < 0.001$) and AUC ($p = 0.004$) than the study group. In the ovulation phase, the control group had a significantly higher endometrial PI ($p < 0.001$) and AUC ($p = 0.021$), as well as subendometrial PI ($p < 0.001$) and AUC ($p = 0.003$). During the implantation window, there were no significant differences between the two groups. Only subendometrial PI underwent a significant periodic change during the menstrual cycle in both groups. This finding was further confirmed by evaluation of the microvessel density of endometria. In conclusion, CEUS can be used to assess endometrial and subendometrial perfusion to evaluate uterine receptivity. Subendometrial PI was the most sensitive index compared with endometrial PI, endometrial AUC and subendometrial AUC. (E-mail: hongmeiliu3@163.com) © 2015 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Infertility, Endometrium, Subendometrium, Perfusion, Contrast-enhanced ultrasound.**

INTRODUCTION

Uterine receptivity is a crucial factor in human reproduction. The human endometrium undergoes a series of morphologic and functional changes to provide the most suitable conditions for embryo implantation. The endometrium is receptive for an embryo only for a limited period, called the implantation window, considered to be days 5–9 post-ovulation (Bonilla-Musoles et al. 2013). Various methods are used to assess endometrial receptivity, such as transvaginal ultrasonography, endometrial biopsy and immunohistochemical analysis. However, because endometrial biopsy damages the endometrium, it is not widely accepted in clinical practice. Therefore, transvaginal ultrasonography may serve as an ideal non-invasive tool for assessing endometrial receptivity.

Because angiogenesis, (sub)endometrial vascularization and perfusion have been found to play critical roles during embryo implantation (Abulafia and Sherer 2000; Chwalisz and Garfield 2000; Demir et al. 2006), many researchers began to focus on the quantitative assessment of (sub)endometrial blood flow. Conventional color

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Doppler, pulsed Doppler and the novel 3-D power Doppler angiography (3-D-PDA) have been used to assess (sub) endometrial blood flow. However, conflicting results have been reported (Kim et al. 2010; Ng et al. 2009; Sardana et al. 2014; Schild et al. 2001). As basal and spiral arteries are tiny vessels with low-velocity blood flow and different ultrasonic instruments differ in sensitivity to these tiny vessels, a definitive method for assessing endometrial and subendometrial perfusion has not yet been established.

With the advent of second-generation contrast agents, which can visualize the capillary net of the examined tissue, contrast-enhanced ultrasound (CEUS) has been widely used to evaluate blood flow perfusion of various organs (Henri et al. 2014; Saini and Hoyt 2014). Because the diameters of contrast agent bubbles are much smaller than those of red blood cells, use of CEUS with the contrast agent could greatly improve the level of detection of tissue microvascularization, even for microvessel diameters <40 μm (Chaudhari et al. 2000). Thus, CEUS might be a better way to investigate the microcirculation of the endometrium. This has been proved during early implantation in the macaque uterus (Keator et al. 2011), but not in human beings. Among the main CEUS parameters, peak intensity (PI) and area under the curve (AUC) are indications of enhancement intensity and are used to evaluate blood perfusion (Wang et al. 2011).

Previous studies found that impaired uterine perfusion might be a cause of unexplained infertility (Goswamy et al. 1998; Uysal et al. 2012), and (sub) endometrial perfusion plays a critical role in embryo implantation (Demir et al. 2006). Thus, a population with unexplained infertility was selected to represent suboptimal endometrial receptivity in this study. Our aim was to compare the uterine receptivity of women normal fertility and those with unexplained infertility during natural cycles by assessment of endometrial and subendometrial perfusion using CEUS to determine which index is more accurate: PI or AUC.

METHODS

Participants

Thirty fertile women and 30 women with unexplained infertility who visited the Obstetrics and Gynecology Outpatient Clinic of the Third Affiliated Hospital of Southern Medical University from March 2012 to December 2012 were recruited into this study. Fifteen women with normal fertility and 18 women with unexplained infertility agreed to endometrial biopsy; the remaining women declined this procedure. The study was approved by the ethics committee of the Third Affiliated Hospital of Southern Medical University, and patient consent was obtained.

The criterion for inclusion in the study group was inability to conceive after 1 y of regular unprotected intercourse. Unexplained infertility was diagnosed according to the guidelines published by the Practice Committee of the American Society for Reproductive Medicine (2006).

The criteria for inclusion in the control group were a history of a normal delivery within a year, no specific gynecologic complaints or checkups and no breastfeeding for 3 mo.

In addition, all participants in the two groups had to have regular menstrual cycles of 26–32 d and should not have used hormones or taken any medication that could possibly interfere with the pelvic blood supply in the 3 mo before the study. Coagulation deficiency or hemorrhagic disorders were ruled out. All participants were required to use non-drug contraception during the testing period.

Ultrasound examination and setting of endometrial biopsy date

Ultrasound examinations (color Doppler flow imaging [CDFI] and CEUS examinations, respectively) and biopsy were both performed on days 10–12 (late proliferative phase), days luteinizing hormone (LH) to day between Day LH and two days after Day LH (LH + 2) (ovulation period) and days LH + 8 to LH + 10 (implantation window) of a menstrual cycle; ultrasound examinations were performed before biopsy. All ultrasound scans and measurements were performed between 9 and 12 am. The date of ovulation was confirmed by transvaginal ultrasonography and urinary luteinizing hormone (LH). After the first CEUS examination and biopsy on days 10–12 of menstruation, patients were told to test their urinary LH twice daily (10 am and 10 pm) with the LH diagnostic kit (Hemtrue, Shanghai Kaichuang Biological Technology, Shanghai, China) until the day on which LH surge (with the occurrence of T > C). Transvaginal ultrasonography was then performed daily after the LH surge to monitor for follicular rupture. Only when the follicular rupture actually occurred on days LH to LH + 2, did the study continue during the implantation window.

CDFI examination

A Philips IU22 ultrasound system (Philips, Amsterdam, the Netherlands) with C8-4v transvaginal transducer (5–7.5 MHz) was used. A true longitudinal view of the uterus was first obtained on the static gray-scale images, and then color Doppler mode was activated to assess the endometrial–subendometrial blood flow on a longitudinal scan of the uterus. The color sample frame was placed to include the entire endometrium. The velocity scale was set as 2.1 cm/s, and color gain was set as 80 ± 2%. On the basis of its distribution pattern,