Taiwanese Journal of Obstetrics & Gynecology 53 (2014) 360–365

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

Detection of endometrial and subendometrial vasculature on the day of embryo transfer and prediction of pregnancy during fresh *in vitro* fertilization cycles

Ari Kim [a], Hyuk Jung [b], Won June Choi [c], Sung Nam Hong [d], Heung Yeol Kim [e, *]

[a] Department of Obstetrics and Gynecology, Institute of Wonkwang Medical Science, College of Medicine, Wonkwang University, Iksan, Republic of Korea
[b] Department of Obstetrics and Gynecology, College of Medicine Chosun University, Kwangju, Republic of Korea
[c] Department of Obstetrics and Gynecology, College of Medicine, Kyung Sang University, Jinju, Republic of Korea
[d] Department of Obstetrics and Gynecology, Samsung Changwon Hospital, School of Medicine, Sungkyungkwan University, Changwon, Republic of Korea
[e] Department of Obstetrics and Gynecology, College of Medicine, Kosin University, Busan, Republic of Korea

ARTICLE INFO

Article history:
Accepted 27 May 2013

Keywords:
endometrial blood flow
endometrial receptivity
IVF
pregnancy
three-dimensional power Doppler

ABSTRACT

Objectives: Successful implantation depends on interaction between a blastocyst and a receptive endometrium. Endometrial vasculature is important in the early endometrial response to blastocyst implantation, and vascular changes can affect uterine receptivity. This study aims to investigate whether vascular parameters measured using three-dimensional power Doppler ultrasound (3D PD-US) could predict pregnancy following fresh *in vitro* fertilization and embryo transfer (IVF–ET) using a gonadotropin releasing hormone (GnRH) agonist long protocol.

Materials and methods: This prospective observational study enrolled 236 nulliparous women who underwent a first IVF–ET using a GnRH long protocol with stimulation by recombinant FSH (rFSH) from May 2009 to April 2012. After excluding two cases of tubal pregnancy, 234 women were in either a pregnant group ($n = 113$) or a nonpregnant group ($n = 121$). Color Doppler ultrasound and 3D PD-US examinations were performed on the day of embryo transfer. Main outcomes were pulsatility index (PI), resistance index (RI), systolic/diastolic ratio (S/D) of the uterine artery, vascularization index (VI), flow index (FI), and vascularization flow index (VFI) of the endometrium and subendometrial region. Measurements were analyzed relative to IVF–ET outcome (pregnant vs. nonpregnant).

Results: No significant differences were observed in patient age, infertility duration, body mass index (BMI), basal FSH levels, number of retrieved oocytes or good quality embryos, or endometrial thickness or volume between the two groups. The pregnant group had higher endometrial VI, FI, and VFI scores than the nonpregnant group ($p = 0.001$, $p = 0.000$, $p = 0.021$, respectively). By contrast, neither subendometrial region VI, FI, and VFI scores ($p = 0.770$, $p = 0.252$, $p = 0.451$), nor uterine artery PI, RI, or S/D scores ($p = 0.256$, $p = 0.527$, $p = 0.365$) differed between groups. Cut-off values of endometrial VI, FI, and VFI scores were 0.95, 12.94, and 0.15 for pregnancy achievement.

Conclusion: Three dimensional PD-US was a useful and effective method for assessing endometrial blood flow in IVF cycles. Good endometrial blood flow on the day of embryo transfer might be associated with high pregnancy success with a GnRH long protocol, because this is indicative of endometrial receptivity in fresh IVF cycles.

Introduction

Favorable maternal conditions and embryo quality are important for successful implantation. Problems that can originate from the host environment include abnormal uterine anatomy, maternal medical conditions, and a nonreceptive endometrium; all can have adverse effects on the cross-communication between the embryo

* Corresponding author. Department of Obstetrics and Gynecology, College of Medicine, Kosin University, 34 Amnam-dong, Seo-gu, Busan 607-702, Republic of Korea.
E-mail address: ttochil@hanmail.net (H.Y. Kim).

http://dx.doi.org/10.1016/j.tjog.2013.05.007

and the endometrium [1]. The endometrium is critical for successful implantation through interaction with the embryo [2]. Endometrial–embryo interactions can be altered if the embryo is defective, which can result from either paternal sperm factors or oocyte abnormalities.

Uterine receptivity is defined as the endometrial conditions for blastocyst attachment and implantation that occur during limited periods. The endometrium undergoes a complex series of changes during the menstrual cycle, with a short period of receptivity [3]. In this endometrial transition period, embryo implantation is supported by the ovarian hormones estrogen and progesterone, which modulate endometrial events in a spatiotemporal manner. The hormones affect numerous factors involved in implantation in humans [4]. In spite of our poor understanding of the molecular mechanism involving endometrial–embryo interactions, some interactions might influence endometrial neovascularization [5].

To increase advantageous endometrial–embryo interactions, the endometrium must become thicker, with richer vascularity. Endometrial blood flow reflects uterine receptivity because the endometrium is the site of embryonic implantation [5]. During *in vitro* fertilization (IVF) and embryo transfer (IVF–ET) cycles, implantation is a major determinant of success or failure. Up to two-thirds of implantation failures are estimated to be caused by defects in endometrial receptivity [6]. Efforts have been made to evaluate endometrial receptivity in endometrial and subendometrial blood supplies, especially during intrauterine insemination and IVF–ET cycles [7]. This study evaluated whether endometrial, subendometrial, and uterine blood flow parameters measured using three-dimensional power Doppler ultrasound (3D PD-US) and color Doppler ultrasound were useful in predicting pregnancy in fresh cycles using a GnRH long protocol. In comparison with previous studies, the uterine receptivity with vasculatures targeting restricted protocol and purified women experienced IVF–ET were evaluated for the first time.

Materials and methods

Patient population

A total of 236 infertile women, aged 26–41 years old, who underwent a first IVF–ET with a GnRH long protocol with stimulation by recombinant follicle stimulating hormone (FSH) (rFSH) from May 2009 to April 2012 were enrolled in the fertility centers of three university hospitals. Couples were considered for inclusion after chart review, excluding the following conditions: (1) infertility attributed to endocrine abnormalities such as hyperprolactinemia, polycystic ovarian syndrome, and hyperthyroidism; (2) other diagnosed chronic diseases; (3) previous gynecologic operation for ovarian or uterine pathology; or (4) inadequate data for analysis.

The inclusion criterion was that the infertile couple had no known infertility factors. Women with endometriosis, ovarian cyst, tubal obstruction, severe adenomyosis, uterine fibroid, endometrial polyp, intrauterine adhesion, severe pelvic adhesion, or low resolution on ultrasound examination were excluded. All patients were included for only a single cycle to avoid selection bias. In this prospective observational study, no therapeutic interventions except routine procedures were performed on patients. A careful ultrasound evaluation just before embryo transfer (ET) was performed. Ethics approval was granted by the Institutional Review Board of Kosin University, Busan, Korea.

Controlled ovarian stimulation and IVF–ET procedures

All patients underwent a GnRH agonist (Lucrin; Abbott, Rungis Cedex, France) long protocol for controlled ovarian stimulation (COS) with daily injection of rFSH (Gonal-F; Serono, Geneva, Switzerland or Puregon; Organon, Oss, The Netherlands). After proper pituitary regulation and desensitization using the GnRH agonist during the previous midluteal phase, COS was initiated at the beginning of menstrual cycle Day 3. The daily dose of rFSH was customized according to serum E2 concentration and the follicular growth and number assessed by transvaginal ultrasound. Final oocyte maturation was induced by intramuscular administration of 10,000 IU of hCG (Pregnyl; Organon) when at least two follicles reached >18 mm in mean diameter on transvaginal ultrasound.

Oocyte retrieval was carried out under transvaginal ultrasound-guided aspiration at 36 hours after hCG injection. Retrieved oocytes were fertilized by either conventional insemination or intracytoplasmic sperm injection (ICSI). Fertilization was performed 3–6 hours after oocyte retrieval in IVF medium (Quinn's Advantage media; SAGE BioPharma, Badminster, NJ, USA) with either ICSI or conventional insemination according to the presence or absence of male factor infertility. At 24 hours after oocyte retrieval, normal fertilization was confirmed by the presence of two pronuclei with two distinct or fragmented polar bodies.

Embryos were cultured in culture medium (Sage sequential media; SAGE BioPharma) to Day 3 after fertilization. After assessing the quality of cultured embryos, two or three good-quality embryos were transferred using a catheter (Cook catheter; Cook Medical, Bloomington, IN, USA) with abdominal ultrasound guidance on Day 3 after fertilization, adjusted for patient age, individual history, or preferences. Embryo quality was measured according to the following parameters: number of blastomeres, rate of fragmentation, and multinucleation of blastomeres. Other embryos were transferred from cleavage medium to blastocyst medium.

All patients received daily progesterone support (IM P 50 mg or vaginal 600 mg of micronized progesterone) from the day of oocyte retrieval.

Ultrasound investigation

Except for routine serial ultrasound examination, adjunctive 3D PD-US (ACCUVIX XQ, Medison, Seoul, Korea) and color Doppler evaluation by vaginal 3-D probe were performed to assess endometrium, subendometrium, and uterine arterial vascularity on the day of ET. All 3D PD-US and Doppler evaluation scans were carried out by uniform ultrasound modes and given to a single investigator for volume and Doppler analysis. The results of adjunctive ultrasound assessment did not affect subsequent clinical management procedures because the clinicians did not know the ultrasound evaluation results.

With color Doppler in the two-dimensional (2D) mode, flow velocity waveforms were obtained from the ascending main branch of the uterine artery on the left and right sides of the cervix in a longitudinal plane. The cursor of the Doppler was positioned where vessels with good color signals were identified on the screen. The PI, RI, and S/D ratio of the uterine artery were calculated electronically. Almost no differences in uterine PI, RI, and S/D ratios were seen between the left and right sides. Thus, analyses used the vascular indices of the right side uterine artery.

When a longitudinal view of the uterus was obtained, the power Doppler mode was turned on. The areas of interest were the endometrium and subendometrial regions within 5 mm of the echogenic endometrial borders. The setting conditions were: frame average, 5; balance, 16; sensitivity, 15; scale, 0.6 kHz; filter, 1; density, low; gray, 60. The 3D mode was activated and the area of interest was adjusted, and 3D volume data were obtained by automatic sweep with angle set to 90° to ensure inclusion of a