ASSESSMENT OF ENDOMETRIAL-SUBENDOMETRIAL BLOOD FLOW DETECTED BY COLOR DOPPLER SONOGRAPHY AND UTERINE RECEPTIVITY IN INFERTILE WOMEN

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Abstract- Endometrial vasculature has been shown to play an important role in the early endometrial response to the implanting blastocyst, and vascular changes may contribute to uterine receptivity**.** The aim of this study was to investigate the role of endometrial and subendometrial vascularity and thickness in uterine receptivity in infertile women treated with assisted reproductive technologies (ART). A total of 175 women were recruited from our ART program from June 2005 to May 2006. In the first stage, down regulation was done using GnRH-a and then ovarian hyperstimulation was initiated with human menopausal gonadotrophin and in vitro fertilization (IVF) procedures were performed. Then, the endometrial–subendometrial blood flow distribution pattern was determined by color Doppler ultrasonography and its characteristics were compared between pregnant (n = 56) and non-pregnant (n = 119) women**.** Zones 1, 2 and 3 of vascular penetration were found in 15.4%, 22.3%, and 62.3% of patients, respectively. The number of implanted embryos was 62 and implantation rate was 11.61%. There was no significant association between implantation rate and endometrial thickness or zone of vascular penetration. Among all women, fertility occurred in 56 (32.2%) women. Zone 1 of vascular penetration was found in 10.71% and 10.08% (P = 0.898), zone 2 in 16.07% and 13.44% (P = 0.643), and zone 3 in 41.07% and 41.17% (P = 0.989) of pregnant and non-pregnant groups, respectively. Endometrial–subendometrial blood flow (according the findings of Doppler ultrasonography) in women undergoing ART could not predict endometrial receptivity and IVF outcome.

Acta Medica Iranica 2008; 46(6): 461-466.

Key words: Uterine blood flow, color Doppler sonography, uterine receptivity

INTRODUCTION

There are several causes for infertility including gamete quality, congenital anatomical abnormalities and surgical complications. Previous studies have demonstrated that the poor uterine blood flow is also a cause of infertility (1).

Received: 13 Mar. 2007, Revised: 30 May 2007, Accepted: 1 Aug. 2007

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Embryo quality and endometrial receptivity are two of the parameters which determine the outcome of *in vitro* fertilization (IVF) (2). The condition of the uterus is critical to the process of embryo implantation, and among uterine conditions, endometrial development is the most important. Endometrial vasculature has been shown to play a prominent role in the early endometrial response to the implanting blastocyst, and vascular changes may contribute to the uterine receptivity (3, 4). It was first reported in 1988 that impaired perfusion of the uterine arteries may be a cause of infertility and may be related to unsuccessful IVF treatment (5).

Evaluation of endometrial receptivity remains a challenge in clinical practice. The condition of the uterus during IVF treatment is mainly assessed by ultrasonographic examination. Sonographic evaluation of endometrial thickness and texture has been used for endometrial assessment. The introduction of transvaginal Doppler ultrasound makes the measurement of uterine artery blood flow possible, even it was supposed that uterine arterial resistance changes might reflect uterine receptivity (6, 7).

The aim of this study was to assess the role of endometrial–subendometrial vascularity pattern and thickness detected by color Doppler ultrasonography in uterine receptivity in infertile women treated with assisted reproductive technologies (ART).

MATERIALS AND METHODS

Patients characteristics

From June 2005 to May 2006, consecutive patients attending the Assisted Reproduction Unit at the Department of Obstetrics and Gynecology, Shariati Hospital, for IVF treatment were recruited. Inclusion criteria were: 1) having at least one good quality embryo, as defined by the morphology criteria, for transfer on the 2nd or 3rd day after oocyte retrieval, and 2) no uterine abnormalities confirmed by hysterosalpingography.

Exclusion criteria were: 1) distortions of the uterine cavity detected during transvaginal ultrasound examinations, 2) unsuitable uterine cavity during hysterosalpingography, and 3) fertilization aided by donor. All patients were included in the study only once to avoid selection bias. The study was approved by the institutional review board of Shariati Medical University Hospital, and each woman gave verbal informed consent.

Ovarian hyperstimulation and IVF procedures

In the first stage, on the basis of Long protocol (8), down regulation was performed using gonadotrophin releasing hormone agonist (GnRH-a) and then ovarian hyperstimulation was initiated with administration of human menopausal gonadotrophin (HMG) (Pergonal; Serono, Geneva, Switzerland) at a dose of 10,000 IU IM on the 2nd day of the menstrual cycle.

When there were at least two leading follicles with a diameter of > 17 mm, oocytes were retrieved by transvaginal ultrasound-guided follicular aspiration 34-36 hours after hCG injection. The embryos were evaluated before transfer based on the fragmentation pattern previously outlined by Alikani *et al.* (9), with fragmentation pattern I exhibiting minimal fragments and pattern V exhibiting extensive fragmentation. Embryos with fragmentation pattern I to III were defined as good quality in this study. Micronized vaginal progesterone (Utrogestan; Laboratories Piette International, Brussels, Belgium) was used for luteal support. Serum β-hCG levels were measured 14 days after embryo transplantation (ET), and if positive, micronized progesterone was continued for 4 weeks.

Ultrasound investigation

On the day of ET, transvaginal sonography examination was performed with patient in the lithotomy position using a 10-MHz transvaginal transducer with color Doppler facility (Acuson sequoia 512). All ultrasound scans were performed by L.W.C. When a longitudinal view of the uterus was obtained, the color Doppler mode was activated. The endometrial–subendometrial blood flow distribution pattern was determined by demonstrating pulsatile color signals in the subendometrial and endometrial regions. For those with vascularization penetrating the subendometrial area, we adopted the definition from Applebaum (10), summarized as follows: zone 1, vessels penetrating the outer hypoechogenic area surrounding the endometrium but not entering the hyperechogenic outer margin; zone 2, vessels penetrating the hyperechogenic outer margin of the endometrium but not entering the hypoechogenic inner area; and zone 3, vessels entering the hypoechogenic inner area.

The blood flow distribution pattern was observed at the beginning and the end of the ultrasound examination. If different patterns were observed, the pattern with deeper vascular penetration into the endometrium was adopted for analysis. Doppler sonography was then performed on the vessels with

the highest color intensity within the innermost endometrial-subendometrial area. The insonation angle was kept at 0° because the course of the small spiral arteries could not be determined. After confirming that waveforms were continuous, an average of three to five cardiac cycles was selected for calculation of resistance index (RI), pulsatility index (PI), maximum peak systolic blood flow velocity (V max), and time-averaged peak systolic blood flow velocity (V mean). The vessel with the lowest PI was considered for further statistical analysis. Uterine circulation was assessed simultaneously in each examination; bilateral uterine arteries were sampled lateral to the cervix near the internal os. Mean levels of both uterine RI and PI were used for analysis. The temporal average intensity of the spatial peak of ultrasound for B-mode and Doppler examinations was < 50 mW/cm, and the total examination time was kept to ≤ 15 minutes. The intraobserver variation of Doppler flow measurement was 7.8% ± 2.5%.

The endometrial pattern visualized was designated as a multilayered or a non-multilayered endometrium (11). A multilayered endometrium presented as a triple-line pattern in which hyperechogenic outer lines and a well-defined central echogenic line were seen with hypoechogenic or black areas between these lines. A non-multilayered endometrium consisted of homogeneous endometrial patterns characterized by either hyperechogenic or isoechogenic endometrium.

Diagnosis of pregnancy

Clinical pregnancy was defined as the presence of gestational sac by ultrasound with appropriate rise in β-hCG levels. The diagnosis of extrauterine pregnancy was confirmed by laparoscopy. Miscarriage was defined as pregnancy loss before 20 weeks of gestation.

Statistical analysis

Continuous data has been presented as mean ± SD. Comparisons among the groups for pregnancy outcome variables were made by Fisher's exact test or χ^2 test, adjusted for multiple comparisons using the Bonferroni technique. For comparisons among the groups for prognostic factors, the Mann-Whitney U test and Kruskal-Wallis test were used where appropriate. Multiple linear regression techniques were performed to adjust the confounding factors that may affect the pregnancy outcome between patients with presence or absence of subendometrial flow. Odds ratio (OR) and 95% confidence intervals (CI) were calculated.

A *P* value of < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS software version 13 and SAS version 9.1 for windows.

RESULTS

A total of 175 women were recruited. The mean age of the women was 31 ± 5.4 years (range, 18-45 years), and 148 (84.6%) of them had primary infertility. The mean body mass index (BMI) was 26.4 ± 3.8 kg/m^2 (range 16-38 kg/m^2). The mean duration of infertility was 9.1 ± 5.3 years.

The causes of infertility are shown in Table 1. The most common cause of infertility was male factor (41.7%). Among the etiologies of infertility, only tubal factor was significantly more common in non-pregnant women (*P* = 0.006).

Histories of previous interventions are summarized in Table 2. Among the study population, 54.3% had no previous interventions.

Table 1. The causes of infertility in infertile women (pregnant and non pregnant) treated with assisted reproductive technologies*

Causes	Total patients (n =175)	Pregnant group (n =56)	Nonpregnant group (n =119)	*P* value
Male factor	41.7	50.0	37.81	0.127
Tubal factor	23.4	10.71	29.41	0.006
Age	14.3	16.07	13.44	0.643
Ovarian factor	11.4	16.07	9.24	0.185
Unexplained	7.4	5.35	8.40	0.473
Age and tubal factor	1.7	1.78	1.68	0.999

*Data are given as percent.

Table 2. History of previous interventions in infertile women treated with assisted reproductive technologies

Interventions	Percentage (n = 175)
None	54.3
IUI	16.0
ZIFT	13.7
IVF	8.6
ZIFT/IUI	3.4
IVF/ZIFT	1.7
GIFT	1.1
IVF/GIFT	0.6
ICSI	0.6

Abbreviations: IUI, intrauterine insemination; ZIFT, zygote intrafallopian transfer; IVF, In-vitro fertilization; GIFT, gamete intrafallopian transfer; ICSI, Intra cytoplasmic sperm injection.

Table 3. The number of transferred embryos in infertile women treated with assisted reproductive technologies

Number of transferred embryos	Percentage (n = 175)
1	5.7
2	16.0
3	33.7
4	18.3
5	24.6
6	1.1
8	0.6

Endometrial pattern was triple-line in 58.9%, solid in 29.1%, and semi + solid in 3.4%. One, 2 and 3 zones of vascular penetration were found in 15.4%, 22.3%, and 62.3% of patients, respectively.

The numbers of transferred embryos are shown in Table 3. Altogether, 606 embryos were transferred. Also, the number of implanted embryos was 62 and implantation rate was 11.61% (95% CI: 8.19-15.04).

Implantation rates in relation to zone of vascular penetration and endometrial thickness are shown in Table 4. There was no significant association between implantation rates and endometrial thickness (P = 0.698) and zone of penetration (P = 0.558). Among all studied women, fertility occurred in 56 (32.2%) women.

After adjustment for the age (P = 0.243), BMI (P = 0.617), and duration of infertility (P = 0.543), we compared the characteristics of pregnant (as study group; n = 56) and non-pregnant women (as control group; n = 119). The mean endometrial thickness in pregnant and non-pregnant groups were 11.84 ± 2.88 and 11.95 ± 3.49, respectively that was similar between groups (P = 0.600). Also, no significant difference was found in different endometrial diameters between 2 groups (Table 5).

Findings of Doppler ultrasonography were also compared between two groups. Zone 1 of vascular penetration were found in 10.71% and 10.08% (P = 0.898), zone 2 in 16.07% and 13.44% (P = 0.643), and zone 3 in 41.07% and 41.17% (P = 0.989) of study and control groups, respectively. Also endometrial triple-line pattern were found in 42.85% and 39.49% (P = 0.673), solid pattern in 17.85% and 20.16% (P = 0.719), and semisolid pattern in 1.78% and 3.36% (P = 0.999) of study and control groups, respectively, that were similar between groups.

Table 4. Implantation rates in relation to zone of vascular penetration and endometrial thickness

Criteria	Odds Ratio	95% Confidence Interval		*P* value
Endometrial thickness				
6-8 mm	6.666	-1.513	14.846	0.698
8-10 mm	13.633	7.068	20.197	
10-12 mm	12.817	4.640	20.995	
> 12 mm	10.666	5.130	16.203	
Doppler findings				
Zone1	10.370	3.865	16.875	0.558
Zone 2	15.128	6.164	24.092	
Zone 3	10.672	6.388	14.957	

Table 5. The endometrial thickness in pregnant and nonpregnant groups*

Endometrial thickness	Pregnant group (n = 56)	Nonpregnant group (n = 119)	*P* value
6-8 mm	5.35	12.60	0.141
8-10 mm	35.71	25.21	0.151
10-12 mm	25.0	23.53	0.832
> 12 mm	33.92	38.65	0.546

* Data are given as percent.

DISCUSSION

Infertility may have different prevalence rates in different populations (12). World Health Organization (WHO) has estimated that there are 50 million to 80 million infertile couples worldwide (13). Studies on infertility prevalence are rare in Iran (14). Furthermore, there is a significant difference in infertility rate between geographic parts of the province (15).

In our study, the most common cause of infertility was male factor (41.7%). In Chien *et al.* (4), Schild *et al.* (16), and Ng *et al.* (17) studies, the predominant diagnoses were male-factor infertility (41.1%, 59.2%, and 51.8%, respectively), whereas in Aflatoonian *et al.* study, the most common cause of infertility was female factor (57.5%) and male factor was reported in only 25.3% of couples (15). Also, in our and Chien *et al.* studies, primary infertility was found in 84.6% and 47.7% of women, whereas in Aflatoonian study, only 3.2% of women had primary infertility (14). This rate in Schild *et al.* (16) and Ng *et al.* (17) studies was 68.9% and 68.3%, respectively.

In our study, the mean endometrial thickness in pregnant and non-pregnant groups was similar. Similar to our study, in Chien *et al.* study, no significant difference was found in endometrial thickness between two groups (4). In their study, the thickness of the endometrium significantly differed with the pattern of endometrial–subendometrial blood flow distribution. Also intraendometrial vascular penetration was associated with a thicker endometrium, suggesting a correlation between blood perfusion and endometrial development (4). Also in Schild *et al.* study, neither endometrial thickness nor endometrial volume were correlated with the likelihood of successful implantation (16).

We were unable to find any significant association between implantation rates and zone of penetration (P = 0.558). In Chien *et al.* study, although pregnancy and implantation rates were significantly higher in patients with zone 3 compared with zone 1 or 2 penetration, there was no significant difference between the groups with zone 1 and 2 penetrations (4).

We found that the endometrial patterns were similar between pregnant and non-pregnant women. However, multiple studies have confirmed a lower implantation rate in women who exhibit a homogeneous pattern (pattern 2) of the endometrium compared to a triple-line pattern on the day of hCG administration. Endometrial pattern, rather than thickness, on the day of oocyte retrieval appears to be an important prognosticator of endometrial receptivity (18). However, in several studies it has been shown that endometrial thickness and pattern have low positive predictive value and specificity in the prediction of IVF outcome (19-22).

In conclusion, according to our findings, endometrial–subendometrial blood flow distribution pattern and thickness based on findings of Doppler ultrasonography in women undergoing ART, could not predict endometrial receptivity and IVF outcome.

Acknowledgements

This research project has been supported by Shriati Hospital and Tehran University of Medical Sciences and Health Services grant.

Conflict of interests

The authors declare that they have no competing interests.

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