

## Correlation between Endometrial Parameters in Doppler Sonography and Success Rate of Implantation in Assisted Reproductive Programs

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### Abstract

**Background:** A good blood supply towards the endometrium is usually considered to be an essential requirement for implantation. Evaluating the role of endometrial and sub-endometrial blood flows detected by color Doppler sonography at the day of embryo transfer, as a predictor of pregnancy rate during IVF/ICSI programs was our main goal in this study.

**Materials and Methods:** Seventy three infertile patients aged <38 years with basal serum FSH level <12 mIU/ml who had less than two failed attempts of ART cycles were prospectively evaluated. The cases with more than two failed IVF cycle and uterine disorders were excluded. All procedures were performed by one expert and two good quality embryos were transferred. Vaginal power color Doppler sonography was performed at the day of embryo transfer and endometrial characteristics including endometrial vascularization, area of vascularization distribution and pulsatility index were registered. Then pregnant and non-pregnant groups were compared for ultrasonographic parameters of endometrium.

**Results:** Pregnancy occurred in 28 patients. Mean age, duration and etiology of infertility, baseline mean FSH and estradiol level at the day of Human chorionic gonadotropin (HCG) injection, amount of drug administered, number of retrieved oocytes and embryos, also number and quality of transferred embryos in the pregnant and non-pregnant groups showed no statistically significant difference. On the other hand, none of the sonographic parameters reported in two groups indicated a statistically significant difference.

**Conclusion:** Doppler ultrasonographic indices at the day of embryo transfer are not considered appropriate criteria for prediction of success rate or failure of embryo implantation.

**Keywords:** Doppler Sonography, Implantation, Endometrial Vascularization Vessels, Assisted Reproductive Techniques

### Introduction

Vaginal sonography is an effective method for evaluating uterine and ovarian changes during cycles of assisted reproductive technique (ART). Recently particular attention has been focused upon the value of endometrial Doppler sonography indices for predicting success rate of assisted reproductive programs (1, 2). An appropriate endometrium is one of the major factors in success of ART programs and embryo implantation and failure may be due to

insufficient endometrial circulation (3-5). Studies based on Doppler sonography have revealed considerable difference in pattern of endometrial circulation alteration during days of natural menstrual cycle and stimulated cycles (4, 5). Raine- Fenning et al performed a study on 27 women and concluded that endometrial vascularity alters considerably along a menstrual cycle; the highest rate of changes were seen three days before ovulation and the lowest, 5 days

Received: 11 April 2007, Accepted: 2 June 2007

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Royan Institute  
Iranian Journal of Fertility and Sterility  
Vol 1, No 2, Summer 2007, Pages: 63-68

after ovulation and near implantation period (5). In another study, Ng. et al examined 67 cases with normal and stimulated cycles using Doppler sonography and presented their vascularization index (VI), vascularization flow index (VFI) and flow index (FI). They showed that during stimulated cycles endometrial blood flow is significantly less than normal cycles (4). Various reports have evaluated the role of endometrial circulation index in endometrial receptivity and concluded that success rate during assisted reproductive cycles depends on it (3, 6, 7). Dietterich et al evaluated endometrial parameters using ultra-sonography and reported that in patients with an endometrial thickness < 9mm, implantation rate is significantly decreased. Also, they observed the same cases had the least subendometrial circulation (3).

In another study by Contart et al endometrial vascularity was evaluated in 185 infertile patients using ultra-sonography on the day of HCG injection. Results showed that this method was not conclusive in predicting pregnancy during intra-cytoplasmic sperm injection (ICSI) program (6). Puerto et al (7) and Salle et al (8) have found no positive correlation between blood circulation, endometrial morphology, and successful implantation of embryo. On the other hand Zaidi et al have reported that rate of implantation of embryo is significantly decreased. (2). Wu et al conducted a study on 54 patients during their first IVF cycle and at the day of HCG injection in order to define the criteria of endometrial receptivity based on sonographic findings of endometrial circulation. Based on their study, these sonographic findings have an important role in predicting success rate of in vitro fertilization (IVF) (9).

Due to the controversies about the value of sonographic criteria in predicting the possibility of pregnancy and considerable number of ARTs performed in Royan Institute, this study was conducted to define the correlation between endometrial

vascularity using power Doppler sonography and successful implantation of embryo during the year 2005.

## Materials and Methods

In this prospective observational study, 73 infertile patients who referred to Royan Institute (Infertility Clinic & Reproductive Biomedicine Center) and underwent IVF/ICSI programs were studied from July to October 2005. The study was approved by the Royan research center's ethics committee and written informed consent was obtained from each participant. The inclusion criteria were age <38 years, basal serum FSH level <12 mIU/ml and history of maximally two failed attempts of IVF/ICSI in preceding cycles. The cases with more than two failed IVF/ICSI cycles, endometriosis and uterine disorders were excluded.

For all patients GnRH agonist (Suprefact Hoechst, Germany) 500µg/day subcutaneously was prescribed from the 21st day of the cycle for 12-14 days to suppress ovarian function. After ovarian suppression which was confirmed by vaginal ultrasonography (negative for follicles > 8 mm) and serum Estradiol level on day 2 of cycle ( $LH \leq 5IU$ ,  $E_2 \leq 50pg/ml$ ) gonadotropin therapy was started using highly purified FSH (150-225IU/d; Menopur, Ferring Pharmaceuticals, Denmark) from the second day of cycle. GnRH analogue (200µg/day) was continued up to the day of hCG administration. Follicular development was monitored by serial transvaginal ultrasonography (Aloka 1000, Japan, 7.5 MHz probe) and serum  $E_2$  levels. All of sonographic evaluations were done by one expert. OCP-LD was administered from 5th day of their preceding cycle.

When at least two follicles reached 18 mm in diameter, hCG (10.000 IU; Serono; Pregnyl, Organon) was administered, and then ultrasound-guided transvaginal oocyte pick-up was performed 34-35 hours later. Embryo transfer was performed 48-72 hours

after oocyte pick-up and according to patient age, indications for IVF or ICSI, number of previous treatment cycles, and number and quality of embryos, 2-4 good quality embryos (using Veek system grading) were transferred.

Endometrial color Doppler sonography (Aloka 1700, Japan, 4-7.5 MHZ probe) was performed 2-4 hours before embryo transfer to obtain endometrial parameters.

Color mapping of endometrial vascularity, according to the degree of penetration into the endometrial thickness were classified as follows:

- Zone 0 (absent) Absence or negative flow: when only surrounding myometrial vessels are seen without reaching the endometrium.
- Zone 1 (sub-endometrial) or peripheral flow: color signals reach the hyperechogenic outer layer of the endometrium
- Zone 2 (outer hyperechogenic Zone) or intermediate: when color mapping occupies the outer half of the endometrial hypoechogenic thickness
- Zone 3 (inner hypoechogenic Zone) or central flow: vessels reach the endometrial cavity invading the entire endometrial thickness and therefore penetrate all layers of endometrium (6).

The luteal phase was supported by vaginal Progesterone (Vag. Supp., Cyclogest, 400 mg, BD, Alpha, Barnstaple, UK). Clinical pregnancies were detected by increasing serum  $\beta$ -hCG levels in at least two determinations 12–14 days after ET and were confirmed by ultrasonographic screening of the gestational sac showing embryo activity 14 days after the last  $\beta$ -hCG determination. Then pregnant and non pregnant cases were compared for endometrial thickness, endometrial pattern and endometrial vascularization.

The data were analyzed with the use of Student's *t*, Chi-square, and Fisher's Exact tests and  $p < 0.05$  was considered as statistically significant.

## Results

The demographic and clinical characteristics of the patients are shown in Table 1.

**Table 1: Comparison between patient characteristics and treatment cycle in pregnant and non-pregnant groups.**

<b>Age (Year)</b> mean $\pm$ SD	30.4 $\pm$ 5.2	30.1 $\pm$ 4	t-test: NS*
<b>Duration of infertility (Year)</b>	9.1 $\pm$ 5.5	10.2 $\pm$ 4.3	t-test: NS
<b>Number of cycles performed</b>	1.5 $\pm$ 0.5	1.5 $\pm$ 0.7	t-test: NS
<b>Infertility etiology:</b>			t-test: NS
<b>Frequency (percent)</b>			
<b>Male factor</b>	17(37.8)	9(32.1)	
<b>Ovulation</b>	7(15.6)	4(14.3)	
<b>Unexplained</b>	5(11.1)	6(21.4)	
<b>Others</b>	16(35.6)	9(32.1)	
<b>Type of cycle: frequency (percent)</b>			t-test: NS
<b>IVF</b>	4(14.3)	4(8.9)	
<b>ICSI</b>	17(60.7)	34(75.6)	
<b>IVF+ICSI</b>	7(25)	7(15.6)	
<b>Baseline FSH level (mlu/ml)</b>	6 $\pm$ 2.8	6.6 $\pm$ 2.7	t-test: NS
<b>Baseline Estrogen level (pg/ml)</b>	98.9 $\pm$ 31.1	78 $\pm$ 89.7	t-test: NS
<b>Estrogen level on day of HCG injection (pg/ml)</b>	977.2 $\pm$ 690.3	971.8 $\pm$ 3.6	t-test: NS
<b>Number of Gonad-F administration</b>	21.9 $\pm$ 10.6	24.5 $\pm$ 13.5	t-test: NS
<b>Number of retrieved oocytes</b>	9.6 $\pm$ 6.2	9.6 $\pm$ 4.4	t-test: NS
<b>Number of produced embryo</b>	4.3 $\pm$ 2.1	5.7 $\pm$ 3.2	t-test: NS
<b>Number of transferred embryos</b>	3.4 $\pm$ 0.9	3.4 $\pm$ 0.7	t-test: NS
<b>Number of transferred embryos with good quality</b>	3.1 $\pm$ 1	3.7 $\pm$ 1.5	t-test: NS

NS: non-significant

There was no statistically significant difference in pregnant and non-pregnant

groups regarding age, duration of infertility, number of cycles performed, basal FSH, basal Estradiol, Estradiol level at the day of HCG injection, number of HMG used, number of oocytes retrieved and number of embryos transferred ( $p>0.05$ ). Also, both groups had similar frequencies of infertility etiology and types of treatment cycle.

**Table2: Comparison between Doppler sonography results in pregnant and non-pregnant cases.**

Variable	Group pregnant	Non-pregnant	P-value
<b>Development and Penetration of vessels in endometrium: number (percent)</b>			<b>Fishers Exact test: NS*</b>
Absent	3(10.7)	1(2.2)	
Zone 1	7(25)	25(55.6)	
Zone 2	16(57.1)	16(35.6)	
Zone 3	2(7.1)	3(6.7)	
<b>Vascularity Area: Frequency (percent)</b>			<b>Fishers Exact test</b>
EPDA	1(3.8)	4(9.1)	NS
>5mm <sup>2</sup>	25(96.2)	40(90.9)	NS
<5mm <sup>2</sup>			
<b>Pulsatility index (PI)</b>	2±0.2	2.1±0.4	t-test: NS

\*NS: non-significant

Table 2 demonstrates color Doppler characteristics of the endometrium in two groups. Endometrial thickness ranged from 8 to 25 millimeters in both groups. Comparing vascular pattern of the two groups revealed that in 4 patients endometrial vessels were absent, in 43.8% vessels were extended to zone A and in a similar number (32 patients) this extension was up to the middle of the endometrium (zone B). In only 6.8% were the vessels extended up to the interface of the two endometrial layers (zone C). Vascularity of the endometrium was not statistically different among the two groups (table 2). In 92.9% of the patients endometrial vascular area was reported more than 5 square millimeters. Although most of the patients in the non-pregnant group had a vascular area less than 5 mm<sup>2</sup> (9% compared to 3%) this

difference was not statistically significant among the two groups. The pulsatility index (PI) ranged from 1.18 to 3 and there was not statistically significant difference in PI between the groups.

## Discussion

In the present study, the mean age of the study group was 30 years which is considered a young population and success rate was 38.2%.

Some researchers have reported that endometrial blood flow is considered more important than other sonographic indices such as pattern and thickness of endometrium (2). For example, Chien et al have found a positive correlation between vascular penetration into the endometrium and occurrence of pregnancy (1). On the other hand Zaidi et al measured sub-endometrial blood flow and intraendometrial vascularization on the day of HCG injection among 96 patients undergoing IVF cycles. In eight (8.3%) of non-pregnant patients, subendometrial color flow and intraendometrial vascularization were not detected. This absence of blood flow was associated with failure of implantation ( $p<0.05$ ). These patients were devoid of sub-endometrial color flow and intra- endometrial vascularization. Although the incidence of pregnancy increased with penetration of vessels into the superficial layers of endometrium (zone1> zone2> zone3) difference between the layers was not statistically significant (2). Considering the negative correlation between vascular penetration and pregnancy occurrence, the present study was in accordance with the above-mentioned studies but in cases with absent penetration the situation was different. In the latter cases 3 pregnancies occurred in spite of absent endometrial vascularity which is in accordance with results obtained by Salle et al and Puerto et al (7, 8). Studies using Doppler sonography have shown that endometrial and uterine arterial resistance decreases in the luteal phase in which embryonal implantation occurs (10).

reaches to maximum when the pulsatility index ranges 2 to 3 and has a dramatic decrease when it reaches 3 or 4 (11). Despite various studies still no consensus has been reached considering the importance of studying these arteries during ART cycles (10).

This means that a large number of researchers have reported a significant difference considering uterine artery resistance between successful and unsuccessful cycles (12). Results of the present study were quite similar to numerous other studies (7, 8, 13, 14) and showed no significant difference considering mean pulsatility index in two groups. None of our patients had pulsatility indices more than 3. A group of researchers have concluded that rate of pregnancy is significantly increased in women with higher endometrial blood flow (15).

## Conclusion

In present study no correlation was found between vascular signals and pregnancy rate.

In summary, Doppler sonography parameters at the day of embryo transfer have no positive predictive value considering success of assisted reproductive techniques leading to pregnancy.

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