Endometrial thickness and Blood Supply evaluation by 3D and Dopplar U/S as a Predictor of IVF Outcome

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Abstract: Background: Despite the fact that top-quality embryos may be available for transfer during in-vitro fertilization (IVF) intracytoplasmic sperm (ICSI) cycles, only a maximum of one third of the embryos transferred finally implant. Many factors contribute to obtaining a successful pregnancy. Understanding these factors can help in counseling patients regarding their chances of success and the possible predictors of pregnancy that have been evaluated are endometrial thickness endometrial pattern and endometrial blood flow. **The aim of is study:** the study designed to evaluate the relation between endometrial thickness and vascularity measured by 3D ultrasound, Doppler and pregnancy outcome in IVF cycles. **Patients and methods:** 120 infertile women were included in this study all of them were subjected to controlled ovarian hyperstimulation using slandered long protocol and all of them had three-dimensional ultrasound and Doppler ultrasound on the day of HCG injection of stimulated cycle. **Results:** out of the study 52 women become pregnant with mean of endometrial thickness and endometrial volume 13.03±1.63 and 6.75±1.95 respectively in comparison to non-pregnant women that was highly significant decrease . Also pulsatility index and resistance index of uterine artery were lower in pregnant women (1.67±0.33 and 0.83±0.05 respectively) while flow index of uterine artery and subendometrial vascularization flow index were higher in pregnant women (25.44±2.1 and 0.33±0.21 respectively) in comparison to non-pregnant women. **Conclusions:** although3D ultrasound and power Doppler angiography aremore expensive they may offer useful tool to assess endometrial receptivity in IVF/ICSI and embryo transfer especially with minimal number of embryo transferred. That may give big help in programs that tend to minimize embryo transfer up to single embryo transfer .
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1. Introduction:

Successful embryo implantation is a crucial event in natural and assisted human reproduction. Blastocyst implantation is a dynamic process, involving embryo apposition, attachment to the maternal endometrial epithelium, and invasion into the endometrial stroma ***(Hanna and Ariel,* 2006).**With in vitro fertilization (IVF), implantation failure can occur due to several Factors ***(Levi et al., 2004),*** including poor embryo quality which is identified as a major cause of implantation failure ***(Urman et al., 2005).***

Another widely acknowledged barrier to successful blastocyst implantation is an inappropriately developed endometrium. It is well established that embryos cannot implant in a poorly matured endometrium ***(Hanna and Ariel, 2006)*** and this may be responsible for low implantation rates with transfer of "good quality" embryos. Moreover the success of embryonic implantation further relies upon cross talk between the embryo and a receptive endometrium ***(Hanna and Ariel, 2006).***

Implantation failure remains an unsolved problem in reproductive medicine and is considered as a major cause of infertility in otherwise healthy women. Indeed, the average implantation rate in IVF is around 25% ***(De Los et al., 2003)***Inadequate uterine receptivity is responsible for approximately two-thirds of implantation failures, whereas the embryo itself is responsible for only one-third of these failures ***(Ledee-Bataille et al., 2002; Simone et al., 1998).***

Three dimensional endometrial volume assessment allows the information gathered to be manipulated by a variety of techniques ***Jones 2001)*** the most common are **Multiplanar reformatting**, when the image is presented as the three orthogonal planes of the acquired volume (longitudinal, transverse and coronal). The view can then be rotated or repositioned (by parallel shift) in any of the three spatial axes **(Figure 1)** and **Volume rendering,** often used with power Doppler, where the (3D) image is projected onto a (2D) plane and each voxel is weighted and summed either to produce maximum intensity projection or minimum intensity for translucency rendering.

Power Doppler, developed in the 1990s ***(Rubin and Adler, 1993).*** is based upon the integrated power (intensity, strength, energy or amplitude) of the Doppler received ultrasound signal (corresponding to the zero lag of autocorrelation) rather than the mean Doppler frequency shift ***(Rubin et al., 1995).*** As a result all frequency information including flow direction and velocity is eliminated *(Weskott, 1997).* Signal is thus governed by the volume of moving blood rather than its velocity *(Evans, 2010)* and it is proportional to the number of moving scatters within an isolated volume *(Adler et al., 1995).*

Signal is displayed as differing hues of a single colour, often ranging from yellow through orange to red, the lighter the hue the greater the intensity of the signal *(Martinoli et al., 1998).* The hue is approximately proportional to the log of the reflector concentration within the region of interest *(Adler, 2001).*Computer software programs have been developed in order to quantify blood flow three-dimensionally; the method currently used is VOCAL (Virtual Organ Computer-aided Analysis) within the 4D View software program *(Gumar et al., 2008).* *(Figure 2).*

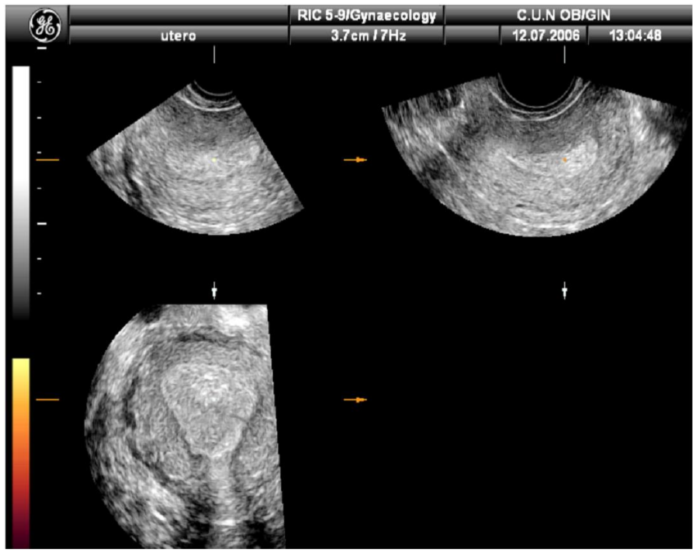

Figure (1): Three-dimensional ultrasound depicting multiplanar display of the uterus. All three orthogonal planes can be displayed using this technique ***(Alcazar, 2006).***

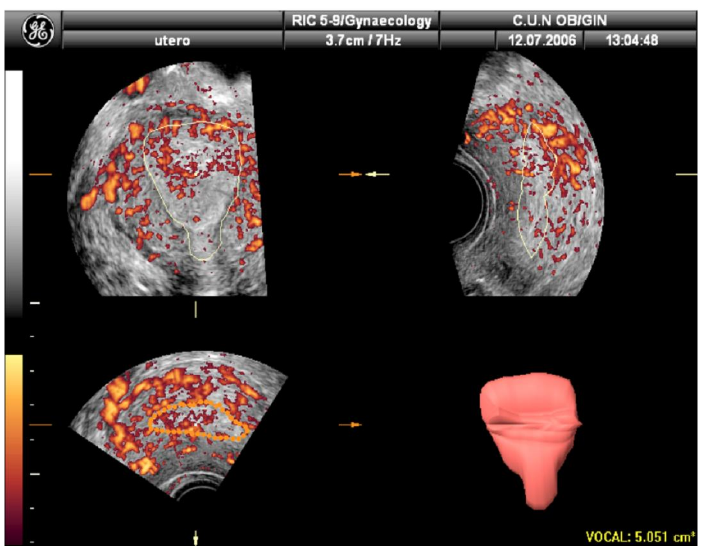

Figure (2): Endometrial volume calculation by using the VOCAL software after three-dimensional ultrasound ***(Alcazar, 2006).***

The power Doppler signal is quantified through the `histogram facility', which generates the vascular indices Vascularization Index (VI), Flow Index (FI) and Vascularization-Flow Index (VFI) ***(Pairleitner et al., 1999).*** **(Figure 3).**

Ultrasound parameters of the endometrium and the evaluation of uterine and endometrial blood flow have long be considered as implantation markers in In vitro fertilization (IVF) and embryo transfer cycles *(Merce, 2002).*A triple-layer endometrial pattern and an endometrial thickness greater than 7 mm have been proposed as markers of endometrial receptivity but have yielded a high percentage of false-positive results ***(Friedler et al., 1996).*** However uterine receptivity improves when blood flow increases under hormonal replacement therapy, an elevated pulsatility index of the uterine arteries is associated with low implantation and pregnancy rates ***(Merce, 2002).*** Though an adequate Doppler velocimetry of the uterine arteries is only slightly specific for predicting gestation ***(Friedler et al., 1996).***

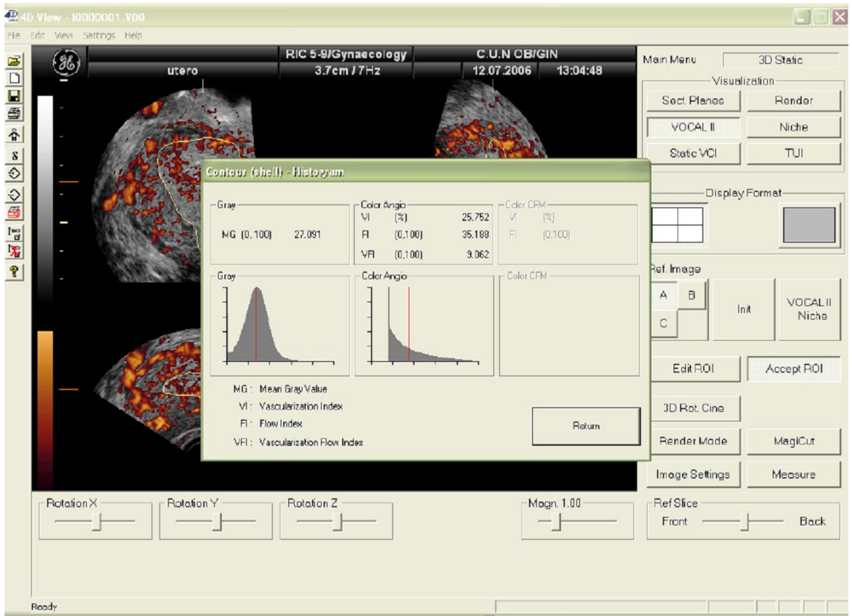

Figure (3): 3D-Power Doppler indexes for assessing endometrial vascularization by means of the three-dimensional ultrasound *(Alcazar, 2006).*

2. Patients and methods:

This study is prospective observational, single center study that was conducted at Ain Shams University Maternity Hospital in the period from September 2012 to Jun 2013. 120 infertile women who were referred to Assisted Reproduction center to perform ICSI cycle all of them known to had not uterine pathology and all patient subjected to standard long protocol of ovarian hyperstimulation after baseline hormonal profile assessment.

*Controlled Ovarian Stimulation Protocol:*was performed according to a long GnRH agonist protocol starting in the midluteal phase. Seven days after ovulation, daily subcutaneous injections with triptoreline acetate (Decapeptyl 0.05 mg/day; Ferring pharmaceuticals, Kiel, Germany) was started ***(McLachlan et al., 1986)*** On day 3 of the next cycle, ovarian stimulation was started with daily IM injections of a dose of 150 – 225 I.U. HMG (Menogon 75 IU /ampoule; Ferring pharmaceuticals, Kiel, Germany). The starting dose of the gonadotropins was prescribed according to the age, body built of the subjects. Then the duration and daily doses were adjusted according to serum E2 levels and follicular number and size in an ultrasound scan of the ovary .Ovarian stimulation was continued until the largest follicles reach a diameter of ≥18mm. The maximum duration of HMG administration was not allowed to exceed 16 days. If these criteria was met, Menogon and Decapeptyl was discontinued and 10.000 IU of HCG (Pregnyl. 10.000 IU/ampoule: Organon, Oss, Netherlands) was administered.

All women had their ultrasound scans at the same day of HCG administration with two dimensional (2D) and three dimensional (3D) ultrasound using the Voluson E6BT12 with Transvaginal RIC5-9 endocavity probe transducer. First, the uterus will be visualized in the B mode. All data of the uterus and uterine arteries Doppler were obtained. The endometrial thickness was obtained was defined as greatest distance between both myoendometrial interfaces, uterine arties Doppler parameters, Pulsatility index (PI), Resistance index (RI).

Through the VOCAL program (virtual Organ Computer Aided Analysis) the endometrial area was manually achieved from the coronal or "C" plane.Applying the rotational technique with 9 steps.12 endometrial ‘‘slices’’ were obtained outlining the endometrium at the myoendometrial junction from the fundus to internal cervical os. The VOCAL program calculates automatically the EV and three angiographic power Doppler indexes: vascularization index (VI), Flow index (FI), Vascularization Flow index (VFI) which represent the number of vessels, blood flow, and endometrial perfusion, respectively.

Vascularization index (VI) measuring the ratio of the number of colour voxels to the number of all the voxels is thought to represent the presence of blood vessels (vascularity) in the endometrium and is expressed as a percentage (%) of the endometrial volume. Flow index (FI), the mean power Doppler signal intensity inside the endometrium, Vascularization Flow index (VFI) combination of vascularity and flow intensity***(Pairleitner et al., 1999).***

The study group was divided into two subgroups as regard of pregnancy outcome which was confirmed by positive pregnancy test done after 14-18 days of embryo transferred.

3.Results:

120 women were included in the study grouped as regard pregnancy test done 14-18 day after embryo transfer into 52 pregnant women (43.3%) and 68 non-pregnant women (56.7%).

In pregnant group age was 25.8 ± 2.3 while BMI was 28.2 ± 2.6 which was statistically lower than non-pregnant group (29.8 ± 3.7 and 32.7 ± 3.6 respectively). On the other hand mean of basal FSH in pregnant group was 5.9 ± 1.4 and 7.0 ± 1.8 in non-pregnant group with statistically high significant decrease (table 1).

Table 1: demographic and basal hormonal characteristics.[a]

	Pregnant group n= 43.3%	Non-pregnant group n= 56.7%	p-value
Age (year)	25.8 ± 2.3	29.8 ± 3.7	> 0.001
BMI	2.8.2 ± 2.6	32.7 ± 3.6	>0.001
Duration of infertility	6.9 ± 2.8	5.8 ± 3.1	0.068
FSH	5.9 ± 1.4	7.07 ± 1.8	0.003
LH	6.9 ± 3.3	8.05 ± 4.4	0.153
E_2	19.6 ± 12.9	42.05 ± 32.5	> 0.001
prolactin	13.7 ± 4.5	15.5 ± 4.2	0.028

a Values are expressed as mean ± standard of deviation, n (%)

Pregnant group showed much increase in both endometrial thickness and endometrial volume (13.03 ± 1.63 and 6.75 ± 1.95 respectively), comparing to non-pregnant group (11.18 ± 1.633 and 5.39 ± 1.11 respectively) with high statistical significant difference. (table 2)

With Doppler and Power Doppler study uterine artery pulsatility index (PI) showed high statistical significant decrease in pregnant women comparing to non-pregnant women (1.6 ± 0.3 and 2.3 ± 0.5 respectively), on the other hand uterine artery resistance index (RI) was higher in non-pregnant women than those pregnant but without statistical significant difference (0.83 ± 0.05and 0.85 ± 0.06 respectively). (table 2)

As regard Doppler assessment of endometrial blood flow , flow index (FI) , vascularization index (VI) and vascularization flow index (VFI) were higher in pregnant group (25.4 ± 2.1, 0,6 ± 0.3 and 0.3 ± 0.2 respectively) than in non-pregnant group (24.1 ± 2,4 , 0.5 ± 0.3 and 0.1 ± 0.1 respectively) with high statistical significant difference . (table 2)

Table 2: ultrasound and Doppler study parameters

	Pregnant group n= 43.3%	Non-pregnant group n= 56.7%	p-value
• Endometrial thickness(ET)	13.03 ± 1.6	11.1± 1.6	> 0.001
• Endometrial volume (EV)	6.7 ± 1.9	5.3 ± 1.1	>0.001
• Uterine artery pulsatility index (PI)	1.6 ± 0.33	2.3 ± 0.5	>0.001
• Uterine artery resistance index (RI)	0.83 ± 0.05	0.85 ± 0.06	0.083
• Endometrial flow index (FI)	25.4 ± 2.1	24.1 ± 2.4	0.003
• Endometrial vascularization index (VI)	0.68 ± 0.39	0.51± 0.31	0.008
• Endometrial vascularization flow index (VFI)	0.33± 0.2	0.15 ± 0.1	0.028

a Values are expressed as mean ± standard of deviation, n (%)

4. Discussion:

This study is prospective observational, single center study that was conducted at Ain Shams University Maternity Hospital in the period from September 2012 to Jun 2013.120 infertile women participate in the study 43.3 % out of them become pregnant (n=52) while 56.7 % had now pregnancy (n=68).

No doubt, age and BMI have great impact on success rate of ICSI cycle that has been shown in the final result as the age and BMI were higher in non-pregnant group(29.8 ± 3.7 and 32.7 ± 3.6 respectively)that was powered by *(*Andersen et al., 2008)who evaluated the clinical efficacy of body mass index (BMI) and age as predictor of (IVF-ET) and he founded the pregnancy rate tended to be lower when age and BMI increase.

On the other hand, the study shows that women with successful ICSI outcome have higher both endometrial thickness and endometrial volume (13.03 ± 1.63 and 6.75 ± 1.95 respectively), comparing to women have not get pregnant after ICSI cycle (11.18 ± 1.633 and 5.39 ± 1.11 respectively) that point was agreed by previous study ***(Achache and Revel, 2006)*** who evaluated the relationship between endometrial thickness on day of human chorionic gonadotropin (HCG) administration and in vitro fertilization intacytoplasmic sperm injection (IVF-ICSI). In prospective cross-sectional study included a total of 593 women. This endometrium thickness on the day of HCG administration was measured by Transvaginal ultrasonography the chance of pregnancy appears to be lower (TV-USG) in individual with endometrial thickness loss than 7 mm compared with those of higher value. Also that mentioned by previous study held in 2010 ***(Schild et al., 2010)*** which evaluated the combined effects of endometrial thickness and pattern on clinical outcome in patients undergoing invitro-fertilization (ICSI/IVF). The endometrial thickness and pattern on the day of HCG administration was better predictors of outcome of IVF/ICSI-ET and may be more helpful for patients counseling than the separate analysis. That put same light on the role of endometrial thickness assessment on the success of ICSI cycle with no much data available on the role of endometrial volume.

The study also evaluated uterine artery blood flow on the day of HCG administration on the stimulated cycles of all patients using color Doppler in two dimensional mode (2D) in form of pulsatility index (PI) and resistance index (RI).pulsatility index (PI) showed high statistical significant decrease in pregnant women comparing to non-pregnant women (1.6 ± 0.3 and 2.3 ± 0.5 respectively), on the other hand uterine artery resistance index (RI) was higher in non-pregnant women than those pregnant but without statistical significant difference (0.83 ± 0.05 and 0.85 ± 0.06 respectively),and these agree with *(23)*.In which the evaluated 106 patients aged 24-42 years were included in the study. The patients were divided into two groups according to successful outcome defined as positive pregnancy test. These were statistically significant lower mean uterine artery PI in the pregnant group with no difference in RI. On the opposite hand one study disagree with these data ***(Ng et al., 2006)*** who have pointed out that uterine artery (PI) and (RI) are similar in non-conceptional and conception.He was included women in spontaneous cycles and stimulated cycles and he measured uterine artery (PI) and (RI) on the day of oocyte retrieved in stimulated cycle, and LH surge day in spontaneous cycles.

By using of 3D ultrasound and power Doppler angiograph to assess flow index (FI), vascularization index (VI) and vascularization flow index (VFI) at level of endometrium in studied women we found that all parameter were much higher in pregnant group (25.4 ± 2.1 , 0,6 ± 0.3 and 0.3 ± 0.2 respectively) in comparison to non-pregnant group(24.1 ± 2,4 , 0.5 ± 0.3 and 0.1 ± 0.1 respectively), that was agreed with another study ***(Merce et al., 2007)*** in which they conducted on Eighty women who underwent IVF cycles using Endometrial 3D ultrasound evaluated by VOCAL software To evaluate whether endometrial parameters by three-dimensional ultrasonography and power Doppler angiography (3D US-PDA) can predict in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) outcome, the results showed that, in the pregnant group, FI and VFI were statistically significantly higher. Moreover, The results of the present study partially agreed with the results obtained by Wu and coworker, ***(Wu et al., 2003)*** who measured endometrial and subendometrial blood flow on the day of hCG in 54 patients. Endometrial VFI on the day of HCG was significantly higher in the pregnant group while endometrial VI and FI were similar between pregnant and non-pregnant cycles.

We consider the interpretation and clinical application of our results interesting, and hope that future studies confirm our findings. In this context, we believe that the new 3D markers of endometrial receptivity could be useful for selecting which cycle is more suitable for the transfer of a single embryo.

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2/3/2014