The Role of Detection of Endometrial and Subendometrial Vasculature on the Day of Embryo Transfer in Prediction of Pregnancy During ICSI Cycles

Prof. Dr. Asem Anwar Mousa[1], Prof. Dr. Abd El-Satar Mohamed Farhan[1], Dr. Mohamed Ibrahim Mostafa[2], Mostafa Mahmoud Monib El-Shazly[2]

[1]Department of Obstetrics and Gynaecology, Faculty of Medicine, Al Azhar University, Egypt
[2]Department of Obstetrics and Gynaecology, El Galaa Teaching Hospital, Egypt.
shazly-junior@hotmail.com

Abstract: Objectives: Successful implantation depends on interaction between a blast cyst and a receptive endometrium. Endometrial vasculature is important in the early endometrial response to blast cyst 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 intracytoplasmic sperm injection and embryo transfer (ICSIe ET) using a gonadotropin releasing hormone (GnRH) agonist long protocol. **Materials and methods:** This prospective observational study was carried out on 100 infertile women recruited from those attending the outpatient clinics of assisted reproduction unit in El Galaa Teaching hospital during the period between June 2017 and December 2018.3D PD-US examinations were performed on the day of embryo transfer. Main outcomes were endometrial volume (EV), vascularization index (VI), flow index (FI), and vascularization flow index (VFI) of the endometrium and subendometrial region. Measurements were analyzed relative to ICSIeET outcome (pregnant vs. nonpregnant). **Results:** No significant differences were observed in patient age, infertility duration, body mass index (BMI), basal FSH levels, number of transferred embryos, or endometrial thickness between the two groups. The pregnant group had higher endometrial V, VI, FI, and VFI scores than the nonpregnant group. By contrast, subendometrial region V, VI, FI, and VFI scores were same for both groups. **Conclusion:** Three-dimensional PD-US was a useful and effective method for assessing endometrial blood flow in ICSI 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 ICSI cycles.

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1. Introduction:

The pioneering of intracytoplasmic sperm injection (ICSI) has been heralded as one of the major breakthroughs in the field of reproductive medicine. In the years since the first live births with ICSI were reported in 1992 (Palermo et al., 1992), this technique has been deployed as a powerful tool to treat almost all forms of male infertility, and also to overcome fertilization failure (Pereira et al., 2017).

One of the main factors responsible for a successful in vitro-fertilization (IVF) outcome is uterine receptivity. In IVF or intra-cytoplasmic sperm injection (ICSI) cycles, up to two-thirds of implantation failures are estimated to be caused due to defects in endometrial receptivity (kalmantis et al., 2012).

Past studies related to endometrial receptivity were mainly focused on histopathological investigation of the endometrium, presented as endometrial dating by Noyes dating (Noyes et al., 1975), or investigation of the receptors of receptors for estrogen or progesterone. However, these methods were invasive, a waste of time and not accepted by patients (Wang et al., 2010).

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 (Merce et al., 2008).

Ultrasound parameters of the endometrium and the evaluation of uterine and endometrial blood flow have been considered as implantation markers in (IVF-ET) cycles (Merce et al., 2008).

The advent of transvaginal ultrasound with 2D and 3D power Doppler has provided a perfect non-invasive tool to assess endometrial receptivity. Measurement of endometrial and sub-endometrial blood flow using 3 D power Doppler in IVF cycles and their role in predicting IVF cycle outcome has attracted a lot of attention across the world in recent years (Mishra et al., 2016).

An advantage of power Doppler ultrasound is extreme sensitivity to low or slow vascular flow, which can identify overlapping vessels. This technique displays total flow in a confined area, providing images similar to angiography (Kim et al., 2014).

2. Subjects and Methods:

A total of 100 infertile women, aged 20-35 years old, who underwent a first ICSI-ET with a GnRH long protocol with stimulation by highly purified human menopausal gonadotrophin (Merional) from June 2017 to December 2018 were enrolled in assisted reproduction unit in El Galaa Teaching hospital.

Inclusion criteria were infertile couples due to male, tubal, ovarian factors or unexplained infertility, all women underwent a first ICSI-ET with GnRH long protocol for controlled ovarian stimulation and ll women had at least one good quality embryo on the 2nd and 3rd day after oocyte retrieval. The patients were excluded if they had endometrial polyps or uterine fibroids, distortion of uterine cavity, uterine anomalies or previous operations on the uterus.

Complete personal and medical history was taken to certain inclusion criteria and exclude unsuitable patients and each patient received explanation regarding the procedure, its safety, success rate and verbal consent was taken.

General and local examinations were performed, hormonal Profile: Serum FSH, LH, Estradiol and prolactin concentrations were determined on day 3-5 of the cycle and a transvaginal ultrasound (TVS) to detect the antral follicle count (AFC), and any uterine abnormalities.

The long protocol was commenced by pituitary suppression with Decapeptyl 0.1mg once daily subcutaneous starting on day 21 of the previous menstrual cycle. Pituitary suppression and down regulation was confirmed by using (TVS) through measuring endometrial thickness which should be less than 5mm. Also, down regulation was confirmed by hormonal profile measured on the first day of the treating cycle by measuring E2, LH which should be below 50pg/ml and 2mIU/ml respectively. Once patients were down regulated, Merional (highly purified human menopausal gonadotrophin) was started intramuscular. The dose was determined by the patient's age, BMI, basal serum FSH level and ovarian size.

Ultrasound monitoring of follicular response from day 7 of gonadotropins stimulation was then performed. HCG (Choriomon) 10000IU was then administered intramuscularly when at least three leading follicles were ≥ 18 mm in diameter.

The patient reported to the assisted conception unit on the next morning of the procedure 34-36 hours after HCG administration. Oocyte retrieval by (TVS) guided aspiration of follicles under general anaesthesia was done using Cock needle for suction. The oocytes were placed in culture medium and intracytoplasmic sperm injection was performed using inverted phase microscope and micro- manipulating equipment. The injected oocytes were incubated. Fertilization was diagnosed by the presence of two pronuclei in the injected oocyte.

Embryo transfer was done 3 days after oocyte retrieval when fertilized oocyte reached at least 4 or 8 cell stage. Embryo transfer was done with no anaesthesia in lithotomy position. A maximum of three good quality embryos was transferred (grade 1 and grade 2).

Ultrasound Investigations were done on the same day of embryo transfer by using a GE Voluson 730 PRO ultrasound system equipped with a transvaginal multi-frequency (5-9 MHz) transducer.

The power Doppler sample was placed over this uterine longitudinal section, including the whole endometrial and subendometrial regions. The same power Doppler characteristics were applied in all examinations: normal quality of color, color gain 3.4, pulse repetition frequency of 600 Hz, and wall motion filter of 50 Hz. Using a scanning angle of 90˚, the volume box was placed over the previous power Doppler image.

3D volume data were obtained from the areas of interest which were the endometrium and sub-endometrial regions within 2 mm of the echogenic endometrial borders by automatic sweep with angle set to 90 to ensure inclusion of a complete uterine volume encompassing the entire sub-endometrium. The manual mode of the VOCAL (Virtual Organ Computer- Aided Analysis) Contour Editor was used to cover the entire 3D volume of the endometrium with a 15rotation step. A total of 12 endometrial slices were obtained outlining the endometrium at the myoendometrial junction from fundus to internal Os (Fig. 1)

Built-in VOCAL software for 3D power Doppler histograms was used to measure blood flow indices (Fig. 2). Vascularization index (VI), Flow index (FI) and Vascularization flow index (VFI).

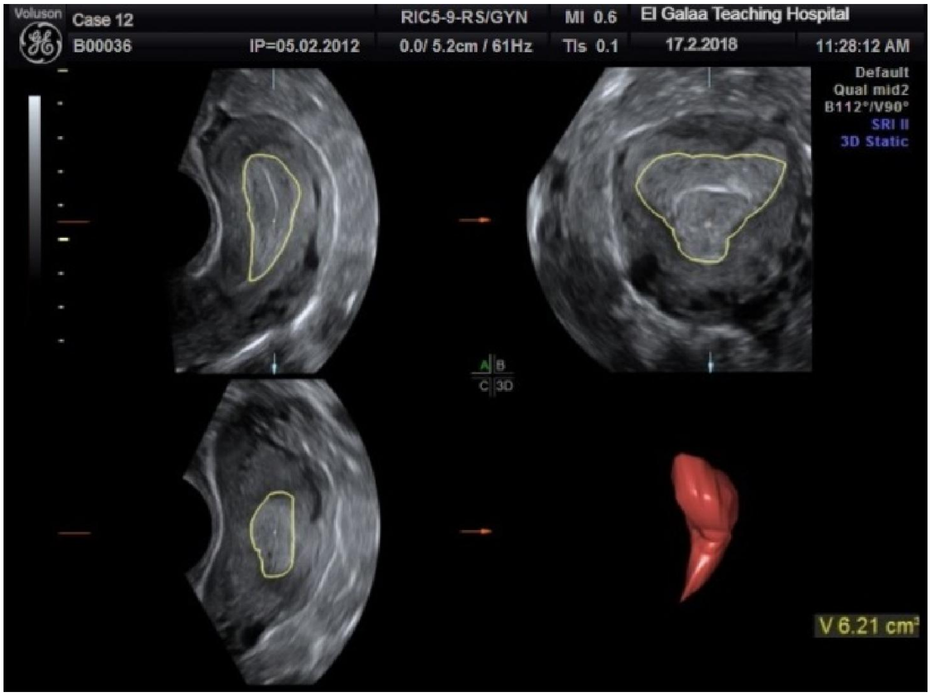

Figure 1: Calculation of endometrial volume using (VOCAL) imaging program

Quantitative β-hCG was done 14 days after the embryo transfers for diagnosis of pregnancy. Women proved to be pregnant by quantitative β-hCG, scheduled for TVS 20 days after the quantitative β -hCG for detection of gestational sac or sacs.

Studied women were classified according to the outcome of the current ICSI trial into; successful ICSI group (pregnant women), and unsuccessful ICSI group (non-pregnant women). Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation, student t- test, Chi-square by SPSS V20.

Figure 2: Automatic calculation of vascular indices of power Doppler using the histogram facility of (VOCAL) program

3. Results:

The mean age of the studied women was 27.740±4.059years, the mean BMI of the studied women was 30.940±4.447 kg/m2, and the mean duration of the infertility was 5.340±2.483 years. The ICSI trials were indicated in 54% (54/100) of the studied women because of primary infertility, and in 46% (46/100) of the studied women because of secondary infertility.

The etiologies of infertility among the study population were; male factor in 28% (28/100), unexplained infertility in 22% (22/100), tubal factor in 26% (26/100) and ovarian factor in 24% (24/100).

The outcome of the current ICSI trials was successful in 33% (33/100) of the studied women and unsuccessful in 67% (67/100) of the studied women.

The studied women classified according to the outcome of the current ICSI trials into; successful ICSI group (pregnant women), and unsuccessful ICSI group (non-pregnant women).

No significant differences were observed in patient age, infertility duration, body mass index (BMI), basal hormonal profile levels, number of transferred embryos, or endometrial thickness between the two groups (Table. 1).

Endometial volume (V), Flow Index (FI), Vascularization index (VI) and Vascularization flow index (VFI) were significantly higher in cases within pregnant group than those within non-pregnant group while Subendometrial (V), (VI), (FI) and (VFI) were similar for the two groups with no significant difference (Table. 1)

Table (1):

		Pregnancy Test						T-Test	
		Pregnant			Non-Pregnant			t	P-value
Age (Years)	**Range**	22	-	35	21	-	35	0.030	0.976
	Mean ±SD	27.758	±	3.750	27.731	±	4.230		
Duration (Years)	**Range**	2	-	8	1	-	11	-0.531	0.597
	Mean ±SD	5.152	±	1.839	5.433	±	2.754		
BMI	**Range**	25	-	42	25	-	40	0.811	0.420
	Mean ±SD	31.455	±	5.130	30.687	±	4.087		
Basal E2	**Range**	18	-	67	10.6	-	53	1.749	0.083
	Mean ±SD	40.806	±	11.266	36.528	±	11.612		
Basal FSH	**Range**	4.5	-	10.5	4.5	-	11.4	0.418	0.677
	Mean ±SD	7.476	±	1.701	7.312	±	1.908		
Basal LH	**Range**	2.4	-	8.4	2.1	-	7.4	1.067	0.289
	Mean ±SD	5.018	±	1.716	4.684	±	1.343		
E Thickness	**Range**	7.4	-	11.6	5.4	-	11.1	1.653	0.101
	Mean ±SD	9.655	±	1.270	9.232	±	1.167		
Endometrial V	**Range**	3.4	-	8.6	2.4	-	6.8	6.323	<0.001*
	Mean ±SD	6.118	±	1.328	4.581	±	1.042		
Endometrial VI	**Range**	2.126	-	3.984	1.325	-	3.525	3.190	0.002*
	Mean ±SD	3.145	±	0.503	2.809	±	0.491		
Endometrial FI	**Range**	23.432	-	27.523	22.44	-	27.523	6.759	<0.001*
	Mean ±SD	26.058	±	1.272	24.130	±	1.374		
Endometrial VFI	**Range**	1.002	-	1.561	0.732	-	1.452	14.284	<0.001*
	Mean ±SD	1.309	±	0.136	0.902	±	0.133		
Sub endometrial V	**Range**	1.77	-	2.99	1.88	-	2.69	0.745	0.458
	Mean ±SD	2.424	±	0.387	2.376	±	0.246		
Sub endometrial VI	**Range**	1.325	-	3.715	1.325	-	4.316	0.564	0.574
	Mean ±SD	2.958	±	0.600	2.883	±	0.636		
Sub endometrial FI	**Range**	22.321	-	27.523	22.231	-	27.523	0.374	0.709
	Mean ±SD	24.539	±	2.056	24.397	±	1.652		
Sub endometrial VFI	**Range**	0.876	-	1.986	0.853	-	1.986	0.734	0.464
	Mean ±SD	1.138	±	0.298	1.097	±	0.242		

Three-dimensional ultrasound and power Doppler angiography parameters of the endometrium were lower in the nonpregnant patients compared to the pregnant patients. Endometrial V, VI, FI, and VFI were all significantly higher in the pregnant group, whereas subendometrial V, VI, FI, and VFI were similar for the two groups. The receiver operating characteristic curve (ROC) was analyzed for endometrial and sub-endometrial three-dimensional

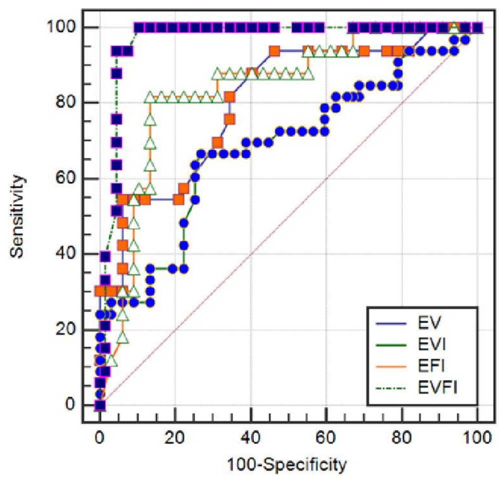

Figure (3): ROC curve for EV, VI, FI and VFI in prediction of endometrial receptivity in ICSI patients

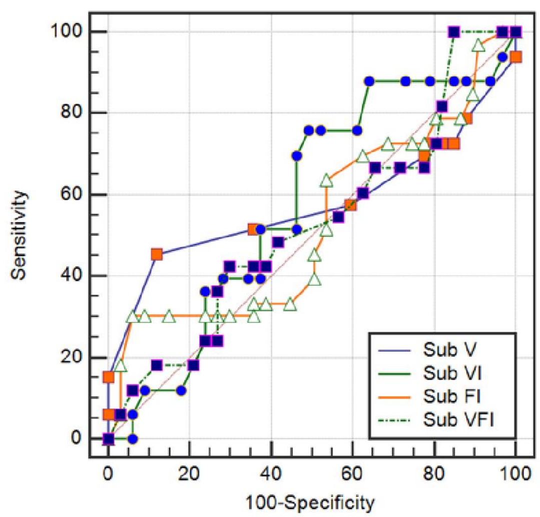

Figure (4): ROC curve for Subendometial V, VI, FI and VFI in prediction of endometrial receptivity in ICSI patients.

4. Discussion:

One hundred (100) women undergoing ICSI included in this study to evaluate the endometrial and sub-endometrial parameters as predictors of ICSI success. Studied women were assessed using the 3D ultrasound for detection of the endometrial and sub-endometrial volume, and the 3D power Doppler angiography to evaluate the endometrial and sub-endometrial vascularity on day of the embryo transfer. ultrasound and power Doppler angiography parameters to assess their predictive values for pregnancy. The area under the curve was significantly different for endometrial V, VI, FI, and VFI (Fig. 3), but not for subendometrial V, VI, FI, and VFI (Fig. 4).

The endometrial volume must be at least 2.0-2.5 ml for achieving a pregnancy (Zollner et al., 2003). In our study, endometrial volume of all the patients was more than 2cm 3.

Although Schild et al., found that the endometrial volume failed to predict the outcome of the IVF cycles (Schild et al., 2000) and Yaman et al., concluded that the endometrial volume did not differ significantly in women that became pregnant from those who did not (Yaman et al., 2003), The EV in this study done on day of embryo transfers was significantly higher in pregnant compared to non-pregnant ICSI group.

Alcázar et al., reported that the endometrial volume was ineffective for predicting pregnancy in IVF program in the majority of the published studies (Alcazar et al., 2005). In addition; J¨arvel et al., found no difference in the endometrial volume between conception cycles, and non-conception cycles, 36 hours after oocytes retrieval (J¨arvel et al., 2002).

Moreover, Abuelghar et al., found that EV measured before embryo transfers was higher in successful ICSI compared to unsuccessful ICSI group but without any significant difference (Abuleghar et al., 2018) and Mishra et al., found endometrial thickness and endometrial volume were comparable between the two groups and but not predictive of pregnancy (Mishra et al., 2016). These results are similar to a study which showed that the endometrial volume measured by 3D ultrasound is not predictive of pregnancy (kim et al., 2014).

However, Kupesic et al., agreed with our study when they found that the pregnancy and the implantation rates were significantly lower in patients with endometrial volume <2 mL, and no pregnancy achieved in patients with endometrial volume <1 mL or >8 mL (Kupesic et al., 2001).

Ng et al., in his study showed that in IVF treatment, endometrial volume measured by three-dimensional 3D ultrasound was significantly comparable for pregnant and non-pregnant women (Ng et al., 2006). In a study by Merce et al., it was seen that endometrial volume measured on the day of hCG was significantly higher in the pregnant group as compared to the non-pregnant group (Merce et al., 2008).

Raine-Fenning et al., found that the endometrial, and the sub-endometrial blood flows increased during the proliferative phase, reaching the maximum peak three days before ovulation and decreased again five days' post-ovulation (Raine-Fenning et al., 2004).

Raine-Fenning et al. in another study concluded that the endometrial, and the sub-endometrial blood flows reduced in women with unexplained infertility irrespective the serum levels of estrogen and/or progesterone, and endometrial morphology (Raine-Fenning et al., 2004).

In the current study endometrial VI, FI, and VFI were all significantly higher in the pregnant group, whereas subendometrial VI, FI, and VFI were similar for the two groups. The receiver operating characteristic curve (ROC) for endometrial and subendometrial power Doppler angiography parameters used to assess their predictive values for pregnancy revealed that the area under the curve was significantly different for endometrial VI, FI, and VFI, but not for subendometrial VI, FI, and VFI.

Kim et al., completely agreed with our study as they found that endometrial blood flow measured on day of embryos transfer was lower in the nonpregnant patients compared to the pregnant patients. Endometrial vascular indices by power Doppler were predictive for ICSI success, whereas subendometrial indices were not (Kim et al., 2014).

Merce et al., found that the endometrial vascular flow indices (VI, FI, and VFI) were significantly high on the day of HCG in the pregnant compared to the non-pregnant group, and concluded that the endometrial blood flow reflects the uterine receptivity accurately (Merce et al., 2008).

In a further study, sub-endometrial VI, FI, and VFI measurements were performed before embryo transfers and found to be higher in successful ICSI compared to unsuccessful ICSI group without any significant difference (Abuleghar et al., 2018).

Jarvela et al., found no difference in the endometrial/subendometrial VI, FI, and VFI, 36 hours after oocyte retrieval between conception, and non-conception cycles (J¨arvel et al., 2002), and Ng et al., found no differences in the sub-endometrial VI, FI, and VFI on day of oocyte retrieval between conception, and non-conception cycles (Ng et al., 2006).

Dorn et al. found no difference in the sub-endometrial VI, FI, and VFI on the day of oocyte retrieval between pregnant, and non-pregnant women (Dorn et al., 2004).

Mishra et al., agreed with our study as they found endometrial VI, FI and VFI were significantly higher in the pregnant as compared to the non-pregnant group; however, Among the sub-endometrial indices, VI and VFI were found to be significantly higher in the pregnant group, while there was no difference in FI between the two groups (Mishra et al., 2016), and similarly Wu HM et al. found that sub-endometrial VFI may be useful in predicting implantation and pregnancy rates in IVF (Wu et al., 2003).

A study of 89 patients by Kupesic et al., where they found that sub-endometrial FI on the day of embryo transfer was significantly higher in pregnant as compared to non-pregnant patients, whereas sub-endometrial VI and VFI were similar between the two groups (Kupesic et al., 2001).

On the other side, Ernest in his study of 293 patients undergoing the first IVF cycle showed that endometrial and sub-endometrial blood flow on the days of HCG and embryo transfer were not predictive of pregnancy (Ng et al., 2009).

Also, Zackova et al., concluded that assessment of endometrial characteristics using 3D power Doppler is not helpful in predicting the response in Frozen embryo transfer (FET) cycles (Zackova et al., 2009). Similarly, Check et al., did not find any relationship between endometrial and sub-endometrial blood flow and pregnancy rates in FET cycles (Check et al., 2003).

In a study of FET and IVF–ET groups by Tekay et al., it was seen that the Doppler velocimetry measurements between conception and non-conception cycles were not significantly different. They concluded that impaired uterine blood flow negatively affects implantation, while an adequate uterine blood may not necessarily result in pregnancy (Tekay et al., 1995).

However, Ng et al., found that endometrial and sub-endometrial blood flow was significantly higher in pregnant females with live birth than those with a miscarriage (Ng et al., 2007).

Chein et al., suggested that the development of the endometrial vascular network is important for the support of the first stages of pregnancy when he discovered that when pregnancy is achieved but endometrial and sub-endometrial flow on the day of ET cannot be seen, more than half of these pregnancies will end in spontaneous miscarriage (Chein et al., 2002).

Although the majority of investigators assessed the subendometrial vascularization as predictors of IVF/ICSI success (Ng et al., 2006), Merce et al., decided to calculate and investigate the endometrial 3D power Doppler indices because the endometrium is the actual place of implantation (Merce et al., 2008).

In conclusion, these findings suggest that endometrial evaluation with 3D Power Doppler US can be a useful prognostic protocol for pregnancy in infertile women undergoing a fresh GnRH agonist long protocol. However, further randomized large-scale studies are required on IVF success measuring multiple vascular parameters in several endometrial and sub-endometrial sections on different days, using various COS protocols.

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