Role of measuring the endometrial thickness and assessment of sub-endometrial vascularity by power Doppler ultrasound in prediction of pregnancy rate in patients undergoing IVF/ICSI cycle

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Abstract: Background: A favorable endometrial environment is necessary for successful implantation. use of ultrasonic technology has increased as a way to measure possible predictors of endometrial receptivity, such as endometrial thickness endometrial blood flow and blood flow in the (sub) endometrial arteries*.* Aim of the Work: Is to study the correlation between (endometrial thickness, and assessment of sub-endometrial vascularity, by power Doppler ultrasonography), and uterine receptivity in infertile women treated with IVF/ICSI. And the pregnancy outcome. Patietns and Methods: The study included 50 infertile patients suffering from various durations of both primary and secondary infertility, and undergoing IVF/ICSI, endometrial thickness was measured and sub-endometrial blood flow was assessed by power Doppler ultrasound on the day of hCG triggering. Results: There were no significant differences between no pregnancy group and pregnancy group regarding their age, infertility duration, type of infertility, level of E2 on day of hcg, hCG injection day, number of follicles and their diameter also there were also no difference regarding FSH, LH, TSH. There was a statistical significant difference between the two groups regarding: prolactin, the mean RI, the mean PI, The mean S/D ratio of the subendometrial blood flow, the mean endometrial thickness number of oocytes and number of available and transferred embryos.Conclusion: Transvaginal color Doppler examination of the endometrial–subendometrial blood flow distribution provides a simple and effective method to evaluate endometrial receptivity. The presence of both endometrial and subendometrial blood flow is indicative of good endometrial receptivity, whereas the absence of both represents a poor uterine environment. This approach may be helpful in deciding the number and timing of ICSI in IVF treatments.

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Key words: Power Doppler ultrasound, endometrial thickness, subendometrial vascularity, pregnancy rate, IVF/ICSI

1. Introduction

Despite recent advances in IVF technologies and ovarian stimulation regimens, the pregnancy rates have not increased accordingly, Multiple factors responsible for a successful IVF outcome have been described, not the least of which is uterine receptivity (Coulam et al, 1994)**.**

It appears that a favorable endometrial milieu is necessary for successful implantation in the menstrual cycle, the endometrium has no adhesive qualities until the implantation window phase, during which for a very short time, the endometrium allows the implantation of gestational sacs. This feature is referred to as endometrial receptivity (Dominguez et al, 2003)**.**

With the advance of diagnostic ultrasonography, clinical use of ultrasonic technology has increased as a way to measure possible predictors of endometrial receptivity, among them are uterine predictors ofimplantation, such as endometrial thickness, in the assessment of the developmental potential of the basal layer of the endometrium. With the increased resolving power and sensitivity of ultrasonography, more studies were conducted on the use of endometrial blood flow and blood flow in the (sub) endometrial arteries in predicting endometrial receptivity (Schild et al, 2000)**.** To date, the advantages of ultrasonography include its non-invasiveness, repeatability, real-time monitoring and predictability (Wang et al, 2010)**.**

Endometrial thickness measurement is used as a clinical tool to predict implantation following ovarian stimulation for IVF. Endometrial thickness is defined as the minimal distance between the echogenic interfaces of the myometrium and endometrium measured in the plane through the central longitudinal axis of the uterine body. There is possible interaction between overall blood supply in the sub endometrial area and pregnancy rate (Yu Ng et al., 2006)**.**

Using a transvaginal transducer with power Doppler facility, when a longitudinal view of the uterus is obtained, sub-endometrial blood flow can be studied measuring the following parameters:

1. Resistance index (RI) unitless and angle independent: the difference between maximal systolic blood flow and minimal diastolic flow divided by the peak systolic flow (S-D/S).
2. ii. Pulsatility index (PI) unitless and angle independent: the difference between maximal systolic blood flow and minimal diastolic flow divided by the mean flow throughout the cycle (S – D/ mean).
3. The ratio between peak systolic flow and lowest diastolic flow (S/D).

These three parameters express the resistance to flow from the point of measurement downstream. The value increases when resistance increases, and vice versa. The diastolic flow is considered to be influenced by resistance to a greater extent than the systolic flow. All parameters are also examined by using the power Doppler system.

**Aim of the work**

Is to study the correlation between (endometrial thickness, and assessment of sub-endometrial vascularity, by power Doppler ultrasonography), and uterine receptivity in infertile women treated with IVF/ICSI. And the pregnancy outcome.

**2. Materials and methods**

Patients’ characteristics

From June 2015 to june 2016, fifty infertile patients candidate for IVF/ICSI treatment were recruited.

Inclusion criteria:

* Age 25 -35 years.
* Infertile patients' candidate for IVF treatment.
* Normal size and shape of the uterus, as evidence by trans-vaginal ultrasound and hysterograghy.

Exclusion criteria:

* Age >35 years and <25 years.
* Uterine factor of infertility, uterine myomas.
* Patients with history of surgical procedures involving the uterus.
* Excluding any Male factor of infertility.

All patients were included in the study only once to avoid selection bias. The study was approved by the local Ethical Committee of Department of Obstetrics & Gynecology, Alazhar University hospital. The procedure and its safety were explained shortly, and each woman gave verbal informed consent.

Ovarian hyperstimulation and IVF procedures:

In the first stage, on the basis of Long protocol, down regulation was performed using gonadotrophin releasing hormone agonist (GnRH-a): 0.1mg/day starting 7-8 days after estimated ovulation in the previous cycle, and on doses of 0.05 mg GnRHa/day, and then ovarian hyperstimulation was initiated with administration of human menopausal gonadotrophin (HMG): 150-225IU/day, on the 2nd day of the menstrual cycle, ultrasound evidence of down regulated endometrium (<5mm) was assessed on the 3rd or 4th day of the cycle. Serum E2 <50.

* History taking and pelvic examination were done.
* When there were at least two leading follicles, patients were examined after spontaneous emptying of the urinary bladder, lying supine with the knees slightly bent (lithotomy position) and with a small pillow under the buttocks, using a transvaginal transducer with power Doppler facility, when a longitudinal view of the uterus was obtained, the Power Doppler mode was activated.. Sub- endometrial blood flow was studied.
* The parameters assessed were:
  1. Resistance index (RI): (S-D/S).
  2. Pulsatility index (PI): (S – D/ mean);
  3. The ratio between peak systolic flow and lowest diastolic flow (S/D).
* Endometrial thickness was measured.

Outcome:

Patients were divided into two groups:

* **Group A:** successful outcome (pregnancy), documented by positive pregnancy test, documented by Serum β-hCG levels measured 14 days after embryo transplantation (ET), and ultrasound showing intrauterine gestational sac, six weeks following ET embryo transfer.
* Group B: failure of pregnancy, documented by a negative pregnancy test.

Statistical analysis

Data were entered checked and analyzed using Epi-Info version 6 and SPP for Windows version 8 (Dean, 2006).

Data were summarized using:

The arithmetic mean:

As an average describing the central tendency of observations:



Where:

Σ = Sum of

X = Individual data

n = Number of individual data

The Standard Deviation (SD):

Asa measure of dispersion of the results around the mean:



Student t test:

It was used when comparing two means.



1 = Mean of group I

2 = mean of group II

SD1 and SD2 = the corresponding standard deviation.

n1 and n2 = Number of observation in group I and group II respectively.

**NB:** Paired t test was used for comparison of paired observation.

X2 (chi-squared) (test of significance):

Σ =



It is used for difference between two or more qualitative variable.

DF = (r – 1) (c – 1)

*Where,*

Σ = The summation

O = Observed value

E = Expected value =

R = Row total

C = Column total

DF = Degree of Freedom

Level significance:

For all above mentioned statistical tests done, the threshold of significance is fixed at 5% level (p-value).

The results was considered:

* Significant when the probability of error is less than 5% (p < 0.05).
* Non-significant when the probability of error is more than 5% (p > 0.05).
* Highly significant when the probability of error is less than 0.1% (p < 0.001).

The smaller the p-value obtained, the more significant are the results.

3. Results

The study included 50 infertile patients suffering from various durations of both primary and secondary infertility, and undergoing IVF/ICSI, it evaluated the role of the endometrial thickness and subendometrial blood flow measured by 2D power Doppler ultrasound performed on the day of hCG triggering in the prediction of pregnancy during IVF treatment.

**Table (1):** Subendometrial blood flow

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Non-pregnant** | **Pregnant** | **t** | **p** |
| **RI** |  |  |  |  |
| Mean ± SD  Range | 0.93± 0.14  0.65-1.1 | 0.76 ± 0.2  0.45-1.02 | 4.2 | < 0.001  (HS) |
| **PI** |  |  |  |  |
| Mean ± SD  Range | 2.5 ± 1  1.02-3.67 | 1.53 ± 1.1  0.67-3.6 | 3.15 | 0.002  (S) |
| **S/D** | 2.296 | 2.591 | 0.64 | 0.186 (NS) |

Table (3) shows the mean subendometrial blood flow in pregnant and non-pregnant groups.

**Resistance index**

the mean RI in pregnant and non-pregnant groups, the Mean RI in no pregnancy group is higher than in pregnancy group, the difference, is statistically highly significant, P value for pregnancy group: <0.001

**Pulsatility index PI:**

the mean PI in no pregnancy and pregnant groups; the Mean PI in no pregnancy group is higher than in pregnancy group, the difference is statistically significant (P value for pregnancy group: 0.002).

**S/D Ratio:**

The mean S/D ratio in pregnant and no pregnant groups; the Mean S/D ratio is higher in no pregnancy group, the difference is statistically insignificant (P value for pregnancy group: 0.186).

**Table (2):** Endometrial thickness

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Non-pregnant** | **Pregnant** | **t** | **p** |
|  | | | | |
| Mean ± SD | 9.46 ± 1.4 | 11.2 ± 2.67 | 3.01 | 0.004  (S) |
| Range | 8-12 | 8-16 |  |



**Figure (1):** Subendometrial blood flow

Table (2) shows the mean endometrial thickness in pregnant and non-pregnant groups.

**Endometrial thickness**

The mean endometrial thickness in pregnant and non-pregnant groups, The mean endometrial thickness in no pregnancy group is lower than in pregnancy group, the difference is statistically significant (p = 0.004).



**Figure (2):** Endometrial thickness

**4. Discussion**

The condition of the uterus during IVF treatment at present mainly is assessed by ultrasonographic examination. Sonographic evaluation of endometrial thickness and texture has been used for endometrium assessment, but it is not possible to predict the likelihood of pregnancy based solely on this method. The introduction of transvaginal Doppler ultrasound makes the measurement of uterine artery blood flow possible, and at one time it was hoped that uterine arterial resistance changes might reflect uterine receptivity (Steer et al., 1992).

Although pregnancy outcome tended to be poor in patients with higher mean uterine arterial impedance indices, the predictive value of using a specific resistance index (RI) or pulsatility index (PI) variable in assessing endometrial receptivity seems to be limited. One of the explanations is that the major uterine compartment is the myometrium and not the endometrium, and thus most of the blood passing through the uterine arteries never reaches the endometrium. A more logical approach would be to evaluate the vascularization around the endometrium directly in an attempt to assess endometrial receptivity (Tekay et al., 1996).

Endometrial blood flow studies, either with conventional color Doppler sonography or the newer techniques of power and three-dimensional (3-D) power Doppler sonography, have become clinically feasible in recent years. Although initial studies showed promising results in predicting the pregnancy outcome of IVF/ET treatments (Kupesic et al., 2001), how ever **Schild et al** failed to demonstrate significant differences between pregnant and nonpregnant patients. **(Kupesic et al., 2001), ( Schild et al., 2001).**

Raine-Fenning et al., (2004b) demonstrated that Endometrial and subendometrial vascularity are significantly reduced (decrease in endometrial perfusion, and possible endometrial receptivity) in 29 women with unexplained subfertility during the mid-late follicular phase irrespective of estradiol or progesterone concentrations and endometrial morphometry.

Different approaches in measuring endometrial blood flow in the published studies may have contributed to the inconsistent conclusions. Blood flow to the endometrium comes from the radial artery, which divides after passing through the myometrial–endometrial junction to form the basal arteries that supply the basal portion of the endometrium, and the spiral arterioles that continue up toward the endometrial surface. At the myometrial–endometrial junction, a specific subendometrial area can be identified as a thin hypoechoic layer between the echogenic endometrium and myometrium on ultrasound examination (Tetlow et al., 1999).

It has been described as the subendometrial halo or the junctional zone of the myometrium. Histological studies have confirmed that the subendometrial halo surrounding the endometrium represents the innermost layer of the myometrium, and compared with the outer myometrium, it consists of a distinct compartment of more tightly packed muscle cells with increased vascularity (Tetlow et al., 1999). **Lesny et al.** (1999) have shown that interactions between the junctional zone and the endometrium may play an important role in the implantation process.

The condition of the uterus is critical to the process of embryo implantation, and among uterine conditions, endometrium 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 uterine receptivity (Rogers, 1996).

This study evaluated the the correlation between (endometrial thickness, and assessment of sub-endometrial vascularity, by power Doppler ultrasonography), and uterine receptivity in infertile women treated with IVF/ICSI and the pregnancy outcome.

The study included 50 infertile patients suffering from various durations of both primary and secondary infertility, and undergoing IVF/ICSI, endometrial thickness was measured and sub-endometrial blood flow was assessed by power Doppler ultrasound on the day of hCG triggering.

In our study, The mean age ranged from 22 to 35 years with mean ± SD of 30.1 ± 0.4 years in non-pregnant patients (n = 22) and 28.97 ± 4.9 in pregnant patients (n = 28). There is no statistical difference between the two groups (p = 0.55). **Chien et al.** (2002) investigated the correlation of blood flow in the endometrial–subendometrial region detected by color Doppler sonography with pregnancy outcome of an IVF-ET program. They found that the mean age of the women who became pregnant was lower than that of those who did not achieve pregnancy.

In our study, there were no significant differences between no pregnancy group and pregnancy group regarding follicle stimulating hormone, luteinizing hormone, E2 and thyroid stimulating hormone and there were a statistical significant difference regarding prolactin. where it is level was lower in pregnant group than the non pregnant ones.

The mean RI in no pregnancy group is higher than in pregnancy group; the difference is statistically highly significant. The mean PI in no pregnancy group is higher than in pregnancy group; the difference is statistically significant.

**Zaidi et al.** (1995) assessed the presence or absence of subendometrial or intraendometrial color flow, intraendometrial vascular penetration, and subendometrial blood flow velocimetry on the day of hCG administration and related the results to pregnancy rates. They found no significant difference in subendometrial peak systolic velocity or subendometrial PI between conception and nonconception cycles. The absence of both subendometrial and intraendometrial vascularization, however, was shown to be associated with failure of implantation.

**Ng et al.** (2006) recruited 451 patients in their first IVF cycle who received a standard long protocol of pituitary down-regulation, in their study to evaluate the role of the endometrial and sub-endometrial blood flows measured by 3D power Doppler ultrasound in the prediction of pregnancy during IVF treatment, on the day of oocyte retrieval, they determined endometrial thickness, endometrial pattern, pulsatility index (PI) and resistance index (RI) of uterine vessels, endometrial volume, vascularization index (VI), flow index (FI) and vascularization flow index (VFI) of endometrial and sub-endometrial regions, they found that the pregnant group had significantly lower uterine RI, endometrial VI and VFI than the non-pregnant group, however, Receiver operator characteristic curve analysis revealed that the area under the curve was ~0.5 for all ultrasound parameters for endometrial receptivity, thus they concluded that Endometrial and subendometrial blood flows measured by 3D power Doppler ultrasound were not good predictors of pregnancy if they were measured at one time-point during IVF treatment.

In our study, the mean endometrial thickness in no pregnancy group is lower than in pregnancy group, the difference is statistically significant.

Raga et al (1999) in their study to investigate the role of endometrial volume by 3D US in relation to endometrial receptivity in 72 patients undergoing IVF cycles, found that implantation and pregnancy rates were significantly lower when endometrial volume was less than 2, no pregnancy occurred when endometrial volume was less than 1.

E2, hCG, number of follicles and their diameter were not significantly different between pregnancy and no pregnancy groups. But, there were statistically significant differences between no pregnancy group and pregnancy group regarding number of oocytes and number of available and transferred embryos.

**Salle et al.** (1998) examined the ability of an ultrasonography score to assess the uterine receptivity. A total of 96 women undergoing in-vitro fertilization (IVF) treatment were examined by transvaginal ultrasonography with colour and pulsed Doppler ultrasound on the 22nd day of the menstrual cycle preceding IVF. The overall pregnancy rate was 30.2%, and there was no difference between the pregnant and non-pregnant groups with regard to any of the ultrasonographic and Doppler parameters when examined separately. However, the uterine score was significantly higher in the pregnant group. No pregnancy occurred if the score was between 0 and 10. With a score of 11-15 there was a 34.7% chance of pregnancy, and scores >16 had a 42% chance of pregnancy. In conclusion, individual ultrasonographic and Doppler parameters are not of sufficient accuracy to predict uterine receptivity. The uterine score calculated prior to IVF cycles appears to be a useful predictor of implantation.

In an attempt to use endometrial vascular resistance measured by Doppler spectral analysis to predict the success rate of in vitro fertilization, **Yuval et al.** (1999) failed to find significant differences between pregnant and nonpregnant patients.

**Schild et al.** (2000) investigated the role of 3-D power Doppler sonography of the subendometrial area on the 1st day of ovarian stimulation in predicting the outcome of an IVF program and demonstrated that neither endometrial measurements nor uterine blood flow were correlated with the pregnancy rate.

**Kupesic et al.** (2001) demonstrated that quantitative analysis of blood flow could be obtained by implementing the color histogram mode and calculations by the built-in computer for 3-D power Doppler examinations. A significantly higher flow index on the day of ET was found in pregnant patients by using these new and complicated techniques. Patients who became pregnant were characterized by a significantly lower RI, obtained from subendometrial vessels by transvaginal color Doppler ultrasonography.

**Kupesic et al.** (2001) compared the 2-D and 3-D ultrasonographic scoring systems by combining parameters including endometrial thickness, volume, echogenicity, and subendometrial blood flow and found the two systems had similar efficiencies in predicting pregnancy outcome of IVF/ET procedures. They showed that the resistance to endometrial blood flow is more indicative than the presence or absence of subendometrial blood flow alone.

**Schild et al.** (2001) noticed that those combining the endometrial and subendometrial flow parameters showed significant differences between pregnant and nonpregnant patients. In contrast, there was no significant difference if attention was only focused on intraendometrial or subendometrial blood flow. These results implied that the endometrial–subendometrial area must be considered as a whole in evaluating endometrial perfusion.

**Chien et al.** (2002) stated that there were no significant differences between the pregnant and nonpregnant groups with regard to the duration of infertility, duration of gonadotropin stimulation, endometrial thickness, or serum estradiol or progesterone concentrations on the day of ET. The mean number of oocytes aspirated and mean number of embryos transferred were significantly higher in pregnant patients than in nonpregnant patients. Subendometrial blood flow was detected in 477 (76.6%) cases; pregnancy and implantation rates were significantly higher for these than for those with no detectable flow. After adjustment for the age and the number of embryos transferred, patients with presence of flow were estimated to be 5.9 times as likely to become pregnant as were those with absence of flow. Among patients with the presence of subendometrial flow on the day of ET, there were no significant differences in the subendometrial blood flow parameters including RI, PI, Vmax, and Vmean between the conception and nonconception groups. They concluded that endometrial–subendometrial blood flow distribution pattern assessed by transvaginal color Doppler before ET is correlated with the implantation and pregnancy rate of IVF treatment.

**Mercé et al.** (2007) using three-dimensional ultrasonography and power Doppler angiography, on the day of hCG, to examine 80 cycles found that In the pregnant group, endometrial volume EV, vascularization index VI, flow index FI, and flow vascularization index FVI were statistically significantly higher than in non pregnant, but triple-line pattern and endometrial thickness ET were not statistically significantly correlated. 3D power Doppler indices seem to be useful for evaluating endometrial receptivity.

**Aghahoseini et al.** (2008) investigated the role of measurement of endometrial thickness and assessment of sub-endometrial blood flow in prediction of pregnancy rate in 175 patients undergoing IVF/ICSI cycles by long protocol, by color Doppler ultrasound on the day of ET, they found no significant difference found in endometrial thickness or the zone of vascular penetration between pregnant and nonpregnant groups.

The thickness of the endometrium significantly differed with the pattern of endometrial–subendometrial flow distribution. Intraendometrial vascular penetration was associated with a thicker endometrium, suggesting a correlation of blood perfusion with endometrial development. Patients with no detectable endometrial–subendometrial flow demonstrated a higher uterine arterial resistance than those with the presence of flow. This finding is consistent with the notion that changes in uterine arterial resistance may reflect peripheral endometrial blood flow, yet it may not be sensitive enough to indicate endometrial receptivity. A trend of increasing pregnancy rates with deeper intraendometrial vascular penetration was noted in a previous study (Zaidi et al., 1995), but pregnancy rates were not significantly different in that report.

We found that patients with the presence of endometrial flow had significantly higher pregnancy rates than did those without endometrial flow. Endometrial assessment was done on the day of ET in this study instead of the day of hCG injection as in the previous studies. According to **Chien et al.** (1998), endometrial–subendometrial flow distributions can be different before and after hCG administration. Therefore, it may be necessary to examine the patient until the day of ET for a better evaluation of endometrial receptivity.

Advancements in in vitro culture systems and cryopreservation techniques for preimplanted embryos have allowed greater flexibility in the timing and number of ET. A more effective approach to assessing the uterine condition is necessary for making clinical decisions concerning ET. Because the color Doppler mode has become a standard component in most current ultrasound machines, we suggested that it should be added to the routine examination of the endometrium during IVF/ET treatments. However, the appearance of blood flow within the endometrial–subendometrial area is influenced by the quality of the equipment, the settings of ultrasound, the position of the uterus, and the experience of the operators.

**Conclusion**

There is still no consensus when the ultrasound examination for assessing endometrial receptivity in IVF treatment should be done. However, one point of time measurement may not predict implantation and pregnancy rate; it is proposed that measurement of endometrial and subendometrial blood flow during the follicular phase and early luteal phase to determine the changes may reflect better the role of endometrial and sub-endometrial bloodflows.

In conclusion, transvaginal color Doppler examination of the endometrial–subendometrial blood flow distribution provides a simple and effective method to evaluate endometrial receptivity. The presence of both endometrial and subendometrial blood flow is indicative of good endometrial receptivity, whereas the absence of both represents a poor uterine environment. This approach may be helpful in deciding the number and timing of ICSI in IVF treatments.

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