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Original Research Article

Prediction of pregnancy in artificial reproductive techniques through evaluation of thickness, morphology and vascularity of endometrium

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ABSTRACT

Background: Prediction of pregnancy in Artificial Reproductive Techniques through evaluation of thickness, morphology and vascularity of endometrium.
Methods: Endometrial thickness, morphology and sub endometrial blood flow were assessed using trans-vaginal ultrasound on the day of HCG in 200 undergoing IVF/ICSI treatment in the period between October 2009 and December 2014. Statistical analysis was done.
Results: There was no difference in the demographic features between pregnant and non-pregnant women. Overall, 80 patients conceived; 46 (57.5%) of them had blood flow in zone III and 30 (37.5%) in zone II. All patients achieved pregnancy had endometrial thickness >8 mm. There was no significant difference in Doppler indices between pregnant and non-pregnant women.
Conclusions: When the endometrial thickness is <8 mm, and if there are non-triple endometrial line, pregnancy rate decreases and the absence of colour mapping of the endometrium and subendometrial areas means and absolute implantation failure or a significant decrease of the implantation rate. Conversely, the pregnancy rate increases when the vessels reach endometrium.

Keywords: Artificial reproductive techniques, Endometrial vascularity, Pregnancy

INTRODUCTION

The endometrium is a commonly used non-invasive tool to assess endometrial receptivity during IVF treatment. Endometrial receptivity is defined as a temporary unique sequence of factors that make the endometrium receptive to the embryonic implantation. It is the window of time when the uterine environment is conductive to blastocyst acceptance and subsequent implantation. The process of implantation may be separated into a series of developmental phases starting with the blastocyst hatching and attachment to the endometrium and culminating in the formation of the placenta. The steps start with apposition, and progress through adhesion, penetration and invasion. Evaluation of endometrial receptivity remains a challenge in clinical practice. Ultrasonography has an important role in the evaluation and treatment of infertility patients. It is an efficient and cost-effective modality for studying the female reproductive organs.

Study of endometrium includes its thickness, pattern and vascularity. Endometrial thickness is defined as the maximal distance between the echogenic interfaces of the myometrium and the endometrium, measured in the plane through the central longitudinal axis of the uterus.

Endometrial pattern is defined as the relative echogenicity of the endometrium and the adjacent myometrium as demonstrated on a longitudinal ultrasound scan. During the proliferative phase of the menstrual cycle the

endometrium achieves triple line morphology. In principle, the central echogenic line represents the uterine cavity; the outer lines represent the basal layer of the endometrium, or the interface between the endometrium and myometrium. During the secretory phase of the menstrual cycle, the endometrium acquires a hyper echogenic morphology that is due to stromal oedema, spirilization and secretion of the endometrial glands caused by the action of progesterone. However, since a correlation between echogenicity and progesterone has not been demonstrated other factors such as androgen and gonadotropin effects could explain these changes.

Classification of the types of appearance of the endometrium has been simplified over time. Nowadays, intermediate patterns are often discarded and the endometrium is simply described as multi-layered or non-multi layered. The triple line endometrial pattern has high sensitivity (79-100%) but an elevated percentage of false positives (57-91%) also; subsequently it has an additional interest by its high negative predictive value (75-100%). Although achieving a pregnancy with a non-triple-line pattern is possible, its frequency is low. A good blood supply to the endometrium is an essential requirement for implantation and assessment of endometrial blood flow in IVF treatment has attracted a lot of attention in recent years.[1]

Doppler study of uterine arteries does not reflect the actual blood flow to the endometrium. Endometrial and sub endometrial blood flows can be more objectively and reliably measured with three-dimensional power Doppler ultrasound. The study of endometrial thickness, pattern, volume, Doppler and sub-endometrial blood flow by ultrasound has been used to assess endometrial receptivity during IVF treatment. The measurement of endometrial blood flow as a physiologic dimension in addition to the anatomic parameters gained by the ultrasound had played an important role in the expectation of pregnancy outcome in IVF/ICSI cycles.[2-4] Present study aimed to assess pregnancy rate in ART by measurement of the endometrial thickness, pattern and endometrial blood flows by 2D Doppler ultrasound.

METHODS

This study is a prospective observational study including 200women undergoing IVF/ICSI treatment at a Rama medical college and Raj infertility center, Kanpur. All the patients gave a written informed consent before enrolment in the study.

Inclusion criteria

Age between 20 and 39 years and IVF/ICSI treatment due to many factors including tubal, male, PCOS or other unexplained factors.

Exclusion criteria

Patients under 20 years or above 39 years and (b) patients who were unable to give a written informed consent. Follicular study was performed using trans-vaginal ultrasound scan starting from day six of stimulation and every other day afterwards till the day of HCG. One expert radiologist evaluated each patient on day of HCG using 2D trans-vaginal ultrasound machine (Sonoline G 50, Siemens) with 4-9 MHz-convex array transducer as follows:

- We use the gray scale function of the ultrasound machine to study and measure the endometrial thickness as the thickest part of the endometrium between the highly reflective echogenic lines in the true longitudinal scan of the uterus.
- In this true longitudinal scan of the uterus, we note the endometrial pattern as either triple-line (described as hypo echoic endometrium surrounded by hyper echoic zones) or non-triple-line.
- After then, we activate the Doppler function of the ultrasound machine to evaluate the endo-sub-endometrial blood flow or vascularization and is either, zone I in which the blood flow reached only sub-endometrial region, zone II in which the blood flow reached the outer hyper-echoic region or zone III in which the blood flow reached the inner hypo-echoic zone.
- To evaluate the Doppler indices of the endometrial vasculature.

We activate the pulsed power Doppler function of the machine and applied the Doppler gate over the appropriate color area then tried to have five or more consecutive Wave forms for the study to be satisfactory (each wave represented maximum Doppler shift). Then we measure the resistive index= Peak systolic velocities – Peak diastolic velocities/Peak systolic velocities and standard of deviation ratio (S/D ratio) was calculated on three consecutive uniform waveforms.

Then we gave 10,000 HCG IU intramuscular when there were minimum of three mature follicles measuring 17-18mm in dimensions and after 36 h the ovum was picked up under intra-venous sedation. Two or three embryos were placed inside the uterine cavity of the patient on days 4–5 after the retrieval and good quality embryos were frozen. On the luteal phase we gave 800 microgram of micronized progesterone vaginally from day of embryo-transfer (ET) till 12 weeks of pregnancy. A serum pregnancy test was done two weeks after embryo transfer.

Study outcome

The primary outcome of the study was clinical pregnancy rate that defined as the presence of one or more gestational sac by ultrasound scan two weeks after a positive serum pregnancy test.

RESULTS

The current study included 200 patients who underwent IVF/ICSI treatment. Pregnancy was achieved in eighty patients. There was no difference in the demographic features, hormonal milieu between pregnant and non-pregnant women as shown in (Table 1).

Table 1: Demographic characteristic in the pregnant and non-pregnant women.

	Pregnant (n=80)	Non-pregnant (n= 120)	P value
Age (yrs)	30.6± 2.1	28.2 ±1.8	0.9
BMI (kg/m^2)	29.2± 0.5	28.8 ±0.4	0.5
Duration of infertility (yrs)	9.1± 1.3	8.5 ±0.7	0.7
Cause of infertility			0.8
Male factor	32 (40%)	50 (41.7%)	
Tubal factor	18 (22.5%)	20 (27%)	
Endometriosis	6 (7.5%)	10 (8.3%)	
Unexplained	17 (21.2%)	30(25%)	
Anovulation	6 (7.5%)	8 (6.6%)	
Combined	3 (3.7%)	2 (1.7%)	
Basal FSH (IU/L)	6.7±0.5	6.4±0.5	0.6
Basal LH (IU/L)	4.2±0.3	4.7±0.5	0.7
Basal AMH (ng/ml)	2.3±0.3	2.9±0.3	0.9
AFC	12.8±1.1	13.9±1.1	0.5

Data are presented as mean±SEM or number (%). FSH= follicle stimulating hormone, LH= luteinizing hormone, AMH= anti-Mu¨ llerian hormone, AFC=antral follicle count.

There was no statistically significant difference in the Doppler indices of the sub-endometrial blood vessels between pregnant and non-pregnant women as shown in (Table 2).

Table 2: Doppler indices and pregnancies.

	Pregnant (n =80)	Non-pregnant (n =120)	P value
Resistance index (RI)	0.93±0.69	0.93±0.86	0.2
Pulsatility index (PI)	1.06±0.54	1.06±0.99	0.1
S/D ratio	18.4±6.2	9.9±4.3	0.7

There was a direct correlation between endometrial blood flow and IVF/ICSI cycle outcome as more pregnancies were achieved when endometrial blood flow was in zone III (57.5%) as compared with 41% and 1.4% when the blood flow was in zone II and zone I respectively (Figure 1-3). These results are shown in (Table 3). Endometrial thickness was found to be strongly correlated with successful pregnancy in IVF/ICSI cycles. No pregnancies were achieved when endometrial thickness was less than 8 mm (Table 4).

Table 3: Endometrial blood flow zone and pregnancies.

Endometrial blood flow	No. of cases (n=200)	No. of pregnancies (n=80)	(%)
Zone I	40	1	1.4
Zone II	75	33	41
Zone III	85	46	57.5

Pregnancy rate is highest when blood flow is in zone III

Table 4: Endometrial thickness and number of pregnancies.

	No. of cases (n=200)	No. of pregnancies (n=80)	(%)
<8 mm	8	0	0
8-10mm	80	42	52.5
10-12 mm	52	19	23.8
12-14 mm	41	18	22.5
>14mm	29	1	1.3

Maximum pregnancy rate is when endometrium is 8-10mm thick and no pregnancy was achieved with endometrium <8 mm.

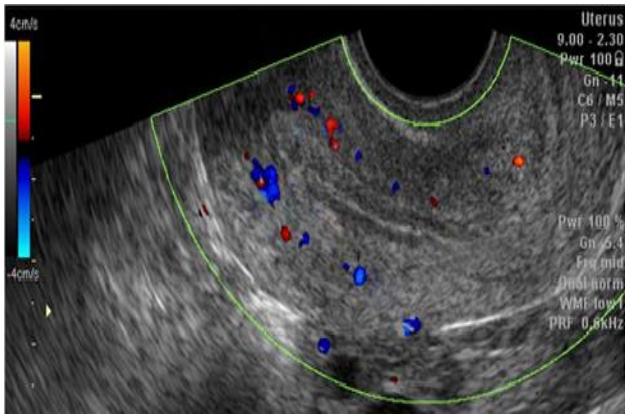

Figure 1: Endometrial vascularity: Zone 1.

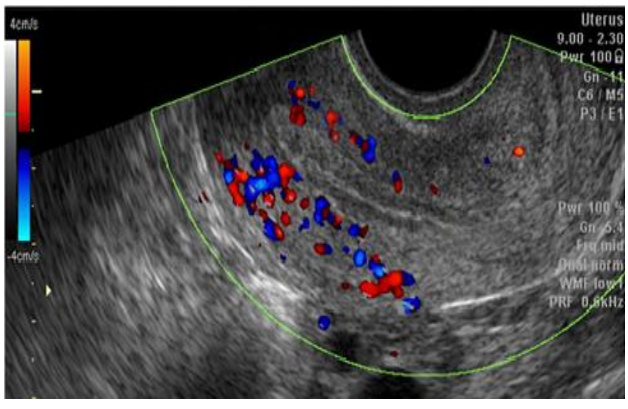

Figure 2: Endometrial vascularity- Zone 2.

Regarding endometrial morphology, all cases who achieved pregnancies had a triple-line pattern of the endometrium on day of HCG. Classification of the types of appearance of the endometrium has been simplified over time. Nowadays, intermediate patterns are often discarded and the endometrium is simply described as multi-layered (Figure 4) or non-multi layered pattern (Figure 5) to be more predictive of implantation.

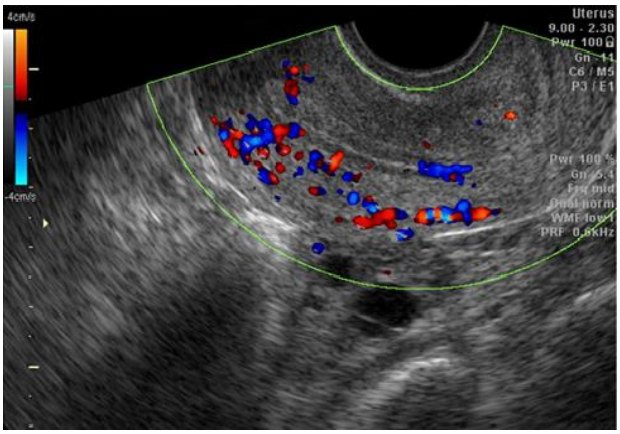

Figure 3: Endometrial vascularity- Zone 3.

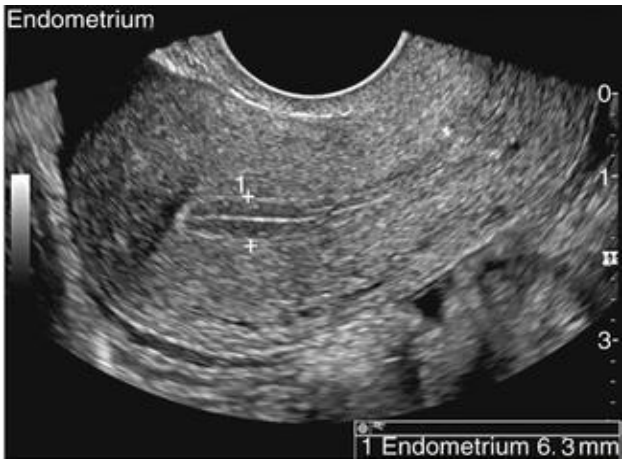

Figure 4: Multi layered endometrium.

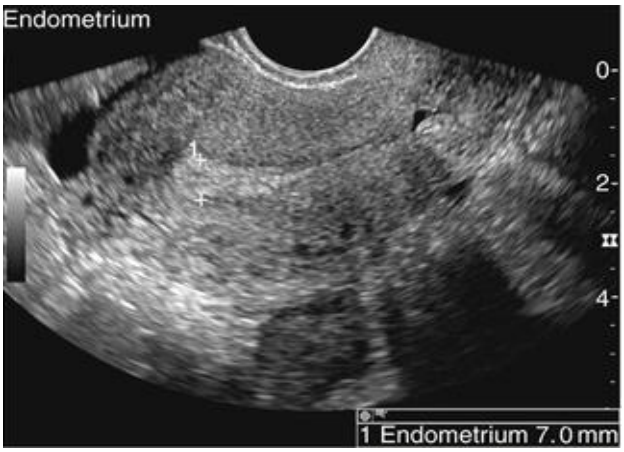

Figure 5: Non-multi layered endometrium.

In eight cases, there was gapping between the endometrium and fluid inside. None of these cases achieved pregnancy.

DISCUSSION

The assessment of the endometrial receptivity is mandatory for the success of all IVF/ICSI procedures. Angiogenesis plays important role in various female reproductive processes as maturation of dominant follicle, endometrial growth and implantation process.[5-7] Here, there was no difference in the demographic features between pregnant and non-pregnant women. In this study, there were no statistically significant differences in the Doppler indices of sub-endometrial blood vessels between pregnant and non-pregnant women. Raine-Fenninget al. stated that endometrial and sub endometrial blood flows by ultrasound increased during the proliferativephase, peaking around 3 days prior to ovulation thendecreased 5 days post-ovulation. Therefore, there was a period of relatively reduced perfusion in the immediate post ovulatory period, extending to the implantation period inspontaneous cycles.[8]

Ernest Hung et al, concluded that endometrial and sub-endometrial blood flows were not good predictors of pregnancy if they were measured at onetime-point during IVF treatment.[4] This debate suggests that further longitudinal studies in the late follicular phase and early luteal phase are needed to reach a firm conclusion. So we suggest that the endometrial and sub-endometrial S/D, RI and PI could not be used alone to predict endometrial receptivity. A reduced endometrial blood flow after ovulation could be related to an increased uterine contractility and may lead to endometrial hypoxia during the implantation period.

In this study, the endometrial thickness was found to be strongly correlated with successful pregnancy in IVF/ICSI cycles. The most suitable endometrial thickness for pregnancy was 8–10 mm (50% of them achieved pregnancy), followed by endometrial thickness of 10-12 mm (25% of them achieved pregnancy). The least of them is when the endometrial thickness is more than 14 mm (only 2.5% of them achieved pregnancy). No pregnancies were achieved when endometrial thickness was less than 8 mm.

Singh et al. reported that, largest number of pregnancies occurred when the endometrial thickness is 8-10 mm. He also, postulated that no pregnancies reported when endometrial thickness is less than 5.8 mm.[2] Weissman et al reported the lowest percentage of conception when endometrial thickness is more than 14 mm.[9] (Figure 1). Dynamic change in endometrial thickness in assisted conception cycles was first described by Rabinowitz et al.[10] Using trans-vaginal scanning, Gonen et al. suggested that endometrial thickness, on the day before oocyte recovery, was significantly greater in pregnant than in non-pregnant women, and postulated that it may predict the

likelihood of implantation.[11] During the secretory phase of the menstrual cycle, the endometrium acquires a hyper echogenic morphology that is due to stromal oedema, spirilization and secretion of the endometrial glands caused by the action of progesterone.[12] However, since a correlation between echogenicity and progesterone has not been demonstrated, other factors such as androgen and gonadotropin effects could explain these changes.[13]

In our research, all cases who achieved pregnancy had a triple-line pattern of the endometrium on day of HCG. In eight cases, there was gapping between the endometrium and fluid inside, and none of these cases achieved pregnancy. Classification of the types of appearance of the endometrium has been simplified over time. Nowadays, intermediate patterns are often discarded and the endometrium is simply described as multi layered or non-multilayered.[14] In a prospective study, Serafimi et al. found the multi layered pattern to be more predictive of implantation than any other parameter measured.[15] The "triple line" endometrial pattern has high sensitivity (79-100%) but an elevated percentage of false positives (57-91%) also, subsequently it has an additional interest by its high negative predictive value (75-100%).

Although achieving a pregnancy with a non-triple-line pattern is possible, its frequency is low. El-Zenneni et al, stated that the triple layer endometrial pattern was the most suitable for conception.[16] Singh et al proved that the triple layer endometrium was good prognostic factor for occurrence of pregnancy.[2] Jarvela et al. considered the endometrial thickness and morphology correlated to IVF/ET outcomes, as 44.8% of their patients with triple line pattern before HCG injection achieved pregnancy; 80% of patients with triple-line pattern on the day of ovum collection achieved pregnancy.[17] Contrary to our research results, Ng et al, reported, no relationship between endometrial thickness, morphology and pregnancy outcomes.[4] Regarding the endometrial blood flow zones, in this study, 57.5% of the pregnant women had zone III of endometrial blood flow, 37.5% had zone II of blood flow while only 5%of the pregnant women had zone I of blood flow. Ng et al. found that the endometrial and sub-endometrial blood flow was not a good predictor of pregnancy.[18] In this study, the clinical pregnancy rate reached 40%. Other investigators stated that the clinical pregnancy rate was 26.73% and noted a higher pregnancy rate when the blood flow to the endometrium was in zone III (51.8%) compared to (14.8%) when in zone I.[19,20] Dechaud et al, considered that, the endometrial pattern, thickness and end-diastolic blood flow were shown to be the most effective combination for the evaluation of uterine receptivity.[21] Wang et al, and Nygren et al, had suggested positive correlation of the endometrial and sub-endometrial blood flows to the outcome of IVF/ET.[22,23] while Aghahoseini et al had suggested negative correlation.[24] Divya Sardana et al, suggested that a combination of endometrial thickness and Doppler analysis of endometrial blood flow was a simple and effective tool to improve the outcome of IVF/ET and should be incorporated into routine clinical practice.[25] This was suggested by our study results in addition to the endometrial pattern.

CONCLUSION

With endometrial thickness less than 8 mm and no-triple-line pattern on HCG day in IVF/ICSI cycles, there is a poor chance of achieving pregnancy. Average endometrial line thickness of 8-12 mm and triple line (good morphologic texture) are good prognostic values if good quality embryos are transferred. Maximum pregnancy was achieved when blood supply was in zone III and no pregnancy was achieved when there was no blood flow in Doppler ultrasonography.

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