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Original Research Article

A study of correlation of endometrial morphology and subendometrial vascularity with pregnancy rate in fresh and frozen embryo transfer cycles

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ABSTRACT

Background: Aim of the study was to evaluate the correlation of endometrial morphology and sub endometrial vascularity with pregnancy rate in fresh and frozen embryo transfer cycles.
Methods: Prospective, non-randomized observational study done on 76 women undergoing *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) treatment at a tertiary health care center. Woman with tubal factor, male factor and unexplained infertility were included in the study. Parameters like endometrial thickness, endometrial pattern, endometrial blood flow were correlated to clinical outcomes such as implantation and clinical pregnancy rates. Independent two-tailed t-test and chi-square test was used as a part of statistical analysis.
Results: There is no significant difference in distribution of mean age, mean duration of infertility, cause of infertility, mean basal follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), progesterone (P4), anti-Mullerian hormone (AMH), total gonadotropin dose, median E2 (on day of trigger), median P4 (on day of trigger), number of embryos transferred and endometrial thickness between group of cases with positive and negative pregnancy in our study group. Whereas body mass index (BMI), blastocyst quality of embryo transferred, triple-line pattern of endometrium and endometrial blood flow reaching zone 4 were found to be significant in the study group.
Conclusions: A combination of endometrial thickness and Doppler analysis of endometrial blood flow was a simple and effective tool to improve pregnancy rates in hormone replacement treatment-frozen embryo transfer (HRT-FET) cycles and should be incorporated into routine clinical practice. In addition, this can help us reduce the number of embryos to be transferred and hence, the multiple pregnancies and the associated complications.

Keywords: Frozen-thawed embryo transfer, Power Doppler, Subendometrial-endometrial blood flow

INTRODUCTION

Successful implantation depends on a close interaction between a good quality embryo and the receptive endometrium.[1] The implantation potential of good quality embryos remains low during *in vitro* fertilization (IVF) treatment, despite advances in ovarian stimulation regimens, the method of assisted fertilization and improved culture conditions.

It is established that the endometrium is a key factor during the so-called "implantation window", a short period of time of maximal endometrial receptivity to blastocyst signals.[2,3] In humans, this period begins 6–10 days after the luteinizing hormone (LH) surge and lasts for nearly 48 hours.[4] Several diagnostic tools have been proposed to 'measure' or 'estimate' the endometrial receptivity which include sonographic measures like endometrial thickness, endometrial pattern as well as sub endometrial blood flows. Assessment of endometrial blood flow adds a

physiological dimension to the anatomical ultrasound parameters.[5-8]

In combination with 3D ultrasound, power Doppler provides a non-invasive tool with which to examine the blood supply to the whole endometrium and the subendometrial region.

However, the role of endometrial and subendometrial blood flow in IVF outcomes remains controversial. Endometrial or subendometrial blood flow was significantly higher in pregnant patients.[9-11]

The present study has been emphasized so that even though good quality embryos have been transferred in a morphologically good endometrium results may be negative. In this study, we attempted to predict the outcomes of conception cycles. Unsuccessful cycle rates could be decreased if we try to optimize the time of embryo transfer based upon sonographic signs of endometrial preparedness.

Further, if the likelihood of an unsuccessful cycle existed, the patient could be informed prior to the time of transfer of embryos, the embryos could be frozen and thus the patient would not be disappointed either by the failure of the current cycle or the wastage of embryos.

METHODS

Study type

It was a prospective observational type of study.

Study place

The study was conducted at Tata Centre for Reproductive Health, K. E. M. Hospital, Pune, Maharashtra, India.

Study period

The duration of the study was for 21 months (October 2017 to May 2019).

Inclusion criteria

Patients undergoing IVF/intracytoplasmic sperm injection (ICSI) cycles for tubal factor infertility, male factor infertility, polycystic ovarian syndrome, endometriosis or other unexplained factors were included in the study.

Exclusion criteria

Patients with congenital uterine anomalies, severe adenomyosis, uterine fibroid, endometrial polyp, intra uterine adhesions, endometrial Koch's, distorted endometrial cavity, low resolution on ultrasonography which are liable to affect the outcome, were excluded from the study.

Procedure details

Fresh embryo transfer cycle

In fresh cycles, basal levels of E2, progesterone, follicle stimulating hormone (FSH), and LH were measured on day 2 of menstrual cycle. Stimulation was done with gonadotropins using antagonist protocol. Stimulation was started with gonadotropins on day 2/day 3 of cycle. Dose of gonadotropins depended on age, antral follicular count (AFC), anti-Mullerian hormone (AMH), response to previous stimulation and body mass index (BMI). Folliculometry was performed using transvaginal ultrasound scan starting from day 4 to 5 of stimulation and every other day afterwards till the day of human chorionic gonadotropin (hCG). One expert sonologist evaluated each patient on day of hCG using 2D transvaginal ultrasound machine (VOLUSON E6 GE Medical Systems) with transvaginal volume probe as follows.

We used 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. It was measured from the myometrial endometrial junction to the endometrial myometrial junction in sagittal plane.

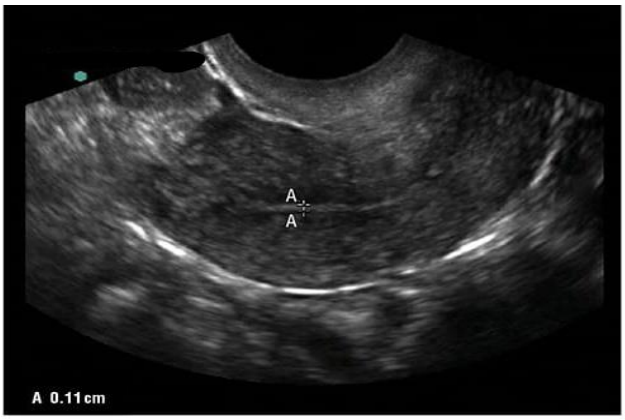

Figure 1: Endometrial thickness.

In this true longitudinal scan of the uterus, we noted the endometrial pattern as either triple-line (described as hypo echoic endometrium surrounded by hyper echoic zones) or non-triple-line.

After then, we activated the Doppler function of the ultrasound machine to evaluate the endo-sub endometrial blood flow or vascularization. The endometrial and peri endometrial areas are divided into the following four zones: zone 1- a 2 mm thick area surrounding the hyper echoic outer layer of the endometrium; zone 2 - the hyper echoic outer layer of the endometrium; zone 3 - the hypo echoic inner layer of the endometrium; and zone 4 - the endometrial cavity.

On pulse Doppler, these blood vessels should show RI 0.49-0.59 and PI 1.1-2.3. These blood vessels should cover 5 mm^2 area of a particular 3,4 zone of endometrium. The power Doppler characteristics applied in all examinations were as follows: normal quality of color, color gain-3.4, pulse repetition frequency of 600 Hz and wall motion filter of 50 Hz.

We have given recombinant hCG (6500 IU) as trigger when there were minimum of three mature follicles measuring 17–18 mm in dimensions and serum progesterone was measured on the day of hCG. Ovum pickup was done 35-36 hours after trigger. Progesterone gel was started vaginally once a day for the patients from the day of pick up. Two or three morphologically good embryos were transferred inside the uterine cavity of the patient on day 3 or on day 5 after the retrieval. In the luteal phase we have given progesterone gel 8% vaginally twice a day from day of embryo-transfer (ET) till 12 weeks of pregnancy. A serum β-hCG test was done two weeks after embryo transfer. If serum progesterone was >1.5 U/ml all embryos were frozen.

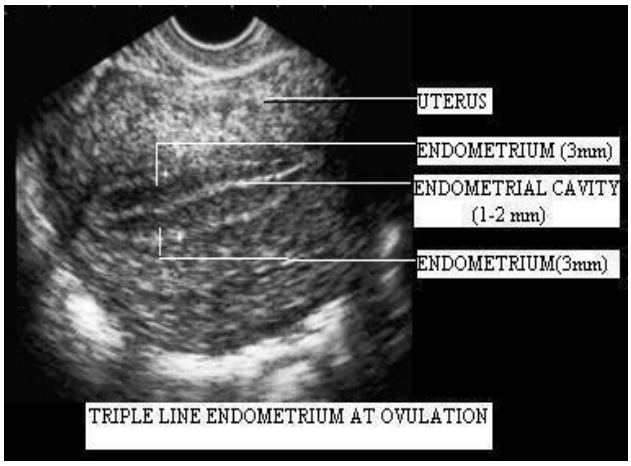

Figure 2: Triple layered endometrium.

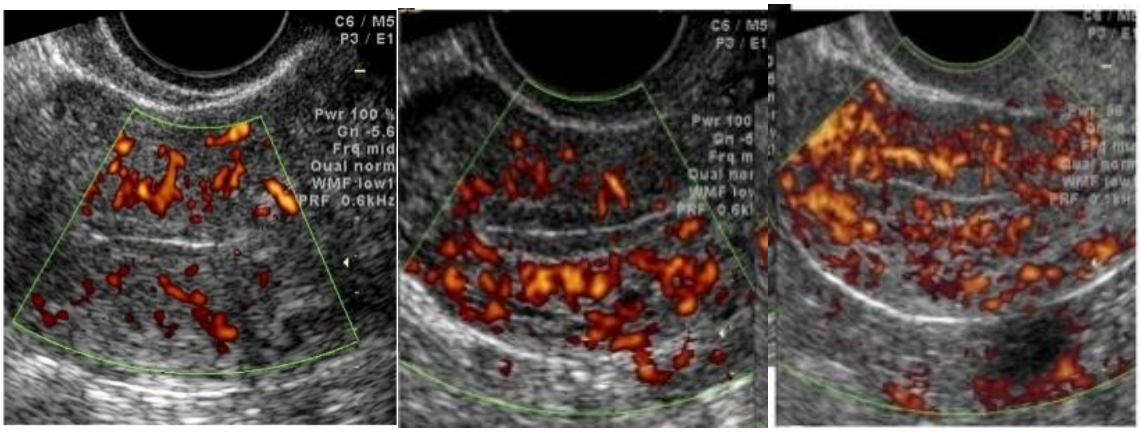

Figure 3: Doppler blood flow in zone II, zone III, and zone IV.

Frozen embryo transfer cycle

Patients were given injection lupride depot 3.75 mg on day 21 of previous. When endometrial thickness was <5 mm and no ovarian cyst was seen on ultrasound, patients were started on estradiol supplementation (tablet estradiol valerate 2 mg BD for 5 days then TDS) from day 2 or day 3 of cycle. All patients received folic acid supplements. Ultrasound was repeated after a week of Estradiol valerate and endometrial thickness, endometrial pattern and endometrial Doppler ware evaluated. Once endometrial thickness was >7 mm with triple line and Doppler reaching zone 3,4 embryo transfer was planned. Progesterone supplement (progesterone 8% gel vaginally for 3 to 5 days) was added and cleavage stage or blastocyst embryo transfer (2 or 3 good quality embryos) was done after 3 to 5 days under ultrasound guidance. All patients were prescribed vaginal progesterone suppositories and estradiol valerate tablets for luteal phase support after ET along with folic acid supplements for 2 weeks after which blood was tested for β-hCG. β-hCG >50 IU/l was considered as positive for pregnancy. Clinical pregnancy was defined by the appearance of the gestational sac on ultrasound with appropriately rising hCG levels. When no gestational sac could be seen with positive β-hCG by 6 weeks, it was defined as a biochemical pregnancy.

Ethical approval

The ethical approval for the study was obtained from the institute's ethical committee. All the patients gave a written informed consent before enrolment in the study.

Sample size calculation

Sample size was determined by using the effect sizes from the previously published study by Scioscia et al with the help of following formula given below. [12]

$$n = z^2 \frac{pq}{(me)^2}$$

Where p is 0.269 (26.9%) (approximate estimate of the incidence of clinical pregnancy among a group of women

undergoing fresh and frozen ET cycles with higher ET (>7 mm)); q is 0.731 (73.1%) (approximate estimate of the incidence of clinical non-pregnancy among a group of women undergoing fresh and frozen ET cycles with higher ET (>7 mm)); Z is 1.96 (score at 95% confidence interval); me is 0.10 (margin of error); and n is given by the following formula.

$$n = 1.96^2 \times 0.269 \times 0.731/(0.10^2) = 75.54$$

Thus, the minimum sample size required according to this formula is 75.54 OR 76.

Statistical analysis

The data on categorical variables were presented as N (% of cases) and the values on continuous variables were presented as mean±standard deviation (SD). The significance of difference of distribution of incidence of clinical pregnancy across various groups of interest (such as age groups, and ET groups) were tested using Chi-square test/Fisher's exact probability test (39, 40). Independent sample 't' was used to test the significance of difference in the continuous variables across two groups. The underlying assumption of normality was tested before subjecting the study variables to t test.

P values less than 0.05 was considered to be statistically significant. All the hypotheses were formulated using two tailed alternatives against each null hypothesis (hypothesis of no difference). The entire data was statistically analyzed using statistical package for social sciences (SPSS version 21.0, IBM Corporation; NY, USA) for Microsoft Windows.

RESULTS

Among 76 patients who underwent IVF treatment, 75% had primary infertility and 25% had secondary infertility. Out of the 76 patients, 40 patients underwent fresh cycle embryo transfer and 36 patients underwent thaw cycle embryo transfer. Pregnancy was achieved in 49 patients.

Table 1: Correlation of different parameters between pregnant and non-pregnant groups.

Parameters	Pregnant (n=49)	Non pregnant (n=27)	P value
Age (years)	30.5 (SD 3.8)	30.1 (SD 3.9)	0.63^{NS}
BMI (kg/m^2)	26.48 (SD 3.44)	28.55 (SD 3.70)	0.017^{***}
Duration of infertility (years)	5.39 (SD 2.43)	5.30 (SD 1.94)	0.867^{NS}
Tubal factor (%)	16.3	29.6	0.517^{NS}
Male factor (%)	24.5	25.9	
PCOD (%)	4.1	3.7	
Unexplained (%)	55.1	40.7	
Fresh cycle day 2 FSH (mIU/ml)	6.53	6.71	0.631^{NS}
Fresh cycle day 2 LH (IU/lit)	4	4.49	0.153^{NS}
Fresh cycle day 2 E2 (pg/ml)	44	39.50	0.516^{NS}
Fresh cycle day 2 P4 (ng/ml)	0.10	0.22	0.441^{NS}
AMH (ng/ml)	3.40	2.59	0.153^{NS}
Thaw cycle day 2 LH (IU/lit)	4.10	4.10	0.575^{NS}
Thaw cycle day 2 E2 (pg/ml)	46	44	0.321^{NS}
Total gonadotropin dose (mIU/ml)	2675	2850	0.317^{NS}
E2 on day of trigger (pg/ml)	2070	1840	0.493^{NS}
P4 on day of trigger (ng/ml)	0.80	0.70	0.424^{NS}
1 embryo transferred, N (%)	2 (50)	2 (50)	0.197^{NS}
2 embryos transferred, N (%)	34 (72.3)	13 (27.7)	
3 embryos transferred, N (%)	13 (52)	12 (48)	
3 celled stage embryo transfer, N (%)	19 (48.7)	20 (51.3)	0.004^{**}
Blastocyst embryo transfer, N (%)	30 (81.1)	7 (18.9)	
Endometrial thickness <7 mm, N (%)	1 (25)	3 (75)	0.125^{NS}
Endometrial thickness >7 mm, N (%)	48 (66.7)	24 (33.3)	
Endometrial pattern C (triple line), N (%)	45 (67.2)	12 (32.8)	0.001^{***}
Endometrial pattern B, N (%)	4 (21)	15 (79)	
Endometrial Doppler zone 2 (%)	3 (37.5)	5 (62.5)	0.001^{***}
Endometrial Doppler zone 3, N (%)	6 (26.1)	17 (73.9)	
Endometrial Doppler zone 4, N (%)	40 (88.9)	5 (11.1)	

BMI=body mass index, PCOD=polycystic ovarian disease, FSH=follicle stimulating hormone, LH=luteinizing hormone, P4=progesterone, E2=estradiol.

DISCUSSION

The current study included 76 patients who underwent IVF treatment. 57 patients (75%) had primary infertility and 19 patients (25%) had secondary infertility. Out of the 76 patients, 40 patients underwent fresh cycle embryo transfer and 36 patients underwent thaw cycle ET. Pregnancy was achieved in 49 patients (64.5%) and 27 (35.5%) patients did not get pregnant.

Correlation of endometrial thickness between pregnant and non-pregnant groups

In our current study, of 4 patients with endometrial thickness <7 mm, 1 patient (25.0%) conceived and 3 patients (75.0%) did not conceive. Of 72 patients with endometrial thickness 7-14 mm, 48 patients (66.7%) conceived and 24 patients (33.3%) did not conceive. Distribution of pregnancy status did not differ significantly according to level of endometrial thickness in the study group (p value >0.05). In a study by Moustafa et al no pregnancies were achieved when endometrial thickness was <8 mm.[13] They found out that endometrial thickness was strongly correlated with successful pregnancy in IVF cycles. In a study by Yuan et al, the mean endometrial thickness (±SD) in patients who achieved a clinical pregnancy was 11.5 (±2.4) mm, which was significantly thicker (p<0.001) than the mean (±SD) endometrial thickness of 10.8 (±2.4) mm in women who did not conceive.[14] No pregnancy occurred when the endometrial thickness was below 4 mm. Among those who conceived, the mean (±SD) endometrial thickness in live birth group is 11.6 (±2.4 mm). No significant difference were found in pregnancy rates based on endometrial morphology. Kasius et al in their study found out that an endometrial thickness 7 mm or less is associated with a significantly reduced chance of conception after IVF and embryo transfer treatment.[15] In their meta-analysis, the conception rate in women with an endometrial thickness 7 mm or less was 23.3% and 48.1% of those whose endometrial thickness was greater than 7 mm. Our observations were strikingly similar, with a conception rate of 25.0% (4/76) in women with an endometrial thickness less than 7 mm (lower 5th centile), and significantly increased to 66.7% (72/76) for women whose endometrial thickness was 8 mm or greater.

Correlation of endometrial pattern between pregnant and non-pregnant groups

A triple-line pattern at ultrasound examination reflects endometrial proliferation. In the present study, of 19 cases with endometrial pattern B, 4 (21.0%) had positive outcome and 15 (79.0%) did not have positive outcome. Of 57 cases with endometrial pattern A, 45 (67.2%) had positive outcome and 12 (32.8%) did not have positive outcome. Distribution of pregnancy status differed significantly according to endometrial pattern in the study group (p value <0.001). Endometrial pattern on the day of hCG administration is correlated with clinical pregnancy rates. The present data, was in accordance with the results described by Sher et al and Bohrer et al.[16,17] Sher et al found a significant difference in pregnancy rates (33% versus 7%) in favour of the triple-line endometrium associated with an endometrial thickness of over 9 mm. Bohrer et al concluded that a homogeneous endometrium (pregnancy rate, 2.9%) that failed to convert to the trilaminar pattern (pregnancy rate, 23%) was less receptive for implantation. Chen et al found a significant higher clinical pregnancy rates in patients with three-layer pattern on the day of hCG administration among IVF cohorts, but not necessarily throughout all of the endometrial thickness range.[18] In a study by Bourgain and Devroey, the presence of such a pattern on the day of hCG injection has been found to be associated with a higher pregnancy rate than is the absence of this pattern.[19] The absence of a triple-line pattern may be a sign of premature secretory changes in the endometrium, and that the time of maximal endometrial receptivity had passed. Conversely, Friedler in his study found out that the assessment of endometrial characteristics (thickness and pattern) on the day of hCG administration to be of poor predictive value.[20]

Correlation of endometrial Doppler between pregnant and non-pregnant groups

Several studies have evaluated the correlation between subendometrial and endometrial blood flow and the pregnancy rates in IVF cycles. Endometrial-subendometrial blood flow distribution pattern assessed by transvaginal color Doppler before embryo transfer was correlated with the implantation and pregnancy rate of IVF treatment. Quantitative assessment of vessel density and perfusion within subendometrial area using three-dimensional-power Doppler (3D-PD) was found to be the strongest predictive factor of IVF success among various sonographic parameters.[21]

Studies done by Merce and Chien have suggested positive correlation of PRs with the subendometrial and endometrial blood flows.[11,22] Similarly, the present study showed that the presence of endometrial blood flow on 2D-PD in IVF cycles was associated with significant improvement in the cycle outcome. Sardana et al, in their study found out that the patients with presence of subendometrial-endometrial blood flow were estimated to be 2.48 times as likely to become pregnant when compared to those with the absence of flow (odds ratio [OR]=2.48; 95% confidence interval=1.03-5.98; p=0.06).[8] Though the OR was not statistically significant for patients with subendometrial-endometrial blood flow group but, a clinical trend towards increased pregnancy outcome is seen in this group. Studies done by Jaffe and Abulafia found out that most patients without diagnosed infertility (presumed normal) usually demonstrated flow into zone III by the mid-cycle.[23,24] Vascular penetration towards the endometrial canal differed among patients. They found out that inadequate vascular penetration of endometrial blood flow (not within zone 3) prior to transfer had been associated with an unfavourable outcome.

Limitations

One limitation of our study is that the number of subjects in the study population were too small to make a definitive statement. Further studies are needed to clarify the biological modifications that underlie the endometrial growth. Future research should be directed towards understanding the pathophysiology of the endometrial receptivity and how it can be successfully managed.

CONCLUSION

We conclude that a combination of endometrial thickness and Doppler analysis of endometrial blood flow was a simple and effective tool to improve pregnancy rates in HRT-FET cycles and should be incorporated into routine clinical practice. Apart from endometrial thickness and endometrial pattern, the endometrial vascularity had a useful predictive value on the implantation rate in IVF cycles irrespective of the morphological appearance of the endometrium. When a thinner endometrium (≤7 mm), poor endometrial Doppler and no-triple-line endometrial pattern coexist in an IVF/ICSI candidate, cryopreservation should be recommended. In addition, this can help us reduce the number of embryos to be transferred and hence, the multiple pregnancies and the associated complications. If the likelihood of an unsuccessful cycle existed, the patient could be informed prior to the time of transfer of embryos, the embryos could be frozen and thus the patient would not be disappointed either by the failure of the current cycle or the wastage of embryos

Recommendations

3D PD-US (3D power doppler-ultrasonography) can be a useful prognostic protocol for pregnancy in infertile women. We call for studies that will shed additional light on the role of sonographic evaluation of the endometrium during infertility evaluation and throughout fertility treatments.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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