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Radiology

EMERGING ROLE OF COLOUR DOPPLER IN INFERTILITY AND ASSISTED REPRODUCTION-A PUBLIC HEALTH PERSPECTIVE

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ABSTRACT **Purpose:** To find out the efficacy and usefulness of the TVS colour Doppler,for the assessment of perifollicular vascularity,endometrial biophysical scoring in positive intrauterine insemination (IUI) outcome in patients with infertility.

Methods: A total of 40 patients were included in this study undergoing IUI procedures and were evaluated.Transvaginal sonography done on 10th day of menstrual cycle for uterine scoring system for reproduction (USSR) scoring .USSR scoring includes parameters such as endometrial thickness,endometrial layering,myometrial contractions,myometrial echogenicity,uterine artery Doppler flow,endometrial blood flow,myometrial blood flow.

Results: Perifollicular PI <0.5 was found in 30 females out of which 24(80%)females conceived.18 cases had biophysical scoring ≥17 in which 15(57.6%) females conceived.Among 35 females with endometrial thickness 7-14 mm 66% females conceived.75.27% (22/29), 40% (4/10), females showed positive outcome in distinct trilaminar,indistinct trilaminar,respectively. 67.85% (19/28) females conceived with pulsatility index (PI) value 2.1-2.49.

Conclusion: There are many transvaginal sonographic(TVS) indicators that can predict the successful implantation by the Doppler study of the perifollicular blood flow, endometrial growth pattern and its perfusion.Evaluation of Perifollicular vascularity and uterine biophysical profile by colour doppler is reliable indicator of IUI outcome.More perfusion indicates more chances of likelihood of intrauterine implantation.Colour Doppler evaluation of uterine artery also showing low resistive index and high flow index or PI signifies favourable outcome.

KEYWORDS : Colour Doppler,Perifollicular Vascularity,Endometrial Receptivity,Intrauterine Insemination

INTRODUCTION

In Today's scenario Colour Doppler is emerging as a valuable diagnostic imaging modality in the field of reproductive medicine, primarily infertility. Reproductive disorders, including infertility, spontaneous abortions/ miscarriages,have emerged as a major public health problem(s) worldwide. With the advances in ultrasound (US) technology the management of infertility has changed.With the advent of colour Doppler,pulse Doppler,three-dimensional (3D) US and 3D power Doppler, the previously unexplained causes of failure of fertilization and implantation can now be explained. Infertility (clinical definition) is currently defined as 1 year of unwanted non conception with unprotected intercourse in the fertile phase of the menstrual cycle [1].Treatment for infertility may be medical, surgical or involve assisted conception.Successful implantation during in vitro fertilization(IVF) depends on many factors including embryo quality and uterine receptivity. Endometrial receptivity is defined as a temporary unique sequence of factors that make the endometrium receptive to embryonic implantation.Regulation of uterine and ovarian blood flow is an important aspect of reproductive physiology.Colour Doppler technology have been used to show that blood flow directed to the uterus and ovary undergoing important modification in relation to cyclic hormonal changes and can be easily detected using colour Doppler techniques.Ultrasound with colour doppler can give better idea about the functional maturity of the follicle and the endometrium, to decide the timing of human chorionic gonadotropin(hCG) administration for all assisted reproductive techniques for better pregnancy rates.The accuracy of diagnosis and monitoring of infertility treatments, such as ovulation induction,has greatly increased because of the availability of sophisticated US technology and equipments.[2]

Assessment of the follicular maturity and endometrial receptivity and for the time of hCG is one of the key factors for success of all ART procedures.The follicular maturity can be assessed by oestradiol levels,but regular assessment of blood oestradiol level is cumbersome. Since,the advent of the transvaginal ultrasound this has been a preferred method for assessment of follicle and endometrium.Earlier, the size of mature follicle of 16 to 18 mm and the endometrial thickness of 8 mm were considered optimum parameters for beta hCG administration for ovulation trigger.But this assesses only the anatomical maturity of follicle and endometrium.A more logical approach would be to analyse all the ultrasonographic and Doppler parameters together in order to assess uterine receptivity.It is now only implantation,which eludes scientific basis.To overcome this, various centres are increasing the number of IUI,which often leads to multiple gestations and painful embryo reductions, and spontaneous early pregnancy loss.It becomes an absolute necessity to evaluate the uterus and endometrium by colour Doppler imaging,so we can perform ART in only favourable uteri. IUI should be performed only in a favourable cycle based on the various scoring criteria like the uterine biophysical profile (UBP) and uterine scoring system for reproduction (USSR) [3] Our study is an endeavour to examine the ability of an ultrasonographic scores to assess uterine receptivity and perifollicular vascularity. Nowadays, ultrasonography plays a vital role in tracing development of follicle and endometrial in patients receiving ovulation. The maximal follicle size is the main point to consider giving betahCG.The sonographic findings which may help to predict the outcome have been weighed by many,but the uterine scoring system for reproduction ("USSR") by Michael[5]has been extensively used in assisted reproductive technique cycles.[4]

MATERIALS AND METHODS

Data Collection

Hospital-based prospective observational study was conducted during

controlled ovarian stimulation-intrauterine insemination (COS-IUI) treatment cycles for couples visiting to the infertility clinic of obstetric and gynaecology department at Chhatrapati Shivaji Subharti Hospital with the complaints of inability to conceive even after 1 year of regular unprotected coitus and with all baseline investigations (TVS,tubal patency test, day 2 follicle stimulating hormone(FSH) ,luteinizing hormone(LH) ,semen analysis,serum prolactin,and thyroid stimulating hormone)within normal limits. A total of forty cycles were studied.

Inclusion Criteria :

Forty Women aged <42 years, primary or secondary infertility lasting for at least 12 months, tubal patency assessed by hysterosalpingo graphy and/or laparoscopy, with at least one patent fallopian tube, normal semen analysis. Patients with normal ovarian function, regular spontaneous ovulatory menstrual cycles.

Exclusion Criteria :

Patients with coexisting disease such as bilateral tubal block, congenital uterine anomalies, associated male subfertility and infertility, patients who were started on ovulation induction regime but were not subjected to IUI due to inappropriate follicular growth (follicular size less than 16 mm till 22nd day of the cycle),patients with multiple dominant follicles (four or more) were excluded from our study considering the risk for multifetal pregnancies. Patients with prior ovarian surgery, endometriosis ,PCOD, poor ovarian reserve, smoking ,alcohol abuse were also excluded.

Method Of Data Collection

This prospective study approved by the IEC of Subharti Medical college & Hospital

Following a written informed consent from couples willing to be a part of the study, the history, examination and investigation findings were noted. All ultrasound measurements were performed using 6.5Hz transvaginal transducer with doppler facility (HS70,HS50 Samsung).The ultrasound examination was performed after the patient had emptied her bladder, and in lithotomy position.A baseline TVS was performed on day 2 of spontaneous cycle. In the event of a normal day 2 scan,she was stimulated with clomiphene citrate (50 or 100 mg)depending on antral follicle count.She was re-evaluated on day 5 of the cycle for recruitment and dominance of follicles. Subsequently,follicular growth was monitored every 2 days until the lead follicle was 15 mm in diameter and then on a daily basis till the 18 mm was attained.(figure1).

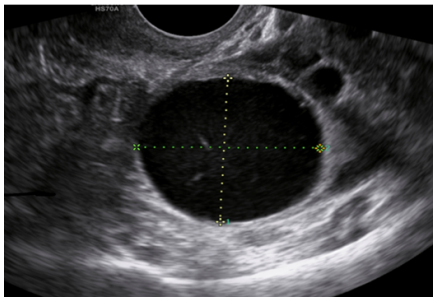

Figure 1 - Mature follicle on day 12 of a 30 year old female on ovulation induction

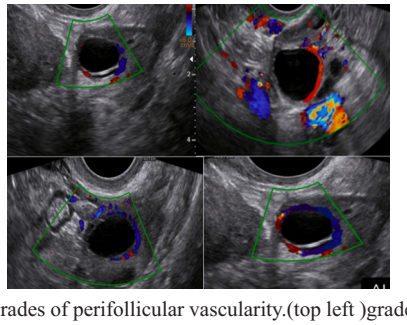

Figure 2 Grades of perifollicular vascularity.(top left)grade 1 (<25% of circumference) (top right)grade 2 (25%-50%) (bottom left)grade3 (50%-75%)(bottom right)grade 4 (>75% of circumference).

Perifollicular vascularity(figure2) and uterine biophysical profile was noted.The maximum thickness of the endometrium on both sides of the midline was measured in a longitudinal plane and was designated as distinct five-line appearance,hazy five-line appearance,or no layering(figure 3).

Figure 3 TVS images of uterus showing pattern of endometrium, indistinct endometrium(top),hazy 5 layered endometrium (middle),distinct 5 layered endometrium(bottom)

In principle,the central echogenic line represents the uterine cavity; the outer lines represent the basal layer of the endometrium or endo-myometrial interface.The relatively hypo-echogenic regions between these two represents the functional layer of the endometrium. Endometrial vascularization was assessed by placing the colour gate over the thickest part of the endometrium. The presence or absence of colour in zone3 of the endometrium (hypoechoic inner layer) was determined (figure 4).

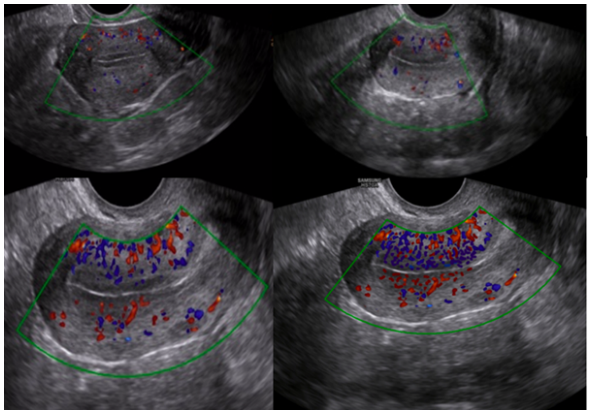

Figure 4 TVS images of uterus showing zonal vascularity of endometrium ,(top left) zone 1,(top right) zone 2,(bottom left) zone 3,(bottom right) zone 4

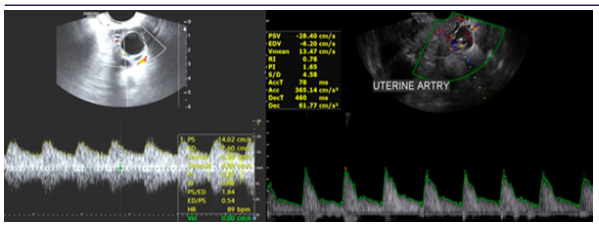

Figure 5 Preovulatory follicle with pulse doppler showing low resistance flow(left),uterine artery pulse doppler showing low pulsatality index and low resistance flow

Using colour Doppler,flow velocity waveforms (figure 5) were obtained from uterine artery on both sides of the cervix in a longitudinal plane.The pulsatlity index (PI) is measured from the flow velocity waveforms as the systolic peak velocity minus end diastolic velocity divided by the mean. The USSR score was evaluated, and injection of human chorionic gonadotrophin 10,000 units (HCG) was administered as ovulation trigger.IUI was planned 34–36 h post trigger. The USSR score was re-evaluated just before the IUI and was done with prepared semen samples with at least 15 million motile sperms. Post-IUI luteal support with micronized progesterone was provided. On the 14th post-IUI day, beta HCG values were estimated and repeated on day 16, and values of 25 IU/ml and above were considered pregnancy positive.If it was positive, ultrasound was performed 10–14 days later to confirm intrauterine pregnancy.

Doppler Features of a Good pre-hCG Follicle

Increase in perifollicular vascularity of dominant follicle in theca layer starts developing as early as 8th day of the cycle. Fall in perifollicular RI starts 2 days before ovulation, reaches nadir at ovulation, remains low for 4 days and then with gradual rise reaches 0.5 in midluteal phase.[5]When functionally mature,on colour Doppler, the follicle shows blood vessels covering at least 3/4th of the follicular circumference.Chui et al graded the follicular flow on the day of oocyte collection as grade 1 to 4 when in a single cross area slice the flow covered <25, 25 to 50, 50 to 75 > 75% of follicular circumference.The conception was related to grade 3 to 4 vascularity.[6]

Endometrium

The Uterine Scoring System for Reproduction ("USSR") was done and total score as per Applebaum criteria [3] was calculated.This comprised of evaluation of the following parameters:

Each parameter was scored as follows:

1. Endometrial thickness: (full-thickness measured from the myometrial-endometrial junction to the endometrial- myometrial junction)
a. < 7 mm = 0,
b. 7 - 9 mm = 2,
c. 10 - 14 mm = 3,
d. > 14 mm = 1

2. Endometrial layering: (i.e., a 5-line appearance)
a. no layering = 0,
b. hazy 5-line/ tri laminar appearance = 1,
c. distinct 5-line / tri laminar appearance = 3

3. Endometrial vascularization within zone 3:
a. absent = 0,
b. present but sparse = 2
c. present multifocally = 5

4. Myometrial echogenicity:
a. coarse/inhomogeneous echogenicity = 1,
b. relatively homogeneous echogenicity = 2

5. Uterine artery Doppler flow evaluation [Pulsatlity Index – PI]:
a. PI > 3.0 = 0,
b. PI< 2.5 - 2.99 = 0,
c. PI < 2.2 - 2.49 = 1,
d. PI < 2.19 = 2

6. Endometrial blood flow within Zone 3:
a. absent = 0,
b. present, but sparse = 2,
c. present multifocally = 5

7. Myometrial blood flow internal to the arcuate vessels seen on grey-scale examination:
a. absent = 0,
b. present = 2

Total USSR of all the patients were calculated using above parameters and timed intercourse was advised accordingly. According to Applebaum, the endometrial and periendometrial areas are divided in terms of endometrial vascularity into the following four [7]

Zones:

Zone 1 -- 2 mm thick area surrounding the hyperechoic outer layer of the endometrium
Zone 2 -- hyperechoic outer layer of the endometrium
Zone 3 -- hypoechoic inner layer of the endometrium
Zone 4 -- endometrial cavity

Colour Doppler has become a high-throughput, sophisticated imaging technique for the assessment of uterine anomalies, intrauterine pathology, tubal patency, polycystic ovaries, ovarian follicular monitoring,endometrial receptivity, failed pregnancy and/or ectopic pregnancy, male infertility, and uterine,endometrial,and ovarian vascularity[8-13]. Human chorionic gonadotropin (hCG) and power Doppler assessment of the follicle has been implicated in improving pregnancy rates in IUI[14]. Ectopic and multiple pregnancies are emerging as one of the leading causes of morbidity and mortality in women of childbearing age and untreated, ectopic pregnancy can lead to massive haemorrhage and infertility, eventually resulting in death;[15] So high-resolution colour Doppler imaging has become a boon for early prevention of pregnancy related deaths in women worldwide. [16]

RESULTS

The perifollicular vascularity was associated with better pregnancy rate. No pregnancy was achieved with perifollicular vascularity with grade 1 and grade 2 (which is highly significant $p < 0.001$). The pregnancy rate was 19% with grade 3 perifollicular vascularity (not significant) and was 68% with grade 4 perifollicular vascularity (which is highly significant $p < 0.001$).Our data[table 1,2]

Table1

Follicular PSV	</=9	>10
conception	7(26.6%)	19(73%)
no conception	7	7

Table 2

Follicular RI	<0.5	0.50-0.56
conception	24(92.3%)	2(7.6%)
no conception	6	8

shown that when the perifollicular RI > 0.53 and PSV < 9 cm/sec, 12 hours before hCG injection, the conception rates were only 7.6% and 26.6% respectively as compared with 92.3% and 73.0% respectively and individually when perifollicular RI <0.50 and PSV >11 cm/sec.

A total of 40 female patients were included in this study for both primary and secondary infertility excluding uterine abnormalities causing distortion of endometrial cavity or tubal block. The mean age of all cases was 29.12 years (range = 19-42 years). The youngest patient was 20 years old, with 2 years duration of infertility. The eldest female was 41 years old with 10 years of infertility. Among 40 females, 26(65%) conceived [Figure 6]

Chart -1 Number Of Pregnancies.

and was confirmed by rising beta-HCG level and/or presence of

gestational sac by ultrasonography. Two patients had abortion at 5-7 weeks of pregnancy with unexplained etiology.18 cases had biophysical scoring ≥17 in which 15(57.6%) females conceived [Chart -1].

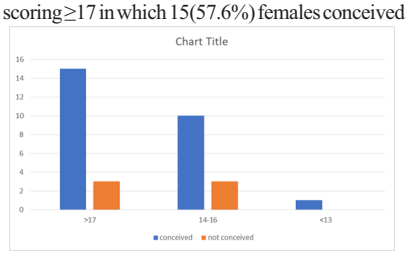

Chart 2 -pregnancy Count With Relation Of USSR Score .

13 patients had biophysical scoring 14-16 and 17 patients had BPs ≤13 in which 38.4% (10/13) and 3.8% (1/9) conceived, respectively. Out of Twenty-six pregnant patients, 11 patients had endometrial thickness 7-10 mm, 13 patients had endometrial thickness 10-14 mm, 2 patient had endometrial of >14 mm[Chart 2].

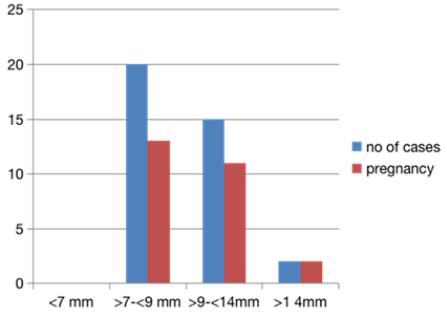

Chart 3 Endometrial Thickness In Conceived Patient.

We found significant relation between endometrial thickness and pregnancy rates, highest (50%) with endometrial thickness ranging from 10 to 14 mm ($p < 0.05$). However not all patients with thicker endometrium had positive outcome. In case endometrial thickness was 6-8 mm, it showed a significantly lower rates of pregnancy ($p < 0.01$). Correlation: Pearson coefficient between endometrial thickness and pregnancy is 0.31, it is a positive correlation which suggest that more the endometrial thickness more the pregnancy rate. There is significant correlation between pregnancy rate and endometrial vascularity. Of 23 patients with multifocal vascularity in zone 3/4, 20 patients (86.5%) had positive outcome(highly significant $p < 0.001$)[Chart 3].

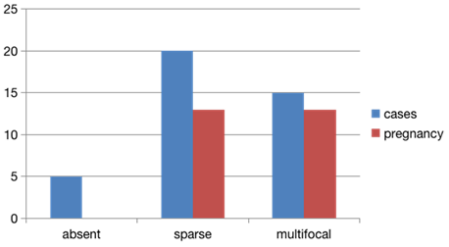

Chart- 4 Multifocal vascularity

Whereas only 37% (6 out of 16) patients had conception with sparse vascularity in zone 3/4. No conception was reported in women having zone 1/2 endometrial vascularity (highly significant $p < 0.001$,significantly lower chances of pregnancy if in zone 2). Statistically higher pregnancy rate (81%) was reported in women with distinct five line appearance ($p < 0.05$). Patients with no endometrial layering had no conception and 36% pregnancy was seen with hazy five line appearance[Chart- 4].

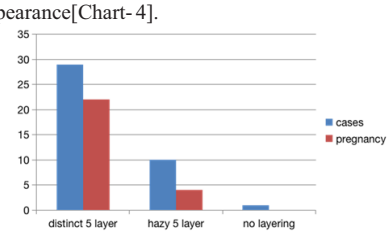

Chart- 5 Endometrial Morphology

Lesser the uterine artery impedance, more the number of pregnancies reported. No pregnancy was reported in women when uterine artery impedance PI was more than 2.99 (statistically significant $p < 0.05$).There was significant difference in pregnancy rates when uterine arterial PI was low, 67.85% (19/28) females conceived with pulsatility index (PI) value 2.1-2.49.

DISCUSSION

In the field of assisted reproduction, transvaginal ultrasonography is often used to examine endometrial thickness, morphology and blood flow status to predict uterine receptivity [17]. However, many issues, such as time of ultrasonography examination and its value in predicting IUI outcome remain uncertain.In our study we found that the perifollicular vascularity was associated with better pregnancy rate. No pregnancy was achieved when perifollicular vascularity was grade 1 or grade 2. The pregnancy rate was 19% with grade 3 perifollicular vascularity (not significant) and was 68% with grade 4 perifollicular vascularity (which is highly significant $p < 0.001$). Bhal et al in their study reported that the pregnancy rate was low in the low-grade perifollicular vascularity, and it was higher in cycles with grade 3 and grade 4 perifollicular vascularity. [18] Stefano et al also confirmed that the perifollicular vascularity is related to good-quality oocytes. [19]Endometrial receptivity plays an important role in the favourable outcome of IUI. In this study, we investigated for the USSR by TVS with Doppler supplementation which improved the predictability of endometrial receptibility.The mean age of female was 29.12 years (range = 19-42 years) in our study. The mean age of women was 31 ± 5.4 years (range = 18-45 years) in a study conducted by Aghahoseini *et al*. [20]In this study, 65% (26/40) female conceived. Miscarriages were found in 2 patients at 5-6 weeks of pregnancy due to unexplained aetiology. No ectopic pregnancy was found in our study group. According to Aghahoseini *et al*., pregnancy rate occurred in 32.2% of female.[20] Kupesic *et al.* found that fertility occurred in 31.5% women. [21]In our study, biophysical score between 17 and 20 was associated with 57.6% pregnancy rate. Score between 14 and 16 and 13 or less was associated with 38.45% and 3.8% pregnancy rate, respectively. In some study, it has been found that score between 17 and 19 was associated with 77% pregnancy rate and between 14 and 16 associated with 60% pregnancy rate and below score 13 or <13 were associated with poor pregnancy outcome.[3]In our study,87.5% of patients had endometrial thickness between 7 and 14 mm and among them,71.4% of females conceived. 20% (1/5) females were conceived when the endometrial thickness is <7 mm. Hence, significant association was found between implantation rates and endometrial thickness.Glissant *et al*. found that endometrial thickness was significantly greater in cycles resulting in pregnancy than those, which did not. [21 Welker *et al*. were unable to relate endometrial thickness to outcome.[22]These confusing results may be due to: (1) Different sonographic techniques such as transabdominal and TVS, (2) different ovarian stimulation protocols. In this study, endometrial pattern was found to be triple line showing conception in 81% and it drops down suddenly to 36% in indistinct trilaminar endometrium. Kupesic *et al.* also found that endometrial pattern was triple line in 58.9%, indistinct in 3.4% and homogeneous in 29.1%. Success rate of implantation were significantly more in endometrium with triple-line pattern. Welker *et al*. found that triple-line appearance of endometrium was more likely to be associated with implantation. [22] Multiple studies showed lower implantation rate in female exhibiting homogeneous endometrium pattern. However, another study by Kupesic *et al.* showed that the homogeneous hyperechoic endometrial texture is favourable for implantation.[23]In this study, 86.5% (20/23) females showed positive outcome with abundant flow in zone 3 and 37.6% (6/16) female only if zone 3 showed sparse flow. Absent endometrial blood flow has been associated with no conception in spite of good scoring for other parameters. According to Zaidi *et al*., absence of endometrial blood flow was associated with failure of implantation in IVF-cycles.[24]In our study, 67.85% (19/28) females conceived with PI value 2.1-2.49; 25%(1/4) and 66.6% (4/6) females with PI value 2.5-2.95 and <2,respectively. No pregnancy occurred with PI value above 3. Steer *et al*. demonstrated that patients with low PI at the expected time of ovulation were more likely to conceive than those with high PI.[25]Battaglia *et al*. also showed that gradual and progressive decline in PI at mid-cycle have been associated with better outcome of assisted reproductive techniques.[26] Cacciatore *et al.* also found that implantation became very unlikely when PI was >3.3.[27]

Future Perspectives:

Future research and development efforts in the field of reproductive

medicine are warranted further to provide more meaningful interpretation of the sophisticated colour Doppler-based diagnostic imaging, thereby significantly increasing the success rates of IVF among infertile couples. Moreover,multicentric and/or multinational research and development efforts may incorporate case-control/gene epidemiology, animal model, in vitro cell-based assays, and clinical trials for biomarker development utilizing genomics, proteomics, transcriptomics, and metabolic approaches for efficient management of infertility patients of ethnically disparate populations worldwide.

CONCLUSION

Ultrasound has actually become one of the indispensable diagnostic tools in the field of obstetrics, gynaecology and the clinical application in infertility as well. Colour Doppler is a high-throughput sophisticated imaging technique for the assessment of ovarian follicular monitoring uterine and endometrial vascularity.TVS and power doppler ultrasound are highly reproducible technique for the clinical stage of infertility.

Conflict Of Interest

The author have no competing interest to declare.

Ethical Approval

This research received the local Ethical Committee approval.

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