Perifollicular vascularity as a potential variable affecting outcome in stimulated intrauterine insemination treatment cycles: by using transvaginal power Doppler

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الخلاصة

اجريت هذه الدراسة لمعرفة العلاقة بين نسبة سريان الدم في البيضة الناضجة واحتمالية حدوث الحمل بعد عملية تنشيط البيضة و التلقيح الاصطناعي.

خمسة و سبعون مريضة تم تنشيط بيوضهن باخذ مادة الكلومفين ستريت 100 مل غم من ثاني يوم الدورة ولمدة خمسة ايام و تمت متابعة تنشيط البويضات بواسطة السونار المهبلي وبعد ان تصبح البيضة ناضجة يتم زرق المريضة بعقار ا ل اج . سي . جي ، بعدها باربع و عشرين ساعة نقوم بعمل الدوبلر التراساوند المهبلي لقياس نسبة جريان الدم في البيضة الناضجة وبعد ستة و ثلاثون ساعة من تاريخ زرق عقار ا ل اج سي جي ونقوم بعملية التلقيح الاصطناعي للمريضة وبعد ذلك باسبوعين نقوم بعمل تحليل الحمل ومعرفة النتائج.

تم دراسة نسبة جريان الدم لخمسة و سبعون بيضة ناضجة وتم تقسيم الحالات حسب نسبة الجريان الى ثلاث مستويات : المستوى الاول تكون فيه نسبة جريان الدم منخفضة (13.3 %)، المستوى الثاني تكون فيه نسبة جريان الدم متوسطة (32 %) و المستوى الثالث تكون فيه نسبة جريان الدم عالية (54.6 %). في هذه الدراسة وجدنا ان بسبة حدوث الحمل اكثر في المستوى الثالث الذي تكون فيه نسبة جريان الدم عالية (19.5 %) مقارنة بالمستوى الثاني (12.5%) و المستوى الاول (صفر %) . بالاضافة الى ذلك وجدنا ان نسبة فقدان الحمل في مراحله الاولى تكون اقل كلما كان مستوى جريان الدم عال (12.5 للمستوى الثالث و 33.3% للمستوى الثاني لجريان الدم). كما اننا وجدنا ان نسبة جريان الدم تقل مع زيادة العمر والفترة الزمنية لعدم او تاخر الانجاب .

نستنتج من هذه الدراسة اننا يمكننا الاستعانة بالدوبلر التراساوند المهبلي في تشخيص و متابعة حالات العقم و تاخر الحمل .

Abstract

The aim of study was to asses any potential relationship between perifollicular vascularity and fetal outcome in an invivo environment following ovarian stimulation and intrauterine insemination.

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A total of 75 unselected consecutive patients undergoing stimulated intrauterine insemination cycles (with clomid) was recruited where the perifollicular vascularity of the follicles ≥16 mm was studied using subjective grading system by transvaginal power Doppler ultrasonography 24 h after HCG administration.

36 hours after H.C.G administration we did intrauterine insemination and two weeks later we did pregnancy test for our patients.

A total 75 mature follicles (> 16 mm) was studied. According to the result of Doppler study we found that we had 54.6% of follicles having grade III perfollicular vascularity , 32% having grade II , and 13.3% grade I. The follicles of high grade vascularity were associated with higher pregnancy rate (grade 3= 19.5%) than cycles with low grade vascularity (grade2 =12.5%), with no pregnancy occur in grade 1 vascularity group. Early pregnancy loss rate was significantly higher in grade2 follicular vascularity (33.3%) than grade 3 (12.5%) .

The mean age and duration of subfertility were significantly higher ($P<0.05$), with low follicular vascularity grades compared with grade II, III.

These data would suggest that perfollicular vascularity and PI (pulsatility index) of uterine artery has an important role to play in the outcome of IUI cycles. And that the power Doppler has the potential to refine the management of assisted reproduction treatment cycles.

Introdution

Infertility defines as Inability to conceive after one or two years of unprotected intercourse. Data from population- based studies suggest that 10-15% of the couples in the western world experienced infertility. Half of them 8% will subsequently conceive without the need for specialist advice and treatment remaining 8% require input from fertility clinics.[1].

Fecundity: is the probability that a single cycle will result in alive birth.[2]

Fecundability: is the probability that a single cycle will result in pregnancy.[2,3].

Male factor: No demonstrable cause , varicocoele, Idiopathic oligozoospermo , Isolated seminalplasma abnormalities , suspected immunological infertility , congenital abnormalities systemic disease , sexual inaclequacy , obstructive azoospermia , pituitary lesions Gonadotrophin deficiency.[1-4].

Female factor: causes

Ovarian factors: Ovulatory dysfunction this condition may be severe enough by itself to prevent conception (an ovulation) or simply be a contributing factor (oligoovulation). Different methods can be used to determine if and when ovulation occurs Directly or Indirectly these are:

1- Menstrual History: menstrual inistory alone after sufficient to establish a diagnosis of anovulation. Menes in normally ovulate women generally are regular. 2-Basal body Temperature. 3-Serum progesterone concentration. 4-Urinary LH Excretion. 5-Endo material biopsy. 6-Ultra sound.[1-5]

Cervical factor: Abnormalities of sperm test used post Goital test.[5]

Uterin factor: Anatomic and functional Abnormalities, it is uncommon cause of infertility but should always be considered. Investigation used :

1- Hysterosalpingography.2-Trans vaginal ultrasound and sonohysterography. 3-Hysteroscopy [6] .

Tubal factor: Tubal occlusion and adnexal Adhesions.[6,7]

Test used in diognesis [1] HSG [2] Laparoscopy.

Unexplained infertility: is diagnosed when all of the standard element, of infertility evaluation yield normal results.[8]. At a minimum the diagnosis of unexplained infertility implies anormal semen analysis, objective evidence of ovulation. Normal uterine cavity and bilateral tubal patency [9].

Treatment of infertility: All couples trying for pregnancy will benefit from some general advice such as cessation of smoking and limiting alcohol intake also involve weight loss in women with

B.M.I over 30. The most important cause an ovulation treated by Induction of ovulation**:** Drugs used are :

Clomiphene citrate : is an orally active synthetic non steroidal compound with oestrogenic as well as antioestrogenic properties which has traditionally been treatment of choice in women with anovulatory PCOS. It displaces Oestrogen from its receptors in the hypothalamic- pituitary axis reduce the negative feed back effect of oestrogen and encourages GnRH secreation. It administered in an initial daily dose of 50mg on day 2-6 of aspontaneous or induced menstrual period. The dose can be increased by 50 mg per day till ovulation is achieved up [10].

Gonadrophins : used when no respond to 6-12 ovulatory cycles, preparations in common use include recombinant F.SH, or purified urinary human menopausal which contains F.SH and LH.[11].

Assisted reproductive technologies:

ART encompass all techniques involving direct manipulation of occytes outside the body.

Types:

1- In vitro fertilization I.V.P. 2-Intra cytoplasmic sperm injection (ICSI).3-Gamete intrafallopian transfer.(GIFT). 4-Zygot intrafallopian transfer (ZIFT).[12,13].

Other method help in treatment of infertility is I.U.I. especially in treatment of male factors causes [13, 14] .

I.U.I

Artificial insemination, has been used to treat infertile couples for almost 200 years, and is an accepted form of treatment for men with sever hypospadias, retrograde ejaculation, neurologic impotence and sexual dysfunction. Artificial insemination has also been used as ameans to overcome oligospemia, athenospermia. Low ejaculate volumes, Antisperm antibiodies and cervical factors, treatment for sever uncorrectable male factor infertility, inherited gentic disorolered in the male partner, and single or lesbian women who desir pregnancy, before and advent of I.V.F and ICSI [15,16]. The rational of intrauterine insemination (I.U.I) with washed

spermatozoa involves by passing the cervical mucus barrieng resulting in an increased gamete density at the site of fertilization. Better washing procedures are believed to enhance sperm fertilization ability both in vitro and environment which may lead to higher success rates after I.U.I in male factor causes I.U.I has significant cost savings and less invasive, without necessarily a reduction in effectiveness, compared with other form of assisted reproduction treatment such as I.V.F , or gamete intrafallopian transfer (GIFT) in particular for nontubal infertility [17,18]. So artificial insemination may be performed by despositing sperm into cervical os or directly into the uterus, but I.U.I is now almost universally performed for several reasons. First when trying to over-come the limitations of decreased sperm density or motility in treatment of male factor infertility, cervical. Insemination offers no significant advantage over whole can be achieved by intercourse second where as the potential for reactions to proteins, prostaglandin, and bacteria in semin severely limits the volume of untreated semen (and thus the numbers of sperm) that can be delivered to the upper female genital tract I.U.I with ' washed' sperm constrate (devoid of seminal plasma) delivers most of the sperm in an ejaculate, most importantly,[19,20] .

It is difficult to gauge the effectiveness of I.U.I using the sperm of the infertile male because almost all of the many published series examining I.U.I cycle out comes have included couples with a variety of infertility factors and have employed combined treatment with I.U.I an empiric ovarian stimulation. There are ample data from retrospective studies of out comes in therapeutic donor insemination cycles (I.U.I with or without ovarian stimulation), but the results achieved using infertile partner sperm cannot be expected to equal those using normal alone sperm. Considering all of relevant variable, the available data suggest that cycle fecundity range between 3% and 10% when I.U.I is performed using infertile partner sperm [6,7,21] and is approximately 3 times higher (9.30%) when donor sperm are used.[8,9,22].

The use of ovarian stimulation by gonadotrophins or clomiphene citrate in I.U.I is now wide spread and has shown to improve significantly the odds of pregnancy [4,23] so super ovulation combined with washed I.U.I has been advocated for the treatment of various forms of infertility when more traditional therapy has failed (4,6,24) .

It has long been recognized that neovascularization may be of prime important in the growth and selection of ovulatory follicles. (7,8,25) . During menstrual cycle LH-driven luteinization is marked by a profound increase in the vascularization of pre-ovulatory follicle through out this process, some granulosa cell derived products are likely to promot blood vessles out-growth. So it possible that vascularization status of ovarian follicles influences their reproductive competence by regulating oxygen supply to the oocytes [9,26]. Several studies have examined the cyclical variation of female pelvic haemodynamics and angiogenesis using Doppler.u/s. (27).

Color Doppler

Color Doppler imaging has had a great impact on ultrasound . This technique depicts local flow by encoding an estimate of the mean Doppler frequency shift at a particular position in color [28].

With this form of display areas of blood flow are represented as color within the image , it has become common practice represent flow toward the transducer as red and flow a way as blue .

Pulsed Doppler u /s aimed directly to measure the speed of blood flow in particular uses.

power Doppler: advantages over color Doppler its higher sensitivity to flow and depiction of continuity of flow . The display is thus a map of the distribution of moving red cells [29].

The RI and PI (resist and pulsatiliy) are commonly used as Doppler indexes of vascular impedance

$$RI = \frac{\text{maximum velocity - minimum velocity}}{\text{maximum velocity}}$$

$$PI = \frac{\text{maximum velocity - minimum velocity}}{\text{Time avaraged maximum velocity of the cycle}}$$ [27,30]

The wave forms from the uterine and ovarian arteries can be studied in most women especially if transvaginal route is used . They normally slow periodic changes during the menstrual cycle , but these are modified or lost in some an ovulatory cases of infertility.

Successful treatment of the underlying cases of the infertility may be confirmed by a return of the normal changes . Similarly a much reduced peripheral resistance can be detected in the artery supplying an ovary that is about to ovulate . [31,32]

So Doppler us used to confirm the possible relation ship between vascularization reproductive competence of ovarian follicles most of them show appositive association between follicle vascularization and ovarian responsiveness to controlled ovarian stimulation (33,34) so Doppler u\s use to assess uterine and ovarian blood flow patterns. In ART cycles statistical models have been reported for predicting success rates which suggested that follicle number, duration of subfertility were the most significant variables in predicting outcome. The application of Doppler u/s in I.U.I has tended to relate uterine perfusion to outcome. So the aim of the present study was to asses any potential relationship between the perifollicular vascularity following ovarian stimulation and outcome in an invivo environment using the subjective grading system of power Doppler u/s.

Materials and Methods

This was a prospective cross-sectional study of unselected consecutive stimulated I.U.I treatment cycles carried out at fertility center in Al-Saddar hospital between January 2009-November2009. All patients had tubal patency tests in the form of laparoscopy or hysterosalpingography , to confirm tubal patency.

A total of 75 treatment cycles was studied . The main exclusion criteria included couples with a severe male factor where the sperm wash preparation was $<20\times10^6$ motile spermatozoa on the day of I.U.I patients recruited into the study provided verbal

consent to partake in this study , clomiphine citrate 100 mg / day was given day 2 of the cycle for 5 days under went pelvic ultrasonography scanning to exclude pelvic abnormalities such as ovarian cyst before commencing ovarian stimulation.

The standard starting dose for stimulation was clomid 100 mg at day 2 for 5 days with frequent vaginal ultrasound measurement for the size of ovarian follicle till it become mature where human Chorionic Gonadotrophin (pregnyl) in a dose of 10000 iu/ IM was given. Trans vaginal Doppler assessment (By fukoda .powerdoppler (u/s) was performed 24 hours following the administration of HCG when the lead follicle was ≥16-18 mm in diameter .

The largest or mature follicles was graded using power Doppler based on a subjective grading system . Variables used to quantify vascularity have changed from mathematical variables such as pulsatility or resistance index of the intra- ovarian or ovarian artery blood flow through semi-quantitative evaluation of follicular vascularity at present the most widely used quantification grading is based on follicular circumference in which flow is identified . This grading system was based on % of perifollicular circumference (in 25% increment) that depicted an echo signal and ranged from grades (1-4). In our study we divide our patients into three groups grade I ,grade II and grade III according to perifollicular vascularity grading. Other parameters measured included mean uterine pulsatility indices . According to (PI). Also the patients divided to three groups: first group < 2, second group (2-3), third group > 3.

Then I.U.I using prepared washed semen (the solution used in washing sperm is global solution) was carried out. In term of evaluating outcome in relation to follicular vascularity treatment cycles this was based on the vascularity grades and PI indices .

Statistical analysis: The SPSS (statistical package for social sciences) 15 for Windows was used for all analysis. Descriptive statistics (mean, standard deviations and percentage), and t-student test were used to demonstrates differences between means.

One Way ANOVA was assessed for comparison of the three studied groups of grades. $P \leq 0.05$ was considered statistically significant. Chi ^{2} test was used to compare categorized variables.

Results

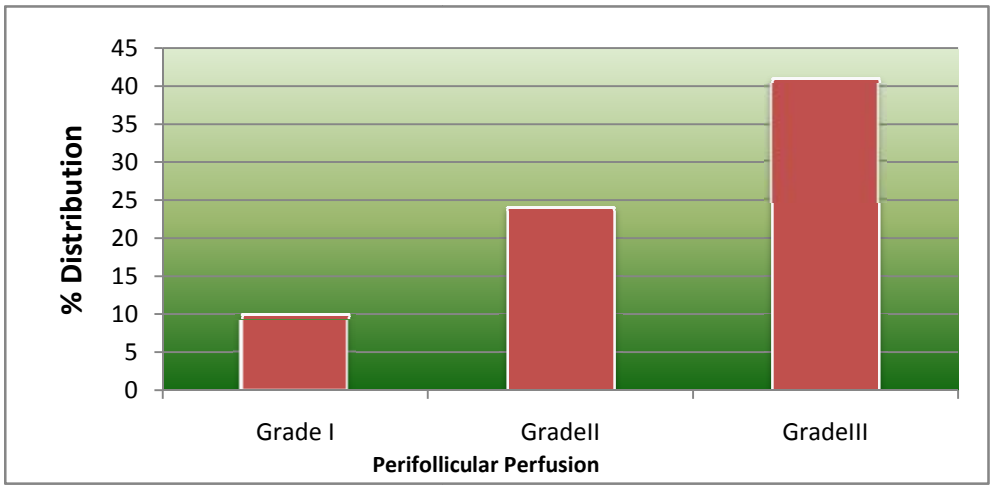
Seventy five patient were recruited distributed according to the grade of follicular vascularity as shown in table 1, figure1:

Grade 1, n=10 (13.33%), grade2, n=24 (32%), grade 3, n=41 (54.67%), grade 4, n=0.

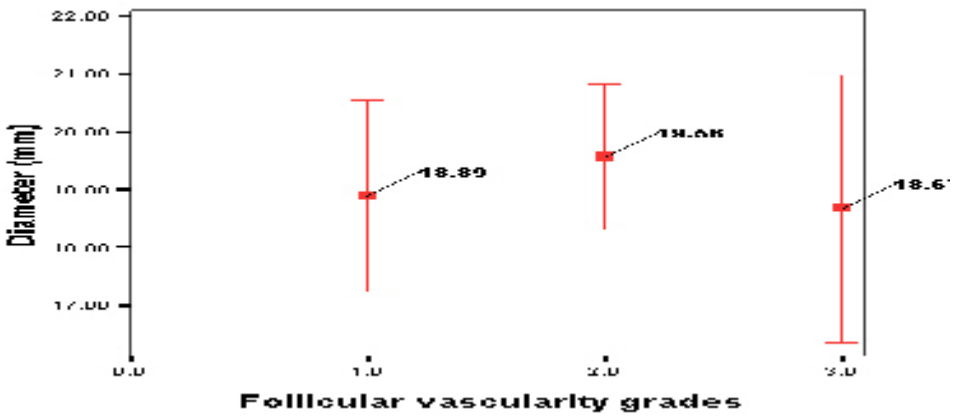
The distribution of all follicles studied (all ≥ 16mm in diameter) is depicted in figure2. Vascularity grading was seen to be independent of follicular size among follicles ≥ 16mm on the day of IUI.

Table(1)Number of Cycles and mean of ova diameters according to Perifollicular Perfusion grades among 75 patients studied

Parameters Grades	Grade I	Grade II	Grade III
No. of Cycles	10(13.33%)	24(32%)	41(54.67%)
Ova Diameter(mean)	18.89±2.028	19.56±2.331	18.67±6.115



Figure(1). Distribution of Perifollicular Perfusion grades among 75 patients studied.



Figure(2). Mean follicular diameter (mm, ± 95%CI) in relation to follicular vascularity in the intrauterine insemination study population.

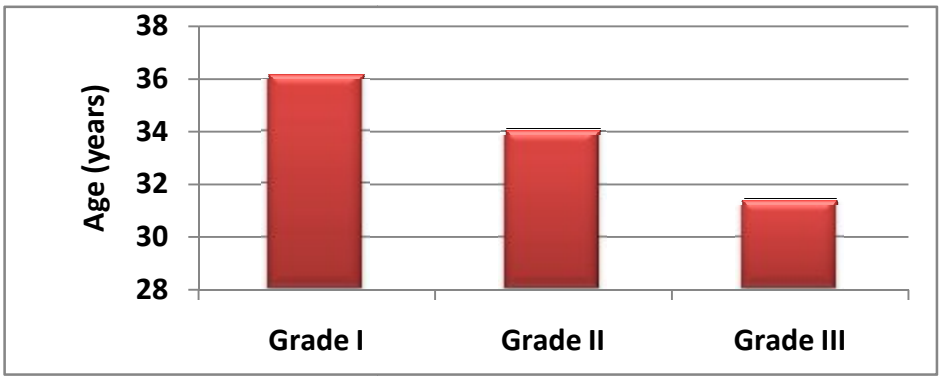
An unpaired t-test was used to compare mean diameters between the three grades, all comparisons are not significant.
Base line clinical characteristics according to the grade of follicular vascularity are summarized in table 2:

Table (2). Data on patient characteristics

	Grade I (N0.10)	Grade II (N0.24)	Grade III (N0.41)
Age of Female Partener(Years)	36.17±0.69	34.06±0.24	31.40±0.46*[a]
Duration of Subfertility	8.11±0.42	6.98±0.80	5.10±0.10*[a]
FSH (IU/I)	7.52±0.96	8.25±0.20	6.42±0.59N.S
Primary type infertility(%)	7(70%)	16(66.67%)	23(56.09%) N.S

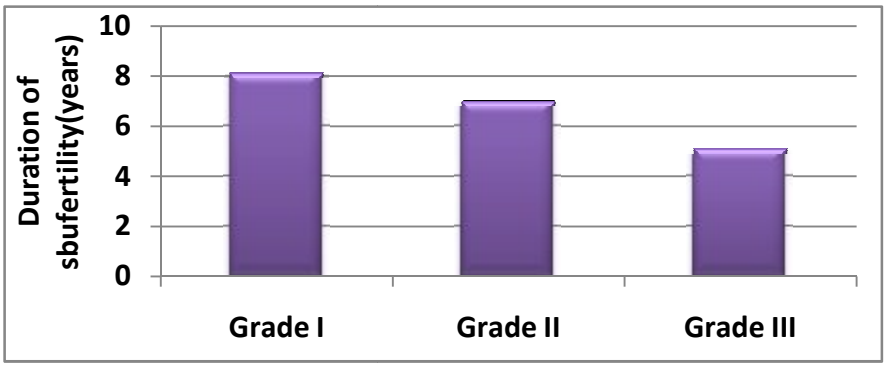
Values are mean ±S.E , values between parentheses are percentages , *[a] significant differences between Grade III and Grade I at P<0.05, N.S not significant.

The mean age was higher within low grade follicular vascularity (grade 1, 36.17 ± 0.69 and grade 2, 34.06 ± 0.24) compared with the higher grade follicular vascularity (grade 33, 31.40 ± 0.46) with $p < 0.05$ which is significant as shown in figure 3.



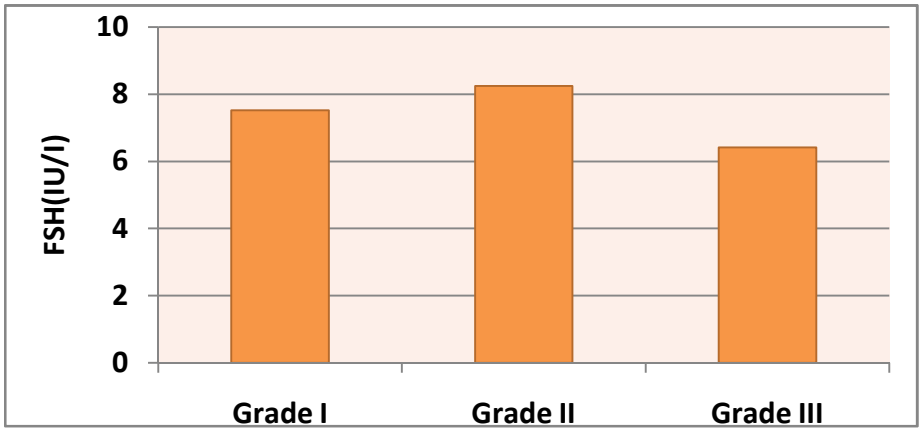
Figure(3) Mean of Age of Female Partner according to Follicular Vascularity Grades.

Also duration of subfertility were significantly higher ($p< 0.05$) in the group of treatment cycle where all follicle ≥ 16 mm had lower grade vascular perfusion compared with high grade population, table2, figure 4.



Figure(4) Mean of Duration of subfertility of Female Partner according to Follicular Vascularity Grades.

The mean base line serum FSH concentration also tended to be higher in low grade vascularity cycle compared with high grade cycle (7.5 ± 0,96, 8.25 ± 0.20 compared with 6.42 ± 0.59) respectively. But this difference was not significant (table 2, figure 5).



Figure(5) Mean of FSH (IU/I according to Follicular Vascularity Grades.

The patient characteristics, included parity or type of sub fertility were similar between all three vascularity cohorts. (primary type of infertility percent found to be 70%, 66.67%. 56.09% in grade 1,2 and 3 respectively), table 2.

There were 11 pregnancies, giving a pregnancy rate 14.66%, of which 2 pregnancy loss occur giving pregnancy loss rate 18.18% .

The follicles of high grade vascularity were associated with higher pregnancy rate (grade 3= 19.5%) than cycles with low grade vascularity (grade2 =12.5%), with no pregnancy occur in grade 1 vascularity group, as shown in table 5. this result is significant (as $p< 0.05$).

Early pregnancy loss rate was significantly higher in grade2 follicular vascularity (33.3%) than grade 3 (12.5%) with $p< 0.05$ which is significant as shown in table 5.

Table (5). Clinical pregnancy and early pregnancy loss rates of treatment cycles.

Parameters	Grade III	Grade II	Grade I	Chi²*
Pregnant cycles	8(19.5%)	3(12.5%)	0(0%)	P<0.05**
Pregnancy losses	1/8(12.5%)	1/3(33.3%)	0(0%)	P<0.05**
Ectopic Pregnancies	0(0%)	0(0%)	0(0%)	
No. of Cycles	41	24	10	

*Chi2/ Fisher's Exact test , ** significant differences at P<0.05 (df,2)

Mean uterine artery pulsatility index (UAPI) of < 2 associated with high fecundability rate (25%) while fecundability rate was lower (6.66%) in the group where the mean UAPI was 2-3 compared with those group when UAPI of >3 was associated with no pregnancy ,as shown in table 6 and figure (8)and figure (9).

Table (6). Relation between Pulsatility index of the ascending branch of the uterine arteries and fecundability rate

Pulsatility index	Less than 2	2-3	More than 3	Chi²
Fecundability	25%	6.66%	0	0.002**
Grade I	5(45.45%)	3(27.27%)	2(18.18%)	0.002*
Grade II	11(45.83%)	10(41.66%)	3(12.5%)	0.000**
Grade III	20(78.78%)	17(41.46%)	4(9.76%)	0.000**

* significant differences at P<0.05, ** significant differences at P<0.01

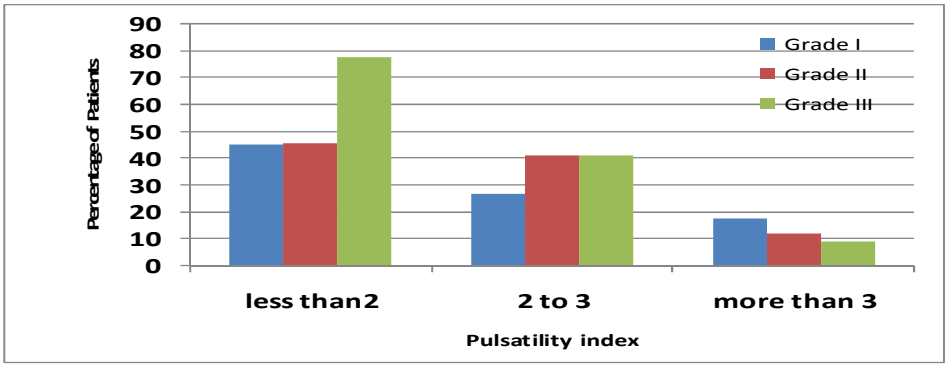


Figure (8). Relation between Pulsatility index of the ascending branch of the uterine arteries and Percentage of patients according to Follicular Vascularity Grades.

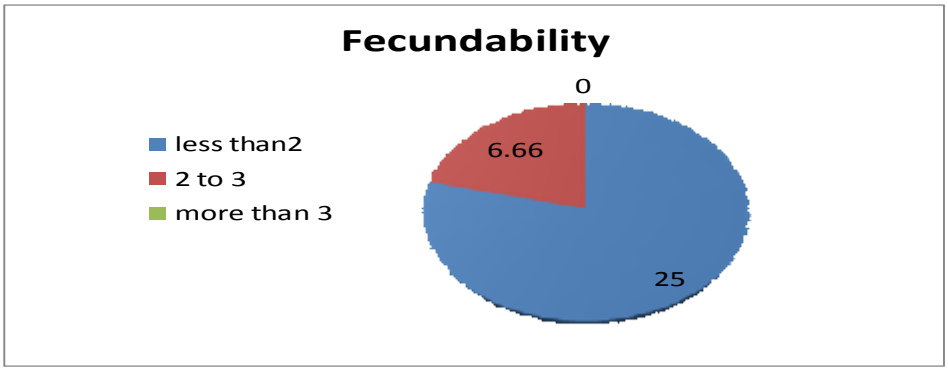


Figure (9). Relation between Pulsatility index of the ascending branch of the uterine arteries and Fecundability

Discussion

In this study we evaluate the perifollicular vascular perfusion and uterine artery PI as prognostic indicator that influence pregnancy outcome and therefore provide another prognostic indicator for success.

We use the developed grading system to obtain a semiquantative assessment of the presence of an echo signal detected by Doppler (grade1-4 vascularity).

Despite its subjectivity it achieve a good interobserver agreement with a good applicability and reproducibility (33,34) .

Our result have only 3 grades of perifollicular vascularity (grade1 , 2 and 3) . The reason may be the type of ovulation induction used as we use only clomiphene citrate 100mg per day for 5 days not like other studies which used controlled hyperstimulation program for ovulation induction and some studies was for I.V.F treatment. In our study we find no relationship have been confirmed between follicular size and vascularity of follicles, this result correlate well with Daniel et al; 2007, Bhal et al; 2001(34,35) and in contrast with Bhal et al;1999 and Balakier and Stronall ;1994 (36). One explanation could be that the coefficient of variation for inter and intraobserves error in relation to follicular diameter measurement in some study were 4-6% and 2-9%

resgrectively (37, 38) . Further causes could be related to the instrument used to asses follicular vascularity and diameter.

It is now recognized that advancing maternal age (feedback et al; 1994; Campona et al; 1996; Brezecgffa and Buylaos 1997; Legro et al) (40) and duration of subfertility (Tucker et al ; 1990; Tomolinson et al; Noujua, Huthunen et al 1999) (39) have major impact as important predictive variable in the success of I.U.I treatment cycles.

There is a significant relationship between maternal age and duration of subfertility with perifollicular vascularity grades with high mean age (36.17 ± 0.69) among grade I follicular perfusion compared with high grade vascularity (grade III) in low mean age (31.40±0.46). Therefore it could be postulated that the changes which affect follicles number , oocyt or emberyo quality in relation to age /duration of subfertility might affect follicular vascularity in a similar manner (40,41).

In addition , there was a correlation between mean baseline FSH concentration and low grade vascularity (7.52±0.96 in grade 1 compared to 6.42±59 in grade 3) even non significantly , This suggest that the postulated link between reduced ovarian reserve and responsiveness to stimulation with elevated based FSH concentration noted by other study (Balasch et al;1996) (42 , 43) might have an ovarian vascular component.

The difference in pregnancy rate between treatment cycles in correlation with perifollicular vascularity grades was significant($P<0.05$) (pregnancy rate was 19.5% , 3% , 0% in grade 3,2,1 respectively) this support the hypothesis that hight grade follicular blood flow may be necessary for implantation and continuation of pregnancy. This results were correlated to there studies like Bhal et al;1999 , Bhal et al;2001, Daniel et al;2007) but in contrast with (44,45).G.Ragni.2005

The main reason for this difference was that the baseline characteristics of the 3 study groups were not completely similar in all studies(age, duration and other characteristics) it might be that these differences may have influenced the chance of pregnancy

perse thus making a possible in dependent effect of follicular vascularity.

Uterine artery pulsatility index may be other parameter represent vascularity , a relationship between uterine PI and pregnancy outcome was found Significant. Mean uterine artery pulsatility index (UAPI) of < 2 associated with high fecundability rate (25%) while fecundability rate was lower (6.66%) in the group where the mean UAPI was 2-3 compared with those group when UAPI of >3 was associated with no pregnancy this result correlate with other results (Tsai et al;1996) , Thomo et al; 1997 , Salati et al;2003) (46) while in contrast with Bhal et al;2001(47) .

Conclusions

Perifollicular vascular perfusion appear to be an important factor in determining the outcome of stimulated IUI cycles, and may have clinical implication in assisted reproduction therapy. As there were no pregnancy in the group of women with uniformly low grade vascularity, the identification of these cycles would be valuable in terms of counseling them with regard to the potential outcome in that cycle.

Ideally, the Identification of these women earlier in the cycle (before HCG) would be helpful and could be cost- effective, both financially and emotionally.

PI can use in determining the outcome of stimulated I.U.I cycle but this needs further studies .

However, further longitudinal data would be needed before this form of prospective management could be applied clinically.

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