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Assessment of Different Methods of Ovulation Induction on Endometrial and Ovarian Blood Flow by Transvaginal Ultrasound Doppler

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ABSTRACT

The study was performed in a prospective randomized fashion in order to compare the value of tansvaginal ultrasound, and the value of combined colour Doppler imaging of uterine, ovarian blood flow and transvaginal folliculometry in assessment of ovulation induction of infertile women. Ovulation rate was taken as the gold standard for assessment of ovulation induction result Analysis of the results provided the following information: A) Better sensitivity & specificity of colour Doppler indices in detection of ovulation. B) Better timing of human chorionic gonadotrophin administration & better ovulation rate when colour Doppler was used in cycle monitoring.

Conclusion: It can be concluded from the results of this study that combined use of transvaginal sonography& colour Doppler imaging is more reliable in assessment of ovulation & is more useful in monitoring of follicular growth and vascularity in clomiphene citrate induced cycles than the use of transvaginal ultrasound alone.

The study opens a new field of ongoing research on the valuable application of colour Doppler studies in the management of gynecologic infertility due to ovarian cause.

Keywords: Transvaginal ultrasound, colour Doppler, ovulation induction clomiphene citrate.

INTRODUCTION

The standard definition of infertility is considered as failure to conceive after 12 months of unprotected regular intercourse. Statistically, it affects almost 10% of the couples, with 40% of the cases related to female pathology, disorders of ovulation account for about 30% to 40% of all cases of female infertility. These disorders are generally among the most easily diagnosed and most treatable causes of infertility [(1)]. Ovulation induction is the most common method of infertility treatment in which the ovaries are stimulated to produce multiple follicles. The most commonly used oral agent for induction of ovulation is Clomiphene Citrate which is a nonsteroidal triphenylethylene derivative that exhibits both estrogen agonist and antagonist properties, i.e. selective estrogen receptor modulating activity [(2)].

FSH is available mixed with LH activity in various gonadotropins including more purified forms of urinary gonadotropins, as well as without LH activity recombinant FSH. It is used commonly in infertility therapy to stimulate follicular development, notably in IVF therapy, as well as with intrauterine insemination (IUI). Gonadotropin preparations as HMG (Human Menopausal Gonadotrophins), FSH and LH prepared from human urine collected from postmenopausal women that was extracted in 1953 and injected intra-muscularly (IM) or subcutaneously (SC) [(3)]. A good blood supply towards the endometrium is usually considered to be an essential requirement for normal implantation. Endometrial microvascular blood flow determined by an intrauterine Doppler technique has been shown to be predictive of pregnancy and superior to other conventional parameters predicting endometrial receptivity [(4)].

The endometrium colour Doppler gives direct information about estradiol and progesterone levels and in turn also about functional maturity of the follicle and endometrium. Colour Doppler mapping and sampling of flow velocity waveforms proved that the peak systolic velocity appeared to follow the mean rise in circulating LH by approximately 12 hours [(5)].

Transvaginal ultrasonography with colour Doppler imaging and pulsed Doppler spectral analysis have been used to measure follicular volume and derive indices of blood flow. The end points for each follicle include: the volume, peak systolic velocity and pulsatility index. The value for peak systolic velocity, before the administration of human chorionic gonadotropin (HCG), can be used to identify follicles with a high probability of producing an oocyte and a high grade preimplantation embryo [(6)].

Since the advent of the transvaginal ultrasound, this has been a preferred method for the assessment of the follicle and the endometrium. The assessment of follicular maturity at the time of human chorionic gonadotropin (HCG) is one of the key factors for the success of all assisted reproductive technique procedures [(7)].

AIM OF THE WORK

The aim of this study is to evaluate the value of trans-vaginal ultrasound Doppler in assessment of endometrial and ovarian blood flow in women undergoing induction of ovulation and detect pregnancy outcome among different protocols of ovulation induction.

MATERIALS AND METHODS

This is prospective randomized control study which was done at Elsayed-Galal University hospital from the period between March 2018 to December

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2018 and the study was included 150 patient divided into 90 study group and 60 control group.

The study was approved by the Ethics Board of Al-Azhar University.

Inclusion criteria:

1- Age: 20-35 y.
2- BMI: 20-25 y.
3- Ovarian factor of infertility.
4- History and clinical data suggestive of PCO e.g. obesity, hirsutism, oligomenorrhea…. etc.
5- Both primary and secondary infertility.

Exclusion criteria:

a. Any patient who failed to fulfill any of the above criteria.
b. History of chronic cardio vascular disease.
c. Known allergy to any of medications used in induction of ovulation.

Women were divided into 4 groups:

Group (A): (30 women) were prescribed clomiphene citrate (clomid tablets, 50 mg, Aventis co, Cairo) administered orally, starting on the third day of the menstrual cycle. Treatment began with a 50 mg tablet daily for 5 consecutive days, increasing by 50 mg in subsequent cycles until ovulation is induced for at least 3 Consecutive cycles.

Group (B): (30 women) were prescribed clomiphene citrate (clomid) combined with highly purified urinary follicle stimulating hormone (fostimonvial, IBSA Co, Cairo) containing urofollitrophin 75 IU. It was taken in the form of intramuscular injection forat least 3 Consecutive cycles.

Group (C): (30 women) were prescribed clomiphene citrate (clomid) combined with human menopausal gonadotropin (pergonal ampule, Epico Co, Cairo) containing 75 IU FSH+75 IU LH. It was taken in the form of intramuscular injection, administered daily from day 3 to day 7 of menstrual cycle in a daily dose of 2 ampoules forat least 3 Consecutive cycles.

Group (D): (60 women) control group with normal menstrual cycles. They were selected according to clinical and ultrasonographic findings of normal uteri and ovaries with documented ovulation and menstrual cycle length of 21-35 days for at least the previous 3 cycles. The control group was selected from patients coming to the out-patient clinic seeking contraception.

Each woman was submitted for:

Full obstetric, gynecological history and clinical examination. Baseline vaginal ultrasound at the start of the cycle to provide information on uterine and ovarian morphology and to delineate pelvic structures.

Transvaginal ultrasound folliculometry:

Monitoring was started on the 8th day of the cycle on an every other day basis after we started induction of ovulation by different drug protocols as mentioned above. The woman was placed in the supine position with flexed legs and an empty bladder. The vaginal probe was covered with coupling gel and introduced into one of the digits of a sterile surgical glove that was lubricated with coupling gel, vaginal probe (Medison Co. Accuvix V20, 7.5 MHz, South Korea). Using the B-mode transvaginal sonography, morphology of the uterus, endometrial thickness and ovaries were explored (folliculometry). The dominant follicle was measured in three planes as follows: after obtaining the roundest possible image using B-mode gray-scale transvaginal ultrasound, in two perpendicular dimensions at the center; cranio-caudal and transverse, all measurements were expressed in mm.

The mean follicular diameter was then calculated by taking the mean of three serial measurements of the three follicular diameters. The monitoring was started on the 8th day of the cycle on an every other day basis till the dominant follicle reached 14 mm diameter when daily follow up was then performed. Human chorionic gonadotropin injection 10,000 IU IM (Pregnyl Ampoule 5000 IU, Nile Co, Cairo) was given to the patient when the dominant follicle reached 18mm in diameter. At that time, the endometrium was evaluated as regards both endometrial thickness and pattern.

***Colour Doppler ultrasound:**

The Doppler study was done using the transvaginal transducer with pulsed and colour Doppler.The Doppler studies are better done at the same time everyday as circadian rhythm is seen in uterine artery flow in periovulatory phase.

The pulsatility index (PI) and resistance index (RI) of the uterine and ovarian arteries were calculated electronically when similar consecutive waveforms of good quality are obtained.

Doppler flow parameters of uterine and ovarian vessels were then measured. Flow velocity waveforms were obtained from the ascending main branch of uterine artery on the right and left side of the cervix in a longitudinal plane before it entered the uterus, the "gate" of the Doppler was positioned when the vessel with good color signals was identified on the screen.

First a sagittal section was obtained to gain information about the position of the uterus. Then the head of the transducer was directed into the lateral fornix of the vaginal vault and rotated 90 degrees. Therefore, a coronal section could be obtained and we were able to scan the ovaries. Lateral to and below the ovaries, a shape of a longitudinal vessel could be identified, the internal iliac artery. Thereafter, colour Doppler equipment was added for monitoring ovarian blood flow on the dominant side. 10,000 IU of HCG was given to the patient IM when the dominant follicle reached 18mm in diameter and the blood flow indices of the active ovary; showing the dominant follicle were good i.e. around ± 0.54 for RI, ± 0.9 for PI, ±0.10 for PSV. The follicular diameter at the time of HCG

administration was noted. All patients were advised to have a sexual intercourse 36 &48 hours after the HCG injection to detect pregnancy outcome.

Midluteal (day 21of the cycle) serum progesterone was done for patients to reveal the presence or absence of ovulation, and to evaluate the presumed role of colour Doppler, if any, in assessment of ovulation manifested by a better ovulation rate.

Statistical methods used in the study

Data were statistically represented in terms of mean, median, frequency tables and standard deviation where appropriate. For statistical analysis of categorical data, the Fisher exact test or the chi^2 test was used where appropriate. Continuous data were analyzed with the unpaired (two sample) student's t test where appropriate. A probability value (P value) less than or equals 0.05 was considered significant. Accuracy was represented using the terms sensitivity, specificity, +ve predictive value and –ve predictive value.

All statistical calculations were done using computer programs Microsoft excel version 5 and SPSS (statistical package for the social science) statistical programs.

RESULTS

By comparing the control group (4) with the first 3 groups (1, 2 and 3) regarding the clinical data (age, BMI and the duration of infertility), the following results were obtained:

There was a statistically significant difference between group (4) and the other three groups concerning the mean age of the patients who participated in the study, but; fortunately this significant difference is in favor of the benefit of using clomid for super induction as the control group has the youngest mean age of the four groups so they have the best probability of regular and proper ovulation when compared to the older mean age of the rest of the groups i.e they have a better chance of ovulation than patients receiving treatment.

At the same time, the P value for the other groups together i.e P (1#2)=1.0, P (1#3) =0,729, P (2#3) =0,746 was statistically non significant which means that there is no special preference of patients enrolled in the study as shown in table (1)**.**

Table (1): The age of the study groups

Group	Mean (year) ± SD	P value
Group I:(on clomid)	25.2 ± 2.5	P(1#4)=**0.009**
Group II:(on clomid + FSH)	25.2 ± 2.86	P(2#4)=**0.014**
Group III:(on clomid + HMG)	24.9 ± 2.7	P(3#4)=**0.027**
Group IV: (Control)	23.42 ± 2.59	

S.D = Standard deviation, P value is significant if <0.05

Concerning the body mass index (BMI) of the patients, the only statistically significant difference was between group (3) and group (4); it was noted that the higher mean BMI of patients in group (4) compared to the lower one of group (3) gave them better chance of ovulation success. At the same time, there was no significant difference between the other groups which gave them equal chances to respond to induction of ovulation as shown in table (2).

Table (2): The body mass index (BMI) of the study groups.

Group	Mean ± SD	P value
Group I: (on clomid)	22.75 ± 1.33	P(1#4)=;0.101 P(1#2)=0.807
Group II: (on clomid + FSH)	22.65 ± 1.79	P(2#4)=0.075; P(2#3)=0.717
Group III: (on clomid + HMG)	21.85 ± 1.63	P(3#4)= 0.**023**; P(3#1)=0.479
Group IV: (Control)	23.75 ± 1.45	

There was no statistically significant difference concerning the duration of infertility among different groups and this indicates that there is no special preference of one of groups than the others as shown in table (3).

Table (3): The duration of infertility among the study groups.

Group	Mean (year) ± SD	P value
Group I:(on clomid)	3.12 ± 1.14	P(1#2)=0.735
Group II:(on clomid + FSH)	3.02 ± 1.13	P(2#3)=0.707
Group III:(on clomid + HMG)	3.01 ± 1.25	P(3#1)=0.957
Group IV:Control)		

S.D = Standard deviation.

Concerning the endometrial thickness, there was a statistically significant difference between group 1 and 2 (P =0.001) and, between group 1 and 3 (P =0.006) and between group 1 and 4 (P =0.006). This indicates that induction of ovulation with the use of CC seriously affects the endometrial thickness and also indicates that the use of FSH or HMG can partially reverse this effect.

However, the P value for group 2 and 3 (P =0.547) was statistically insignificant which indicates non superiority of either mode of treatment upon the other as shown in table (4).

Table (4): The endometrial thickness among the study groups

Group	Mean (mm) ± SD	P value
Group I: (on clomid)	9.57 ± 1.38	P(1#4)=**0.006**; P(1#2)=**0.001**
Group II: (on clomid + FSH)	10.77 ± 1.22	P(2#4)=0.785; P(2#3)=0.547
Group III: (on clomid + HMG)	10.57 ± 1.33	P(3#4)=0.792; P(3#1)=**0.006**
Group IV: (Control)	10.67 ± 1.58	

S.D = Standard deviation.

Induction by CC seriously affected the percentage of trilaminar endometrial pattern and increased the percentage of non-trilaminar pattern. The use of FSH & HMG with CC induction partially corrected these changes and HMG has higher percentage of trilaminar endometrial pattern more than FSH did. However, as shown in table (5).

Table (5): The percentage of trilaminar and non trilaminarendometreium among the different study groups

Group	trilaminar	Non-trilaminar
clomid	10%	90%
clomid + FSH	40%	60%
clomid + HMG	60%	40%
Control	50%	50%

S.D = Standard deviation.

Although the mean day of appearance of dominant follicle was slightly lower in patients receiving HMG than the rest groups; which is probably due to increased blood flow to the pelvic region and subsequently to the ovaries; yet there was no statistically significant difference that can point to the value of using HMG to achieve an earlier ovulation day in different protocols of ovulation induction as shown in table (6).

Table (6): The day of appearance of dominant follicle.

Group	Mean ± SD	P value
Clomid	13.53 ± 1.55	P(1#4)=0.550; P(1#2)=0.925
clomid + FSH	13.57 ± 1.17	P(2#4)=0.559; P(2#3)=0.674
clomid + HMG	13.43 ± 1.28	P(3#4)=0.350; P(3#1)=0.786
Control	13.77 ± 1.45	

S.D = Standard deviation.

As regards the number of dominant follicles in patients treated by clomid, the mean was 1.53 ± 0.59 SD, while in patients treated by clomid + HMG it was 2.00 ± 0.65 SD, and this showed a highly statistically significant difference (P value= 0.001). Also, there was a statistically significant difference between groups 1 and 2 (P value= 0.005), between groups 1 and 4 (P value= 0.006), between groups 2 and 3 (P value= 0.017) and between groups 3 and 4 (P value= 0.002). However, the greatest number of dominant follicles was obtained in patients treated with clomid+HMG as shown in table (7)**.**

Table (7): The mean number of dominant follicles.

Group	Mean ± SD	P value
clomid	1.53 ± 0.59	P(1#4)=**0.006**; P(1#2)=**0.005**
clomid + FSH	1.89 ± 0.67	P(2#4)=**0.004** P(2#3)=**0.017**
clomid + HMG	2.00 ± 0.65	P(3#4)=**0.002**; P(3#1)=**0.001**
Control	1.00±0.02	

S.D = Standard deviation.

As regards the diameter of dominant follicles in patients treated by clomid, the mean was19.05±0.83 SD, while in patients treated by clomid + HMG it was 20.9 ± 0.67 SD, and this showed a highly statistically significant difference (P value= 0.001). Also, there was a statistically significant difference between groups 1 and 2 (P value= 0.005), between groups 1 and 4 (P value= 0.006) and between groups 2 and 3 (P value= 0.017). However, there was no statistically significant difference between groups 3 and 4 (P value= 0.444). The largest diameter of dominant follicles was obtained in patients treated with clomid +HMG as shown in table (8)**.**

Table (8): The diameter of dominant follicles.

Group	Mean (mm) ± SD	P value
clomid	19.05±0.83	P(1#4)=**0.006**; P(1#2)=**0.005**
clomid + FSH	20.01 ± 1.56	P(2#4)=**0.025** P(2#3)=**0.017**
clomid + HMG	20.09 ± 0.67	P(3#4)=0.444; P(3#1)=**0.001**
Control	19.01 ± 0.84	

S.D = Standard deviation.

Regarding the pulsatility index (PI) of the uterine artery, a statistically significant difference was obtained when we compared the pulsatility indices of different groups with each other. First, comparing the PI of groups 1 and 4 (P value=0.004) showed a significant increase in the PI of group 1 which indicates the deleterious effect of CC induction of ovulation on

the uterine blood flow. And more importantly, comparing the other groups together showed that group 3 had the lowest PI among all groups which indicates sure correction of deleterious effects of CC on uterine blood flow.

In addition, although both groups 2 and 3 showed improvement of the PI with the addition of FSH and HMG, respectively, yet the upper hand and the best improvement remains related to the use of HMG rather than FSH. It is the effect of FSH that revert the CC induced changes in uterine pulsatility index more or less to be close to that of the control group or slightly increased.

A statistically significant difference was obtained when we compared the pulsatility indices of groups 1 and 2 (P value=0.003), groups 2 and 3 (P value=0.001), groups 1 and 3(P value=0.005) and groups 4 and 3 (P value=0.003) as shown in table (9).

Table (9): The pulsatility index "PI" of the uterine artery.

Group	Mean ± SD	P value
clomid	2.87 ± 0.05	P(1#4)=**0.004;** P(1#2)=**0.003**
clomid + FSH	2.59 ± 0.07	P(2#4)=0.212; P(2#3)= **0.001**
clomid + HMG	2.43 ± 0.03	P(3#4)=**0.003**; P(3#1)=**0.005**
Control	2.54 ± 0.07	

There was no statistically significant difference between the different groups concerning the RI of the uterine artery although the lowest mean RI 0.65 ± 0.02 SD remained for those patients who used HMG indicating better blood flow to uterine artery as shown in table (10).

Table (10): The resistance index "RI" of the uterine artery.

Group	Mean ± SD	P value
Clomid	0.66 ± 0.03	P(1#4)=0.282; P(1#2)=0.522
clomid + FSH	0.67 ± 0.01	P(2#4)=0.635; P(2#3)= 0.242
clomid + HMG	0.65 ± 0.02	P(3#4)=0.095; P(3#1)=0.699
Control	0.67 ± 0.02	

S.D = Standard deviation.

A statistically significant difference was obtained when we compared the pulsatility indices of the ovarian arteries of different groups with each other. First, comparing the PI of groups 1 and 4 (P value=0.001) showed a significant increase in the PI of group 1 indicates the deleterious effect of CC induction of ovulation on the ovarian blood flow. And more importantly, comparing the other groups together showed that group 2 had the lowest PI among all groups which indicates correction of deleterious effects of CC on ovarian blood flow as shown in table (11).In addition, although both groups 2 and 3 showed improvement of the PI with the addition of FSH and HMG, respectively, yet the upper hand and the best improvement remains related to the use of FSH rather than HMG.

Table (11): The pulsatility index "PI" of the ovarian artery.

Group	Mean ± SD	P value
clomid	1.09 ± 0.1	P(1#4)=**0.001;** P(1#2)=0.124
clomid + FSH	0.94 ± 0.1	P(2#4)=0.199; P(2#3)= 0.114
clomid + HMG	0.99 ± 0.13	P(3#4)=0.130; P(3#1)=0.054
Control	1.06 ± 0.13	

S.D = Standard deviation.

As regards the resistance index (RI) of the ovarian artery, there was no statistically significant difference between the different groups except concerning the statistically significant difference which was obtained when we compared the resistance index between group 3 and group 4 (P value=0.002). The lowest mean RI of the ovarian artery 0.46 ± 0.03 SD remained for those patients who used HMG indicating better blood flow to the ovarian artery as shown in table (12) and figure (12).

Table (12): The resistance index "RI" of the ovarian artery

Group	Mean ± SD	P value
clomid	0.53 ± 0.8	P(1#4)=0.273; P(1#2)=0.497
clomid + FSH	0.54 ± 0.07	P(2#4)=0.612; P(2#3)= 0.234
clomid + HMG	0.46 ± 0.03	P(3#4)=**0.002**; P(3#1)=0.683
Control	0.52 ± 0.07	

As regards the midluteal (day 21 of the cycle) serum progesterone level in ng/ml, there was a statistically significant difference between groups 1 and 2 (P value= 0.005), between groups 1 and 3 (P value= 0.004) and between groups 3 and 4 (P value= 0.002). However, there was no statistically significant difference between groups 1 and 4 (P value= 0.154),

between groups 2 and 3 (P value= 0.765) and between groups 2 and 4 (P value= 0.423). The highest serum progesterone level was seen in the CC+HMG group as shown in table (13).

Table (13):The midluteal serum progesterone level in ng/ml.

Group	Mean ± SD	P value
clomid	12.6 ± 6.4	P(1#4)=0.154; P(1#2)=**0.005**
clomid + FSH	14.7 ± 8.9	P(2#4)=0.423; P(2#3)= 0.765
clomid + HMG	16.5 ± 6.1	P(3#4)=**0.002**; P(3#1)=**0.004**
Control	10.5± 5.3	

S.D = Standard deviation.

Concerning the pregnancy rate:

As for the control group, none of the 60 patients enrolled in the follow up program conceived in consecutive cycles.

Only one of those receiving CC alone conceived and this was during her second treatment cycle. Similarly, only one patient of the 30 patients receiving CC with the addition of FSH conceived and this was during her third cycle.

Three patients of group 3 receiving CC induction with the addition of HMG conceived (one of them conceived in her first treatment cycle and another one conceived in her second treatment cycle and third one conceived during her third treatment cycle).

As regard transvaginal ultrasonography in detection of ovulation at cut off value of 18 mm follicular diameter, the sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 39.4%, 28.6%, 72.2%, 9.1%, 37.5% respectively as shown in table (14).

Table (14): The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of transvaginal sonography in detection of ovulation at cut off value of 18 mm follicular diameter.

Cut off value of follicular diameter in mm	Sensitivity	Specificity	(+)ve PV	(-)ve PV	accuracy
18 mm	39.4%	28.6%	72.2%	9.1%	37.5%

As regards using the 4 Doppler indicesused in detection of ovulation, they gave better results regarding the sensitivity, specificity, positive predictive value, negative predictive value and accuracy than using any one of them alone or transvaginal ultrasound alone as the figures were 62.9%, 83.3%, 98.2%, 44.5%, 65% respectively as shown in table (15).

Table (15): The overall sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the 4 Doppler indices alone used in detection of ovulation.

Sensitivity	62.9%
Specificity	83.3%
Positive predictive value	98.2%
Negative predictive value	44.5%
Accuracy	65%

The best results were obtained when we used combined TVS and the 4 Doppler indices in detection of ovulation as the best findings regarding the sensitivity, specificity, positive predictive value, negative predictive value and accuracy were evident than using any one of them alone or transvaginal ultrasound alone as the figures were 69.5%, 88.9%, 95.4%, 55.6%, 77% respectively as shown in table (16) and figure (16).

Table (16):The overall sensitivity, specificity, positive predictive value, negative predictive value and accuracy of combined TVS and the 4Doppler indices used in the study in detection of ovulation.

sensitivity	69.5%
specificity	88.9%
positive predictive value	95.4%
negative predictive value	55.6%
accuracy	77%

DISCUSSION

Successful implantation during in-vitro fertilization and embryo transfer depends on many factors including embryo quality and uterine receptivity. Transvaginal ultrasonography plays an essential role in modern fertility management. In the context of IVF, the transducer allows easy monitoring of follicular and endometrial growth, inspection of the uterine, ovarian and endometrial morphology, and easy access to the ovaries for the procedure of oocyte retrieval**.** Clomiphene citrate**,** which was introduced in 1961, is considered to increase the incidence of spontaneous abortion **[(8)].** This increase has been attributed to several factors, including impaired endometrial development and reduced endometrial thickness**[(9)].**

The success of embryonic implantation relies upon a perfect dialogue between good quality embryos and a receptive endometrium. The main reason for the current disappointing results of embryonic implantation is the quality of the endometrium that is affected during pharmacologic treatments. Also, when implantation starts an even higher amount of early embryonic wastage occurs after ART compared with natural cycles **[(10)].**

For these reasons, the implantation process constitutes the limiting factor to increase pregnancy

rates in ART and the fundamental question is: Could implantation be improved in ART up to physiological levels[10].

In this work, we studied the role of vaginal ultrasonography & color Doppler in examination of the endometrium, uterine and ovarian vasculature in infertile patients treated with clomiphene citrate alone or with addition of either follicle stimulating hormone (FSH) or human menopausal gonadotrophin (HMG) to evaluate their effects on different endometrial parameters, comparing them with other patients not receiving induction and taken as control.

Color Doppler studies were performed when vaginal ultrasonography demonstrate optimum diameter of the leading follicle, i.e at the time of HCG administration.

Although there were no preferences upon classifying the patients among the different groups, the patients chosen to receive CC+HMG induction were those with younger age groups and more or less shorter duration of infertility. This coincidence gave those patients the best chances concerning ovulation and possible pregnancy, as, according to **Abdelmassih *et al.*[11]**, the younger the patient and the shorter the duration of infertility, the better the results during ICSI. This ensures that if there are any benefits of adding FSH or HMG to CC induction, it would be the more power of the drugs to revert the changes of CC rather than a better patient profile.

In this study the mean endometrial thickness for patients receiving clomid was 9.57 mm ± 1.38 SD, while in control group it was 10.67 mm ± 1.58 SD and the difference was statistically significant. This statistically significant data expresses that patients receiving clomiphene citrate have reduced endometrial thickness. Also, the administration of FSH resulted in a mean endometreial thickness of 10.77 mm ± 1.22 SD whereas administration of HMG resulted in a mean endometrial thickness of 10.57 mm ± 1.33 SD. This indicates that the administration of either drug had a reversing power on the unfavorable changes in endometrial thickness due to CC induction. However, only one patient of the thirty patients who were given CC together with FSH got pregnant and this occurred in the third cycle of her treatment. At the same time only one patient of those receiving CC alone got pregnant, and this was also in her third treatment cycle. On the other hand, three of the patients receiving HMG with CC induction could achieve a positive pregnancy test and they were distributed evenly on the number of treatment cycles, i.e one of them got pregnant in the first treatment cycle and one in the second cycle and the last one got pregnant in her third treatment cycle.

The effect of clomiphene citrate on endometrial thickness was first reported by **Fleischer *et al.*[12],** and was found to be inversely proportional to clomiphene citrate dosage.

The only point on which all investigators agree regarding the endometrial thickness is that the endometrium must be sufficiently thick (6-8 mm) to enable embryonic implantation [13].

In the present study, the maximum endometrial thickness in pregnant group was 10.77 mm.

But this does not match with the result of **Dickey et al.[9]** and **Noyes et al.[14]** who found endometrial thickness of ≥14 mm in 9.6 % of IVF-ET cycles which was associated with a normal rate of pregnancy but with a higher rate of biochemical pregnancies, **Noyes et al.[14]** also noted that endometrial thickness of ≥15 mm was found in 11.6% of IVF-ET cycles and resulted in high implantation rates and clinical pregnancies.

In our study, definite affection of the endometrial triple layer appearance occurred in patients receiving CC alone; the percentage ratio of those with triple layered endometrium to non triple layer was 10:90%. The use of FSH corrected this ratio to be 40:60%. Whereas; the use of HMG produced a ratio of 60%:40% exactly. The net result is that the addition of HMG produced the best ratio of trilaminar endometrium which is believed by many authors to be associated with better pregnancy rates than non trilaminar pattern[15].

Although, there is continuing debate over the usefulness of the endometrial thickness measurement, the current literature would suggest that the ultrasonographic texture of the endometrium may have a greater prognostic value for implantation [16].

As regards Doppler study of the uterine artery, in the current study, PI was statistically significant between the group that received clomid only and both groups in which FSH and HMG were added and also a statistically significant difference was found between the group that received clomid only and the control group. However, the difference was statistically non significant between (clomid+ FSH) group and the control group.

From these results, it is clear that vascular impedance is lower in the control group rather than patients receiving induction specially those receiving clomiphene citrate, which signifies the importance of receiving medications as low dose aspirin in addition to induction medication to lower the vascular impedance to enhance the implantation process.

This result matches with the study done by **Mara *et al.*[17]** which signifies the importance of low dose aspirin in reduction of PI values.

In this study, the mean PI of the uterine artery in the third group (clomid+ HMG) was2.43 ± 0.03 (S.D.), this group who received HMG had the greatest number of pregnancy rate and the lowest value of PI of uterine artery.

Tomas *et al.*[18] studied uterine artery PI impedance in women with unexplained infertility receiving supplementation with human menopausal gonadotropins (HMG) as compared to clomiphene

citrate alone. They concluded that Doppler velocimetry and intrinsically poor uterine perfusion was found regardless the method of induction.

These results match with the results of **Tsai et al.**[19] who performed a valuable study to evaluate the prognostic value of uterine perfusion on the day of human chorionic gonadotrophin administration in patients who were undergoing intrauterine insemination. No pregnancy occurred when the pulsatility index (PI) of the ascending branch of the uterine arteries was more than 3. This data suggests that measurement of uterine perfusion on the day of human chorionic gonadotrophin administration may have predictive value regarding fecundity and the continuation of pregnancy.

This is consistent with **Tekay *et al.***[20], who concluded that there were no conception cases in cycles in which the uterine arteries bilaterally had a flow velocity waveform with an absent end-diastolic flow. The uterine blood flow remains one of the most important predictive factors of the success of implantation and proper embryo transfer as concluded by many authors [21, 22].

However, this was in contrary to the results of **Tekay *et al.***[20]who found no relation between PI and implantation rate in their study.

In the present study, the RI of the uterine artery value was statistically non significant among different groups. The lowest mean RI 0.65 ± 0.02 SD was for those patients who used HMG + clomid indicating better blood flow to the uterine artery. The mean RI of the first three groups who conceived was < 0.76, this result matches with **Sefarini et al.**[23] who signified the value of RI <0.76 in pregnant cases in their study.

This result also matches with **Tomaset *et al.***[18] and **Sefarini et al.**[23] who reported that the presence of the end diastolic blood flow is a good predictive factor of clinical pregnancy and normal pregnancy following IVF and embryo transfer and the presence of diastolic flow seems to be important for implantation.

In this study, a statistically significant difference was obtained when we compared the pulsatility indices (PI) of the ovarian arteries of different groups with each other. First, comparing the PI of groups 1 and 4 showed a significant increase in the PI of group 1 which indicates the deleterious effect of CC induction of ovulation on the ovarian blood flow.And more importantly, comparing the other groups together showed that group 2 had the lowest PI among all groups which indicates correction of deleterious effects of CC on ovarian blood flow.

In addition, although both groups 2 and 3 showed improvement of the PI of ovarian arteries with the addition of FSH and HMG, respectively, yet the upper hand and the best improvement remains related to the use of FSH rather than HMG.

As regards the resistance index (RI) of the ovarian artery, there was no statistically significant difference between the different groups except concerning the statistically significant difference between group 3 and group 4. The lowest mean RI of the ovarian artery remained for those patients who used HMG indicating better blood flow to the ovarian artery.

Uterine and ovarian perfusion during the periovulatory period have been assessed by transvaginalcolour Doppler in a study conveyed by **Kupesic and Kurjak**[24] in spontaneous and induced ovarian cycles with confirmed ovulation. Ovarian flow velocity was found to have an RI of 0.52 the day before ovulation in the group with spontaneous cycles and 0.51 in the group with stimulated cycles. The value for RI tended to decrease, whereas blood velocity tended to increase during the day of ovulation. Analyzing the PIs between these two groups, slightly decreased PI levels of follicular and early luteal flow occurred during the spontaneous cycles (mean PI=0.75).

Collins *et al.*[25] suggested that intraovarian vascularization may be a predictor of ovulation**. Campbell *et al.***[5] observed a marked increase of blood flow within the leading follicle during the periovulatory phase in spontaneous cycles. Furthermore, **Kupesic and Kurjak**[24] reported that ovarian blood velocity tended to increase during the day of ovulation. Regarding the relationship between utero-ovarian haemodynamics and follicular development, they stated that the clinical applicability of Doppler ultrasound for noninvasive evaluation of the utero-ovarian circulation usefulness in monitoring follicular development, and the prediction of successful outcome in patients undergoing ovarian stimulation has yet to establish.

A serum progesterone level of less than 3 ng\ml is consistent with follicular phase levels. To confirm ovulation, values at the midluteal phase, just at the midpoint between ovulation and the onset of the subsequent menstrual period, should be at least 6.5 ng\ml and preferably 10 ng\ml or more[26].

In this study, the mean serum progesterone level in all groups that received induction of ovulation was higher than 10 ng/ml, and the highest value was obtained with the addition of HMG to clomid (mean 16.5±6.1 SD).

In this study, all groups that received induction of ovulation showed a higher number of dominant follicles than the control group and the difference was statistically significant. Again, addition of HMG to clomid gave the highest mean number of dominant follicles when compared with the group that received clomid alone and even when FSH was added.

This keeps with the results of the study of **Tsai *et al.***[27] who reported that the dominant follicle number increased with total antral follicle number in women who received CC plus HMG (P < 0.001).

The pregnant group had a higher number of antral follicles and dominant follicles in comparison with the non pregnant group ($P < 0.01$ and $P < 0.02$, respectively). The pregnancy rate was low (2/39) in women aged older than 35 years regardless of the number of antral follicles ($P < 0.05$) and the extent of HMG administration ($P < 0.02$). Women aged older than 35 also produced fewer dominant follicles ($P < 0.001$).

These changes were significantly reduced in the endometrium during the mid-late follicular phase and early luteal phase. There were no differences in endometrial thickness or volume between the groups or in the plasma concentrations of estradiol or progesterone and they concluded that endometrial and subendometrial vascularity are significantly reduced in women with unexplained subfertility during the mid-late follicular phase irrespective of estradiol or progesterone concentrations and endometrial morphometry.

In the current study, it was an interesting finding that using the 4 Doppler indicesused in detection of ovulation gave better results regarding the sensitivity, specificity, positive predictive value, negative predictive value and accuracy than using any one of them alone or transvaginal ultrasound alone. These findings are consistent with those of **Farrag *et al.*** [28] addressing the better detection and assessment of ovulation using Doppler indices.

In this study, the best results were obtained when we used combined TVS and the 4 Doppler indices in detection of ovulation as the best findings regarding the sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 69.5%, 88.9%, 95.4%, 55.6%, 77% respectively. This is in accordance with other previous stories [29,30] suggesting the potential adventitious role of color Doppler imaging of ovarian blood flow in assessment of ovulation in stimulated cycles.

CONCLUSION

It can be concluded from the results of this study that combined use of transvaginal sonography and color Doppler imaging is more reliable in assessment of ovulation and is more useful in monitoring of follicular growth and vascularity in induced cycles than the use of conventional transvaginal folliculometry alone.

RECOMMENDATIONS

From this study, we can recommend the addition of either FSH or HMG to clomiphene citrate in induction of ovulation in order to improve follicular growth, endometrial thickness, endometrial pattern, uterine and ovarian blood flow and hence improve the implantation rate.

It is also recommended to combine color Doppler indices with transvaginal ultrasound in monitoring ovulation.

Larger studies with broader scales and multicentre participation are definitely needed to confirm the beneficial effects of the addition of FSH or HMG to CC in induction of ovulation.

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