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

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ABSTRACT
Background: 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.
Objectives: Ovulation rate was taken as the gold standard for assessment of ovulation induction result.
Patients and Methods: 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.
Results: 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.
Conclusion: 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].

It has been proved that 3-D ultrasound is a very highly reproducible technique. With 3-D ultrasound, a volume of a region of interest can be acquired and stored. 3-D ultrasound, allows for a whole assessment of relevant vessels and quantitative assessment of vessel density and perfusion within a specified area. A whole evaluation is then possible for endometrial and subendometrial vascularization and also for ovarian stromal vascularity [5].

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 [6].

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 [7].

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 [8].

AIM OF THE WORK

The aim of this study is to evaluate the efficacy of 3D trans-vaginal ultrasound & Doppler in assessment of endometrial and ovarian blood flow in women undergoing induction of ovulation and detect pregnancy outcome among different induction drug protocols.

PATIENTS AND METHODS

The study included 150 women who were recruited from the Gynecologic out-patient clinic of Bab EL Shaaria Maternity Hospital and Military Production Specialized Hospital, and during the period from June 2016 to December 2017. **The study was approved by the Ethics Board of Al-Azhar University.**

The patients included in the study were 90 patients presenting with infertility (due to ovulatory defect), and 60 women as control group. At the time of initial recruitment the purpose of the study was adequately explained to each participant and then an informed consent was obtained.

1. **Factors of infertility other than ovarian factor were excluded by:** Male: by normal semen analysis. Uterine: by normal U/S and HSG. Tubal: by normal HSG.

2. **Anovulatory cycles: based on:** Menstrual pattern: suggestive of anovulation. Previous folliculometry: no dominant follicle. Previous premenstrual endometreial biopsy (PEB) or serum progesterone assay at day 21 of the 28 day cycle.

3. History and clinical data suggestive of PCO e.g. obesity, hirsutism, oligomenorrhea …. etc.

Any patient who failed to fulfill any of the above criteria or gave history of ischemic heart disease (IHD) or allergy to any of medications used in induction of ovulation was excluded from the study. Previous infertility treatment was not considered as exclusion criteria. Both primary and secondary infertility were included in our study.

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 fifth 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.

Group (B) (30 women)**:** were prescribed clomiphene citrate (clomid) combined with highly purified urinary follicle stimulating hormone (fostimon vial, IBSA Co., Cairo) containing urofollitrophin 75 IU. It was taken in the form of intramuscular injection, admin

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.

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 26-32 days for at least the previous 3 cycles. Their age ranged from 26-31 years. The control group was selected from patients coming to the out-patient clinic for any gynecological problem other than infertility as vaginal infections, bleeding or pelvic pain.

Each woman was submitted for:

Full obstetric, gynecological history and clinical examination. - Baseline vaginal ultrasound at the start of the cycle to provide baseline information on the ovarian morphology before the initiation of ovulation-induction agent and to delineate pelvic structures.

*Transvaginal ultrasound folliculometry.

*Colour Doppler ultrasound.

Statistical methods:

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 square 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

Table (1): The age of the study groups

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

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

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 (3) and figure (10)*.*

Table (2): 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.

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.

Table (3): 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.

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.

Table (4): 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	

S.D = Standard deviation.

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).

DISCUSSION

Clomiphene citrate**,** which was introduced in 1961, is considered to increase the incidence of spontaneous abortion [9]**.** This increase has been attributed to several factors, including impaired endometrial development and reduced endometrial thickness [10]**.**

Many teams have reported significant correlations between pregnancy rate and endometrial thickness [11] and morphology as defined by ultrasonography, while others have failed to demonstrate such relationships [12]. Recently, the measurement of impedance to uterine artery blood flow in IVF cycles has provided an indirect estimation of endometrial receptivity [13].

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 [14].

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 [14]?

In the present 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.

In a recently carried out study in Tehran, pregnancies occurred only in patients with an endometrial thickness of 9-12 mm (P=0.036) [15]. However, the authors stated that the sonographic features of the endometrium (thickness and pattern) on the day of HCG administration did not differ between pregnant and non pregnant patients and that the pregnancy rate declined beyond two limits of endometrial thickness.

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].

This was also confirmed by Renato [17] who described that there is no relation between endometrial thickness and endometrial pattern.

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.

Doppler echography is an interesting tool for assessing uterine receptivity. However, parameters studied by color Doppler and ultrasonography are neither specific nor sensitive enough when used individually [18].

In this study, it is clear that the best pregnancy rate was achieved with the concomitant use of HMG rather than with the use of FSH, although the mean endometrial thickness with FSH use was greater than with HMG.

Uterine and ovarian perfusion during the periovulatory period have been assessed by transvaginal colour Doppler in a study conveyed by *Kupesic and Kurjak* [19] 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 and colleagues [20] suggested that intraovarian vascularization may be a predictor of ovulation. *Campbell and others* [6] observed a marked increase of blood flow within the leading follicle during the periovulatory phase in spontaneous cycles. Furthermore, *Kupesic and Kurjak* [19] 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 non invasive 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.

However, the study done by *Chui and coworkers* [12] failed to demonstrate any significant difference in the intra-ovarian PI between those women who conceived and those who did not. This is in accordance with the study of *Tekay and colleagues* [22] but disparate with other workers [23].

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 [24].

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 [23] suggesting the potential adventitious role of color Doppler imaging of ovarian blood flow in assessment of ovulation in stimulated cycles.

CONCLUSION

Clomiphene citrate has a negative effect on endometrial thickness which is mostly attributed to its antiestrogenic effect. A triple layered endometrium (trilaminar pattern) is associated with higher pregnancy rates; therefore, patients receiving CC with non trilaminar pattern have reduced pregnancy rates. Thin endometrium (less than 7 mm) is usually associated with failure of implantation. Uterine artery PI less than 3 is needed to have a successful implantation. Uterine artery RI less than 0.76 is needed to have a good prediction of implantation. Ovarian artery PI less than 0.9 is needed to have a successful implantation. Ovarian artery RI less than 0.54 is needed to have a good prediction of implantation. The vascular indices of the uterine artery at the time of hCG administration are markedly increased in patients receiving CC induction denoting compromised endometrial blood flow and hence a narrower window of implantation with reduced pregnancy rates. The Doppler parameters PI of uterine artery are significantly different between clomid & both FSH and HMG with lower Doppler indices in HMG induced cycles similar to those of control group indicating good visualization in HMG induced cycles. The Doppler parameters PI of ovarian artery are different between clomiphene citrate & both FSH and HMG with lower Doppler indices in both FSH and HMG induced cycles indicating good visualization in these induced cycles.

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