
Uterine Receptivity Assessed By Three Dimensional Ultrasound and Power Doppler in Women with Polycystic Ovary Syndrome Treated By Clomiphene Citrate Alone or Combined To Metformin

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Abstract

Objective: A prospective randomized single blinded study was carried out to examine whether metformin when combined with clomiphene citrate (CC) had an added effect, if any, on uterine receptivity.

Patients and Methods: Seventy seven egyptian women with polycystic ovary syndrome (PCOS) aging between 20-35 years were randomized to 2 groups received either 100 mg cc for 5 days alone, or with metformin 850mg bid throughout menstrual cycle. Three dimensional (3D) ultrasound with power Doppler examination was done on day 2 after human chorionic gonadotropin (hCG) injection for both groups and; endometrial volume, vascularization index (VI), flow index (FI), and vascularization flow index (VFI) of endometrial and subendometrial regions were measured.

Results: Patients taking metformin, and those destined to be pregnant had significantly lower VI/FI/VFI in endometrial region. Corresponding decreases of values in subendometrial region were insignificant. Serum estradiol (E2) levels on day of hCG injection were significantly lower in cycles, where metformin was added.

Conclusion: Metformin had significantly lowered VI/FI/VFI in early luteal phase in PCOS affected women.

Key Words: Uterine Receptivity, Three Dimensional Ultrasound, Power Doppler, Polycystic Ovary Syndrome, Clomiphene Citrate, Metformin

Introduction

Polycystic ovary syndrome (PCOS) affects 4-12% of women of reproductive age (1). In May 2003 a joint consensus meeting of the European Society of Human Reproduction and Embryology (ESHRE), and the American Society of Reproductive Medicine (ASRM) held in Rotterdam defined PCOS as the presence of 2 of the following 3 criteria: (a) Oligo or anovulation (b) polycystic ovaries on US with 12 or more follicles measuring 2-9mm in diameter and/or increased ovarian volume >10cm³. (c) hyperandrogenism clinical and/or biochemical with the exclusion of other etiologies (2).

The pregnancy rate among PCOS women is only 40-50% even when other factors are excluded and ovulation induction is successful (3), (4). Moreover PCOS is associated with an increased rate of early pregnancy loss of 30-40% (5), due to luteal phase defect (3). The realization that features of PCOS appear to be caused by insulin resistance led to an explosion of interest in the use of insulin sensitizing drugs like metformin. In 1998 a randomized controlled trial (RCT) which reported high rates of ovulation in women treated with metformin and clomiphene fuelled this interest (6).

In addition recent RCTs have shown that women with PCOS who have reported ovulation after metformin exhibited surprisingly high reproductive potential with lower than expected rate of spontaneous miscarriage (7). Subsequent papers; however, have not shown metformin to be as effective as some of the early reports had suggested (8)(9). Being invasive histological analysis of endometrial biopsy (10), endometrial cytokines in uterine flushing (11), the genomic study of a timed endometrial biopsy (12) as methods of predicting uterine receptivity are impossible to be done in treatment cycles.

As a non-invasive method, Ultrasound examination of uterine receptivity has been widely used in spontaneous or stimulated cycles (13). Unfortunately, when using two dimensional (2D), endometrial thickness, and pattern have low positive predictive value and specificity for IVF outcome (14), (15). On the other hand, endometrial blood flow can be non-invasively evaluated by 2D or 3D ultrasound with color and power Doppler. Power Doppler is more sensitive than color Doppler imaging at detecting low velocity flow and hence improves visualization of small vessels.

Blood flow and vessel patterns are demonstrated by encoding the power in the Doppler signal rather than its mean frequency shift (16), (17). This prospective study was carried

out to study whether metformin when combined to clomiphene citrate had an effect – if any – on uterine receptivity using 3D ultrasound and 3D power Doppler study of the endometrium and subendometrial region.

Patients and Methods

This prospective single blinded study was conducted on Egyptian women attending the infertility clinic in Obstetrics and Gynecology department, Zagazig university from 1/6/2008 till 1/9/2009. The women's age range was 20-35 years. Of all the women attending the infertility clinic; eighty seven women satisfying the ESHRE and ASRM criteria for PCOS were included in this study (2). Ten women failed to ovulate during both arms of the study and were discontinued.

All had normal serum prolactin concentrations and thyroid function tests. Levels of 17 α hydroxy-progesterone were examined to rule out late onset congenital adrenal hyperplasia, all patients had normal values of < 2ng/ml. Waist to hip ratio to detect presence of android obesity was measured in all patients (waist to hip ratio > 0.85) (18). Free androgen index (FAI) was calculated for all women in the study. Women who intended to start a diet or specific physical activity program, affected by organic pelvic diseases, previous pelvic surgery, suspected peritoneal factor infertility, tubal or male factor infertility were excluded.

All participants had normal baseline FSH values and estradiol values on cycle day 2. Women were then monitored in stimulated cycles during which they were randomized to receive either 100mg of clomiphene citrate (Clomid; Merrell Dow, Middlesex, UK) daily for 5 days and metformin (850mg twice daily) with meals throughout menstrual cycle; or clomiphene citrate (CC) alone.

The women were blinded to metformin and placebo which was packaged by US and provided to patients. 3D ultrasound and 3D power Doppler indices on day 2 after hCG injection (hCG +2), and 7 days later serum progesterone levels were obtained. Serum estradiol (E2) values were measured on the day of hCG injection. All ultrasound measurements were performed 2 days after injection of hCG using Voluson 730 PROV (GE, Kretz, Zipf, Austria). Once a longitudinal view of a satisfactory grey scale image of the uterus had been obtained, the uterus was centralized within the 3D sector on the screen. The ultrasound machine was then switched to the 3D mode with power Doppler. The setting conditions were pulse repetition frequency 1, control frequency mid, colour gain 38.4, dynamic set: 2, balance: G>140, wall motion filter 75, frame rate 4-6, rise 0.2 persistence 0.8. These settings were found to offer the best compromise between small vessel detection and Doppler artefact (19). The setting condition for the subpower Doppler mode was gain – 6.0; balance: 140; wall motion filter: low 1.

When the power Doppler mode was switched on, the power Doppler box was positioned to cover the whole uterus and volume mode was then switched on. The resultant truncated sector covering the endometrial cavity in a longitudinal plane of the uterus was adjusted and moved and the sweep angle was set to 90° to ensure that a complete uterine volume encompassing the subendometrium was obtained. The patient and the 3D 7.5 MHz transvaginal probes remained as still as possible during volume acquisition; the volume of the endometrium was determined by manually drawing the endometrial outlines. The integrated VOCAL version 4.0 (Virtual Organ Computer Aided Analysis) – the imaging program for the 3D power Doppler histogram – was employed to calculate the endometrial volume and indices of blood flow within the endometrium. Vascularization index (VI) which is the ratio of the number of colour voxels to the number of all the voxels expressed as a percentage (%) of endometrium volume was used to represent the presence of endometrial vascularity. Flow index (FI) the

mean power Doppler signal intensity inside the endometrium was used to express the intensity of flow, then vascularization flow index (VFI) multiplying VI and FI was calculated to combine the aforementioned indices (18). These parameters are unitless with the exception of the VI that is expressed as a percentage.

The manual mode of the VOCAL contour editor was used throughout analysis and calculation with a 15° rotation step. As a result 12 contour planes were defined for the endometrium of each patient to cover 180°. The shell imaging technique which allows the user to generate a variable contour that parallels the original defined surface contour was used to examine the subendometrium. Subendometrial region was considered to be within 2 mm of the originally defined myometrial – endometrial contour. This is an arbitrary distance but one that reflects the inner third of the myometrium and the region supplied by radial arteries (20). VI, FI and VFI of the subendometrial region were then measured. The bias due to inter-observer error was avoided as ultrasonographic assessments were done by single operator.

To test intraobserver reliability of measures, the intraclass correlation coefficients with 95% confidence interval was determined using one – way random effects model (21). It was calculated by scanning 20 patients twice and analyzing each 3D dataset twice to assess consistency of 3D scanning and data acquisition. For the endometrium, the mean intraobserver correlation coefficient (95% CI) for volume, VI, FI, VFI were as follows; 0.95 (0.86, 0.98), 0.99 (0.97, 0.996), 0.89 (0.72, 0.96) and 0.99 (0.98, 0.997) respectively. The mean (95% CI) intraobserver correlation coefficient and the intraobserver variation was not statistically significant.

A urine pregnancy test was done 18 days after hCG injection. If it was positive, ultrasound examination was performed 10-14 days later to confirm intrauterine pregnancy and to determine the number of gestational sacs present.

Statistical analysis:

Statistical analysis was performed using the Statistical Package for Social Science (SPSS release 15.0, SPSS, Inc., USA). The primary outcome measures were endometrial volume, VI, FI, VFI for endometrial and subendometrial region as well as occurrence of clinical pregnancy. As measures were not normally distributed a non parametric test Wilcoxon signed ranks test or Fisher exact test and X²-test were employed to test association between measures of CC stimulated cycles and cycles when metformin was added. Two-tailed P < 0.05 was taken as significant.

Results

Of the 87 women, who entered the study, 10 failed to ovulate in response to the first CC induction. This left 77 eligible women, 39 of whom were randomized to metformin, and 38 to placebo. All the 77 women ovulated in response to the second CC induction. Baseline characteristics and early follicular (day 3) serum FSH, estradiol levels differences between both groups were insignificant as demonstrated in table (1). Endometrial subendometrial blood flow was totally absent in 3 patients (8%) in CC alone arm versus one patient (3%) in CC –metformin arm. Table (2) illustrates differences in ultrasound parameters on day 2 after hCG injection, estradiol level on day of hCG injection, as well as, pregnancy rate between study groups. Pregnancy rate was significantly higher in metformin receiving PCOS women. Ultrasound parameters between those who became pregnant and those, who failed to conceive on day 2 after hCG, and estradiol levels on hCG injection day are presented in table (3).

Table (1):
Baseline characteristics of participant groups.

Parameter	CC n=38	CC + metformin n=39	P value
Age (yr)	25.8 ± 2.8	26 ± 1.6	0.82
Duration of infertility (yr)	4.2 ± 2.3	3.9 ± 2.5	0.91
History of EPL* (%)	7/38 (19%)	7/39 (18%)	0.97
BMI (kg/m ²)	26.7 ± 2.3	27.1 ± 1.8	0.76
Fasting glucose (mmol/L)	5.3 ± 0.2	4.8 ± 0.7	0.25
Fasting insulin (pmol/L)	308.2 ± 52.1	240.3 ± 67.2	0.33
Free Testosterone (pmol/L)	32.8 ± 7.0	29.8 ± 3.1	0.77
Free Androgen index (%)	13.2 ± 4.8	14.8 ± 4.2	0.63
FSH (IU/L)	7.3 ± 1.7	7.2 ± 2.0	0.97
E ₂ (pmol/L)	359.1 ± 51.1	340.6 ± 54.3	0.87

Values are mean ± SE.
* EPL = Early pregnancy loss.
P<0.05 is considered significant.

Table (2):
Ultrasound parameters, estradiol levels, pregnancy rates in studied patients.

Parameter	CC alone n=38	CC - metformin n=39	P value
Estradiol on day ⁺ of hCG injection (pmol/L)	1897.93±1396	1674.72±1238	NS
Endometrial volume ⁺ (cm ³)	4.42 (1.27-9.52)	5.08 (1.42-11.72)	NS
Endometrial (VI) (%)	0.942 (0-8.421)	0.583 (0-6.321)	P<0.01
Endometrial FI (0-100)	24.463 (0-29.271)	22.613 (0-26.352)	P<0.01
Endometrial VFI (0-100)	0.314 (0 - 3.152)	0.117 (0-2.252)	P<0.01
Subendometrial VI (%)	2.734 (0- 20.871)	2.231 (0-19.789)	NS
Subendometrial FI (0-100)	25.027 (0-37.137)	24.871 (0-32.763)	NS
Subendometrial VFI (0-100)	0.583 (0-4.972)	0.462 (0-3.241)	NS
Pregnancy rate (%)	9/38 (24%)	14/39 (36%)	P<0.05

* Values are mean ± SD.
+ values are median (range)

Table (3):
Comparison of ultrasound parameters and estradiol values between pregnant and non pregnant women.

Parameter	Non pregnant n=54	Pregnant n=23	P value
Estradiol (pmol/L)	1836±1229	1544 ± 1035	P<0.05
Endometrial volume (cm ³)	4.96 ± 1.9	4.62 ± 1.3	NS
Endometrial VI (%)	0.89 ± 3.4	0.56 ± 1.9	P<0.01
Endometrial FI	22.4 ± 1.2	22.5 ± 2.5	NS
Endometrial VFI	0.23 ± 1.8	0.34 ± 2.6	P<0.05
Subendometrial VI (%)	2.73 ± 5.88	2.187 ± 6.73	NS
Subendometrial FI	24.312 ± 10.83	25.75 ± 11.96	NS
Subendometrial VFI	0.468 ± 1.68	0.581 ± 2.31	NS

Values are mean ± SD.

Discussion

Hyperinsulinemia contributes to the increased rate of early pregnancy loss in PCOS (3) (22) (23). Metformin treatment significantly decreased both serum insulin and glucose concentrations, and simultaneously decreased serum androgens and increased serum hormone binding globulin concentrations (24). On the other hand, the overall effectiveness of CC in inducing ovulation is 70% but this rate is reduced in the obese and in those with PCOS (25), and it is generally accepted that CC reduced uterine receptivity and thus the chances of conception (26), with further reduction in conception rate after the 3rd cycle of CC administration (9). We recorded significant reduction in endometrial volume and pregnancy rate in CC alone compared to CC-metformin arm of the study which substantiated previous studies.

In 2005, a randomized controlled trial, CC – metformin administration was associated with a higher improvement of fertility and reproductive outcome (9). In the current study, we hypothesized a specific effect of metformin on uterine receptivity. Blood flow in uterine blood vessels assessed by colour Doppler ultrasound is usually expressed as downstream impedance to flow and assumed by many studies to reflect actual blood flow to the endometrium, although the major compartment of the uterus is the myometrium and there is collateral circulation between uterine and ovarian vessels (27).

The 3D ultrasound and power Doppler provides a unique tool to examine blood supply towards the whole endometrium and the subendometrial region (28). In the present study, it was found that endometrial VI, VFI decreased significantly on the day of hCG injection in CC- metformin cycles compared to CC cycles. In the subendometrial region, there was a reduction in same indices in CC- Metformin group; however, it was not significant. This could be explained by the fact that the period of menstrual cycle when endometrial vascularization is at its lowest i.e. 1 to 5 days after ovulation is the period when morphological changes to prepare the endometrium for blastocyst implantation occur (29), and it is also the period when endometrial receptivity is thought to be at its maximum (30).

Decreased endometrial vascul-arity days after ovulation may lead to endometrial hypoxia. It has been shown in animal studies that

near atmospheric oxygen concentration reduce embryo viability compromise embryo development (31), and that oxygen tension in the uterus is lowest during the implantation period (32). Earlier two-dimensional Doppler studies reported a decline in blood flow velocity and pulsatility index in the uterine arteries 2 days before ovulation and an increase in blood flow velocity and a decline in resistance during the mid-luteal phase (33).

Endometrial hypoxia stimulates vascular endometrial growth factor (VEGF) in endometrial stromal cells. VEGF could explain the increase of VI and VFI values in endometrium and subendometrium 2 days after hCG injection (34)(35). A positive correlation between serum VEGF levels and levels of estradiol (E2) and progesterone has been demonstrated (36). The aforementioned fact agrees with the result of this study, which showed a significantly lower estradiol and endometrial VI 2 days later in women who became pregnant compared to those who were not.

Previous studies noted a nadir of VI and VFI in the endometrium 2 days after ovulation followed by an increase again during luteal phase and that these changes mirror the changes in plasma estradiol during menstrual cycle (37). In The present study, pregnancy rate in CC-metformin group was significantly higher than CC alone group. Metformin could act directly or indirectly on uterine vascularity.

Despite the fact that insulin resistance does not play a key role in reducing uterine perfusion in PCOs (38), it is possible to suppose that metformin acts on uterine perfusion by reducing androgen levels (39) and their vasoconstrictive effect on vascular tissues (38). Besides, metformin was found to increase mid-luteal concentration of serum glycodelin and insulin like growth factor 1 IGF-1, two putative biomarkers of endometrial receptivity of 3-fold and 4-fold respectively (40).

In this study, endometrial volumes were not significantly different between pregnant and non-pregnant women. Similar findings were found in other studies employing 3D ultrasound (41)(42). There is disagreement among studies about the definition of sub-endometrial region. In the studies of Kupesic et al (2001)(43) and Wu et al (2003)(44), 3D power Doppler indices of the endometrial region were not given and the subendometrial region included 5mm of the myometrial-endometrial interface.

In this study, we recorded endometrial and subendometrial region was defined as a shell within 2mm of the myometrial-endometrial so that we may study the most vascularized area of the subendometrium, the 2-mm shell was also chosen by Jakubkein et al (2006)(37), because only the myometrium immediately underlying the endometrium exhibits a cyclic pattern of steroid receptor expression like that of the endometrium (45).

Other authors (26)(44)(46) considered 5mm shell as the subendometrial region, however if 5mm was taken the subendometrial region may extend beyond the uterine contour especially in the corneal region, and fibroids may be included within that shell. Even larger 10mm- shell was defined as subendometrial region by Chien and coworkers 2002 (47). Conversely, Ng et al (2006)(48) used a small 1mm shell from myometrial endometrial interface.

It was found that subendometrial VI, FI, and VFI values were not statistically different between CC alone and CC- metformin treatment groups on the day of hCG injection. Similarly, no significant difference in subendometrial 3D power Doppler values between pregnant women and women who failed to conceive. Other studies measuring subendometrial blood flow on the day of hCG injection (44)(48), reported a significantly higher VFI values in those who became pregnant but, they were excluding PCOS patients, using a long protocol of GnRH agonist and gonadotropin stimulation and the difference in the selection of subendometrial region could explain the difference.

In conclusion, women receiving CC- metformin had significantly lower endometrial VI, FI and VFI than women taking CC alone on the day of hCG injection. Patients in the pregnant group have significantly lower endometrial VI, VFI than those in the non pregnant group. In contrast E2 levels on hCG injection day were significantly higher in women who failed to conceive. Further studies using same; inclusion criteria of patients, ovulation stimulation regimen, the day of ultrasound examination and subendometrial shell selection are required to clarify the role of 3D power Doppler in the prediction of pregnancy in PCOS patients.

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