Effect of Metformin on Endometrial Thickness and Subendometrial Flow Patterns in Anovulatory Patients with Polycystic Ovarian Syndrome

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Abstract

Background: Polycystic ovarian syndrome (PCOS) is considered the commonest endocrinological disorder affecting reproductive aged women. PCOS compromises fertility through various pathways. These pathways include hyperandrogenism, insulin resistance and impedance of the uterine and endometrial blood flow. Metformin improves the blood flow to the endometrium. It acts by reducing androgen level and correction of insulin resistance. Endometrial vascular indices were evaluated in this study to evaluate endometrial receptivity in anovulatory patients with PCOS. **Aim of the Work:** To evaluate the outcome of metformin administration in anovulatory patients with PCOS and its effect on the endometrium. This included its role in ovulation and improvement of pregnancy rates. **Patients and Methods:** This study included 85 patients from Ain Shams University outpatient infertility clinics from 1st of January, 2018 till 30th of June, 2018. We investigated these patients before treatment with ultrasound on day 14, 21. We evaluated endometrial thickness, uterine artery flow pattern, endometrial and subendometrial flow patterns. The patients received metformin 500 mg three times per day for three months. After this duration, we reevaluated them by ultrasound at days 14, 21. **Results:** Metformin therapy resulted in a significant increase of endometrial thickness and had a significant decrease on uterine, endometrial and subendometrial resistance index (R.I) and pulsatility index (P.I) at day 14, 21 compared to pre-treatment values indicating better blood flow. **Conclusion:** Metformin therapy resulted in improvement of endometrial flow patterns. Also, it resulted in increase in endometrial thickness and improvement of uterine artery flow.

1. Introduction

Most women with polycystic ovarian syndrome (PCOS) have some degree of insulin resistance. It is a known fact that hyperinsulinemia augments androgen production in PCOS [1]. Insulin resistance has further been associated with abnormal endometrial development and endometrial defects in women with PCOS. [2]. Abnormal uterine blood flow has been observed in women with PCOS; the subfertile population in general [3] and in women with idiopathic recurrent miscarriage [4].

Infertility associated with PCOS also results from chronic anovulation. There are increasing data suggesting that implantation failure can further reduce pregnancy rates in these women [5]. Although ovulation is readily obtained with medical induction, implantation rates remain lower than fertile controls and early pregnancy loss rates are increased [2].

Metformin, an insulin sensitizer, is widely used in women with PCOS to correct this biochemical disorder helping ovulation [6]. One study reported that insulin reduction with metformin enhanced uterine vascularity and reduced uterine vascular resistance, as demonstrated by a 20% reduction in vascular resistance in spiral arteries after metformin use [2]. Endometrial receptivity could be indirectly assessed by ultrasound. This includes the assessment of uterine artery velocimetry and the endometrial characteristics such as thickness, pattern and vascularity [7].

In the current study, we evaluated the effect of metformin received by anovulatory patients with PCOS on endometrial thickness and endometrial vascular flow patterns, in addition to ovulation and pregnancy rates in these women.

2. Patients and Methods

Eighty five patients complaining of infertility due to chronic anovulation associated with PCOS were recruited from Ain Shams University Outpatient Infertility Clinic from January 1st, 2018 until June 30th, 2018. Patients were between 20 and 35 years of age complaining of irregular menses and fulfilling the following criteria for diagnosis of PCOS according to the Androgen Excess and Polycystic Ovarian Syndrome Society [8]:

- presence of hyperandrogenism in the form of hirsutism and/or hyperandrogenaemia;
- presence of ovarian dysfunction in the form of oligo or anovulation and/or polycystic ovaries by transvaginal ultrasound.
- exclusion of other causes of androgen excess or related disorders like defi-

cient non-classic adrenal hyperplasia, androgen-secreting neoplasms, androgenic/anabolic drug use or abuse, Cushing's syndrome, the Hyperandrogenic-Insulin Resistance-Acanthosis Nigricans Syndrome, thyroid dysfunction and hyperprolactinemia.

Patients with regular monthly menses, receiving weight loss eating programs and/or physical exercising were excluded. Furthermore, patients with chronic medical disorders or with uterine fibroids or endometriosis or patients with endocrinological disorders like diabetes mellitus, hypothyroidism, hyperprolactinaemia, Cushing's syndrome and non-classical congenital adrenal hyperplasia were also excluded.

The current study was approved by the Ethical Committee of the Department of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University. It was registered on Clinical trial.gov with I.D:NCT03486626. Written informed consents were obtained from all participants.

Pretreatment with metformin, all participants were subjected to detailed history taking and examination, including body mass index (BMI) calculation and hirsutism scoring using modified Ferriman Gallwey Index [9]. Hormonal profiles for all patients were determined including follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, thyroid stimulating hormone (TSH), androstienodione, free testosterone and 17-hydroxyprogesterone. Serum progesterone level was also measured on day 21 after withdrawal bleeding due to progestin administration. A value less than 3 ng/ml confirmed anovulation in these patients (Figure 1). Transvaginal ultrasound was done for all participants on days 14 and 21 to determine endometrial thickness, uterine artery flow pattern,
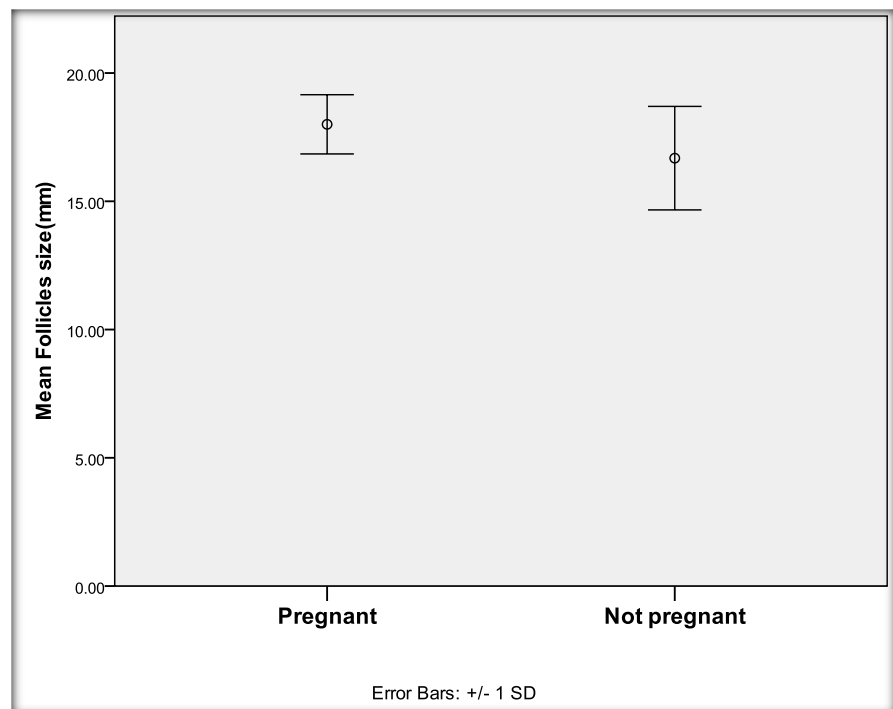

Figure 1. Comparison between pregnant and not pregnant regarding follicle size.

endometrial and subendometrial flow patterns. Samsung R5 ultrasound machine equipped with a 7.5 MHz vaginal probe was used. Uterus was visualized in the longitudinal section. Endometrial thickness was identified as a double layer and maximum distance between each myometrial/endometrial interface was measured. Colour flow images of the ascending main branch of the right and left uterine arteries at the level of the internal os in a longitudinal plane before it entered the uterus were visualized and studied by positioning a sample volume over the area of maximum color and by activating the pulsed Doppler. The mean of three consecutive waveforms of good quality were analyzed. Blood flow impedances of both uterine arteries were calculated and expressed as pulsatility index [PI = (peak systolic velocity − peak diastolic velocity)/mean velocity] and resistance index [RI = (peak systolic velocity − peak diastolic velocity)/peak systolic velocity]. Furthermore, color flow images of endometrial blood vasculature and subendometrial vessels lying within 10 mm of the echogenic endometrial borders were detected by color Doppler energy and PI and RI were calculated electronically when three similar, consecutive waveforms of good quality were obtained.

All participants received metformin 500 mg three times daily for three months. After this duration, transvaginal ultrasound was done on days 14 and 21 of a spontaneous or progestin induced menses. Serum progesterone on day 21 of a spontaneous or progestin induced menses was measured and ovulation was confirmed by a level > 3 ng/ml on day 21 of the cycle.

N.B: We measured serum progesterone on day 21 of progestin induced menses in patients with amenorrhea and in patients who got regular menses every 28 days with metformin treatment. Serum progesterone was measured one week before time of expected menses in patients who got regular cycles longer than 28 days, for example day 28 of 35 day cycle.

During the 3 months period of metformin treatment, we asked the participants to measure serum B-hCG monthly in patients with amenorrhea or one week after missed period in patients who regular menses with metformin treatment.

Data analysis was done using SPSS program version 24. Quantitative variables were presented as mean and standard deviation, while qualitative variables were presented as count and percent. Student t test was used to compare quantitative variables between two independent groups and paired samples t test was used to compare them before and after treatment for the same group. A p-value < 0.05 was considered statistically significant.

3. Results

In our study, 85 patients with anovulatory PCOS were investigated. Their demographic data and hormonal profile are shown in Table 1. Table 2 shows that there is a statistically significant difference between all ultrasonographic results before and after treatment. Pretreatment endometrial thickness on day 14 was

Table 1. Personal data and baseline hormonal profile.

	N	Minimum	Maximum	Mean	SD
Age(years)	85	20.00	36.00	24.95	3.36
BMI(kg/m^2)	85	21.40	43.00	30.23	4.33
Waist/hip ratio	85	0.82	0.90	0.86	0.02
Modified FerrimanGallway score	85	12.00	19.00	16.15	1.59
FSH (mIU/ml)	85	3.60	12.00	5.80	1.33
LH (mIU/ml)	85	2.40	16.80	10.06	2.85
E2 (pg/ml)	85	23.00	78.00	42.80	12.66
Prolactin (ng/ml)	85	3.60	25.00	12.36	4.97
TSH (μIU/ml)	85	0.49	3.00	1.39	0.60
Progesterone (nmol/l)	85	1.25	3.26	2.48	0.50
17-OH-Progesterone (nmol/l)	85	3.10	79.00	6.12	8.10
Androstenedione (nmol/l)	85	5.00	6.00	5.54	0.29
Free Testosterone (pg/dl)	85	0.30	16.00	2.07	1.75

Table 2. Comparison between ultrasonographic findings before and after treatment.

	Pre		Post		t*	Mean diff.	95% CI for diff.		P value
	Mean	SD	Mean	SD			Lower	Upper	
Endometrial Thickness day 14 (mm)	6.67	1.14	11.82	2.25	17.355	−5.15217	−5.74457	−4.55978	< 0.001
Endometrial thickness day 21 (mm)	7.84	1.13	13.21	1.50	23.943	−5.36957	−5.81708	−4.92205	< 0.001
Endometrial vascularity P.I day 14	0.93	0.09	0.80	0.09	8.584	0.13159	0.10100	0.16219	< 0.001
Endometrial vascularity R.I day 14	0.63	0.06	0.54	0.10	7.254	0.09493	0.06881	0.12104	< 0.001
Subendometrial vascularity P.I day 14	0.90	0.07	0.79	0.09	9.010	0.10826	0.08428	0.13224	< 0.001
Subendometrial vascularity R.I day 14	0.91	0.06	0.57	0.06	33.000	−0.33754	−0.35795	−0.31713	< 0.001
Endometrial vascularity P.I day 21	0.96	0.11	0.77	0.08	10.972	0.18812	0.15390	0.22233	< 0.001
Endometrial vascularity R.I day 21	0.64	0.07	0.52	0.07	9.611	0.11638	0.09221	0.14054	< 0.001
Subendometrial vascularity P.I day 21	0.95	0.05	0.77	0.06	18.817	0.18116	0.16195	0.20037	< 0.001
Subendometrial vascularity R.I day 21	0.65	0.06	0.54	0.07	1.580	0.10899	−0.02862	0.24660	0.119
Uterine artery doppler P.I day 14	3.50	0.55	2.75	0.26	10.634	0.74667	0.60656	0.88678	< 0.001
Uterine artery doppler R.I day 14	0.94	0.10	0.86	0.08	6.090	0.08203	0.05515	0.10891	< 0.001
Uterine artery doppler P.I day 21	3.42	0.61	2.70	0.25	8.309	0.72348	0.54973	0.89723	< 0.001
Uterine artery Doppler R.I day 21	0.95	0.08	0.82	0.08	9.038	0.12275	0.09565	0.14986	< 0.001

*Paired samples t test.

6.67 ± 1.14 mm and on day 21 was 7.84 ± 1.13 mm while post-treatment value was 11.82 ± 2.25 mm and 13.21 ± 1.50 mm respectively (p < 0.001). Pretreatment values of uterine artery P.I and R.I on day 14 were 3.50 ± 0.55, 0.94 ± 0.1 and on day 21 were 3.42 ± 0.61, 0.95 ± 0.08 respectively, while post-treatment

values on day 14 were 2.75 ± 0.26, 0.86 ± 0.08 and on day 21 were 3.42 ± 0.61, 0.95 ± 0.08 respectively, (p < 0.001). Pretreatment values of endometrial vessels P.I and R.I on day 14 were 0.93 ± 0.09, 0.63 ± 0.06 and on day 21 were 0.96 ± 0.11, 0.64 ± 0.07 respectively, while post-treatment values on day 14 were 0.80 ± 0.09, 0.54 ± 0.1 and on day 21 were 0.77 ± 0.08, 0.52 ± 0.07 respectively; (p < 0.001). Pretreatment values of subendometrial vessels P.I and R.I on day 14 were 0.90 ± 0.07, 0.91 ± 0.06 and on day 21 were 0.95 ± 0.05, 0.65 ± 0.06 respectively, while post-treatment values on day 14 were 0.79 ± 0.09, 0.57 ± 0.06 and on day 21 were 0.77 ± 0.06, 0.54 ± 0.07 respectively (**Figure 2**).

Spontaneous ovulation occurred in 30.6% of patients. Spontaneous pregnancy occurred in 16 patients (18.8%). Twelve patients got pregnant in the 3rd month of treatment, while 3 patients got pregnant in the 2nd month of treatment and only one patient got pregnant in the 1st month of treatment. **Table 3** shows that there is a statistically insignificant difference between all personal data and hormonal profiles between pregnant and non-pregnant patients. **Table 4** shows that there is no statistically significant difference regarding ultrasound data between pregnant and non-pregnant participants before treatment with metformin (p > 0.05) except for 2 measurements which are endometrial flow R.I on day 21 and subendometrial flow P.I on day 21 which were significantly different (p < 0.001) (**Figure 3**).

4. Discussion

For Implantation to be of great success, it depends on an interaction between the
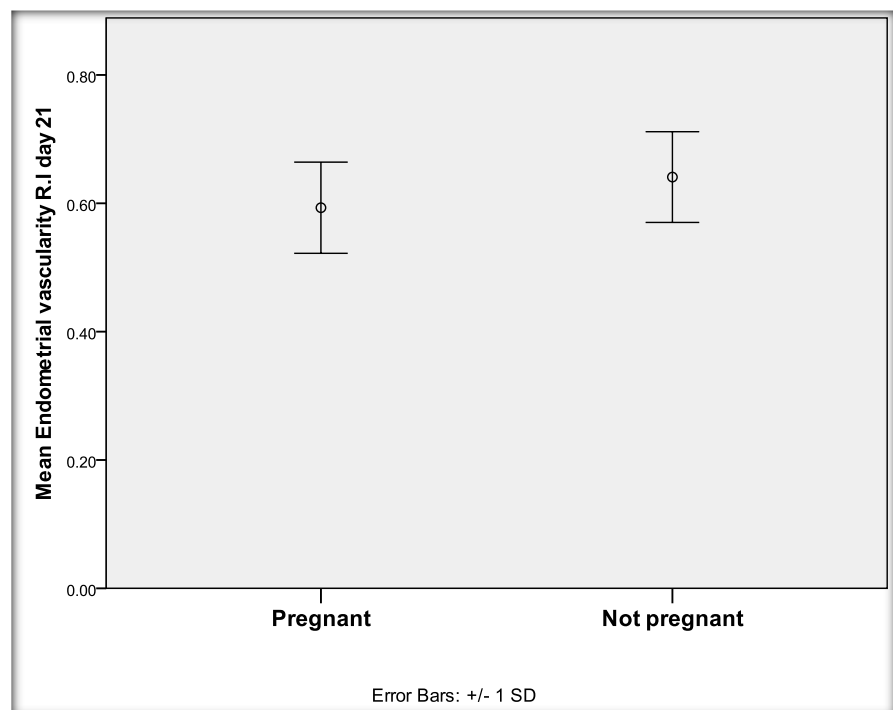

Figure 2. Comparison between pregnant and not pregnant regarding endometrial vascularity R.I day 21 before treatment.

Table 3. Comparison between pregnant and non-pregnant regarding personal data and baseline hormonal profile.

	Pregnant (N = 16)		Not pregnant (N = 69)		t*	Mean diff.	95% CI for diff.		P value
	Mean	SD	Mean	SD			Lower	Upper	
Age (years)	24.63	2.66	25.03	3.51	0.432	−0.40399	−2.26490	1.45693	0.667
BMI (kg/m²)	31.34	3.65	29.97	4.46	1.147	1.37708	−1.01056	3.76473	0.255
waist/hip ratio	0.86	0.02	0.86	0.02	0.453	−0.00222	−0.01195	0.00751	0.651
Modified FerrimanGallway score	16.19	1.87	16.14	1.54	0.096	0.04257	−0.84150	0.92665	0.924
FSH (mIU/ml)	5.42	0.85	5.89	1.41	1.292	−0.47487	−1.20590	0.25615	0.200
LH (mIU/ml)	9.70	1.78	10.15	3.04	0.779	−0.44931	−1.61624	0.71762	0.441
E2(pg/ml)	42.96	11.50	42.77	13.00	0.054	0.19103	−6.83959	7.22165	0.957
Prolactin (ng/ml)	13.66	5.73	12.06	4.78	1.163	1.60076	−1.13786	4.33938	0.248
TSH(µIU/ml)	1.16	0.48	1.44	0.62	1.726	−0.28535	−0.61416	0.04346	0.088
Progesterone (nmol/l)	2.37	0.49	2.50	0.51	0.937	−0.13106	−0.40917	0.14705	0.351
17-OH-Progesterone (nmol/l)	4.83	1.08	6.41	8.96	0.703	−1.58324	−6.06494	2.89846	0.484
Androstenedione (nmol/l)	5.56	0.30	5.54	0.29	0.209	0.01712	−0.14592	0.18016	0.835
Free Testosterone (pg/dl)	1.91	0.79	2.11	1.91	0.424	−0.20679	−1.17650	0.76292	0.673

*Student t test.

Table 4. Comparison between pregnant and non-pregnant regarding ultrasonographic data before treatment.

|	Pregnant (N = 16)		Not pregnant (N = 69)		t*	Mean diff.	95%CI for diff.		P value
	Mean	SD	Mean	SD			Lower	Upper	
Endometrial Thickness day 14 (mm)	6.96	1.46	6.67	1.14	0.869	0.28958	−0.37349	0.95266	0.388
Endometrial thickness day 21 (mm)	8.14	1.65	7.84	1.13	0.875	0.30172	−0.38419	0.98763	0.384
Endometrial vascularity P.I day 14	0.93	0.09	0.93	0.09	0.061	0.00149	−0.04711	0.05010	0.951
Endometrial vascularity R.I day 14	0.63	0.07	0.63	0.06	0.071	−0.00130	−0.03774	0.03513	0.943
Subendometrial vascularity P.I day 14	0.91	0.06	0.90	0.07	0.417	0.00749	−0.02823	0.04321	0.678
Subendometrial vascularity R.I day 14	0.56	0.08	0.57	0.06	0.758	−0.01390	−0.05041	0.02260	0.451
Endometrial vascularity P.I day 21	0.95	0.11	0.96	0.11	0.174	−0.00524	−0.06513	0.05466	0.862
Endometrial vascularity R.I day 21	0.59	0.07	0.64	0.07	2.432	−0.04774	−0.08679	−0.00870	0.017
Subendometrial vascularity P.I day 21	0.91	0.04	0.95	0.05	2.883	−0.03884	−0.06564	−0.01204	0.005
Subendometrial vascularity R.I day 21	0.55	0.05	0.65	0.56	0.676	−0.09480	−0.37372	0.18412	0.501
Uterine artery doppler P.I day 14	3.73	0.54	3.50	0.55	1.507	0.22928	−0.07338	0.53193	0.136
Uterine artery doppler R.I day 14	0.93	0.07	0.94	0.10	0.190	−0.00485	−0.05547	0.04577	0.849
Uterine artery doppler P.I day 21	3.32	0.48	3.42	0.61	0.626	−0.10197	−0.42585	0.22191	0.533
Uterine artery Doppler R.I day 21	0.92	0.08	0.95	0.08	1.338	−0.02955	−0.07346	0.01436	0.184

*Student t test.
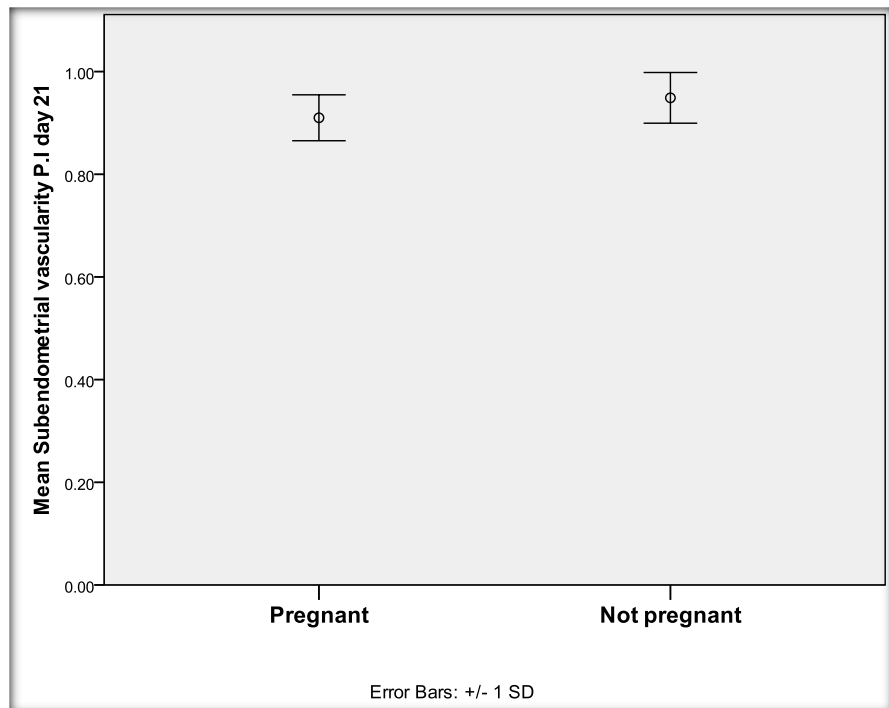
Figure 3. Comparison between pregnant and not pregnant regarding subendometrial vascularity P.I day 21 before treatment.

receptive endometrium and the blastocyst. Endometrial receptivity can be evaluated by histologic evaluation of an endometrial biopsy, endometrial proteins in uterine flushing, or more commonly ultrasound examination of the endometrium. Different ultrasound parameters have been used to assess endometrial receptivity including endometrial thickness, endometrial pattern, endometrial volume, Doppler study of uterine arteries, and endometrial blood flow. [10]

Anovulatory women with PCOS were investigated before and after 3 months of treatment with metformin regarding endometrial thickness, uterine, endometrial and subendometrial vascular indices. Endometrium was significantly thinner before the treatment. All vascular indices were significantly higher before treatment with metformin compared to post-treatment values. These results were the same as *Palomba et al.* [11]

Battaglia et al. concluded that anovulatory patients with PCOS have an alteration in uterine vascularization [12]. In fact, PI and RI values, two measures of blood impedance inversely related to blood flow, were not only unchanged throughout the cycle [13] but also significantly higher before the treatment.

In the current study, it was found that uterine Doppler indices were increased in PCOS patients and this is similar to the study done by *Mohsen et al.* [14]. The uterine artery Doppler indices were reduced upon treatment by metformin as all the patients had hyperandrogensim. It suggested that uterine blood flow was reduced in women with PCOS who had hyperandrogenism or were obese [15]. This indicates that the PCO syndrome with hyperandrogensim can decrease the uterine, endometrial and subendometrial flow patterns which are indirect indi-

cators of endometrial receptivity [16].

In the current study, metformin therapy led to significant increase in endometrial thickness and had a significant effect on uterine RI and PI. On the endometrial level, endometrial and subendometrial R.I and P.I were significantly reduced after metformin treatment indicating better blood flow. In response to metformin therapy, ovulation was achieved in 26 women (30.6%). Pregnancy rate was 18.8%. Twelve patients got pregnant in the 3rd month of treatment. That may be explained by the cumulative effect of metformin action. There were no significant differences between pregnant and non-pregnant women regarding endometrial thickness, personal data nor hormonal profiles. Likewise, pretreatment values of uterine RI and PI were insignificantly different in pregnant compared to non-pregnant women; however, decreased endometrial flow R.I on day 21 and decreased subendometrial flow P.I on day 21 was significantly different.

Metformin did improve the ovulation and clinical pregnancy rates when added to letrozole or clomiphene citrate for ovulation induction in PCOS patients this is attributed to the addition of either letrozole or clomiphene citrate [17]. Endometrial thickness was considered a marker for uterine receptivity and for pregnancy prediction with different results. Although *Kocak et al.* [18] demonstrated a significant effect of metformin on endometrial thickness in PCOS women treated with clomiphene citrate, the study done by *Maged et al.* [19] showed that there was no improvement in endometrial thickness with metformin therapy and that treatment with N-Acetyl Cysteine was superior to metformin. In the current study, metformin treatment increased endometrial thickness significantly. This variation in results could be attributed to the lower Ferriman Gallway starting score used or the different inclusion criteria. The results of the current study demonstrated the vascular effect of metformin on the endometrium and uterine artery flow pattern. It also demonstrated an improvement of endometrial and subendometrial flow patterns after metformin therapy.

Limitation of the Study

Longer duration of treatment might give more conclusive results. Also investigating hormonal profile post treatment could have been done to give an idea on whether metformin has an effect on the hormonal profile in this group of patients. Better assessment of the parameters of the endometrium should have been done by 3-dimensional ultrasound.

5. Conclusion

We can conclude that endometrial vascularity was abnormally reduced in women with anovulatory PCOS. Metformin therapy improves endometrial flow pattern. In addition, it increases endometrial thickness and improved impedance observed in uterine artery flow observed in women with PCOS. We acknowledge that this study suggests that changes in the endometrial and subendometrial blood flow in some women with PCOS are only a preliminary one. The overlap

of the variables also renders it impossible to establish a practical cut-off level.

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Conflicts of Interest

No potential conflict of interest was reported by the authors.

Informed Consent

Informed consents were obtained from all participants.

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