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Plavix Versus Low Dose Aspirin Effect On Uterine Perfusion in Infertile Women With Thin Endometrium: A Randomized Controlled Trial

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Abstract

The endometrium must be at least 9 mm thick to provide a site for proper fetal implantation and also maintains the growing baby in the later stages of gestation. If, due to any reason, this lining becomes not adequate, it becomes impossible for the blastocyst to get implanted to the uterus. It is vital for practitioners to be aware of this issue, so this study aimed to explore the effect of Plavix (75 mg) compared with aspirin (75mg) on improving the endometrial thickness in women with thin endometrium. Prospective observational study ***Setting****: Ain Shams Maternity Hospital and Al-Azhar University hospital (New Damietta).* ***Patients****: 500 women with thin endometrium (≤ 8 ml) were divided randomly into two equal arms of 250 women in each one.* ***Intervention****: Group I administered Plavix while group II received Aspirin. Endometrial pattern and thickness, uterine artery resistance and pulsatility indices, dominant follicles, and pregnancy rates of the two groups were measured. Participants' ages ranged from 20 to 35. There was a significant effect of the two drugs in improving the endometrial thickness and pattern (trilaminar) when compared with the previous untreated cycles but there was no significant difference between Plavix and Aspirin effects as regards these parameters. Also, there was no difference between the two groups as regards uterine artery PI and RI, mean diameter of dominant follicle and pregnancy rates. Low dose aspirin and Plavix had no significant difference in increasing endometrial thickness, PI, RI of uterine artery or increasing pregnancy rates when compared to each other.*

Keywords
Plavix; Aspirin; Infertility, Uterine perfusion; Endometrium

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I. Introduction

In the follicular phase, the endometrium undergoes sufficient proliferation and differentiation under the effect of Estrogen. Then, during the luteal phase, this is followed by timely secretory changes. Implantation is depending on an intimate dialogue between the fertilized ovum and the receptive uterine wall [1]. Endometrial receptivity is a mystery but can be assessed by histological examination of an endometrial sample, endometrial proteins in uterine wash or more frequently an ultrasound assessment of the endometrium [2]. Various ultrasound parameters had been utilized to evaluate endometrial receptivity during induction of ovulation programs and these comprise endometrial pattern, thickness and volume, uterine vascular tree and blood flow of the endometrium by Doppler. Endometrial pattern and thickness have low specificity and positive predictive value in the evaluation of pregnancy outcome [3-4].

Angiogenesis plays a vital role in different processes of female reproductive like growth of an ovarian dominant follicle, corpus luteum, and development of receptive endometrium and implantation. A good blood flow in the endometrium is usually considered a crucial prerequisite for implantation [5-7].

Uterine blood supply is evaluated by color Doppler ultrasound and is expressed, as a downstream resistance to flow due to the assessment of blood flow volume is inaccurate and difficult. This is dependent on the insonation angle, the accurate evaluation of vessels' diameter and the tortuosity of the vessels. Dickey R, 1997 [8] concluded that the implantation in stimulated cycles was less when the uterine artery pulsatility index (PI) was ≥ 3.3-3.5 and the uterine artery resistance index (RI) was ≥ 0.95 at the time of HCG intake.

However, few studies have suggested the value of aspirin in patients receiving induction of ovulation. Aspirin may increase the pregnancy outcomes with artificial reproductive technique (ART) (2,4,5). Kuo et al. [9] suggested that aspirin might increase uterine blood flow in women with unexplained infertility. Wada et al. [10] reported an improvement in uterine blood flow resistance, pregnancy rate, and less miscarriage rate in women with low uterine flow after treatment with aspirin.

Plavix is adenosine diphosphate receptor (ADP) antagonist, a class of oral antiplatelets agents that block P2Y12 component of adenosine diphosphate receptor so it prevents platelet activation and aggregation. It has a rapid action and statistically significant prevention of ADP induced aggregation of platelets is within hours after the first dose and effect is continued during long term treatment [11]. As much as we know, this is the earliest prospective series aiming to evaluate the role of Plavix in patients with thin endometrium and receiving induction of ovulation.

II. Methods

This is an observational prospective clinical trial was carried out at Ain Shams Maternity University Hospital (ASUMH) and Al-Azhar University (New Damietta) involving 500 infertile women with thin endometrium (≤ 8 mm, at the time of HCG intake in the previous cycle) who were undergoing induction of

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ovulation programs; all women were assigned randomly into two groups; a first group of 250 women who received Plavix 75mg and a control group of 250 women who administered aspirin 75mg. This study was conducted during the period from January 2013 to December 2015 and was approved by Ethical Committee of the Faculty of Medicine, Ain Shams University and Al-Azhar University (New Damietta). Explanation of the procedure and verbal consent was taken from all patients participating in the study.

Inclusion criteria:

1. Age: 20-35
2. Infertile women due to ovarian insufficiency with thin endometrium in previous cycles

Exclusion criteria:

1) Couples with female age ≥35 years
2) Poor semen analysis and male factor infertility
3) Hydrosalpinx, uterine cavity abnormality

All patients were subjected to:

1) Full history taking and complete physical examination.
2) Counseling and verbal consent was taken for every patient.
3) All patients received the same induction protocol in the form of: clomiphene citrate (CC) combined with human menopausal gonadotropin (hMG) (Merional, IBSA, pharmaceutici, Italy). CC (150 mg/ day) was given orally on cycle day 2 through cycle day 6 and then four doses of hMG (150 IU/day) were given on cycle day 6, 8, 10 and 12. Ultrasound survey was done on cycle day 10, 12, and 14. Human chorionic gonadotropin (hCG) (5000 IU; Profasi; Serono, Rome, Italy) was given when two follicles ≥16mm in diameter were found.
4) All patients underwent sonography and Doppler surveys on menstrual day 3 and the day of timed intercourse.
5) Sonographic and Doppler assessment were performed transvaginally with a 5-MHz endovaginal probe of VOLUSON, GENERAL ELECTRIC 7300 USA. The endometrial pattern, thickness, pulsatility index (PI) and resistance index (RI) of the uterine artery, and ovarian dominant follicle were evaluated. Endometrial thickness was evaluated at the widest antero-posterior diameter (APD) of the endometrium under a vertical section or the farthest distance between the myometrial–endometrial interfaces through the central longitudinal axis of the uterus.
6) Two endometrial patterns were described: trilaminar and nontrilaminar. A trilaminar pattern was defined as hypoechoic layer with a middle hyperechoic line. A nontrilaminar pattern was known as a single homogeneous layer.
7) For Doppler evaluation, it was focused on the ascending branch of the uterine arteries. Pulsatility and resistance indices were measured for right and left arteries and an average was calculated and used for comparison.

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Evaluations of Uterine Artery Doppler indices

- **Transabdominal Ultrasound Technique:** A 5 or 3.5-MHz curvilinear trans-abdominal transducer is utilized. A mid-sagittal slice of the uterus and cervical canal is taken and the transducer is moved in a lateral direction until visualization of the paracervical vessels. Color flow Doppler is applied. The uterine arteries are seen along the sides of the cervix. Using pulsed wave Doppler, flow velocity waveforms from the ascending uterine artery close to the internal cervical os are obtained, with the Doppler sampling gate set at 2 mm. A precaution is taken to utilize the lowest angle of insonation (<30°) to reach the highest systolic and end-diastolic velocities. When 3 similar consecutive waveforms are taken, the PI can be well evaluated. The mean PI is calculated as the average reading from each side combined. Another site for uterine artery Doppler insonation, is at the level of its crossover with the external iliac artery. Utilizing this technique, the probe is positioned approximately 2-3 cm inside the iliac crests and then moved toward the pelvis and the uterine sides. Color flow Doppler is used to identify each uterine artery. Pulsed wave Doppler is inserted nearly 1 cm above the point at which the uterine artery crosses over the external iliac artery. This confirms that Doppler velocities are taken from the main trunk of the uterine artery [12].
- **Transvaginal Ultrasound (TVS) Technique:** A 4.6–8 MHz trans-vaginal transducer is used. The transducer is placed in the anterior fornix of the vagina and a sagittal cervical section is taken. The vaginal probe is then moved until the paracervical vascular tree is visualized. Colored Doppler is applied and the uterine artery is visualized at the level of the cervicocorporeal junction. Measurements are obtained at this point before the uterine artery divides into the smaller arcuate arteries [13].

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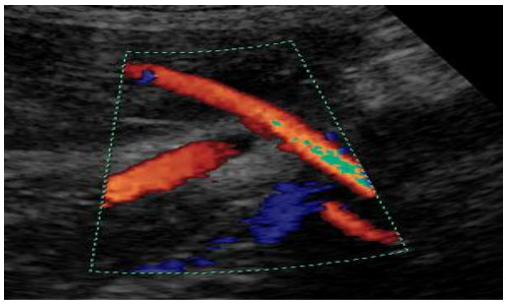
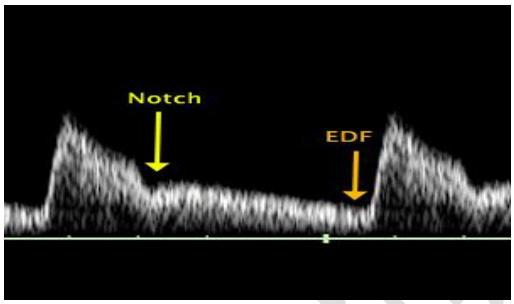
A.  B. 

A) Insonation of the uterine artery at the crossover with the ipsilateral iliac artery.
B) Normal flow velocity waveform from the uterine artery

Changes in Uterine Artery Doppler Waveform

Resistance index (RI)	Maximum – minimum velocity/maximum velocity
Pulsatility index (PI)	Maximum – minimum velocity/mean velocity

In the non-pregnant state, uterine Doppler interrogation typically exhibits a low end-diastolic velocity and an early diastolic notch. Uterine artery impedance might be influenced by many factors such as maternal heart rate, antihypertensive use, and hormonal changes in the menstrual cycle, and the chronic rise in androgen level in the polycystic ovarian syndrome. Resistance to blood flow within the uteroplacental tree is passing upstream to the uterine vessels and can be evaluated as an elevated pulsatility index (PI) or resistance index (RI). Uterine artery PI values are influenced by ethnicity and are less in obese women with a high body mass index (BMI). Uterine artery PI and RI values decrease with pregnancy and increasing gestational age, a change that is known to be a result of a fall in impedance in uterine vessels following trophoblastic invasion [14] abnormal RI (RI > 0.58)

8) If there were more than one follicle ≥16 mm, their mean value represented the diameter of the dominant follicle. If all follicles were <16mm, the largest one was checked and taken as the dominant follicle. Luteal phase support by progesterone was administered 5 days after the timed intercourse.
9) Clinical pregnancy was defined as a positive blood pregnancy test 2 weeks after HCG injection and confirmed by identification of intra-uterine gestational sac by trans-vaginal sonar. If a pregnancy was achieved, patients were instructed to continue the aspirin

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through 8 weeks after the first day of the last menstrual period.

10) Further statistic analyses were involving Patients' age, body mass index, occupation, educational level, endometrial thickness, pattern, uterine artery PI and RI and ovarian dominant follicle, clinical pregnancy rates of both groups.

Ethical consideration:

Institutional review board approval: the ethical scientific committee for approving the study discussed the protocol and informed consent was obtained before participation.

Consent procedure:

The Investigator made certain that an appropriate informed consent process was in place to ensure that potential research subjects, or their authorized representatives, were fully informed about the nature and objectives of the clinical study, the potential risks and benefits of study participation, and their rights as research subjects. The Investigator obtained the written, signed informed consent of each subject, or the subject's authorized representative, prior to performing any study-specific procedures on the subject. The Investigator retained the original signed informed consent form.

Subject Confidentiality:

All laboratory specimens, evaluation forms, reports, video recordings, and other records that leave the site would not include unique personal data to maintain subject confidentiality.

Randomization:

For allocation of the participants, a computer-generated list of random numbers was used and was kept in Ain Shams Maternity Hospital computer and with research supervisors. Participants were randomly assigned following simple randomization procedures (computerized random numbers) to 2 treatment groups modified Lynch and classical Lynch groups. Group assignments were allocated according to a computer-generated randomized series, were kept in sealed envelopes.

Statistical methodology: Retrieved data were recorded on an investigative report form. The data were analyzed with SPSS® for Windows®, version 15.0 (SPSS, Inc, USA). Description of quantitative (numerical) variables was performed in form of mean, standard deviation (SD) and range. Description of qualitative (categorical) data was performed in the form of numbers and percent. Analysis of numerical variables was performed by using student's unpaired t-test (for two groups) or ANOVA (for more than two groups). Analysis of categorical data was performed by using Fischer's exact test and Chi-squared test. Significance level was set at 0.05.

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III. Results

This prospective observational study involved 500 women consented to participate in this study; group I (test group) of 250 cases who administered Plavix and group II (control group) of 250 women who took aspirin, recruited from infertility outpatient clinic (hospital department) over the period from January 2013 till December 2015. Ovarian hyperstimulation programs were given to all patients with follow up by ultrasound for endometrial thickness and pattern, and Doppler was done to evaluate the PI and RI of uterine artery. The mean diameter of the ovarian dominant follicle and pregnancy rate were evaluated. Both groups were comparable in terms of age, body mass index, gravidity, level of education (≤High school or >High school), occupation (house wife or employed/business woman) (Table 1).

Table (2) showed that in group I, the endometrial thickness was 5.9 ± 1.2 mm and 8.1 ± 1.1 mm before and after treatment with Plavix respectively and there was a significant difference between the two measures and the same was in group two in whom the endometrial thickness was 5.7 ± 1.3 mm and 8.2 ± 0.9 and this reflects the significant effect of the two drugs in improving the endometrial thickness but there was no significant difference between Plavix and Aspirin effect as regards this parameter. Also inspite of the significant effect of Plavix and aspirin as regards improving the endometrial pattern (trilaminar) but there was no significant difference between the two drugs. Also, there was no difference between the two groups as regards the uterine artery pulsatility and resistance indices, mean diameter of ovarian dominant follicle and achieved pregnancy rates (table 2).

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Table (1): clinic-demographic data of the population under study

	Group I	Group II	P- value
Age	31.46 ± 3.3	31.3 ± 4.1	> 0.05
Body mass index (kg/m^2)	31.3 ± 5.4	31.1 ± 3.9	> 0.05
Previous gravidity	1 ± 0.8	1 ± 0.9	>0.05
Education			
≤High school	162	167	> 0.05
>High school	88	83	
Occupation			> 0.05
House wife	187	182	
Employed/business Woman	63	48	

Table 2: The Comparison of Endometrial Thickness and Patterns, Uterine Artery PI and RI, Dominant Follicle, and Pregnancy Rate in Plavix and Aspirin Groups

	Group I	Group II	P value
Endometrial thickness			
Before	5.9 ± 1.2	5.7 ± 1.3	**>0.05**
After	8.1 ± 1.1	8.2 ± 0.9	**>0.05**
P value	<0.05*	<0.05*	
Trilaminar pattern			
Before	20.8%	17.6%	**>0.05**
After	66.4%	66.8%	**>0.05**
P value	<0.05*	<0.05*	
Uterine artery			
PI	2.3 ± 0.6	2.4 ± 0.5	> 0.05
RI	0.6 ± 0.2	0.6 ± 0.1	>0.05
Dominant follicle	17.4±2.6	18.2± 2.4	**> 0.05**
Pregnancy			**>0.05**

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rate	30.4	31.6

IV. Discussion

Thin endometrium is associated with poor uterine receptivity and consequently lower embryo implantation rate [15-16]. An increasing amount of data suggested that one of the most important factors involved and regulating the process of uterine receptivity is the uterine artery perfusion, thereby accounting for a major role in human fertility and success of pregnancy [17].

As for treatment of women with thin endometrium, low dose aspirin administration was suggested to improve embryo implantation in animal studies [18]. In fact, the true effect of low dose aspirin is still undetermined in women with thin endometrium and undergoing assisted reproductive techniques.

The purpose of the present study was to confirm the influence of Plavix and low dose aspirin on enhancing the endometrial parameters and pregnancy outcome and to compare their effect. Plavix was approved for use in many fields; however, it is limited to the cardiology branch.

In this study, we evaluated the thickness and pattern of the endometrium, uterine artery PI and RI, mean diameter of ovarian dominant follicle and pregnancy rates in both groups (Plavix and low dose aspirin respectively) we did not find one group superior to the other.

Weckstein et al. [19] suggested that the ovum donation recipients with a thin endometrium got increased embryo implantation rate and improved pregnancy outcomes after the low-dose aspirin administration. They also reported that the suggested value of low dose aspirin is through decreasing the resistance of uterine blood flow in the peri-implantation stage by increasing local formation of prostacyclin on the expense of thromboxanes. In contrast, Check et al. [20] reported the absence of positive value of aspirin therapy on pregnancy rates after the frozen embryo transfer.

Some studies had stated that women with a history of repeated pregnancy loss had higher uterine artery PI and RI in the mid-luteal phase of the cycle in comparison to fertile individuals, thereby, abnormal uterine blood flow may be a vital factor in women with infertility and recurrent miscarriage and negatively influencing the pregnancy outcome, independent from other possible causes [21].

Also, in disagreement to our findings, in a randomized controlled trial in women with recurrent miscarriage, 364 women were divided into 3 groups, group I administered low dose aspirin, group II received low molecular weight heparin (LMWH) and the third group was on placebo. It was found that neither aspirin nor heparin showed significant increase in the birth rate [22].

This study involved a prospective observational study, but it was nevertheless limited in some aspects. To start with, the number of the study population needs to be more, even though all efforts were made to pick up more patients to be able to get better results. Second, the study didn't involve the administration of placebo and therefore not

double-blinded. Future studies should be a multicenter, double-blinded and include more patients.

V. Conclusion

In view of such findings, the aim of the present work was to detect the possible value of Plavix and aspirin in cases of infertility with thin endometrium and compare their effects. We documented higher than imagined, enhanced endometrial thickness and pattern in the two groups when compared with previous cycles. Uterine artery PI and RI, dominant ovarian follicle and pregnancy rates were comparable in both groups. Review of these data led to a change of infertility workup policy in our unit, with the introduction of aspirin and Plavix treatment of infertile women with thin endometrium. We aimed to use the results of this clinical trial to aid design and complete larger studies involving the use of other drugs for management of such clinical dilemma. This might lead to increased characterization and identification of this subgroup of women whose endometrial receptivity is currently poor.

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