

## Uterine Artery Doppler Changes after Vaginal Administration of Isosorbide Mononitrate in Patients with Unexplained Recurrent Pregnancy Loss

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### ABSTRACT

**Background:** Recurrent pregnancy loss (RPL) related to the presence of two or more clinically recognized consecutive pregnancies before the 20th week of gestation (glacial, molar and biochemical pregnancies are not included). It can be primary or secondary. 0.4-1% of women experience three consecutive miscarriages.

**Aim of the work:** To evaluate the Doppler parameters (Resistance Index and Pulsatility index) in the uterine arteries of non-pregnant women with recurrent miscarriage.

**Patients and methods:** This study conducted on 40 non pregnant women complaining of unexplained recurrent pregnancy loss, from those patients attending gynecology clinic of Al -Azhar university outpatient clinic in Assuit. Patients were classified into: IMN group: (20 patient) complaining of unexplained RPL receiving isosorbide mononitrate vaginally (IMN) 20 mg tablet Placebo group: (20 patient) complaining of unexplained RPL receiving placebo.

**Results:** Our results revealed that standard deviation ratio, pulsatility index (PI) and resistance index (RI) of the uterine-artery had fallen in IMN group with no significance difference in placebo group.

**Conclusion:** Isosorbide mononitrate as a nitric oxide donor can be improved Doppler indices, so may be an important drug in treatment of cases of unexplained RPL.

**Keywords:** Recurrent pregnancy loss; Isosorbide Mononitrate; Uterine Artery Doppler.

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**Authorship:** All authors have a substantial contribution to the article.

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### INTRODUCTION

Recurrent pregnancy miscarriage is a serious reproductive health problem, affecting 2% to 5% of couples. The incidence of RPL varies widely due to differences in the definitions and criteria used, as well as in population characteristics.<sup>1</sup> Known common causes of RPL are uterine abnormalities, antiphospholipid antibody syndrome, hormonal and metabolic disorders, and cytogenetic disorders. Other etiologies have been proposed but are still considered controversial, such as chronic endometriosis, hereditary thrombosis, deficiency of the luteal phase and high-level DNA fragmentation of sperm.<sup>2</sup> Nitric oxide (NO) is a gas and free radicals that are now known to play an important physiological role. NO is important in the endothelium-dependent regulation of blood flow and pressure and in the inhibition of platelet activation. Through this process, nitric oxide in the blood activates an enzyme called soluble guanylyl cyclase, which is activated thousands of times to produce the signaling molecule Cyclic GMP.<sup>3</sup> NO plays an essential functional part in the vasculature by stimulating vasodilation, inhibiting platelet aggregation and adhesion to endothelial cells,

inhibiting smooth muscle proliferation, regulating apoptosis, and maintaining the integrity of the endothelial barrier.<sup>4</sup> Transabdominal and transvaginal ultrasound, power and spectrum Doppler is a non-invasive tool for assessing physiological properties and blood flow during pregnancy.<sup>5</sup>

The study aimed to compare uterine-artery blood flow before and after the application of Isosorbide mononitrate as a nitric oxide donor during mid secretory-phase of menstrual-period for women with unexplained recurrent pregnancy-miscarriage.

### PATIENTS AND METHODS

This study was conducted on June 2021 to February 2022, 40 non-pregnant women who had repeated unexplained reports of pregnancy loss from patients attending Al-Azhar University Gynecology Clinic in Assuit. The study was included at ClinicalTrials.gov with the identification code: NCT05341856

Patients were then randomized into 2 groups using a computer-generated random sequence. Isosorbide mononitrate group (IMN group): (20 patients) complaining of unexplained RPL were inserted vaginally (IMN) with a 20 mg isosorbide

mononitrate tablet into the posterior vault and mean values of Doppler indices were taken in the after two specific uterine artery recorded hours of menstruation. of the manufacturer's recommended post-measured drug with maximum effect. Placebo group: (20 patients) complaining of unexplained LPR received a placebo placed in the posterior fornix and the middle uterine artery Doppler indices were remeasured two hours later.

Woman's age was ranged between 20-35 years, history of recurrent miscarriages (two or more consecutive miscarriages) with; Normal HSG or hysteroscopy, normal serum PRL, normal thyroid function, normal HbA1c, normal pelvic ultrasound, and negative tests for antiphospholipid antibody syndrome (LCA, lupus anticoagulant, and anti-B2 glycoprotein 1). Pregnancies, regular menstrual cycles within 3 months prior to the study, no hormonal contraceptive or intrauterine device, and no vasodilators were not included. Age younger or older than 20-35 years, nulliravidae, infertile women, induced abortion, systemic diseases affecting hemodynamic indices such as thrombocytopenia and thyrotoxicosis, cases of uterine abnormalities, uterine fibroids, polyps and adnexal masses were excluded.

All participants have checked; Correct medical history. Exploration: General Exploration, Belly, and VP. Tests: Routine tests (CBC, SBR, liver function, kidney function), 2D transvaginal ultrasound to assess the uterus and adnexa, and uterine artery Doppler examination to assess midmenstrual uterine blood flow using a 7.5 MHz transvaginal convex Probe (GE Healthcare - LOGIQ F6 device). The uterine arteries were identified transvaginally lateral to the cervix and measured and averaged in the

parasagittal plane of the right uterine artery and left uterine artery. Pulsatility and resistance index were calculated.

The angle between the Doppler beam and the blood vessel was as close to 0 as possible. The study was approved by the Ethics Committee of AL Azhar University School of Medicine (Assuit). Receive verbal communications and communications from all participants.

Sample size were calculated with EpiInfo® version 3.01. Confidence interval set at 95%, statistical power at 80%, ratio of unexposed to exposed subjects at 1, percentage of unexposed subjects with result (assumed equal to Type I error 0.05), and percentage of exposed subjects with result specified at 50. The size was 30 patients for a total of 15 patients in each group. Assuming a 10% dropout rate and a 20% rejection rate, the sample size was increased to 40 patients, 20 patients in each group.

Data were analyzed using Statistical Program for the Social Sciences (SPSS) version 15.0. Quantitative data were expressed as mean  $\pm$  standard deviation (SD) (for normally distributed data) and median (IQR) (for anomalously distributed data). Qualitative data were expressed as frequency and percentage. Paired t-test: Used to compare pre- and post-treatment means for the same group. t-significance test for independent samples: used when comparing two means (for normally distributed data). Mann-Whitney U test: Used to compare two means (for abnormally distributed data). Probability (P value): P<0.05 was considered significant, P<0.001 was considered highly significant, and P>0.05 was not significant.

## RESULTS

S/D ratio was the lowest in isosorbide group compared to placebo group and the difference was highly statistically significant (p-value < 0.001). No statistically significant difference (p-value > 0.05) was found between studied groups as regard resistance index after isosorbide mononitrate and placebo. Highly statistically significant difference (p-value < 0.001) was found as regard Pulsatility index in isosorbide mononitrate group ( $2.29 \pm 0.19$ ) when compared placebo group ( $2.88 \pm 0.19$ ) after isosorbide mononitrate and placebo.

|                 |              | IMN group<br>(N = 20) |     | Placebo group<br>(N = 20) |     | Stat. test            | P-value |
|-----------------|--------------|-----------------------|-----|---------------------------|-----|-----------------------|---------|
| Age (years)     | Mean         | 29.8                  |     | 28.8                      |     | T = 0.98              | 0.333   |
|                 | $\pm$ SD     | 3.2                   |     | 2.8                       |     |                       |         |
| Parity          | Mean         | 2.1                   |     | 1.5                       |     | MW = 143              | 0.127   |
|                 | $\pm$ SD     | 0.9                   |     | 1.1                       |     |                       |         |
| Employment      | Employed     | 13                    | 65% | 11                        | 55% | X <sup>2</sup> = 0.41 | 0.518   |
|                 | Not employed | 7                     | 35% | 9                         | 45% |                       |         |
| Education level | Illiterate   | 5                     | 25% | 7                         | 35% | X <sup>2</sup> = 6.5  | 0.087   |
|                 | Primary      | 1                     | 5%  | 6                         | 30% |                       |         |
|                 | Secondary    | 6                     | 30% | 4                         | 20% |                       |         |
|                 | University   | 8                     | 40% | 3                         | 15% |                       |         |
| Residence       | Rural        | 7                     | 35% | 10                        | 50% | X <sup>2</sup> = 0.92 | 0.337   |
|                 | Urban        | 13                    | 65% | 10                        | 50% |                       |         |

**Table 1:** Comparison between studied groups as regard Scio-demographic characteristics.

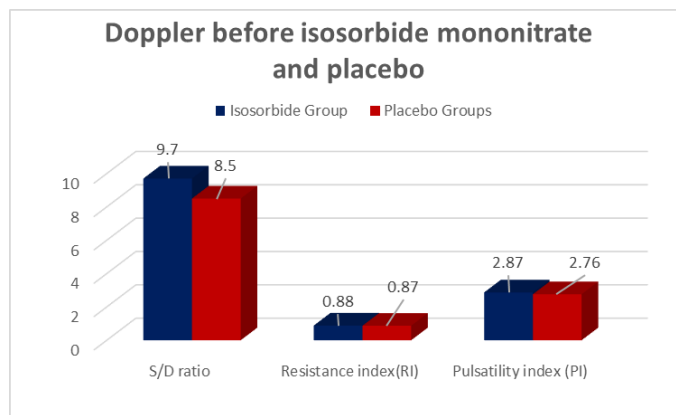
|          |          | IMN group<br>(N = 20) |  | Placebo group<br>(N = 20) |  | Stat. test | P-value |
|----------|----------|-----------------------|--|---------------------------|--|------------|---------|
| Abortion | Mean     | 3.9                   |  | 3.5                       |  | T = 0.99   | 0.327   |
|          | $\pm$ SD | 1.5                   |  | 1.4                       |  |            |         |

|                         |                    |      |     |      |     |              |       |
|-------------------------|--------------------|------|-----|------|-----|--------------|-------|
| <b>BMI</b>              | <b>Normal</b>      | 15   | 75% | 12   | 60% | $X^2 = 1.14$ | 0.565 |
|                         | <b>Underweight</b> | 2    | 10% | 4    | 20% |              |       |
|                         | <b>Overweight</b>  | 3    | 15% | 4    | 20% | $T = 0.27$   | 0.785 |
|                         | <b>Mean</b>        | 22.1 |     | 21.8 |     |              |       |
|                         | <b>±SD</b>         | 3.2  |     | 3.6  |     |              |       |
| <b>Mode of delivery</b> | <b>VD</b>          | 7    | 35% | 13   | 65% | $X^2 = 3.6$  | 0.058 |
|                         | <b>CS</b>          | 13   | 65% | 7    | 35% |              |       |

**Table 2:** Comparison between studied groups as regard clinical characteristics.

| <b>Doppler indices before treatment</b> |             | <b>IMN group<br/>(N = 20)</b> | <b>Placebo group<br/>(N = 20)</b> | <b>Stat. test</b> | <b>P-value</b> |
|---|-------------|-------------------------------|-----------------------------------|-------------------|----------------|
| <b>S/D ratio</b>                        | <b>Mean</b> | 9.7                           | 8.5                               | 167 MW            | 0.531          |
|   | <b>±SD</b>  | 4.5                           | 3.5                               |                   |                |
| <b>Resistance index(RI)</b>             | <b>Mean</b> | 0.88                          | 0.87                              | 1.04 T            | 0.301          |
|   | <b>±SD</b>  | 0.05                          | 0.04                              |                   |                |
| <b>Pulsatility index (PI)</b>           | <b>Mean</b> | 2.87                          | 2.76                              | 1.79 T            | 0.081          |
|   | <b>±SD</b>  | 0.18                          | 0.19                              |                   |                |

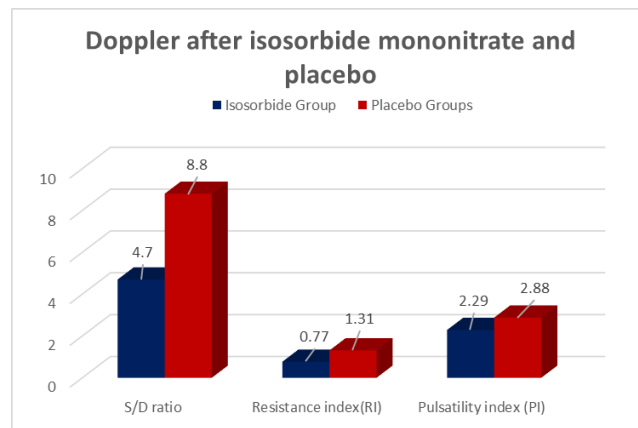
**Table 3:** Pre-treatment doppler indices of both group.



**Fig. 1:** Pre-treatment doppler indices of both group.

| <b>Post -treatment doppler indices</b> |             | <b>IMN group<br/>(N = 20)</b> | <b>placebo<br/>(N = 20)</b> | <b>Stat. test</b> | <b>P-value</b>    |
|--|-------------|-------------------------------|-----------------------------|-------------------|-------------------|
| <b>S/D ratio</b>                       | <b>Mean</b> | 4.7                           | 8.8                         | <b>MW=35</b>      | <b>&lt; 0.001</b> |
|  | <b>±SD</b>  | 1.7                           | 3.9                         |                   |                   |
| <b>Resistance index(RI)</b>            | <b>Mean</b> | 0.77                          | 1.31                        | <b>T=1.21</b>     | <b>0.233</b>      |
|  | <b>±SD</b>  | 0.06                          | 2.00                        |                   |                   |
| <b>Pulsatility index (PI)</b>          | <b>Mean</b> | 2.29                          | 2.88                        | <b>T=9.5</b>      | <b>&lt; 0.001</b> |
|  | <b>±SD</b>  | 0.19                          | 0.19                        |                   |                   |

**Table 4:** Post -treatment doppler indices of both group.



**Fig. 2:** Post -treatment doppler indices of both group.

| IMN group              |      | Before<br>(N = 20) | After<br>(N = 20) | Stat. test | P-value |
|------------------------|------|--------------------|-------------------|------------|---------|
| S/D ratio              | Mean | 9.7                | 4.7               | MW = 25    | < 0.001 |
|                        | ±SD  | 4.5                | 1.7               |            |         |
| Resistance index(RI)   | Mean | 0.88               | 0.77              | t = 6.27   | < 0.001 |
|                        | ±SD  | 0.05               | 0.06              |            |         |
| Pulsatility index (PI) | Mean | 2.87               | 2.29              | t = 9.9    | < 0.001 |
|                        | ±SD  | 0.18               | 0.19              |            |         |

Table 5: Pre &post treatment uterine artery Doppler indices in isosorbide mononitrate group .

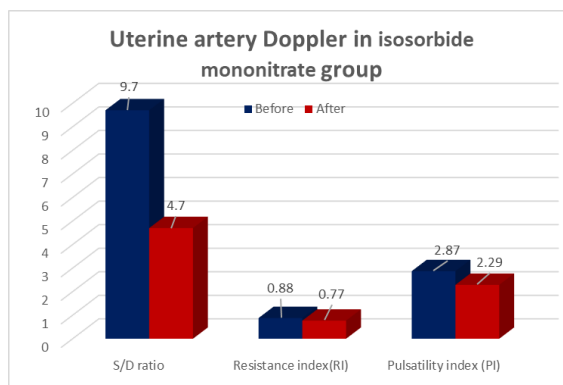


Fig. 3: Pre &post treatment uterine artery Doppler indices in isosorbide mononitrate group .

| Placebo group          |      | Before<br>(N = 20) | After<br>(N = 20) | T         | P-value |
|------------------------|------|--------------------|-------------------|-----------|---------|
| S/D ratio              | Mean | 8.5                | 8.8               | MW= 196.5 | 0.925   |
|                        | ±SD  | 3.5                | 3.9               |           |         |
| Resistance index(RI)   | Mean | 0.87               | 1.31              | t=0.99    | 0.325   |
|                        | ±SD  | 0.04               | 2.0               |           |         |
| Pulsatility index (PI) | Mean | 2.76               | 2.88              | t =1.8    | 0.079   |
|                        | ±SD  | 0.19               | 0.19              |           |         |

Table 6: Pre &post treatment uterine artery Doppler indices in placebo group .

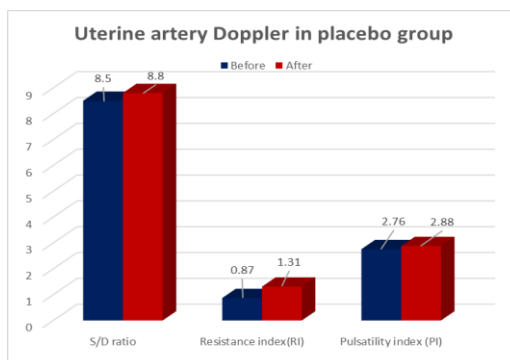


Fig. 4: Pre &post treatment uterine artery Doppler indices in placebo group.

**DISCUSSION**

Recurrent miscarriages are one of the most frustrating and challenging areas of reproductive medicine, as the etiology is often unknown and evidence-based diagnostic and therapeutic strategies are few.<sup>5</sup>

Common methodological weaknesses include failure to meet generally accepted criteria for LPR, confirmation bias, inadequate selection of controls, inconsistent cohort follow-up, failure to exclude aneuploid fetuses, failure to stratify important factors such as number of losses, and early termination.

study after interim analysis. Excessive dropout of patients after randomization.<sup>6</sup>

This study was designed to evaluate the impact of isosorbide mononitrate, a nitric oxide donor administered vaginally through uterine artery blood influx patterns in women with unexplained recurrent pregnancy miscarriage.

Our results revealed that standard deviation ratio, pulsatility index (PI) and resistance index (RI) of the uterine-artery had fallen in IMN group with no significance difference in placebo group. In IMN group, S/D ratio decreased after isosorbide

mononitrate ( $4.7 \pm 1.7$ ) when compared with S/D ratio before isosorbide mononitrate ( $9.7 \pm 4.5$ ), while resistance index (p-value  $< 0.001$ ) decreased after isosorbide mononitrate ( $0.77 \pm 0.06$ ) when compared with RI before isosorbide mononitrate ( $0.88 \pm 0.05$ ), also pulsatility index (p-value  $< 0.001$ ) decreased after isosorbide mononitrate ( $2.29 \pm 0.19$ ) when compared with PI before isosorbide mononitrate ( $2.87 \pm 0.18$ ). In placebo group, S/D ratio has no difference before placebo ( $8.5 \pm 3.5$ ) when compared with S/D ratio after placebo ( $8.8 \pm 3.9$ ), while resistance index (p-value  $> 0.05$ ) had no significance difference before placebo ( $0.87 \pm 0.04$ ) when compared with RI after placebo ( $1.31 \pm 2.0$ ), also pulsatility index (p-value  $> 0.05$ ) had no significance difference before placebo ( $2.76 \pm 0.19$ ) when compared with PI after placebo ( $2.88 \pm 0.19$ ).

According to previous study Bliddal et al.<sup>6</sup> purposes to assess the effect of treatment with N-acetylcysteine (NAC) as a nitric oxide donor in patients diagnosed with recurrent unexplained pregnancy loss (RPL). A group of 80 patients with an unexplained history of recurrent miscarriages were treated with 0.6 g NAC + 500 µg folic acid/day and compared to a group of 86 age-matched patients treated with 500 µg NAC. folic acid alone. Day. NAC + folic acid was associated with a significant increase in the number of children going home compared to folic acid alone, and they concluded that NAC as a nitric oxide donor is a well-tolerated drug that is an effective treatment in unknown patients to be recurrent pregnancy miscarriage.

In agreement to our results; previous study of Amrane and McConnel<sup>7</sup> effect of nitric oxide drug therapy on abnormal post-treatment uterine artery Doppler parameters in unexplained recurrent implant failure and clinical study of post-treatment pregnancy rate.

100 women with a history of ICSI signaling failure, all patients who received a nitric oxide donor (Nitromack retrad 2.5 mg capsule two capsules/day).

Doppler indices show improvement in approximately 69% of cases, with an average IP ( $2.6 \pm 0.92$ ) and an average IR (resistive index) ( $0.87 \pm 0.07$ ) and no improvement in 31% of cases. cases, about 80% of cases received ICSI treatment after treatment. with a clinical pregnancy rate of 59% of cases in this group, approximately 20% did not undergo ICSI treatment, with a spontaneous clinical pregnancy rate of 12%.

Results of the current work with the study of Fredette et al.,<sup>8</sup> who reported that IMN significantly reduced the uterine artery and increased sub-endometrial blood flow indices [p  $< 0.001$ ].

In agreement to our results El-Zahry, et al.,<sup>9</sup> compared between the Uterine artery Doppler indices in women with unexplained recurrent miscarriages. The aim was also to study the effect of intravaginally administered isosorbide mononitrate on the model of uterine blood flow. A higher degree of resistance and pulsation of the uterine artery is observed in women with repeated pregnancies, with a significant decrease in these rates after vaginal use of isosorbide mononitrate in the study group.

## CONCLUSION

Impaired uterine perfusion could play a role in the pathogenesis of recurrent miscarriage. Color Doppler allows a non-invasive method for studying uterine perfusion. Isosorbide mono-nitrate has improved uterine artery Doppler indices in patients with unexplained recurrent miscarriage. Isosorbide mono-nitrate, a NO donor an effective management for improvement of uterine-perfusion in women with unexplained recurrent-pregnancy-loss.

Conflict of interest : none

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