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Nitric oxide donors increase the pregnancy rate in patients with unexplained infertility undergoing clomiphene citrate stimulation and intrauterine insemination: a randomized controlled pilot study

Mohamed Abdel Razik, Seham El-Berry, Ahmed El-Nezamy, Ahmed Saad, and Ahmed Abdel Wahab

Department of Obstetrics and Gynecology, Benha Faculty of Medicine, Benha University, Benha, Egypt

Abstract

This study evaluated the effects of nitric oxide donor's treatment on the pregnancy rate and uterine blood flow in patients with unexplained infertility undergoing clomiphene citrate stimulation and intrauterine insemination. A total of 120 patients were randomly allocated to a control group who received 100 mg clomiphene citrate daily from day 5 to 9 of cycle plus placebo vaginal tablets, and a study group received clomiphene citrate plus isosorbide mononitrate 10 mg vaginal tablets. Vaginal ultrasound was done before treatment and every other day starting from day 12 of cycle to count mature follicles and ovulation was triggered by IM injection of 10 000 IU hCG when one follicle measured 18 ≥ mm followed by intrauterine insemination after 36 h. The endometrial thickness, uterine arteries resistance and pulsation indices, and endometrial vascular flow and vascular flow indices were measured before treatment and at day of hCG injection. Results were analyzed after one cycle treatment using the Mean ± SD, the Student *t* test and the Fisher Exact test. Significant result was considered at *p* values <0.05. The study group had significant higher pregnancy rate/cycle, higher endometrial and lower uterine artery blood flow indices ($p < 0.05$).

Keywords

Unexplained infertility, NO donors, IUI

History

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Introduction

Idiopathic or unexplained infertility (UI) represents about 10–15% of infertility causes and is defined as failure to achieve pregnancy after 12 months of unprotected regular intercourse and basic investigations revealed normal semen analysis, normal regular ovulation with a mid-luteal serum progesterone ≥10 ng/ml and patent fallopian tubes diagnosed by hysterosalpingography and laparoscopy when indicated [1]. Ovarian stimulation and intrauterine insemination (IUI) are an intermediate step of treatment before the application of sophisticated assisted reproductive technologies [2] with a relatively low clinical pregnancy rate/cycle (PR/cycle) of 7% when clomiphene citrate (CC) is used for stimulation [3]. In 1988, Goswamy et al. [4] reported decreased uterine blood flow and uterine receptivity in patients with UI and other studies found that CC decreases uterine blood flow and causes thin endometrium [5–7]. These factors were claimed for the low pregnancy rate and some investigators suggested treatment by using perfusion enhancer drugs [8,9]. Endothelial nitric oxide (eNO) generated *in vivo* from the essential amino acid L-arginine relaxes arterial smooth muscles leading to vasodilatation and increase blood flow [10,11] and has other important roles in the process of reproduction [12–15]. Previous studies reported that genetic impairment of eNO synthesis had adverse effects, which may be a subtle cause of infertility [16–20]. The present study hypothesis that the NO donor isosorbide mononitrate (IMN) administered vaginally as an adjuvant treatment may increase the pregnancy rate in patients with UI undergoing CC stimulation and IUI.

Methods

This pilot study was done at the infertility clinic of Benha University hospital, Egypt, after the approval of the institutional ethics committee and consent of the patients. The study was registered at the Australian New Zealand Clinical Trials Registry (ACTRN 12613001124729). The sample size was calculated using ClinCalc LLC. ©2016, considering the lowest PR/cycle 7% in the control group as reported by ESHRE Capri Workshop Group [4] and an anticipation three-fold increase in the study group. The Alpha error was estimated at 0.05, the Beta error at 0.02 and the power at 0.08, so, a minimum of 100 cases were needed for the study. A total of 140 patients from rural areas of Kalupia governorate, Egypt, house wives and nonsmoker were eligible for the study of which 20 cases were excluded due to treatment with hypertension drugs (5 cases) and refusal of IUI (15 cases). Diagnosis of UI [1] was due to failure to achieve pregnancy after 12 months of unprotected regular intercourse and investigations revealed: (1) Normal semen analysis according to WHO reference values [21]. (2) Normal regular ovulation with a mid-cycle serum progesterone level of > 10 ng/ml. (3) Patent fallopian tubes and normal pelvic cavity on ultrasound examination, hysterosalpingiography and hysteroscopy/laparoscopy when indicated. In all, 120 patients were randomly allocated by computer-generated table in 1:1 ratio to a control group (60 cases) treated with oral CC 100 mg daily from cycle day 5–9 plus placebo vaginal tablets and a study group (60 cases) treated with CC plus

Address for correspondence: Prof. Mohamed Abdel Razik, Department of Obstetrics and Gynecology, Benha Faculty of Medicine, Egypt. Tel: +201223860001. E-mail: prof.mohamedabdelrazik@hotmail.com

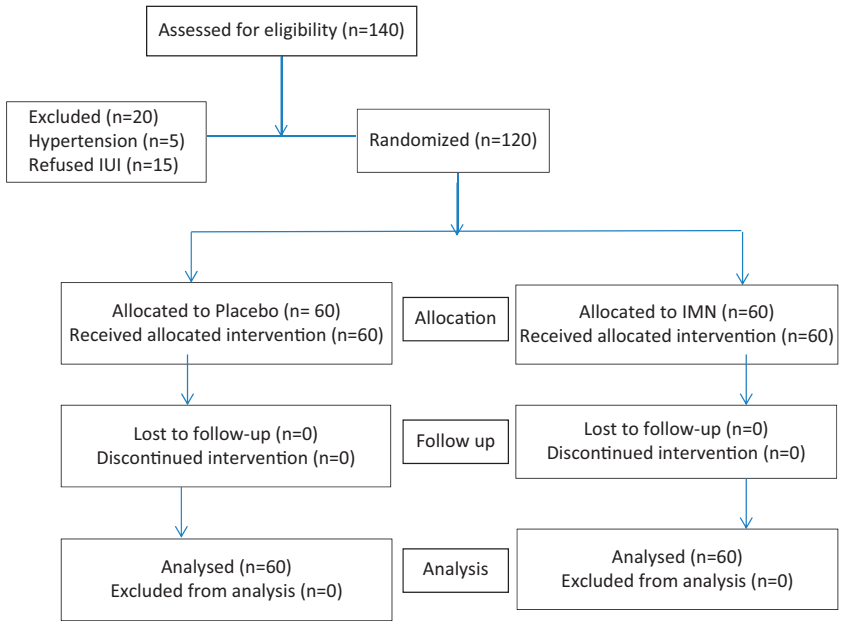

Figure 1. Flowchart of the progress of the study.

IMN 10 mg vaginal tablets. Placebo and IMN were given until diagnosis of pregnancy or occurrence of menstruation. The allocated treatment was put inside a sealed opaque envelop and chosen by the patients while all investigator were blind. Ultrasound examination was done using GE Voluson PRO V 730 by one observer before treatment and at day of hCG injection to count mature follicles $\geq$18 mm and to measure the following parameters: (1) The endometrial thickness: The 2D 7.5 M. Hz vaginal probe displayed the uterus at the longitudinal plane and the maximum thickness of the non-multilayered or multilayered endometrium was measured at its widest diameter on both sides of the midline. (2) Uterine artery blood flow indices: The 2D color Doppler was activated and the ascending branch of the uterine artery was identified parallel to the cervix. Ten identical waveforms with good color signals were obtained and the resistance index (RI) and the pulsation index (PI) of the right and left artery were displayed and the average was calculated. (3) Endometrial blood flow indices: The system was switched into the 3D power Doppler angiography with the following pre-installed settings: frequency ''normal'', pulse repetition frequency 0.6 kHz, gain 4.0, wall motion filter ''low'' (40 Hz). The corpus uteri (without the cervix) was centralized to fill the whole 3D sector and the 3D volume of the endometrium was acquired using the setting ''Quality high 2'' for the 3D sweep speed with an angle of 90. The patient was asked to remain still during examination and more than one volume was acquired and the best one without color artifact was stored for later analysis. The endometrial volume and the three power Doppler indices of the endometrium were calculated using VOCAL^{TM} software (GE Healthcare, Chicago, IL) using rotation steps of 30°. The A-plane (sagittal view of the uterus) was rotated around the y-axis and the vascular index (VI), flow index (FI) and vascular flow index (VFI) were measured. Folliculometry was performed every other day starting from cycle day 12 and ovulation was triggered by IM injection of 10 000 IU hCG when one mature follicle measured $\geq$ 18 mm. The number of mature follicles was recorded and the previous ultrasound parameters were measured. Vaginal ultrasound was done after 36 h of hCG to confirm ovulation by collapse of the follicle and presence of the fluid in Douglas pouch and then followed by IUI. Semen preparation was performed by the swim-up technique with Ham's F10 media and the processed semen was injected through IUI catheter into the fundus. Micronized progesterone 100 mg was given twice daily orally for luteal phase support and pregnancy was diagnosed by serum β-hCG and vaginal ultrasound. Statistical analysis was done after one cycle treatment using Arcus Quickstat version I (Research Solutions Ltd, UK). The mean $\pm$ SD, the Student t test and the Fisher exact test were used for analysis. Result was considered significant at a p values <0.05.

Results

Figure 1 shows the flow chart of the study.

Table 1 shows no significant differences between both groups with regard to patient age, body mass index, duration of infertility, ET, uterine artery RI and PI and endometrial VI, FI and VFI before treatment.

Table 2 shows significantly higher number of mature follicles, thicker endometrium, higher endometrial VI, FI and VFI and significantly lower uterine artery RI and PI at the day of hCG injection in the study group ($p<0.05$).

Table 3 shows significantly higher pregnancy rate/cycle in the study group ($p<0.05$).

Discussion

In the present study, the pregnancy rate/cycle (PR/cycle) in the IMN study group was significantly higher compared to the control group (23.3% versus 8.3%), ($p<0.05$). The ESHRE Capri Workshop Group [3] reported a low PR/cycle of 7% in UI patients treated with CC stimulation and IUI. Past studies [4–6] found that CC stimulation adversely affect uterine artery blood flow and leads to thin endometrium, which impairs endometrial receptivity and lowers the pregnancy rate. Other studies reported 19.9% [22] and an astonishing high rate of 44.1% [23]. At the day of hCG injection, the IMN study group had significant higher mature follicles, ET and endometrial VI, FI and VFI and significant lower uterine artery PI and RI compared to the control group ($p<0.05$). The role of NO in increasing uterine blood flow was proved in a previous study [24] that found N-mono-methyl-arginine (a specific inhibitor of NO) reduces uterine artery blood flow by 60% and in another study Abdel Razik et al., [25] reported that vaginal administration of IMN increased uterine artery and sub-endometrial blood flow during the luteal phase in women with unexplained recurrent abortion. Nitric oxide besides acting as vasodilator and perfusion enhancer plays an important

Table 1. Comparison between bio-data, endometrial thickness, uterine artery and endometrial blood flow indices before treatment in both groups.

	Control group ($n = 60$) Mean ± SD	Study group ($n = 60$) Mean ± SD	p values
Age (years)	26.80 ± 4.11	28.24 ± 6.82	NS
BMI (Kg/m^2)	23.04 ± 2.11	24.34 ± 3.66	NS
Infertility†	6.44 ± 3.67	7.11 ± 4.11	NS
ET (mm)	5.66 ± 1.33	5.33 ± 1.66	NS
RI	0.83 ± 0.20	0.82 ± 0.13	NS
PI	2.49 ± 0.88	2.26 ± 0.91	NS
VI (%)	0.38 ± 0.10	0.40 ± 0.12	NS
FI (0–100)	10.16 ± 3.7	10.04 ± 2.74	NS
VFI (0–100)	0.12 ± 0.06	0.13 ± 0.07	NS

BMI: body mass index.
†Duration of infertility in years; ET: endometrial thickness; RI: resistance index; PI: pulsation index; VI: vascular index; FI: flow index; VFI: vascular flow index; NS: non-significant.

Table 2. Comparison between number of mature follicles, endometrial thickness, uterine artery and endometrial blood flow indices at day of hCG injection in both groups.

	Control group ($n = 60$) Mean ± SD	Study group ($n = 60$) Mean ± SD	p values
No. of mature follicles	1.20 ± 0.5	1.45 ± 0.6	0.01**
ET (mm)	10.32 ± 1.11	11.12 ± 2.19	0.01**
RI	0.84 ± 0.11	0.77 ± 0.22	0.03**
PI	2.49 ± 0.23	2.30 ± 0.66	0.04**
VI (%)	0.38 ± 0.10	0.44 ± 0.18	0.03**
FI (0–100)	10.18 ± 2.78	11.27 ± 2.18	0.02**
VFI (0–100)	0.12 ± 0.06	0.14 ± 0.03	0.02**

ET: endometrial thickness; RI: resistance index; PI: pulsation index; VI: vascular index; FI: flow index; VFI: vascular flow index.
**Significant.

Table 3. Comparison between pregnancy rate/cycle in both groups.

	Control group (No. of cycles = 60)	Study group (No. of cycles = 60)	Fisher exact p values	OR	CI
Pregnancy	5 (8.3%)	14 (23.3%)	0.04**	0.3	0.1–0.9
Single	4 (6.6%)	10 (16.7%)	NS	0.4	0.1–1.2
Twins	1 (1.7%)	4 (6.6%)	NS	0.2	0.03–2.2

**Significant; NS: non-significant; OR: odds ratio; CI: confidence interval.

role in the process of folliculogenesis, follicle maturation, ovulation, fertilization, decidualization, successful implantation and maintenance of early pregnancy [10–14] and was found to increase endocervical secretion at the ovulatory phase [15]. Impairment of NO synthesis adversely affected different stages of reproduction. Deficiency of NO synthase3 enzyme was associated with reduced ovulatory capacity and impaired embryonic viability [16], endogenous NO synthase activity was essential for LH-induced ovulation [17], disruption of eNO gene was found to affect ovulation, fertilization and early embryo survival in experimental mouse [18] and other studies reported the essential role of eNO synthase in follicle maturation and ovulation [19,20]. The use of NO in infertility is limited; oral L-arginine supplementation – a precursor of NO – improved ovarian response to stimulation, endometrial receptivity and pregnancy rate in poor responder IVF patients and was detrimental to embryo quality and pregnancy rate during controlled ovarian hyperstimulation cycles [26,27]. Sildenafil citrate – a selective phosphodiesterase-5 (PDE-5) inhibitor that augments the action of NO by inhibiting its degradation by the enzyme PDE-5- increased significantly uterine blood flow and decreased vascular impedance in non-pregnant, nulliparous women in the luteal phase [28] and successfully treated four patients with unexplained recurrent miscarriage [29]. In another study [30] unexplained recurrent pregnancy loss was treated with N-acetyl cysteine – a NO donor – plus folic acid and reported significant increase in the take-baby home rate compared to treatment with folic acid alone. The increased pregnancy rate with NO donors observed in the present study might not only be due to improved uterine blood flow, increased number of mature follicles and increased ET, but also might be due to offsetting other subtle factors, which are not yet fully investigated in women with UI such as impaired follicle quality, fertilization, implantation and early fetal survival. Vaginal administration of IMN was acceptable and tolerable. Few patients complained of mild headache and palpitation, which needs no special intervention or discontinuation.

Conclusion

Nitric oxide donors may be of value as an adjuvant add-back treatment to improve uterine blood flow and increase the pregnancy rate in women with UI undergoing CC stimulation and IUI. Other randomized controlled studies on a larger sample size are required for definitive results.

Declaration of interest

The authors declare no conflict of interest.

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