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Administration of NTG before embryo transfer does not increase pregnancy rate

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Abstract

Background: Recent studies of uterine contractility in IVF–embryo transfer led us to consider an alternative, and possibly complementary, explanation for the high implantation rates of blastocysts. It has been demonstrated that myometrial contractile activity influences embryo implantation, possibly through mechanical displacement of embryos.

Objective: The aim of this study was to examine the effect of nitroglycerine (NTG) treatment for priming the uterus on the pregnancy outcome of ICSI-ET programs.

Materials and Methods: This study was a prospective, randomized, double-blinded placebo-controlled clinical trial. One hundred consecutive cycles of ICSI-ET on infertile couples were randomly divided into treatment and control groups. The treatment group (50 cycles) received an oral dose of 0.4 mg of NTG, and the control group (50 cycles) received a placebo, 15 minutes before fresh ET. An informed consent from was obtained form each patients. The main outcomes were implantation rate (IR) and pregnancy rate (PR).

Results: The mean age of females in the control group and in the treatment group were 30.1±5.1 and 31±5.5 years respectively. Data showed that the mean duration of infertility was not significantly different between control and treatment groups (6.6±5.8 versus 7.8±5.1 years, respectively). The mean number of oocyte retrieval (metaphase II), 2pn, embryo cleaved, embryo transferred and PR weren't different between two Groups ($p>0.05$).

Overall PR was 36%, it was 38% in treatment group and 34% in control group but there wasn't statistically significant difference between two groups. ($p>0.05$)

Conclusion: NTG didn't increase PR compared to placebo group. These results suggest that NTG treatment before ET isn't effective in the priming of a uterus.

Keywords: *Nitroglycerine(NTG), Embryo transfer(ET), Intracytoplasmic sperm injection (ICSI), Pregnancy rate (PR)*

Introduction

Despite numerous developments in the field of assisted reproduction, the implantation rate remains low. It has been estimated that 85% of the embryos, transferred during IVF or ICSI, fail to implant. Of course, the real cause of this problem is not known but because ICSI procedure and ET into the uterus are carried out in different steps (1), many factors will affect the disparity between embryonic development and pregnancy rates (PR). Uterine contractions is one of these factors (2-7) that has been associated with problematic and unsuccessful embryo implantation. Recent studies of uterine contractility in IVF–embryo transfer led us to consider an alternative, and possibly complementary, explanation for the high implantation rates of blastocysts. It has been demonstrated that myometrial contractile activity

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influences embryo implantation, possibly through mechanical displacement of embryos, in both animals. Data on the relation between uterine contractility and embryo implantation in humans remained scarce because of the invasiveness of traditional methods that required the introduction of pressure probes into the uterine cavity. While uterine contractions have been known for some times to affect embryo implantation in animals (8-9), this topic has only recently been studied in humans. Fanchin *et al* digitized five-minute ultrasound scans to allow objective quantification of frequency of myometrial contractile activity (9). An overall uterine contraction frequency of 4.3 per minute was found. A decrease in PRs and implantation rates was found as the frequency of uterine contraction increased. With a difficult ET, strong random uterine contractions and fundo-cervical uterine contractions were seen. In a separate study, the same group reported that a tenaculum applied to the cervix during mock ET resulted in increased uterine contractions (10). Prostaglandin (PG) which is synthesized from arachidonuic acid by cyclooxygenase (COX), stimulates uterine contraction and may adversely affect outcome following ET. In this respect, treatment, such as uterine relaxants ,should be considered to prime of a uterus suitable for embryo implantation (2,3,11,12) NSAIDs block the action of COX and inhibit the production of PG but have not had any beneficial effect on PR (13,14). Beta sympathicomimetics as well as PG synthetase inhibitors have proposed and used to achieve uterine quiescence at the time of transfer and to improve the PRs. However once more, no proper controlled trials have been performed (15). Nitric oxide (NO) is a potent relaxant of smooth muscle and possibly plays a role in maintaining uterine quiescence during pregnancy. Clinical studies have shown beneficial effects of the stable NO on NTG for the inhibition of pathological myometrial contractility that occurs in preterm labor or dysmenorrheal (16) such as its rapid onset, short half-life, and minimal side effects. NTG is an effective smooth muscle relaxant with a suitable duration of action (half-life of approximately 30-60 minutes) (11,17). Lau *et al* demonstrated that NTG is a potent uterine relaxant in vitro and that the tocolytic effect could be reversed with ease by oxytocics. Data supporting the hypothesis that uterine contractility. Influences IVF-ET PRs encourage further investigation on both the regulation and control of uterine contractions (18). The aim of this study was to examine the effect of NTG treatment for priming of the uterus on the pregnancy outcome of ICSI-ET programs.

Materials and Methods

This study was a prospective, randomized, double-blinded, placebo-controlled, clinical trial. One hundred consecutive cycles of fresh ICSI-ET at the Mehr institute were chosen. The patients underwent ICSI because of female, male, both or unexplained factors. Written informed consent was obtained from all patients.

Monitoring of treatment

All female patients were stimulated with a long protocol and then in 3rd days of the next menstrual cycle HMG (150-225 IU IM) injected which was adjusted with follicular development monitoring by vaginal ultrasound. When at least three follicular diameter were 18 mm, 10000 IU HCG, was given IM and 38 hours later, ovarian puncture was performed. On the day of ovum pick up, spermatozoa retrieval was done by normal ejaculation, testicular sperm extraction (TESE) or precutaneouse epididymal sperm aspiration (PESA) (10). When adequate sperm was retrieved for starting of the ICSI procedure, the ICSI was performed on all normal MII stage (second metaphase) oocytes. Fertilization assessment was performed, 16 to 18 hours later by observation of two pronucli (2pn). ET was performed 48 to 72 hours after ICSI if any normal cleaved embryos were available.

Nitroglycerine (NTG) treatment

Infertile couples were randomly divided into treatment and control groups. All of females who have in ASA I class, (or didn't report history of hypotension (<90/60) and hypertension (>140/90) blood pressure (BP) were measured in supine position, two times with 15 minutes interval before ET. When the mean of Bp was not report 20% lower of baseline, they included in this study. The treatment group (50 cycles) received an oral dose of 0.4 mg of NTG (Zahravi, Iran) 15 minutes before ET and the control group (50 cycles) received a placebo. After embryo transfer BP were measured every 5 minutes for first 20 minutes and then every 15 minutes for 2 hours. Every complication in two groups or side effects of drugs, especially BP variation in NTG, was studied.

Statistical Analysis

The Clinical pregnancy rate was verified by the presence of gestational sac on the 6th week of pregnancy, The PR was evaluated with female age, male age, cause and duration of infertility, mean number of oocyte retrieval, oocyte metaphaseII, 2pn, embryo cleaved, embryo transferred and embryo quality by using χ^2 test and student's T-test. Statistical significant differences were determined at $p<0.05$ levels.

Results

The mean age±SD of females wasn't significantly different in the control group and in the NTG treatment group (30.1±5.1 and 31±5.5 years respectively). The mean age ± SD of males also indicated similar results. Data showed that mean duration of infertility wasn't significantly different between two groups (6.6±5.8 versus7.8±5.1 years, respectively).

In total 698 oocytes was retrieved and 578 (71%) mature oocytes were injected. Fertilization and cleavage rates were 446(82.8%) and 436(97.7%) respectively, also the number of embryo transferred and embryo implantation was 337(77.2%) and 47(13.9%) respectively.

The mean number of oocyte retrieval (metaphase II), 2pn, embryo cleaved, embryo transferred had no statistically significant effect on PR between two groups ($p>0.05$) (Table I).

Overall PR was 36% (19(38%) in NTG and 17(34%) in control group respectively), there wasn't statistically significant difference between two groups ($p>0.05$). In addition, take home baby rate in NTG and control group was 13(26%) and 9(18%) respectively with no statistically significant difference between two groups ($p>0.05$) (Table II).

Our Results indicated that categories of infertility and embryo qualities weren't different in two groups (Table III, IV).

Table I. Patients characteristics in the control and NTG treatments groups

	Control	NTG treatment	T-Test
Female age(y)	30.1±5.1	31±5.5	>0.05
Male age(y)	35.4±6.6	34.7±7.8	>0.05
Duration of infertility	7.8±5.1	6.6±5.8	>0.05
Oocyte retrieved	7.4±4	6.4±3.4	>0.05
Oocyte injected (MII)	6.3±3.4	5.4±3.4	>0.05
2pronucli(2Pn)	4.5±2.4	4.3±2.9	>0.05
Cleaved embryos	4.4±2.4	4.2±2.9	>0.05
Embryos transferred	3.5±1.4	3.3±1.3	>0.05

Table II. ICSI-ET outcomes in the control and NTG treatments groups

Variable	Control (%)	NTG treatment (%)
Biochemical pregnancy	3(6)	3(6)
Abortion	5(10)	3(6)
Clinical pregnancy	14(28%)	16(32)
Take home baby	9(18)	13(26)
Total	17(34)	19(38)
p value was not significant		

Table III. Comparison of infertility causes in the control and NTG treatments groups

Variable	Control (%)	NTG treatment (%)
Female factor	22(45.8)	17(34.7)
Male factor	23(47.9)	28(57.1)
Unexplained	2(4.2)	2(4.1)
Male and female	1(2.1)	2(4.1)
Total	50(100)	50(100)
p value was not significant		

Table IV. Comparison of embryo quality in the control and NTG treatments groups

Variable	Control (%)	NTG treatment (%)
A	40(81.6)	39(78)
B	8(16.3)	7(14)
C	1(2)	4(8)
p value was not significant		

Discussion

The understanding and control of embryo implantation represents the major challenge for success in assisted reproductive technologies. Along with developments in basic research and efforts to optimize embryo quality, the improvement of noninvasive and reliable methods to assess uterine receptivity constitutes an important step toward meeting such a challenge (18).

The contractile activity of the non pregnant uterus plays an important role in the human reproduction process. Today, not only the artificial stimulation of uterine contractions, aiming at promoting sperm transport during the pre-ovulatory phase, but also their attenuation to provide optimum conditions for embryo implantation during the luteal phase, represent innovating, promising issues in the optimization of assisted reproduction treatments(1,12,19-22).

Lesny *et al* (1998) investigated junctional zone contractions (JZ) during cycles of IVF and ET in oocyte donors. After 7 days of superovulation, all patients displayed cervico-fundal, fundo-cervical and random contractions. JZ activity throughout the IVF cycle was more exaggerated when compared to the results reported from observations of the natural cycle but followed a similar pattern. This fact can probably be explained by the vastly different hormone levels. Higher JZ activity and correspondingly increased mobility of the endometrium may impair its receptivity and affect implantation (10). de Ziegler *et al* (2001) assessed the importance of uterine contractility in the implantation of human embryos. Adequate uterine contractility may provide successful gamete/embryo transportation through the utero-tubal cavities and embryo implantation in spontaneous or assisted reproduction. Inadequate uterine contractility may lead to ectopic pregnancies, miscarriages, retrograde bleeding with dysmenorrhea and endometriosis (20).

Uterine relaxation before ET is likely to improve IVF-ET outcome by avoiding the displacement of embryos from the uterine cavity. A few studies used aspirin, another inhibitor of PG, to improve the pregnancy outcome of IVF-ET. They concluded that low–dose aspirin improved implantation rates and PRs in IVF patients. Moon *et al* (2004) examined the effect of beta-cyclodextrin piroxicam treatment for priming of the uterus on the pregnancy outcome of IVF-ET programs. The patients randomly divided into treatment and control groups. In the treatment group, (94 cycles in fresh ET and 39 cycles in frozen-thawed ET) the patients received an oral dose of 10 mg of piroxicam. In the control group, the same number cycles corresponding to the treatment group were treated with placebo. Both groups started piroxicam or placebo treatment 1-2 hours before ET. Piroxicam increased significantly IR (18.7%) and PR (46.8%) compared to the control group (8.6% and 27.6%, respectively) in fresh cycles. With the exception of unexplained factor, patients with the tubal, male infertility, or endometriosis factor had significantly higher PR in the treatment group compared to the control group. The beneficial effect of piroxicam was found in patients less than 40 years old, but it was not found in patients more than 40 years. These results suggest that piroxicam treatment before ET is very effective in the priming of a uterus suitable for embryo implantation. NSAIDs are risk factor C in pregnancy. The use of NSAIDs during pregnancy has not been associated with congenital malformation, preterm delivery, or low birth weight, but two reports showed that adverse effects of NSAIDs might be associated with spontaneous abortion (23). NTG is the most commonly used nitro-vasodilator for this purpose. Its presumed mechanism of action is via nitric oxide and cyclic guanosine monophosphate (cGMP) mediated processes. NTG is known to release nitric oxide to effect smooth muscle relaxation and some dose response data is available for its vasodilator activity. Human myometrium is known to synthesize and respond to nitric oxide, with changes in the production of and sensitivity to nitric oxide being subject to the cyclical and gestational state of the uterus (24). Craig *et al* (1998) conducted a prospective study on the use of sublingual NTG at Caesarean section to induce uterine relaxation. Minimal maternal side-effects were reported. They concluded that NTG is a safe form of uterine relaxant at Caesarean section which may be used in emergency situations and may also be given prophylactically in cases such as breech presentation and in delivery of the preterm infant where fetal trauma is possible. The use of a metered-dose sublingual spray is ideally suited to obstetric practice, being both easy to use and also rapidly administered (25). Experimental data on the efficacy of NTG in reliably producing uterine relaxation is conflicting and inconsistent. Indications for the

use of NTG in achieving rapid uterine relaxation cover the antepartum, intrapartum and postpartum periods. The safety of NTG during obstetric emergencies appears high, with no adverse maternal or neonatal outcomes (24,26-29). David *et al* (2000) compared the relaxation effect of GTN and a beta 2-mimetic substance, fenoterol, in human myometrial tissue. A total of 100 strips from 20 patients were used. GTN demonstrated a significant relaxation effect in the in vitro model on human myometrial strips from pregnant women already treated with oxytocin. Compared to GTN application, muscle strip relaxation was less pronounced under fenoterol; a complete inhibition of myometrial activity was not achieved under fenoterol (26).

Progress in reproductive physiology has shown that NO is involved in the fertilization process and is found in the follicle as well as in the sperm. It also plays a role in decidualization and implantation. Nonetheless, there have been very few clinical studies of NO donors for the treatment of infertility (30).

Shakers *et al* (1993) studied effect of NTG sublingual spray emissions or placebo 3 min before ET. No significant effect was found on any parameter of the transfer procedure (31). Ohl, *et al* (2002) assessed the efficacy of NTG administered to 138 IVF patients with a history of implantation failure. The NTG patch was administered the day before ET and continued until either the results of the pregnancy test were known or until menstruation occurred. Results showed that NTG treatment, the day before ET, was no more effective than placebo in improving the implantation or PRs in a population of IVF patients with a previous history of implantation failures. Only one patient in the placebo group complained about side-effects during the treatment which were probably related to the stress of the IVF procedure. Thus NTG appeared safe and well tolerated (32).

However in present study we didn't observe any statistical significant differences on PR by NTG treatment but differences in abortion and take home baby rate were clinically considerable. It's suggested to perform another study with a larger group before ET and change in the dosage and drug route administration.

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