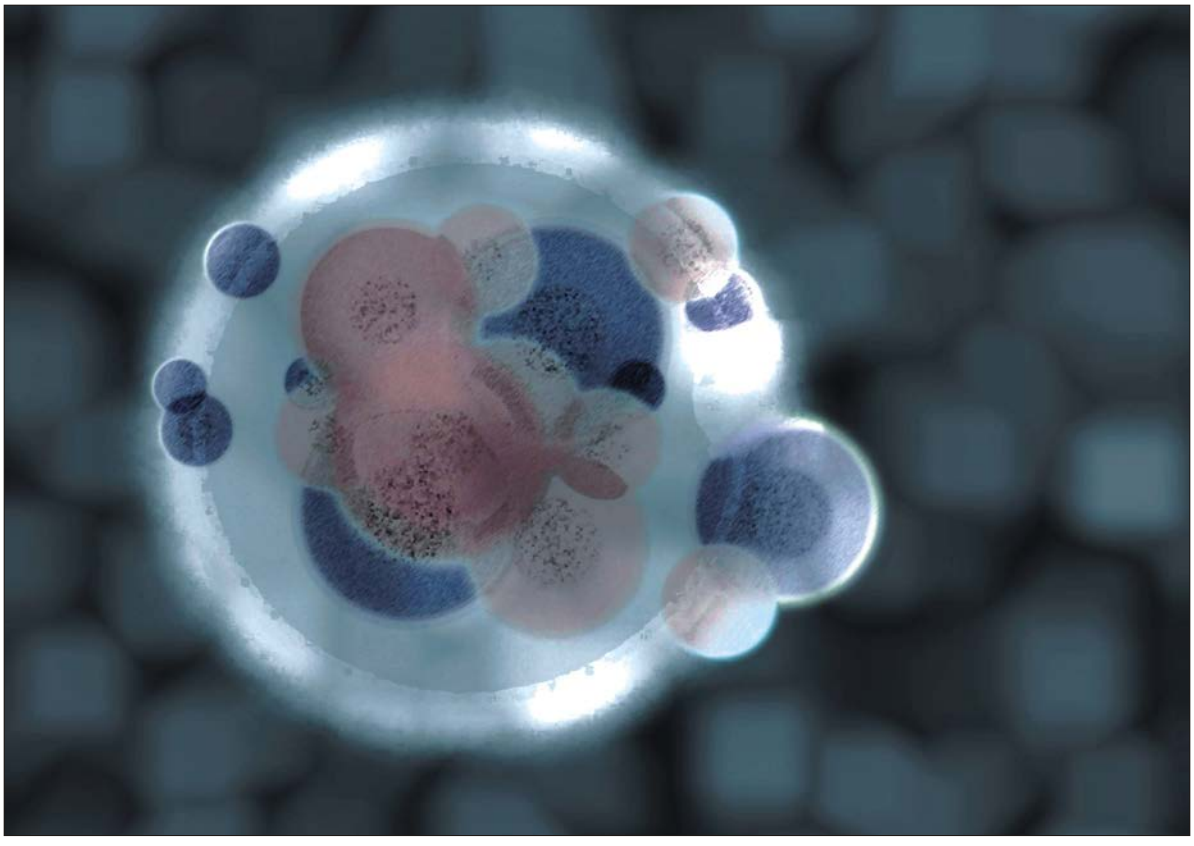

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Effect of Administration of Single Dose Piroxicam Before Embryo Transfer on Implantation and Pregnancy Rates in IVF Cycles

[1]Razieh Dehghani Firouzabadi, [2]Sedighe Ghandi and [3]Naeimeh Tayebi
[1]Department of Obstetric and Gynecology, Clinical and Research Center for Infertility,
[2]Infertility Flowship, Clinical and Research Center for Infertility,
[3]Clinical and Research Center for Infertility, Shahid Sadoughi University, Yazd, Iran

Abstract: The aim of this study was to find out whether the administration of piroxicam prior to embryo transfer might improve implantation and pregnancy rates in patients under IVF therapy. This study was designed as a prospective randomized clinical trial. In total, 180 fresh IVF-ET cycles were randomly divided into treatment and control groups. The treatment group (90 cycles) received an oral dose of 10 mg of piroxicam and the control group (90 cycles) received a placebo, 1-2 h before ET. The woman's age, duration and etiology of infertility, number of oocytes retrieved, number of embryos transferred and the score of embryo transferred showed no significant differences in both groups. The implantation rate and clinical pregnancy rate were significantly higher in the piroxicam treatment group compared with the control group. The implantation rate was 12.3 vs. 7.7% (p-value = 0.04) and the clinical pregnancy rate was 25.5 vs. 10% (p-value = 0.015) in the piroxicam vs. control groups, respectively. The number of miscarriage was one in the piroxicam group and five in the control group (p-value = 0.01). In addition, there were two twins pregnancies in piroxicam group and one in control group. This result proposes that the treatment with piroxicam before ET could prepare a suitable uterus for embryo implantation.

Key words: Piroxicam, embryo transfer, *in vitro* fertilization, implantation failure

INTRODUCTION

Implantation failure is the main problem in Assisted Reproductive Technology (ART) (Van Gestel *et al*., 2003). In order to an embryo implants in the uterus properly and begins a pregnancy, several molecular events occur for preparing the uterus to receive the embryo. If the uterus is not receptive to an embryo, implantation will fail (Imakawa *et al*., 2004).

During the past 20 years, numerous studies have been made to improve the implantation process. Most of their attempts have been focused on the induction and selection of the best quality embryos and the improvement of uterus receptivity (Setter *et al*., 2001). As an aggressive procedure, embryo transfer causes uterine response involving endometrial inflammatory phenomena and increased myometrial contraction (Van Gestel *et al*., 2003) and these presumably hinder implantation. In general, the uterus has typically three patterns of contractility throughout the menstrual cycle that influence embryo implantation (Ijland *et al*., 1996).

Since the introduction of *in vitro* Fertilization (IVF), an increased uterine activity during the procedure was also documented, with its has harmful effects on embryo attachment (Bulletti *et al*., 2000) and therefore a high number of embryos were rejected (Poindexter *et al*., 1986). As a result, treatment with uterine relaxants such as Non-steroidal anti-inflammatory drugs (NSAIDs) before embryo transfer should be considered to reduce of uterine contractility and prepare a suitable uterus for embryo implantation (Bernabeu *et al*., 2006; Fanchin *et al*., 1998).

NSAIDs are the most used drugs in the industrial countries (Brooks, 1998; Hernandez Diaz and Gaeria Rodriguez, 2001). One of the NSAIDs is piroxicam that is prescribed as a therapy for osteoarthritis, rheumatoid arthritis and severe dysmenorrhea. In addition; it may cause the reduction of uterine contractility and increase the success of embryo implantation in IVF procedures (Moon *et al*., 2004).

It is known that prostaglandins increase uterine contractions and decrease receptivity of embryo and since NSAIDs block the production of prostaglandins, the

Corresponding Author: Razieh Dehghani Firouzabadi, Bouali Ave, Safaeyeh, Research and Clinical Center for Infertility, Postal Code: 8916895473, Yazd, Iran Fax: 00983518247087

investigators wanted to find out whether treatment with piroxicam prior to embryo transfer might improve implantation and pregnancy rates in IVF patients or not.

MATERIALS AND METHODS

This study was designed as a prospective randomized clinical trial. The study was performed between February and October 2006 on women who underwent IVF because of tubal, ovarian, male infertility, unexplained and mixed factor. Also, all of the cycles were fresh embryo transfer. The institutional review board gave approval for the study. Written informed consent was obtained from all patients.

The whole patients were treated with long protocol for ovarian stimulation. In long protocol, pituitary down-regulation was achieved by administration buserelin acetate (suprefact; Hoechst AG, Germany) (0.5 mg SC) starting from day 21 of previous menstrual cycle and the dose was decreased to 0.25cc daily when the menstrual bleeding was happened. Then, Stimulation was commenced using Human Menopausal Gonadotrophin (HMG) (Menogon, Ferring, Germany) from the second day of their menstrual cycle with the dose of 150-300 IU daily. Monitoring was carried out by transvaginal ultrasound on day 7 of HMG stimulation. After more than three follicles >18 mm in diameter were observed, 10000 IU of Human Chorionic Gonadotrophin (HCG) (Pregnyl®5000, Organon) was administered intramuscularly and 36 h later oocytes were retrieved under general anesthesia by transvaginal ultrasound-guided aspiration. Mature oocytes were retrieved from follicular fluid and placed in G-fert (version 3; vitrolife, Goteborg, Sweden) and after fertilization, 2PN zygote to G-1 media (G-1 TM version 3; vitrolife, Goteborg, Sweden) was transferred. Embryo transfers were performed 2 days after oocyte retrieval using Labotect catheters (Labor-technik, Germany). A maximum of three embryos was transferred.

The 180 fresh IVF-ET cycles were randomly divided into treatment and control groups by using a computer generated random table. The treatment group (90 cycles) received an oral dose of 10 mg of piroxicam administration and the control group (90 cycles) received a placebo. Both groups started piroxicam or placebo treatment 1-2 h before ET. Patients and staff were blinded to the treatment.

Luteal phase was supported with vaginally Cyclogest (Alpharma, Barnstaple, UK), 800 mg daily, starting on puncture day.

Serum B-HCG was measured two weeks after IVF. Biochemical pregnancy was defined as a serum B-HCG greater than 50 mIU mL^{-1} and clinical pregnancy determined by detection of fetal heart beat by abdominal sonography 8 weeks after IVF.

The data were analyzed with the student's Statistical Package for Social Sciences (SPSS) version13. Rates were compared using the Chi-square test or the exact test when necessary. Groups of values were compared using the t-test. A p-value of<0.05 was considered statistically significant.

RESULTS

In this study, the woman's age, duration and etiology of infertility, number of oocytes retrieved, number of embryos transferred and the score of embryo transferred showed no significant differences (Table 1).

The implantation rate, chemical and clinical pregnancy rates were 7.7, 15.6 and 10% in the control group and 12.3, 26.7 and 25.5% in the piroxicam group. The implantation rate and clinical pregnancy rate were significantly higher in the piroxicam group compared with the control group ($p<0.05$; Table 2)

Also, the number of miscarriage (Termination of pregnancy before 20 weeks based upon the date of the first day of the last normal menses) was five in the control group and one in the piroxicam group ($p = 0.01$). There was one twin pregnancy in control group and two twins in the piroxicam group.

Table1: Comparison of baseline characteristics in the control and piroxicam groups

Variables	Control	Piroxicam	p-value*
No. of subjects	90	90	
Mean (±SD) age of women, years	29.79±5.1	30.28±4.3	NS*
Mean (±SD) duration of infertility, years	7.8±4.4	8.4±4.3	NS
No. of oocytes retrieved	6.12±2.9	6.28±3.9	NS
No. of embryos transferred	2.18±0.5	2.31±0.7	NS
Score of embryos transferred	17.1±1.5	17.14±1.7	NS
Causes of infertility			
Male factor (%)	44 (48.8)	63 (70)	
Tubal factor (%)	16 (17.8)	6 (6.7)	
Ovary factor (%)	10 (11.1)	5 (5.6)	NS
Unexplained (%)	14 (15.6)	11 (12.2)	
Mixed (%)	1 (1.1)	5 (5.6)	

Note: Data are expressed as mean ±standard deviation; *T-test and chi-square test; *NS = Not statistically Significant

Table 2: IVF-ET outcomes in the control and piroxicam group

Variables	Control n = 90	Piroxicam n = 90	p-value*
Implantation rate*	7.7%	12.3%	0.04
Chemical pregnancy rate	15.6% (14/90)	26.7% (24/90)	0.09
Clinical pregnancy rate	10% (9/90)	25.5% (23/90)	0.015

*Number of sacs with FH per number of embryos transferred; All rates are per-embryo replacement; *Exact test

DISCUSSION

Several strategies have been suggested in order to improve uterus receptivity at the time the embryo reaches the endometrial cavity and to minimize the uterine contraction. Reducing cervical stimulation by a careful technique and non traumatic pass of the catheter through the uterine cavity has all shown a beneficial effect. However, apart from the use of progesterone (Fanchin *et al*., 2001) or ritodrine (Pinheiro *et al*., 2003), a pharmacological approach to embryo transfer should be considered.

In this study, piroxicam was used for preparing the uterus to improve the pregnancy rate after IVF-ET. Piroxicam is a NSAID and is in group C (according to classification of FDA) 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 side effects of NSAIDs during pregnancy. One states that NSAIDs block blastocyst implantation by inhibiting blastocyst hatching (Matt and Bozelleea, 1995) and the other reports that the use of NSAIDs might be associated with spontaneous abortion (Neilsen *et al*., 2001). However, there are no reports concerning any adverse effects from one dose (10 mg) of piroxicam during the preimplantation period. Our present study showed a decrease in miscarriage after piroxicam treatment compared with the control group in IVF-ET.

Uterine contractions are shown to affect embryo implantation in animals (Adams, 1980) and in humans (Fanchin *et al*., 2001). Uterine contractility is stimulated by prostaglandins synthesized by cyclooxygenase (COX) (Dawood, 1993). The NSAIDs block the action of COX and inhibit the production of prostaglandins (PGs) (Vane, 1971), probably resulting in the decrease of uterine contractility.

Recently, Maslow and Lyons (2004) have reported an inhibitory action of ibuprofen on mid-cycle myometrial contractions. In addition, indomethacine had been used successfully to reduce uterine contractility (Lenz *et al*., 1991).

It has been shown high frequency contractions of uterus at the time of ET are associated with poorer implantation (Fanchin *et al*., 1998) and poorer IVF-ET outcome (Fanchin *et al*., 2000). These mean that uterine contractility influences embryo implantation.

In this study, oral treatment with single dose piroxicam on the day of ET seems to increase the embryo implantation by calming uterine contraction and it causes to improve clinical pregnancy rate. Similar study was conducted by Moon *et al*. (2004). They have reported that the implantation and clinical pregnancy rates are increased by piroxicam treatment before ET. Also, Elli *et al*. (2001) indicated that a single dose of ibuprofen lysinate before embryo transfer may be an effective adjunctive treatment for assisted reproduction in cattle and implantation and pregnancy rates were higher in the treated groups compared with the control groups, but Bernabeu *et al*. (2006) showed that the indomethacine rectally with three-doses every 12h starting on the night prior to transfer did not increase implantation rate in oocyte recipients.

Although piroxicam decreases uterine contractility, it increases uterine blood flow. It has been shown that aspirin, another inhibitor of PG, improves the implantation and pregnancy rates in IVF patients (Wada *et al*., 1994) by increasing uterine blood flow (Rubinstein *et al*., 1999).

However, the present study could not determine that if piroxicam might decrease uterine contraction or increase uterine blood flow, but it showed that treatment with piroxicam before ET increased the implantation rate and improved the pregnancy outcome after IVF-ET in fresh cycles. Therefore, treatment with this drug before ET should be considered in IVF-ET cycles.

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