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Effects of piroxicam administration on pregnancy outcome in intrauterine insemination (IUI) cycles: a randomized clinical trial

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Summary

Background: Uterus contractibility is considered a powerful prognostic factor in predicting the embryo transfer outcome. Moreover, uterine contractions are known to be stimulated by prostaglandins which are produced by cyclooxygenase from arachidonic acid. As such, suppressing the inflammatory response and contractions using anti-inflammatory and relaxant agents is expected to result in increased success rate of embryo transfer and artificial insemination. *Objective:* To investigate the effect of piroxicam administration on the success rate in intrauterine insemination (IUI) cycles in patients presenting with unexplained infertility. *Materials and Methods:* This randomized, placebo-controlled clinical trial included 260 women with unexplained infertility undergoing IUI cycles. Patients were randomly assigned to receive either piroxicam ten mg/day on days 4-6 after IUI or placebo (control group). The main outcome measures were number of IUI cycles, pregnancy, abortion, and multiple pregnancy rates. *Results:* The pregnancy rate was found to be 25 (19.2%) and 16 (12.3%) in piroxicam and control groups, respectively ($p = 0.039$). Five patients (3.8%) in piroxicam group experienced twin pregnancy whereas only three patients (2.3%) in control group had twin pregnancy ($p = 0.361$). The pregnancy rate per cycle was also significantly higher in those who received piroxicam as compared to controls (11.16 *vs.* 6.66; $p = 0.021$). *Conclusion:* Administration of piroxicam after IUI is associated with decreased number of cycles, as well as increased pregnancy rate and pregnancy rate per cycle in IUI cycles. However, piroxicam did not have any effect on abortion, multiple pregnancy, and ongoing pregnancy rates.

Key words: Piroxicam; Intrauterine insemination; Pregnancy rate; Abortion rate; Multiple pregnancy rate.

Introduction

Artificial insemination which involves injection of washed sperm into the female genital tract without sexual intercourse is a treatment for couples suffering from male factor infertility [1]. So far, many methods have been introduced and used for artificial insemination of which intracervical and intrauterine insemination are extensively employed in clinical practice. Intrauterine insemination (IUI) is the best method of artificial insemination being studied and widely used [2]. In IUI, the processed, washed, and concentrated sperm specimens are placed into the uterine cavity using the transcervical catheterization. IUI possesses the highest success rate amongst all artificial insemination methods [1].

In assisted reproduction techniques including in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), the implantation failure is the main limiting step in successful pregnancy. Furthermore, the implantation failure may result from several factors including the increased uterine myometrial activity. Uterine receptivity is also affected by several factors which cannot be recognized due to its complex nature. These factors include complex morphological and biochemical changes of the endometrium [3]. By direct visualization of endometrium using high resolution probes, uterus is found to have three distinct patterns of contractibility which potentially affect the outcome of IVF after embryo transfer [4]. It has been shown that the embryo transfer as an aggressive method, induces endometrial contractibility which may contribute to implantation failure. Several other factors have been shown to be responsible for uterine response leading to embryo implantation failure. These include the direct myometrial stimulation by drugs, hyperphysiological hormonal levels, endometrial inflammation secondary to direct manipulation of the endometrium, cervical canalization-induced uterus dynamic responses, and the psychological stress during cycles [5].

Uterus contractibility is now considered a powerful prognostic factor to predict the embryo transfer success rate [6]. It is also known that uterine contractions are stimulated by prostaglandins that are produced by cyclooxygenase (COX) from arachidonic acid. As such, suppressing the inflammatory response and contractions using anti-inflammatory and relaxant agents are expected to result in increased success

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rate of embryo transfer and artificial insemination. COX and prostaglandin production can be irreversibly blocked by nonsteroidal anti-inflammatory drugs (NSAIDs) [7]. It is therefore hypothesized that administering NSAIDs in patients who undergo IVF or IUI cycles would suppress the uterine contractibility response of the endometrium and potentially results in an increased success rate. Piroxicam is a member of NSAIDs family which is shown to be effective in alleviating dysmenorrhea [7]. Thus its use is expected to possibly result in favorable effects in patients undergoing IVF or IUI cycles.

Along these lines, Moon *et al.* showed that administering piroxicam increases the implantation- as well as pregnancy rates in patients undergoing IVF and embryo transfer. The favorable effects of piroxicam were more remarkable in subjects younger than 40 years and those who suffered from tubal, male factor infertility or endometriosis [8]. In a similar study, Firouzabadi *et al.* reported that administering a single dose piroxicam improves both implantation and pregnancy rates in IVF cycles [9]. Nevertheless, the possible beneficial effects of piroxicam on the success rate of IUI has not been yet investigated. The above gap prompted the present authors to design and implement the current trial to investigate the effect of piroxicam administration on improving the success rate of IUI cycles in females suffering from unexplained infertility.

Materials and Methods

Patients

This randomized clinical trial was carried out in two tertiary healthcare centers affiliated with Shiraz University of Medical Sciences, over a 20-month period from August 2012 to April 2014. The authors included patients referring to infertility clinics of Ghadir Mother and Child Hospital and Motahari Clinic during the study period. Patients who had unexplained infertility were recruited. Infertility was defined as one year of unprotected intercourse without conception. In general, infertility is described as 'unexplained' when standard investigations including semen analysis, tubal patency tests, and assessment of ovulation fail to identify any abnormalities or a specific diagnosis. In order to find the etiology of infertility, partner's semen analysis, hormonal assay including prolactin, thyroid stimulating hormone (TSH), prolactin (to rule out hypophyseal adenomas), follicle-stimulating hormone (FSH), luteinizing hormone (LH) (to rule out ovarian dysfunction such as premature ovarian failure), hysterosalpingogram (HSG), laparoscopy and hysteroscopy (to rule out uterine/tubal factor including peritubular adhesions and endometriosis) were performed in all patients. All examined women had normal plasma concentrations of LH, FSH, and progesterone; normal renal and hepatic function tests; normal complete blood counts; normal HSG, laparoscopy and hysteroscopy and negative pregnancy tests. The authors excluded subjects with polycystic ovaries in transvaginal ultrasonography, those who had autoimmune disorders, and were found to have endometriosis. Patients who entered this blinded trial were matched for age, body-mass index (BMI), and the duration of infertility.

All participants were asked to sign written informed consents before enrollment. The study protocol was approved by the institutional review board (IRB) of Shiraz University of Medical Sciences under the ethics committee approval code CT-89-5351, assigned in January 2012. The entire protocol was reviewed, approved, and given the Iranian Clinical Trials Code (IRCT) 2013021911790N2.

Study protocol

A total number of 298 women were screened for eligibility to enter the study. All patients underwent complete history and physical examination with all positive findings recorded in their files. The study protocol as well as side effects and benefits were fully explained to all patients and informed written consents were obtained. Patients were then randomly assigned to two groups based on a computer random digit generator using their registration number. Group A (n=130) received ten-mg capsules of piroxicam on days 4-6 after IUI while group B (n=130) received placebo.

Clomiphene citrate (100 mg/PO/day) was administrated for five days from day 5 to day 9 of the cycle, and recombinant FSH was injected intramuscularly at 150 units/day from cycle day 8. Vaginal sonography was performed on the 11th day of the cycle and based on the size and number of stimulated follicles, recombinant FSH was continued until at least one dominant follicle with size of >18 mm was identified. Then, 5,000-10,000 units of human chorionic gonadotropin (hCG) was injected intramuscularly, if serum E2 level was below 1,500 pg/ml. IUI was performed 36 hours after hCG injection. Each patient underwent up to three cycles of IUI. β-hCG was checked if the patient experienced one week missed period. Moreover, pregnancy was documented by transvaginal sonography, at six to seven weeks of gestation. Main outcome measures were the number of IUI cycles, pregnancy rate (detected by positive β-hCG), abortion rate, and multiple pregnancy and ongoing pregnancy rates (calculated by subtracting abortion from pregnancy rates). All investigators except the statistician were blinded to the study protocol.

Statistical analysis

Based on 90% power to detect significant differences between the corresponding variables (p = 0.05, two-sided), 100 patients were required in each group. To compensate the possible none valuable data, the authors enrolled 130 participants in each group. The statistical software package SPSS, version 15.0 was used for data analysis. The paired t-test was employed to compare results within groups, the independent t-test to compare results between the groups, and the χ^2 test to compare proportions. Data were reported as mean ± SD. $P < 0.05$ was considered significant.

Results

A total of 289 patients with unexplained infertility were consecutively selected to undergo IUI cycles and were screened for eligibility out of which 14 were excluded, 13 did not meet the inclusion criteria, and 11 refused to participate in the study. Given the above, a total of 260 subjects were randomly assigned to two study groups, each including 130 patients. All patients followed the study and none of them were lost to follow-up. Therefore, the final number of patients was 260 (Figure 1). The mean age of the patients was 28.9 ± 5.1 (range 18–43) years and the mean duration of infertility was 4.6 ± 4.1 (range 1-23) years.

The overall pregnancy rate and pregnancy rate per cycle were 15.7% and 8.8%, respectively. The pregnancy rate (defined by positive β-hCG) was found to be 25 (19.2%) and 16 (12.3%) in piroxicam and control groups, respec-

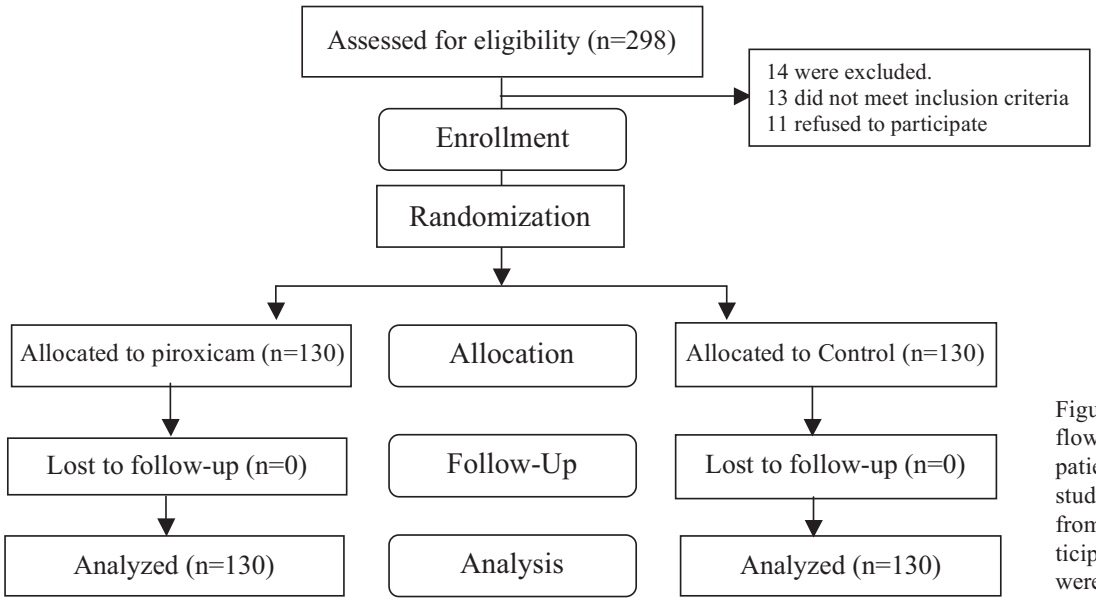

Figure 1. — The study flow chart. All enrolled patients followed the study protocol and data from a total of 130 participant in each group were analyzed.

Table 1. — *The outcomes of IUI cycles in infertile patients treated with piroxicam vs. control subjects.*

	Piroxicam (n=130)	Control (n=130)	*p*-value
Age (years)	28.8 ± 4.7	28.9 ± 5.3	0.873
Infertility duration (kg/m^2)	4.6 ± 3.9	4.7 ± 4.1	0.890
Number of IUI cycles	224	240	0.001
Pregnancy rate (%)	25 (19.2%)	16 (12.3%)	0.039
Single pregnancy (%)	20 (15.3%)	13 (10%)	0.028
Abortion rate (%)	5 (3.8%)	5 (3.8%)	0.823
Twin pregnancy (%)	5 (3.8%)	3 (2.3%)	0.361
Pregnancy rate per cycle (%)	11.16	6.66	0.021
Ongoing pregnancy (%)	20 (15.3%)	11 (8.4%)	0.098

tively. The pregnancy rate was significantly higher in piroxicam group (p = 0.039). The prevalence of abortion was found to be five (3.8%) in piroxicam group and five (3.8%) in control groups (p = 0.823). Five patients (3.8%) in piroxicam group *vs.* three patients (2.3%) in control group had twin pregnancy. However the difference was not statistically significant (p = 0.361). The pregnancy rate per cycle was also significantly higher in patients who received piroxicam as compared to controls (11.16 *vs.* 6.66; p = 0.021). The study results are outlined in Table 1.

Table 2. — *The pregnancy rate in IUI cycles.*

	1st cycle	2nd cycle	3rd cycle	*p*-value
Pregnancy	13 (5%)	26 (10%)	2 (0.7%)	0.013
No pregnancy	92 (35.5%)	84 (32.3%)	43 (16.5%)	

Of those who conceived during the study period (41 patients), 25 (9.6%) conceived in the first cycle of the IUI while 34 (13.1%) during the second cycle of IUI, and seven (2.7%) during the third cycles. Using chi-square test, it was shown that two cycles of IUI is was associated with higher pregnancy rate (p = 0.013) (Table 2). The pregnancy rate in different IUI cycles in both study groups is demonstrated in Table 3.

Discussion

In this randomized clinical study the authors attempted to investigate the effects of piroxicam administration on IUI outcomes. They found that piroxicam administration was associated with higher pregnancy rate following IUI cycles. Piroxicam use resulted in a higher pregnancy rate per cycle of IUI. However piroxicam was not found to have any favorable effect on abortion rate, multiple pregnancy rate, and

Table 3. — *Pregnancy rate in different IUI cycles in both study groups.*

	Piroxicam (n=130)			Control (n=130)		
	1st cycle	2nd cycle	3rd cycle	1st cycle	2nd cycle	3rd cycle
Pregnancy	8 (6.2%)	16 (12.3%)	1 (0.7%)	5 (3.8%)	10 (7.6%)	1 (0.7%)
No pregnant	56 (43.1%)	26 (20%)	23 (17.7%)	36 (27.7%)	58 (44.9%)	20 (15.3%)

the ongoing pregnancy rate following IUI cycles. This is the first study investigating the effect of piroxicam on IUI outcomes, hence no comparison with other studies is possible.

The pregnancy rate and pregnancy rate per cycle of IUI in this study was similar to those previously reported by Alborzi *et al.* [10]. They performed a randomized clinical trial including 110 patients with unexplained, male factor, and cervical factor infertility undergoing IUI cycles using either single or double insemination per cycle methods. The overall pregnancy rate and the pregnancy rate per cycle were reported to be 38.2% and 8.6%, respectively. Comparable to the present findings, most patients conceived in the first two cycles of treatment. The pregnancy rate as well as pregnancy rate per cycle of IUI in this study was comparable to the present results (pregnancy rate and pregnancy rate per cycle of 15.7% and 8.8%, respectively).

Piroxicam is an anti-inflammatory agent and since the uterine contractibility is responsible for implantation failure in IVF and IUI cycles, its administration in patients undergoing IUI and IVF cycles is expected to yield beneficial effects. This hypothesis has been tested by several authors [8, 9, 11]. In this regard, Moon *et al.* performed a prospective, randomized, double-blinded placebo-controlled clinical study including 188 consecutive cycles of fresh IVF-embryo transfer (ET) and 78 cycles of frozen-thawed ET being randomly assigned to receive piroxicam (ten mg piroxicam) and placebo, one to two hours before ET [8]. The primary outcomes were implantation and pregnancy rates. They found that the implantation rate and pregnancy rate increased significantly by 18.7% and 46.8%, respectively, in patients receiving piroxicam as compared to the placebo group (8.6% and 27.6%, respectively). Those who were younger than 40 years of age as well as patients who suffered from tubal, male infertility factor or endometriosis had a significantly higher pregnancy rates with piroxicam. It has been concluded that piroxicam administration before ET increases both implantation- and pregnancy rates. This favorable effect was appeared to be more prominent in patients younger than 40 years and those who suffer from tubal, male factor infertility or endometriosis [8]. Piroxicam is classified as Group C drugs in pregnancy (according to FDA classification). Up to now, no report has warned of the association between the administration of piroxicam or other NSAIDs and preterm birth, low birth weight or congenital malformations. In line with this, earlier reports [8, 9] have shown that piroxicam in limited dose is safe during the pregnancy or implantation period.

Dal Prato and Borini performed another randomized clinical trial in order to determine the effect of piroxicam on the ET outcome [11]. They enrolled 200 women suffering from tubal, male, endometriosis or unexplained factor infertility aging 28-43 years. Patients were randomly assigned to receive ten mg piroxicam or placebo one to two hours prior to ET. The investigation did not find any significant difference between the two study groups in terms of positive βhCG (37% *vs.* 47%) and the pregnancy rate (34% *vs.* 38%). The study also reported no significant difference between groups with regard to the abortion rate. The effect of piroxicam was shown to be independent of age, BMI, the duration and various etiologies of infertility. This report further suggested that single dose administration of piroxicam before ET in patients undergoing IVF is of no beneficial effects [11].

In a similar study by Firouzabadi *et al.*, the effect of single dose administration of piroxicam before ET was examined in 180 fresh IVF-ET cycles in which patients were randomly assigned to receive ten mg piroxicam or placebo one to two hours before ET [9]. According to theirs results, piroxicam administration increased the implantation (12.3% *vs.* 7.7%) as well as pregnancy rates (25.5% *vs.* 10%), as compared to placebo. They also found that abortion rate was significantly lower in those who received piroxicam (1% *vs.* 5%). The beneficial effects of piroxicam in IVF-ET outcomes can be further established in future well-designed studies. Although several studies have investigated the effects of piroxicam administration on IVF-ET outcome, no study has addressed this issue in IUI cycles. To the best of the present authors knowledge, this is the first study to investigate such issue.

The beneficial effects of piroxicam on IVF and IUI outcomes can be summarized in two points. Firstly, the uterine contractibility is reduced by administering piroxicam. Based on the evidence from earlier reports, during the spontaneous cycles, non-conceptional cycles has more endometrial wave-like activity compared to conceptional cycles [12]. In addition, it has also been shown that high frequency contractions of uterus are associated with poorer IVF-ET, implantation and pregnancy outcomes [4]. It can be concluded that uterine contractibility is associated with poorer pregnancy outcomes in IUI and IVF cycles. Prostaglandins, being synthesized by COX, are responsible for stimulating uterine contractions [7]. Therefore, blockade of the COX using NSAIDs can result in decreased uterine contractibility and in turn increases the implantation outcome following IUI or IVF cycles. Secondly, the uterine blood supply and flow is increased following the use of NSAIDs including piroxicam. Previous studies have shown that aspirin administration is associated with increased implantation and pregnancy rates following IVF cycles [13]. Moreover, findings have indicated that the favorable effects of aspirin are attributed to increased uterine blood flow [13].

The utero-placental unit of human is colonized by hematopoietic cells of which 65–70% are natural killer (NK) cells and 10–20%, are antigen presenting cells (APCs). NK cells regulate the invasion of trophoblast. On the other hand, decidual NK cells induce vascular growth needed to establish an adequate decidua. Decidual cells (DCs) begin and coordinate the immune response [5]. These cells are gathered in the pregnant uterus before implantation and will remain in the deciduas until the end of

the pregnancy. A recent study showed that without the uterine DCs, sever impairment of implantation will result [14]. Another study, carried out on a mice model, showed that the spontaneous abortion rate is decreased by therapy with DCs [7]. In conclusion, administration of piroxicam was shown to be associated with increased pregnancy rate in IUI cycles. However, piroxicam did not yield any effect on abortion and multiple pregnancy rates [15]. In other words, the opposition which is necessary for implantation requires an inflammatory- followed by an anti-inflammatory responses. The present evidence further suggests that inflammation is necessary for implantation. Thus, suppressing this beneficial inflammation by administering NSAIDs will result in decreased implantation rate or increased abortion rate. As demonstrated by Bernabeu *et al.*, indomethacin does not affect the initial inflammatory response required for implantation [5]. These results are consistent with animal studies showing that indomethacin does not affect the implantation in rats [16]. However, with piroxicam, as a different NSAID, the present authors observed favorable results suggesting no effect on the inflammatory response of endometrium in such a negative way.

Conclusion

In conclusion, administration of piroxicam after IUI was found to be associated with increased pregnancy rate and pregnancy rate per cycle in IUI cycles. Meanwhile, piroxicam administration yielded no notable effect on abortion, multiple pregnancy, and ongoing pregnancy rates.

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