THE EFFECTS OF LOW-DOSE ASPIRIN TREATMENT IN OVARIAN AND UTERINE RESPONSIVENESS, IMPLANTATION AND PREGNANCY RATES IN PATIENTS UNDERGOING ICSI

F. Akbari Asbagh* and Z. Zahiri Sorouri

Department of Obstetrics and Gynecology, Mirza Kouchek Khan Hospital IVF center, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract- Vascularization of the follicle may play a role in its maturation and aspirin seems to increase this vascularization.In this investigation we have studied the effects of low-dose aspirin on ovarian and uterine response, implantation and pregnancy rates in patients undergoing intracytoplasmic sperm injection (ICSI). This prospective semirandomized, double-blind, placebo-controlled study was undertaken in Mirza Kochek Khan IVF center. Forty four infertile patients who were undergoing ICSI cycle were studied and they were divided in two groups. The mean age of patients and distribution of the cause of infertility in two groups were similar. In the treatment group, 22 patients underwent controlled ovarian hyperstimulation and received a daily dose of 100 mg aspirin. In the control group 22 patients underwent controlled ovarian hyperstimulation in association with placebo. Number of follicles ≥ 15mm in diameter, number of oocytes retrieved, endometrial thickness, cancellation rate, number of transferred embryos and implantation and pregnancy rates were analyzed in two groups.There was no statistically significant difference between the treatment and control groups for each of the above variables ($P > 0.05$). This study showed that low dose aspirin therapy has no added effect to ovarian and uterine response, implantation and pregnancy rates in ICSI patients.Further studies are recommended for confirmation of our results.

Acta Medica Iranica, 42(1): 36-39; 2004

Key words: Aspirin, ICSI- ET, pregnancy rate

INTRODUCTION

Aspirin is a salicylate non-steroidal anti-inflammatory drug (NSAID). Aspirin and other salicylates have analgesic, antiinflammatory and antipyretic properties (1). They act as inhibitors of the enzyme cyclooxygenase (2), which results in the direct inhibition of the biosynthesis of prostaglandins and thromboxanes from arachidonic acid. Aspirin also inhibits platelet aggregation while non-acetylated salicylate do not (1). Aspirin's antiplatelet activity has led to its use in a variety of disorders such as unstable angina, ischemic stroke and anti phospholipid antibody syndrome (1). Aspirin has also been tried in pregnancy- induced hypertension for the prevention of pre-eclampsia and intra-uterine growth retardation (1,3-5). Vascularizatin of the follicle may play a role in its maturation from the early follicular phase. Aspirin seems to increase this vascularization because of a vasodilation effect, therefore, it may improve ovarian and uterine responsiveness.This study was undertaken to determine the effects of low-dose aspirin treatment in infertile patients who were undergoing intracytoplasmic sperm injection (ICSI) cycles.

Received : 6 October 2001, Revised : 7 January 2003, Accepted : 21 May 2003

*** Corresponding Author:**
F. Akbari Asbagh, Department of Obstetrics and Gynecology, Mirza Kouchek Khan Hospital IVF center, School of Medicinc, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 21 8907062, Fax: +98 21 8907241
E-mail: gh_Pourmand@hotmail.com

MATERIALS AND METHODS

This was a prospective semirandomized, double-blind, placebo-controlled study. Randomization was performed by "every other subject" method and each patient received her medication through a third person. Both patients and clinical staff were blinded to treatment. We studied 53 patients whose infertility was due to a tubal factor or male factor other than azospermia or oligoasthenoteratospermia, because

azospermic patients were a limitation for ICSI so they were excluded from the study. The 53 patients were randomly divided into treatment and control groups. All patients were informed and a consent form was taken according to Helsinki protocol. The treatment group (27 patients) received a daily oral dose of 100 mg of aspirin and the control group (26 patients) received placebo. Both groups started aspirin or placebo co-treatment on the 21st day of their preceding menstrual cycle. Controlled ovarian hyper-stimulation was initiated in all patients with the gonadotropine releasing hormone (GnRH) analogue, busereline, started in the midluteal phase (21st day) of the previous cycle with a dose of 0.5 mg/day and decreased to 0.3mg/day from 2nd day of menstrual cycle. Then gonadotropin therapy was given using HMG 3 ampules per day for all patients. GnRH analogue injection was continued up to and including the day of HCG administration and cycle monitoring was performed with serial transvaginal ultrasono-graphy.

For minimizing the measurement bias all scans and measurements were performed by the same operator. Transvaginal follicular aspiration and ICSI embryo transper (ET) were performed after our standard protocol. Pregnancy was detected by increasing serum β-HCG level in at least two determinations (12-14 days after ET) and was confirmed by sonographic screening of the gestational sac one week after the last β-HCG determiation. Pregnant patients continued the medication which included aspirin or placebo co-treatment through 12 week's gestation.

For minimizing the confounding variables, all of the following patients were excluded from the study:

1. All the patients with any known ovulatory dysfunction (such as abnormal menstrual pattern, abnormal hormonal pattern and previous unilateral oophorectomy).

2. All the patients who required TESE (testicular sperm extraction) or PESA. (percutanus epandymal sperm aspiration).

3. All the patients whose treatment protocol fell in a pattern other than our standard protocol (e.g., the patients who needed in increase or decrease in the dose of gonadotropines during follow up or those who received larger dose of busereline during treatment cycle.

All the patients received the same protocol of induction of ovulation. The ovarian and uterine responsiveness and IVF outcome variables were analyzed, including number of follicles ≥ 15 mm, cancellation rate, endometrial thickness on the day of HCG administration, number of oocytes retrieved, number of transferred embryos, implantation and pregnancy rates. The data were analyzed with the use of the two- tailed students's *t*- test, Mannwhitney test and Chi-square test. $P<0.05$ was considered statistically significant.

RESULTS

The 53 patients were randomly divided into treatment (27 patients) and control groups (26 patients). Five patients in treatment group were excluded from study ,one became pregnant spontaneously during GnRH therapy, two due to cyst formation on GnRH therapy and two due to unresponsiveness of ovaries to gonadotropines. Four patients in control group were excluded from study, one due to inability of her husband to give semen sample, one due to cyst formation on suprefact therapy, and two due to ovarian unresponsiveness to gonadotropines. At last 22 patients in each group were analyzed.The results of this study are summarized in tables 1 and 2.The mean (±SD) age was similar in the treatment and control groups (28.41±4.34 versus 28.27 ± 3.45, respectively); all the patients were under age 35.

The cause of infertility was similar in the treatment and control groups (54.5% male factor, 40.9% tubal factor and 4.5% both factors versus 54.5% male factor, 31.8% tubal factor and 13.6% both factors, respectively).

The cancellation rate due to cyst formation or unresponsiveness of ovaries was similar in treatment and control groups (0.18% versus 0.13%, respec-tively).Ovarian responsiveness was expressed as the number of follicles ≥ 15mm on the day of HCG administration and the number of oocytes retrieved.The mean (±SD) number of follicles ≥ 15 mm on the day of HCG administration was 6.82±3.69 versus 5.86±1.96 for the treatment and control groups, respectively ($P>0.05$). The mean (±SD) number of oocyte retrieved was 5.18±3.84 versus 4.73±2.62 for the treatment and control groups, respectively ($P<0.05$). The mean (±SD) thickness of endometrium as a marker for endometrial responsiveness, on the day of HCG administration was 9.61±3.18 versus 10.17±2.15 for the treatment and control groups respectively ($P>0.005$). The mean (±SD) number of transferred embryos was 2.45±2.06 versus 2.36±1.40 in treatment and control groups, respectively ($P>0.05$).

Table 1. ICSI- ET outcome in the treatment and control groups*

Variable	Treatment group	Control group	*P* Value
Age (y)	28.41±4.34	28.27±3.45	NS
Cancellation rate(%)	0.18	0.13	NS
No. of follicles	6.82±3.69	5.86±1.96	NS
End. thickness(mm)	9.61±3.18	10.17±2.15	NS
No. of oocytes	5.18±3.84	4.73±2.62	NS
No. of embryos	2.45±2.06	2.36±1.40	NS
Implantation rate (%)	13.63	27.24	NS
Clinical PR (%)	13.63	27.27	NS

Abbriviations: NS, not significant; PR, Pregnancy rate; End,endometrium

*Data are given as means ± SD except for cancellation, implantation and clinical pregnancy rates.

Table 2. Causes of infertility in the treatment and control groups*

Cause of Infertility	Treatment group		Control group	
	Number	Percent	Number	Percent
Male factor	12	54.5	12	54.5
Tubal factor	9	40.9	7	31.8
Both factors	1	4.5	3	13.6
Total	22	100	22	100

**P* value > 0.05

The implantation and clinical pregnancy rates were 13.63% (3 cases) versus 27.27% (6 cases) for the treatment and control groups, respectively.The difference was not statistically significant (*P*=0.076).

No side effect was observed in patients treated with aspirin;GI symptoms or other types of symptoms and bleeding during aspiration were similar in both groups.

DISCUSSION

Aspirin has several pharmacologic properties. It is an inhibitor of the enzyme cyclooxygenase. In blood platelets this enzyme prevents the synthesis of thromboxane, a compound which is a vasoconstrictor, causes platelet aggregation and is thus potentially thrombotic (1). When this enzyme is irreversibly inhibited by low dose aspirin treatment, platelet aggregation and vasoconstriction may be avoided which in turn may improve folliculogenesis and implantation (6,7). In blood vessel walls, the enzyme inhibition prevents the synthesis of prostacyclin (6,8,9) which is a vasodilator and has anti-aggregating properties and is thus potentially antithrombotic. The duration of these effects, however, may be somewhat different, with the effect on the vascular tissue generally being short lived comparing to the effect on platelets. The different duration of effects may be explained by the fact that vascular cells regain the ability to regenerate prostacycline in a few hours but plateletes are unable to resynthesis cyclooxy-genase thus resulting in no new thromboxane A_2 being produced until after about 24hr (1) and/or even more longer for total life span of platelet, 8-11 days (7,10-12).Aspirin therefore appears to have paradoxical biological effects, both antithrombotic and thrombogenic. The thrombogenic effect occurs only with high doses because platelet cyclooxygnase is more selective than vascular cyclooxygnase therefore low dose aspirin inhibits synthesis of TXA_2 without affecting the excretions of PGI_2 (13-15) thus explaining the increase in blood flow velocity in the uterine and ovarian arteries. PGI_2 has been proposed to modulate the relaxation of vascular smooth muscle of endometrial vessels. As low dose aspirin treatment shifts local production of TXA_2 toward PGI_2, it may improve implantation because of its effect on the endometrium.

Vascularization of the follicle may play a role in its maturation from the early follicular phase. Aspirin seems to increase this vascularization because of a vasodilation effect that could result in preferential delivery of gonadotropin hormones or other growth substances required for steroidogenesis. This may improve folliculogenesis and increase the number of oocytes retrieved. While considering the above explanation it seems logical to use low doses of aspirin as a drug that may improve ovarian and uterine responsiveness as studies performed by Wada et al. (16) and Rubinstein et al. (17), both revealing that aspirin significantly improves ovarian and uterine responsiveness in patients undergoing IVF. The Rubinstein study although was a large RCT but suffers from the effects of some important confounding variables such as nonadjustment of gonadotropin and GnRH agonist dose and the advanced age of the patients. For comparative purposes we performed this placebo-controlled clincal trial but we found no relation between aspirin therapy and improvement in ovarian and uterine responsiveness, implantation and pregnancy. One of the strongest point of our study was its high internal validity. All confounding variables and measurement biases were decreased as low as possible but the

sample size was not large enough to show a definite statistical conclusion.

In conclusion, this study demonstrated that low dose aspirin therapy has no beneficial effect in ICSI results. Which could be due to the small sample size of this study so it seems that further studies are required to determine the exact effect of aspirin on ovarian and uterine responsiveness, implantation and pregnancy rates.

Acknowledgements

Statistical analysis was performed by Mrs. Akhtar Roshan and IVF lab procedures by Dr. Salsabli, Dr. Mostafavi and Dr. Moshtaghi, to whom we are highly grateful, also we would like to thank Mrs Shamiran and Mrs Seifollahi for their valuable help.

REFERENCES

1. Kathleen Parfitt, Martindale the complete drug reference- 1999; p: 16-19.

2. Vane RJ. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin- like drugs. Nat New Biol 1971; 231(25): 232-235.

3. Wallenberg HC, Rotmans N. Prevention of recurrent idiopathic fetal growth retardation by low dose aspirin and dipyridamole. Am J Obstet Gynecol 1987; 157(5): 1230-1235.

4. Yamaguchi M, Mori N. 6- keto prostaglandin F1 alpha, thromboxane B2 and 13,14 dihydro- 15 keto prostaglandin F concentrations of normotensive and preecelamptic patients during pregnancy, delivery and the postpartum period. Am J Obstet Gyneol 1985; 151(1): 121-127.

5. Jouppila P, Kirkinen P, Koivula A, Ylikorkala O. Failure of exogenous prostacyclin to change placental and fetal blood flow in preeclampsia. Am J obstet Gynceol 1985; 151(5): 661-665.

6. Burch JW, Majerus PW. The role of prostaglandins in platelet function. Semin Hematol 1979; 16(3): 196-207.

7. Burch JW, Stanford PW,Majerus PW. Inhibition of platelet prostaglandin synthetase by oral aspirin. J Clin Invest 1978; 61(2): 314-319.

8. Weksler BB, Pett SB, Alonso D, Richter RC, Stelzer P,Subramanian V,Tack-Goldman K,Gay WA. Differential inhibition by aspirin of vascular and platelet prostaglandin synthesis in atherosclerotic patients. N Engl J Med 1983; 308(14): 800-805.

9. Burch JW,Baenziger NL,Stanford N,Majerus PW. Sensitivity of fatty acid cyclooxygenase from human aorta to acetylation by aspirin. Proc Natl Acad Sci USA 1978; 75(10): 5181-5184.

10. Lorenz RL, Boehlig B, Uedelhoven WM, Weber PC. Superior antiplatelet action of alternative day pulsed dosing versus split dose administration of aspirin. Am J Cardiol 1989; 64(18):1185-1188.

11. Clarke RJ, Mayo G, Price P, Fitzgerald GA. Suppression of thromboxane A2 but not of systemic prostacyclin by controlled- release aspirin. N Engl J Med 1991; 325(16): 1137-1141.

12. Majerus PW. Arachidonate metabolism in vascular disorders. J Clin Invest 1983; 72(5): 1521-1525.

13. Smith JB, Willis AL. Aspirin selectively inhibits prostaglandin production in human platelets. Nat New Biol 1971; 231(25): 235-237.

14. Patrignani P, Filabozzi P,Patrono C. Selective cumulative inhibition of platelet thromboxane produation by low-dose aspirin in healthy subjects. J Clin Invest 1982; 69 (6): 1366-1372.

15. Moncada S, Vane JR. Arachidonic acid metabolites and the interactions between platelets and blood-vessel walls. N Engl J Med 1979; 300(20): 1142-1147.

16. Wada I, Hsu CC, Williams G, Macnamee MC, Brindsen PR. The benefits of low dose aspirin therapy in women with impaired uterine perfusion during assisted conception. Hum Reprod 1994; 9(10): 1954-1957.

17. Rubinstein M, Marazzi A, Polak de Fried E.Low-dose aspirin treatment improves ovarian responsiveness, uterine and ovarian blood flow velocity, implantation and pregnancy rates in patients undergoing in vitro fertilization:a prospective, randomized, double blind placebo-controlled assay. Fertil Steril 1999; 71(5): 825-829.