Effect of low-dose aspirin therapy on implantation rate in women undergoing in-vitro fertilization cycles

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ABSTRACT

Objectives: To determine the effect of low-dose aspirin on ovarian response, implantation and pregnancy rates in patients undergoing in-vitro fertilization (IVF) cycles.

Method: We performed a randomized analysis of 145 infertile women with a mean±SD age of 29.6 ± 4.47 years who underwent cyles of IVF. Patients received 100 mg of aspirin (n=72) or placebo (n=73) daily. This study was conducted in Royan Institute, Tehran, Iran from April 2002 to January 2004. Aspirin was started on the 21st of their preceding menstrual cycle and it was continued until menstruation or a negative pregnancy test. Pregnant women received the medication until 12 weeks of pregnancy. The main outcome measures were number of follicles ≥15mm, number of oocytes retrieved, serum E2 levels, cancellation rate, Ovarian Hyperstimulation Syndrome (OHSS) occurrence, number of embryos transferred, and implantation and pregnancy rates.

Results: There were statistically significant differences between the treatment group and the control group in the number of follicles (7.4 ± 4.1 versus 9.0 ± 4.8) and OHSS occurrence (5.6% versus 23.3%) but not in the other measures.

Conclusions: The addition of aspirin low dose (100 mg/daily) to the standard long protocol for oocyte retrieval did not improve implantation and pregnancy rates in unselected patients undergoing IVF cycles.

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In spite of the recent advances in assisted reproductive technologies rates of implantation and pregnancy is still low.[1] The poor quality of oocyte and embryo,[2] decreased uterine receptivity due to ovarian stimulation regimens,[3] and antiphospholipid antibodies (APA)[4] have been proposed to be among the most significant factors responsible for the failure of in-vitro fertilization (IVF)/embryo transfer (ET) cycles. There is a growing body of evidence that the outcome in IVF correlates with the state of blood perfusion in female's reproductive organs[5-8] and impaired uterine blood flow has been proposed as a possible cause of infertility.[9] Aspirin has been utilized as one such potential therapy. It is an irreversible inhibitor, selectively acetylating the cyclooxygenase involved in prostaglandin (PG) synthesis.[10] This may be accomplished by proportionally greater inhibition of vasoconstricting prostaglandins (thromboxane A2) than the vasodilating prostaglandins (prostacyclin).[11] It has been suggested that low-dose aspirin improves ovarian responsiveness and tissue perfusion,[8] increases the embryo implantation and pregnancy rates in infertile women with impaired uterine blood flow[8,12,13] and in APA-positive patients with decreased endometrial receptivity.[14,15] Moreover, it shows that treatment with low-dose aspirin increased the weight of newborn in pregnant patients with fetal growth retardation and improved placenta and fetal blood flow in women with preeclampsia.[16,17] Currently, low dose aspirin is used as an effective therapy for women with antiphospholipid syndrome and recurrent miscarriage.[18,19] However, there are some disagreements between the authors about the positive effects of aspirin therapy on implantation and pregnancy rates following embryo transfer,[20,23] which makes it difficult to come to a certain conclusion in this regard. The aim of the present study was to evaluate whether low-dose aspirin started prior to controlled ovarian stimulation improves ovarian responsiveness, implantation and pregnancy rates in unselected infertile patients in our setting.

Methods. This study was conducted as a prospective, randomized, double-blind placebo- controlled study on 145 patients undergoing conventional IVF, referred to Royan Institute (Infertility Clinic & Reproductive Biomedicine Research Center) from April 2002 to January 2004. The study was approved by the Royan Research Center Ethic Committee and written informed consent was obtained from each participant. The causes of infertility were tubal, male or unexplained factors. The patients with history of endocrine disorders were excluded. All patients had taken no medication for at least 3 months before the study. Hormonal profile including follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, triiodothyronine (T3), thyroxine (T4), thyroid - stimulating hormone (TSH), prolactin, dehydroepiandrosterone (DHEAS), testosterone were measured on day 3 of cycle. The patients were randomly (Randomized Block Design) divided into treatment and control groups. In the treatment group, 72 patients received a daily oral dose of 100 mg of aspirin (Tab-100 mg- Daroo-pakhsh Co. Tehran, Iran) and the control group (73 patients) received placebo. Both groups started aspirin or placebo co-treatment on the 21st of their preceding menstrual cycle and continued until menstruation or a negative pregnancy test. Controlled ovarian hyperstimulation was initiated in all patients with the gonadotropin-releasing hormone (GnRH) analogue (Suprefact: Hoechst, Frankfurt, Germany) 0.5 mg subcutaneous daily, starting in the midluteal phase of the previous cycle. When pituitary desensitization was achieved, which was confirmed by serum E2 levels <40 pg/ml[10] and by ultrasonography, gonadotropin therapy was started using highly purified FSH (150-225 IU; Menopur, Ferring Pharmaceuticals, Denmark). Gonadotropin-releasing hormone analogue injection (0.2 mg/d) was continued up to the day of human chorionic gonadotropin (hCG) administration. Follicular development was monitored by serial transvaginal ultrasonography and serum E2 levels. All scans were carried out by one operator using Aloka SSD 1000 with a transvaginal probe of 7.5 MHz. When at least 2 follicles reached 18 mm in diameter, hCG (10.000 IU; Serono; Pregnyl, Organon) was administered, and then ultrasound-guided transvaginal ovum pick up was performed 34-35 hours later. Embryo transfer was performed 48 hours after ovum pick-up. The luteal phase was supported by vaginal Progesterone (Vag. Supp. Cyclogest, 400 mg, BD, Alpharma, Barnstaple, UK). Clinical pregnancies were detected by increasing serum ß-hCG levels in at least 2 determinations 14 -16 days after ET and were confirmed by ultrasonographic screening of the gestational sac showing fetal heart activity 14 days after the last ß-hCG determination. Pregnant patients continued the medication (Aspirin or Placebo co-treatment), through 12 weeks of pregnancy. The ovarian responsiveness and IVF outcome variables included; number of follicles of ≥15 mm, serum E2 levels on the day of hCG administration, cancellation rate, incidence of ovarian hyperstimulation syndrome (OHSS), number of retrieved oocytes, number of transferred embryos, implantation rate, and pregnancy rate (PR).

The data were analyzed with the use of the 2-tailed Student's t-test, Mann-Whitney rank-sum, and Fisher's exact tests. Data were expressed as means ± SD and $p<0.05$ was considered as statistically significant.

Results. The results of this study are summarized in **Table 1**. The mean (± SD) age was similar in the treatment and control groups. The ovarian responsiveness was expressed as the number of follicles ≥15 mm on the day of hCG administration, the serum E2 levels on the day of hCG administration and number of retrieved oocytes. The mean number of follicles ≥15 mm on the day of hCG administration was significantly lower in the treatment group ($p<0.05$). There were not statistically significant differences in serum E2 level on the day of hCG administration, number of retrieved oocytes and transferred embryos, and cancellation rate between the 2 groups. Although, the implantation rate was higher in aspirin-treated group compared to control group (17.9% versus 12.8%, respectively), the difference was not statistically significant ($p>0.05$). The clinical pregnancy rate was 45.5% versus 33.3% for the treatment and control groups, respectively ($p>0.05$). Also the incidence of OHSS was more frequent in the placebo group compared to the patients who took the aspirin (23.3% versus 5.6% respectively; $p<0.05$).

Table 1 - In-vitro fertilization/embryo transfer outcome in low-dose aspirin-treated patients and controls.

Variable	Treatment group (n=72)	Control group (n=73)	*P* value
Mean age (year)	29.3 ± 4.1	29.9 ± 4.9	NS
Cancellation rate (%)	16.7	23.3	NS
No. of follicles	7.4 ± 4.1	9.0 ± 4.8	<0.05
No. of retrieved oocytes	6.9 ± 5.6	8.6 ± 6.8	NS
E2 level (pg/ml)	953 ± 655.2	957 ± 644	NS
No. of embryos transferred	3.3	3.6	NS
Implantation rate (%)	17.9	12.8	NS
Clinical pregnancy rate	45.5	33.3	NS
OHSS incidence (%)	5.6	23.3	<0.05

Values are expressed as mean±SD unless otherwise indicated.
NS - not significant, OHSS - Ovarian Hyperstimulation Syndrome

Discussion. Oocyte fertilization rates of more than 80% have been reported in IVF patients;[24] however, a large proportion of morphologically normal embryos fail to implant.[25-29] Several studies have been demonstrated that low-dose aspirin,[8,30,31] aspirin/heparin,[32-35] and aspirin plus prednisolone[15,36] are beneficial for infertility therapy. The results of the present study show that low-dose aspirin did not benefit IVF patients in our program. According to our results, aspirin therapy improved the implantation and pregnancy rates in the treatment group compared to control patients, but these differences were not statistically significant. These findings are consistent with some prospective randomized trials performed previously,[22,23,37] and conflict with Rubenstein,[8] Waldenstrom[30] and Weckstein[31] results. Rubenstein et al[8] reported significantly higher ovarian responsiveness, implantation and pregnancy rates using 100 mg aspirin therapy starting in the luteal phase of the preceding cycle. Waldenstrom et al[30] randomized 1380 unselected IVF cycles and reported treatment with 75 mg aspirin daily increased birth rate significantly. Weckstein et al[31] also found enhanced uterine blood flow and higher implantation and clinical pregnancy rates with low does aspirin in women who had a thin endometrium. The result of another study (non-controlled study) showed that IVF outcome was significantly improved when aspirin, heparin, intravenous immunoglobulin therapy was administered to women with repeated IVF failures and antiphospolipid antibodies but not to women with negative antiphospholipid antibodies.[33] Likewise, the number of developed follicles (≥18 mm) and also incidence of ovarian stimulation syndrome were significantly lower in the treatment group compared to control patients whereas there were not significant differences in number of retrieved oocytes, serum E2 level on the day of hCG injection, number of transferred embryos and cancellation rates between the groups. These findings were along with Pakkila[23] and contrary to Rubinstein[8] results. In a similar study carried out by Pakkila et al number of retrieved oocytes, number of transferred embryos and clinical and pregnancy rates were not statistically improved between 2 groups[23] and they reported that aspirin low dose could not improved IVF outcomes in unselected patients. One explanation for all of these findings might be a potential role of prostaglandins in ovulation, fertilization and implantation.[38] It is known that prostaglandin E2 (PGE2) plays a crucial regulatory role in the ovulation process.[39] It may be possible that PGE helps to initiate follicular development by stimulating the appearance of LH and FSH receptors.[40] Also it is a luteotrophic factor. Since aspirin inhibits prostaglandin synthesis, this process could be compromised. The effect of low-dose aspirin on endometrium has been attributed to its potential for peripheral vasodilatation and enhanced tissue (uteroplacental) perfusion.[12] In our study, we did not test uterine blood flow velocity and antiphospholipid antibodies. Therefore, we were not able to determine the women with more responsiveness to aspirin. However, an ASRM Practice Committee Report in 1999 concluded that antiphospholipid antibodies do not affect IVF success and the therapy is not justified,[41] and it seems that implantation and pregnancy rates are more important indicators of IVF outcome compared to indirect measurements such as endometrial blood flow. It has been reported that aspirin and nonsteroidal anti-inflammatory agents increase the risk of miscarriage, although a recent meta-analysis showed no increased risk of miscarriage with aspirin.[42,43] There is at least one reported maternal death due to complications of cerebral hemorrhage in a women treated with aspirin after IVF.[44] Although these risks may be small, treatment with aspirin is not justified in the absence of a proven benefit.

In conclusion, low-dose aspirin therapy did not significantly improve the implantation and pregnancy rates following IVF/ET in our setting. However, finding an ideal protocol for this treatment (namely, patient selection, drug dosage, timing of commencement, duration of therapy) warrants further investigation.

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