ANDROLOGY

Low-Dose Aspirin Does Not Increase Implantation Rates in Patients Undergoing Intracytoplasmic Sperm Injection: A Prospective Randomized Study

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Purpose: *The aim was to evaluate the effect of aspirin on pregnancy and implantation rates in an unselected group of patients undergoing intracytoplasmic sperm injection (ICSI).*
Methods: *Two hundred and seventy-nine patients were randomized to receive 80 mg of aspirin (n = 139) or no treatment (r = 136) starting from the first day of controlled ovarian hyperstimulation.*
Results: *Duration of stimulation, gonadotropin consumption, peak estradiol, number of oocytes retrieved, fertilization rate, cleavage rate, and number of embryos transferred were similar in the two groups. Implantation and clinical pregnancy rates were 15.6% and 39.6% versus 15.1% and 43.4% in aspirin treated and untreated groups, respectively ($P > 0.05$).*
Conclusions: *Low-dose aspirin administration does not improve implantation and pregnancy rates in an unselected group of patients undergoing ICSI.*

KEY WORDS: Aspirin; assisted reproduction; pregnancy.

INTRODUCTION

Numerous attempts have been made to increase implantation and pregnancy rates in couples undergoing assisted reproduction. These include assisted hatching, coculture of embryos, blastocyst transfer, antiprogestins, and measures to increase uterine blood flow.

There are controversial findings regarding the effect of uterine blood flow on the day of human chorionic gonadotropin (hCG) administration or embryo transfer on pregnancy rates after in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). It has been shown that aspirin improves uterine perfusion on the day of luteinizing hormone (LH) peak and in the midluteal phase (1). A few studies concluded that aspirin improves pregnancy rates in patients with positive antiphospholipid antibodies (APAs) treated with assisted reproductive technologies (2–4). Furthermore, it has been demonstrated that aspirin increases pregnancy rates in patients with impaired uterine perfusion undergoing cryopreserved embryo transfer (5) and recipients with a thin endometrium in donor oocyte cycles (6). If increased uterine blood flow improves implantation and pregnancy rates in selected patients, it can be speculated that aspirin resulting in a similar effect may potentially be beneficial to patients undergoing assisted conception regardless of their APA status. Based on this assumption, Rubinstein *et al.* (7), in a randomized double-blind placebo controlled study, showed that aspirin administered at a dose of 100 mg starting at the luteal phase of the preceding cycle improved

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implantation and pregnancy rates in patients undergoing IVF.

The aim of this study was to evaluate the impact of 80-mg aspirin treatment initiated with the onset of controlled ovarian hyperstimulation on implantation and pregnancy rates in an unselected group of patients undergoing ICSI.

MATERIALS AND METHODS

This study included 300 patients undergoing their first ICSI cycle with ejaculated spermatozoa for male infertility between January and August 1998. Since approximately 85% of all initiated treatment cycles in our clinic are ICSI for male infertility, IVF cycles were excluded for the sake of homogeneity. Besides exclusion of the female factor, the patient population was unselected. Patients were randomized into two groups on the third day of their cycle following ovarian down-regulation with gonadotropin-releasing hormone (GnRH) analogues. Group I received 80 mg aspirin beginning on the first day of the gonadotropin stimulation and group II received no treatment. Simple (unrestricted) randomization was achieved using a computer-generated table of random numbers. Concealed assignment was assured by blinding the physician and allocation of the patients by the nurse coordinator. The study was approved by the institutional review board of the American Hospital and each couple undergoing treatment signed an informed consent form.

The patient with an ovarian cyst or inadequate down-regulation on the third day of the cycle remained in the allocated group but aspirin treatment was commenced concurrently with gonadotropin stimulation. Twenty-five cycles were canceled due to inability to achieve adequate down-regulation or poor ovarian response to controlled ovarian hyperstimulation. Of the canceled patients 11 had been randomized to the aspirin and 14 were randomized to the no-treatment group. In patients who proceeded to embryo transfer, 139 had received aspirin and 136 had received no treatment. Aspirin treatment was continued until the ultrasonographic detection of fetal cardiac activity in pregnant patients and was stopped on the day of pregnancy test if the patient did not conceive.

Controlled ovarian hyperstimulation was undertaken using subcutaneous Buserelin acetate (Suprefact proinjection, Hoechst AG, Frankfurt am Main, Germany) in a long protocol combined with pure follicle-stimulating hormone (FSH) (Metrodin, 75, I.F. Serono, Rome, Italy). Buserelin acetate (0.3 mg/day) was commenced on day 20 or 21 of the preceding cycle and continued until the day of hCG. FSH was initiated on the third day of the menstrual cycle with two to six ampules, depeding on the patients' previous or anticipated response. The treatment was then individualized in a step-down fashion. When the leading follicle reached 20 mm in mean diameter with a serum estradiol level of 200–300 pg/ml per mature follicle, 10,000 U hCG (Profasi HP 5000, I.F. Serono, Rome, Italy) was administered. Oocyte retrieval was performed 36 hr after the injection of hCG. Following ICSI, the oocytes were cultured in IVF-50 medium (Scandinavian IVF Science AB, Gotenborg, Sweden). Embryo transfer was performed 48–72 hr after oocyte retrieval. Three or four embryos were transferred according to age and embryo quality. Up to five embryos were transferred in women older than 38 and women with only grade IV embryos available for transfer. Selective assisted hatching was performed in women over 35 years of age and in women with at least one implantation failure in treatment cycles performed elsewhere. Tetracycline 200 mg/day (Monodoks, DEVA, Istanbul, Turkey) and methylprednisolone 16 mg/day (Prednol 16 mg, Mustafa Nevzat Ilaç Sanayi, Istanbul, Turkey) were administered for 5 days starting from the day of oocyte retrieval. Luteal phase was supplemented with intravaginal natural progesterone at a dose of 600 mg/day (Utrogestan, Laboratories Besins Iscovesco, Paris, France) starting on the day of oocyte retrieval. Pregnancy was defined as two β-hCG titers assessed 12 and 14 days after embryo transfer showing appropriate doubling. Clinical pregnancy was defined as the presence of gestational sac(s) with a viable embryo shown on vaginal ultrasonography performed approximately 24 days after embryo transfer.

A power analysis performed at the initiation of the study showed that we needed 146 couples in each group to detect with 80% power a difference of 25% in clinical pregnancy rate per cycle in favor of the aspirin group. Statistical analysis of the results was performed using χ^2 test for comparison of pregnancy rates and independent sample *t*-test for other variables. A P value less than 0.05 was accepted as significant.

RESULTS

There was no difference regarding mean female age, duration of infertility, duration of ovarian stimu-

lation, number of FSH ampules administered, estradiol levels on the day of hCG, endometrial thickness, number of oocytes retrieved, oocyte quality as assessed morphologically, fertilization rate, cleavage rate, and number of embryos transferred (Table I). Implantation and clinical pregnancy rates were 15.6% and 39.6% in aspirin treated and 15.1% and 43.6% in untreated groups, respectively. The difference between the two groups was not significant. Eight (14.5%) abortions and five (9%) tubal pregnancies (bilateral tubal pregnancy in one patient) were observed in group I and seven (11.9%) abortions and one (1.6%) tubal pregnancy were observed in group II patients. In none of the women with a tubal pregnancy was there a history suggestive of tubal disease. Four of the five women with tubal pregnancy in the aspirin group and one woman with tubal pregnancy in the no treatment group had a previous hysterosalpingography showing normal and patent tubes. In the remaining patient the uterine cavity had been evaluated with sonohysterography but there was no information regarding the status of the tubes. All tubal pregnancies were managed by laparoscopic salpingectomy. One patient in the aspirin-treated group showed mild adhesions on the right adnexal area presumably due to a previous appendectomy. The pelvic findings were normal in the remaining patients.

DISCUSSION

Numerous measures have been employed to increase implantation and pregnancy rates in assisted reproduction. However, rendering the endometrium more receptive to the transferred embryo has remained an elusive goal. Embryo manipulation such as zona thinning appears to offer limited benefit and universal agreement regarding the efficacy of this procedure has not been achieved (8). Uterine blood flow at the time of hCG administration has been claimed to be an important marker of success in women undergoing IVF-ET but the subject is still controversial (9–11). Steer *et al.* (9) found that uterine blood flow as assessed by pulsatility index (PI) measurements on the day of embryo transfer could be used to increase implantation rates by indicating the optimal number of embryos that could be safely transferred at a time when the uterus is most receptive. In a later study they also showed that women who achieved pregnancy during assisted reproduction have a significantly lower PI and there is a significant correlation between PI and immunohistochemical markers of endometrial receptivity (10). However, two recent studies concluded that uterine blood flow measurement cannot predict the likelihood of pregnancy in stimulated cycles of IVF or ICSI (11,12). Aspirin has been shown to increase uterine blood flow (1), and it is logical to assume that aspirin administration may increase endometrial receptivity and the success of embryo transfer. Aspirin and heparin were administered to patients with positive APAs undergoing IVF with resulting increase in implantation and pregnancy rates (2–4). Furthermore, Sher *et al.* (2) showed that heparin/aspirin treatment improved pregnancy rates in pa-

Table I. Cycle Characteristics and Pregnancy Outcome of Patients Receiving Aspirin or No Treatment

	Group I (ASA+)[a] (n = 139)	Group II (ASA−) (n = 136)	P value[b]
Age (years)	32.5 ± 4.8	32.4 ± 4.7	NS
Duration infertility (years)	8.6 ± 5.4	9.1 ± 5.5	NS
Fertilization rate	74.3 ± 19.2%	73.6 ± 17.3%	NS
No. of FSH ampules	43.0 ± 17.1	44.0 ± 17.8	NS
Duration of stimulation (days)	10.7 ± 1.6	10.9 ± 1.6	NS
Estradiol level on the day of hCG (pg/ml)	2107.8 ± 1293.9	2405.8 ± 1562.3	NS
No. of oocytes	10.9 ± 6.0	11.7 ± 6.5	NS
Endometrial thickness (mm)	11.1 ± 2.3	11.1 ± 2.3	NS
Cleavage rate	96.3 ± 16.6%	99.1 ± 8.5%	NS
No. of embryos transferred	3.1 ± 1.5	3.2 ± 1.4	NS
Implantation rate/embryo	15.6%	15.1%	NS
Clinical pregnancy rate	39.6% (55/139)	43.4% (59/136)	NS
Abortion rate	14.5%	11.9%	NS
Ectopic pregnancy rate	9.1% (5/55)	1.6% (1/59)	NS

[a] Acetyl salicylic acid 80 mg.
[b] NS, not significant.

tients with positive as well as negative APAs. In two recent studies aspirin has been shown to improve pregnancy rates in patients with decreased uterine blood flow undergoing cryopreserved embryo transfer and in recipients with thin endometrium undergoing oocyte donation (5,6).

Recently, Rubinstein *et al.* (7) reported on the results of a prospective randomized double-blind placebo-controlled study that showed a beneficial effect of low-dose aspirin treatment on response to controlled ovarian hyperstimulation and pregnancy rates in an unselected group of patients undergoing IVF. The authors clearly demonstrated that in women 100 mg aspirin initiated in the luteal phase of the preceding cycle increases the number of recruited follicles and retrieved oocytes. Furthermore, implantation rate per embryo and clinical pregnancy rates were significantly increased. Transvaginal Doppler ultrasonography showed an increased blood flow velocity in the group that received aspirin. The PI of the uterine and ovarian arteries were significantly lower on the day of hCG in the group that received aspirin compared to the group that received placebo. Furthermore, when pregnant and nonpregnant patients were analyzed with respect to changes in the PI, this was significantly lower in the pregnant patients. The authors concluded that (a) aspirin increases ovarian blood flow and improves folliculogenesis, and (b) aspirin increases uterine blood flow, which is associated with increased implantation and pregnancy rates.

The aim of our prospective randomized study was similarly to evaluate the effect of low-dose aspirin treatment on implantation and pregnancy rates after ICSI in an unselected patient population. As opposed to the study by Rubinstein *et al.* (7), low-dose aspirin treatment during the phase of controlled ovarian hyperstimulation and after embryo transfer did not increase the success of ICSI. The explanation for discordant results may lie within the dosage (100 vs. 80 mg) and the duration (initiation at the luteal phase vs. initiation with controlled ovarian hyperstimulation) of aspirin administration.

In our study, it may be argued that Doppler measurement of uterine blood flow and depiction of APA status should have been undertaken to better define the subgroups of patients that may have been affected favorably by aspirin treatment. However, given the conflicting results in the literature regarding Doppler blood flow measurements and APA status, we did not embark upon these tests. Although the difference in pregnancy and implantation rates was not significant, the higher incidence of ectopic pregnancy (although statistically insignificant) needs to be evaluated in larger studies. There is no reasonable explanation for the higher incidence of ectopic pregnancy in the aspirin-treated group. It may be due to a higher incidence of tubal mucosal abnormalities in this group of patients despite them having normal and patent tubes at hysterosalpingography and normal pelvic findings at laparoscopic salpingectomy. It also may be speculated that aspirin alters tubal receptivity to implantation in an unknown manner. Furthermore, aspirin treatment may alter tubal motility by inhibition of the prostaglandin cascade, which then may cause retention of embryos that enter the fallopian tube.

In conclusion, 80 mg aspirin initiated at the start of controlled ovarian hyperstimulation appears to have no beneficial effect on implantation and pregnancy rates in an unselected group of patients undergoing ICSI. Further prospective randomized studies are required to clarify the role of aspirin in patients with positive APAs. Until the incidence of ectopic pregnancy is better defined in further trials, aspirin should only be used in the presence of an established indication.

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