CLINICAL ASSISTED REPRODUCTION

A Matched Study to Determine Whether Low-Dose Aspirin Without Heparin Improves Pregnancy Rates Following Frozen Embryo Transfer and/or Affects Endometrial Sonographic Parameters

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Purpose: *The objective of the matched, controlled study was to determine whether low-dose aspirin therapy without heparin improves pregnancy rates following frozen embryo transfer.*
Methods: *Thirty-six women who did not achieve a pregnancy following fresh embryo transfer and who had frozen embryos available for another transfer were included. Eighteen women were treated with 81 mg aspirin from day 2 of the cycle through pregnancy testing. If the β-human chorionic gonadotropin level was positive, aspirin was continued through the pregnancy. Eighteen women were not given aspirin. The mean outcome variables were pregnancy and implantation rates.*
Results: *The clinical pregnancy rate in the aspirin group was 11.1%, compared with 33.3% for the controls, and implantation rates were 2.9 and 10.9%, respectively.*
Conclusions: *No positive effects of low-dose aspirin therapy on pregnancy rates following frozen embryo transfer were observed.*

KEY WORDS: aspirin; endometrial architecture; frozen embryos; pregnancy; transvaginal sonography.

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INTRODUCTION

There have been several studies suggesting that treating women with heparin and aspirin may improve pregnancy rates (PRs) with in vitro fertilization (IVF)–embryo transfer (ET) (1–3). A study by Sher *et al.* (1) found that 53% of women undergoing IVF-ET for organic pelvic pathology (which represented approximately 85% of their IVF cases) were positive for antiphospholipid antibodies (APAs). The PR in 169 women treated with heparin and aspirin was 49%, compared with only 16% in 25 untreated controls (1). Although Kutteh *et al.* (3) found positive APAs in only 11% of patients undergoing IVF-ET, they concurred that there was a trend for a higher ongoing PR in those treated with heparin/aspirin (6 of 10; 60%) vs. no treatment (2 of 12; 16.7%) (P = NS). Schenk *et al.* found a similar frequency of positive APAs as did Sher *et al.* (48%), even though their requirement was that to be considered positive, two APAs had to be present (2). Although their study was not randomized, based on the fact that the implantation rate (though not the PR) was significantly higher (20%) in the seropositive group rather than the seronegative group (9.7%), they concluded that heparin and aspirin may improve implantation rates in patients undergoing IVF-ET (2).

Although the above studies were suggestive that heparin and aspirin combination improved pregnancy and implantation rates in patients seropositive for

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APAs, none of them were prospective, randomized clinical trials. Thus, definite conclusions about the efficacy of this therapy cannot be reached as of yet. Furthermore, if the treatment is found to be effective in improving implantation rates, then it is not clear whether the combination of heparin and aspirin is needed or whether the results were related to either the use of heparin or aspirin.

The possibility exists that the benefit of heparin and/or aspirin might be through some other mechanism than interfering with the adverse effects of APAs. A study by Wada *et al.* found a higher PR following frozen ET (47%) in patients with previous poor uterine perfusion, as assessed by Doppler ultrasound imaging, when using low-dose aspirin from day 1 of the cycle compared with starting the drug on day 13 (17%) (4). The higher PR correlated with improved uterine perfusion when starting low-dose aspirin on day 1 of the cycle.

The study presented herein is a matched, controlled study to evaluate the efficacy of low-dose aspirin to improve PRs following frozen ET. It was hypothesized that aspirin therapy would improve uterine blood perfusion and provide an improved uterine environment that was more conducive to embryo implantation. Midcycle transvaginal sonography with color Doppler imaging to evaluate endometrial thickness and echo pattern and uterine blood flow impedance was measured, and the effect of aspirin on these parameters was also evaluated.

MATERIALS AND METHODS

Originally a multicycle, prospective, randomized study that would compare pregnancy and implantation rates following heparin, aspirin, and heparin and aspirin in combination following fresh and frozen ETs was proposed to the Institutional Review Board at Cooper Hospital University Medical Center. Furthermore, the study would determine the relative benefits of these therapies in patients seropositive vs. seronegative for APAs and determine the effect on endometrial thickness and echo patterns and uterine blood flow impedance at midcycle. The committee had concerns with (a) having patients pay for APA panels (which would have to be sent to a reference lab), because it was not known that these levels are associated with poor IVF PRs; (b) the use of heparin, in view of its potential risk for patients; and (c) the risk of aspirin causing bleeding following retrieval. Therefore, the study was limited to the efficacy of aspirin therapy in frozen ET. No APA levels were obtained at the patient's expense and heparin was not used.

Study Design

Previous studies have reported an increase of at least 35% in PRs of patients taking anticoagulation therapy (1,3). This study was designed to test the one-sided alternative hypothesis, that aspirin would similarly increase pregnancy rates. Power analysis found that a study with a sample size of 18 subjects per group would have 80% power to detect a difference of 35% in the pregnancy rates at the 0.05 level of significance.

Based on the findings of the power analysis, a matched controlled study with 18 pairs of subjects was conducted. All the subjects enrolled in the study were at most 42 years old, had not conceived following transfer of embryos in the retrieval cycle, were using their own oocytes, and were not taking any concomitant medications that would increase blood flow such as heparin. Each woman eligible to participate in the study was matched with another eligible subject to control for the confounding effects of age, stimulation protocol, and number of embryos transferred. Once the pair was determined, one member of the pair was randomly allocated to the aspirin group, and one was allocated to the control group. Patients in the aspirin group took 81 mg of aspirin daily beginning on day 2 of the frozen ET cycle. Patients in the control group received no aspirin. The study was conducted between January 1, 1996, and August 31, 1996.

Protocols for Frozen ET Cycles

Protocols for frozen ET included natural cycles and hormone replacement cycles with graduated rising dosages of oral estradiol followed by intramuscular progesterone (P) with and without luteal-phase downregulation with leuprolide acetate. All embryos were cryopreserved and thawed using a simplified freezing and thawing method (5). Embryos cryopreserved at the pronuclear stage were thawed 48 hr before transfer. Embryos cryopreserved at the multicell stage were thawed 24 hr before transfer. Three-day-old embryos were used for transfer; assisted embryo hatching was performed on all of them (6).

Endometrial and Uterine Artery Assessments

Patients were asked to undergo transvaginal sonography to evaluate the endometrial thickness and echo pattern along with color Doppler imaging to measure

uterine artery blood flow impedance at midcycle prior to the start of P supplementation and in the midluteal phase 3 days following ET. Endometrial thickness was measured in millimeters by placing electronic calipers on the outer wall of the endometrium as seen in the longitudinal axis of the uterine body. Echo patterns were classified as trilaminar (triple-line or isoechogenic) or homogeneous hyperechogenic. Uterine blood flow impedance was measured by obtaining color Doppler signals from the right and left ascending branches of the uterine arteries lateral to the cervix. A pulsed Doppler range gate was placed over each artery to obtain flow velocity waveforms. Calculations of pulsatility index (PI) and resistance index (RI) were obtained electronically by tracing the waveform and using the formulas: PI = $(A - B)$/mean FD and RI = $(A - B)/A$, where A is the maximum systolic velocity, B is the end diastolic velocity, and FD is the frequency shift. Recordings of each artery were considered suitable for interpretation when multiple consecutive waveforms of equal intensity were demonstrated. All endometrial grading and Doppler indices were performed by one sonographer to reduce interrater variability. The sonographer was blinded to the nature of the study.

Outcome Measures

The main outcome measures were implantation and clinical PRs following frozen ET. A clinical pregnancy in the uterus was confirmed by sonographic evidence of a gestational sac. Implantation rates were computed as number of gestational sacs per embryo transferred.

Statistical Analysis

The PRs for the matched samples were compared using McNemar's test. A P value of 0.05 was considered significant.

RESULTS

The mean numbers of embryos transferred were 3.8 ± 0.9 in the aspirin group and 3.5 ± 0.6 in the control group. There were two clinical pregnancies (11.1%) in the study group, compared with six clinical pregnancies in the control group (33.3%) ($P < 0.05$; McNemar test). The implantation rates were 2.9% (2 of 68) in the aspirin group and 10.9% (7 of 64) in the control group ($P = 0.064$).

Doppler results were only available for 14 patients at midcycle and 12 in the luteal phase in the aspirin group; in the control group, Doppler results were available for nine patients at midcycle and seven in the luteal phase. Failure to obtain these values in all cycles was related to poor patient compliance, especially in those coming from greater distances. Therefore, no inferential statistics were performed. Descriptive statistics are presented in Table I to illustrate possible trends that are worth further investigation.

The mean endometrial thickness, echo patterns, and mean RI were similar in both groups in midcycle. The mean PI was higher for the controls (3.0 ± 0.7 vs. 2.5 ± 0.5). However, in the luteal phase, the endometrial thickness was on average 2 mm thicker in the aspirin group and there was more variation. Fifty-eight percent (7 of 12) of the aspirin group did not have a homogeneous hyperechogenic pattern in the luteal phase, compared with only 28.6% of the control group (2 of 7).

DISCUSSION

The data presented herein do not support the general use of low-dose aspirin to improve PRs following frozen ET. Although an APA panel was not obtained, if there was an approximately 50% frequency as suggested by Sher *et al.* (1) and Schenk *et al.* (2), then one could conclude that the aspirin portion of the heparin–aspirin treatment is not the important part of the therapy improving PRs but the success may be related to the heparin component. However, the possibility exists that these patients only had a low frequency of seropositivity for APAs similar to the findings of Kutteh *et al.* (3).

The study by Wada *et al.* found 37% of the patients on hormone replacement therapy for frozen ET to have poor uterine perfusion and concluded this must be a fairly common problem. We did not find uterine perfusion defects to be a common problem, and perhaps that is why these data did not show any beneficial effect of aspirin. Also, we used a lower dose of aspirin (81 mg) than did Wada *et al.* (150 and 300 mg) (4).

Recently Weckstein *et al.* found that low-dose aspirin improves implantation rates in oocyte donation recipients with a thin endometrium (7). However, similar to the results of our study, there was no increase in the endometrial thickness despite aspirin

Table I. Comparison of Sonographic Measurements by Therapy[a]

	Group 1: aspirin therapy (n = 18)	Group 2: controls (n = 18)
Endometrial thickness (mm)		
Midcycle	11.6 ± 2.2	11.2 ± 2.7
Luteal phase	12.6 ± 3.2	10.4 ± 1.1
Pulsatility index		
Mid cycle	2.5 + 0.5	3.0 + 0.8
Luteal phase	2.9 ± 0.5	2.9 ± 0.7
Resistance index		
Midcycle	0.90 ± 0.08	0.90 ± 0.06
Luteal phase	0.90 ± 0.03	0.90 ± 0.03

[a] Data presented as mean ± standard deviation.

therapy (7). Similar to the study presented here, Weckstein *et al.* did not measure APAs, but they speculate that perhaps the benefit was related to negating adverse effects of APAs (7). However, because donor oocyte recipients are usually devoid of pelvic pathology, they would be expected to have a low frequency of positive APAs (1). Thus, this does not appear to be a plausible explanation to explain the improvement noted in aspirin-treated recipients. One could hypothesize that the benefit might have been related to the improvement in uterine blood flow as measured by color Doppler, but this was not measured by Weckstein *et al.* (7); however, our data did not show any change in blood flow following aspirin therapy. Furthermore, although previous studies in our own group also found an adverse effect of a thin endometrium on subsequent PRs in IVF-ET cycles (8), we did not find a similar adverse effect with thin endometria in donor oocyte recipients (9).

Weckstein *et al.* (7) used the low-dose aspirin 1 week longer than it was used in the study presented here. Possibly that could explain the marked opposite conclusions compared with those in our study. However, it is difficult to reconcile how taking low-dose aspirin for 3 weeks is helpful but 2 weeks is harmful.

Criticisms of previous studies suggesting improved PRs following ET with low-dose aspirin have been that they were not randomized. The study by Weckstein *et al.* reports the first randomized study demonstrating the benefits of low-dose aspirin on subsequent implantation rates. We believe that the data presented herein is the first matched study casting dispersions on the benefits of low-dose aspirin for IVF.

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