Does endometrial integrin expression in endometriosis patients predict enhanced in vitro fertilization cycle outcomes after prolonged GnRH agonist therapy?

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Objective: To determine whether endometrial expression of the integrin $\alpha_v\beta_3$ vitronectin can predict which endometriosis patient subgroup will benefit from pre-IVF cycle prolonged GnRH agonist (GnRHa) therapy.
Design: Prospective randomized institutional review board approved pilot trial.
Setting: Private assisted reproductive technology program.
Patient(s): IVF candidates with regular menses, surgically confirmed endometriosis, and normal ovarian reserve.
Intervention(s): All patients underwent endometrial biopsy 9 to 11 days post-LH surge to evaluate $\alpha_v\beta_3$ integrin expression. Patients were randomized either to receive depot leuprolide acetate 3.75 mg every 28 days for three doses before controlled ovarian hyperstimulation (COH) or to proceed directly to COH and IVF. Group 1: integrin-positive controls (N = 12); group 2: integrin-positive administered prolonged GnRHa (N = 8). Group A: integrin-negative controls (N = 7); group B: integrin-negative administered prolonged GnRHa (N = 9).
Main Outcome Measure(s): COH responses, ongoing pregnancy and implantation rates.
Results: There were no significant effects of GnRH agonist treatment in either of the integrin expression strata regarding ongoing pregnancy or implantation rates, although these outcomes were more frequent in group 2 vs. 1 (62.5% vs. 41.6% and 35% vs. 20.6%, respectively). This effect may have because of limited sample size. The value of a negative integrin biopsy in predicting an ongoing pregnancy after prolonged GnRH agonist therapy was only 44.4%.
Conclusion(s): Endometrial $\alpha_v\beta_3$ integrin expression did not predict which endometriosis patients would benefit from prolonged GnRHa therapy before IVF. (Fertil Steril® 2010;93:646–51. ©2010 by American Society for Reproductive Medicine.)

Key Words: Implantation, integrin, endometriosis, GnRH agonist, infertility, in vitro fertilization

It has been previously demonstrated that endometrial expression of the integrin subtype $\alpha_v\beta_3$ vitronectin and its receptor appears to occur 6 to 10 days after ovulation, the presumed window of implantation (1, 2). This cell adhesion molecule has also been demonstrated on the surface of embryos (3). It has been proposed that this integrin and its receptor may act not only as a site of interaction between the embryo and the endometrium, but may also play a role in initiating trophoblast invasion of the endometrium (4, 5).

Lessey et al. (6) reported that aberrant endometrial $\alpha_v\beta_3$ integrin expression in 39% of women with unexplained infertility and "in-phase" endometrial biopsies. Most of the patients in this trial were noted to have stage I and II endometriosis. These same investigators demonstrated aberrant expression in 44% of endometriosis patients (7). These findings, however, have not been consistently reported (8, 9).

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We have recently described a 48.6% prevalence of absent integrin expression in a case–control study of 74 consecutive IVF candidates felt to be at high risk for implantation defects because of prior IVF failure but adequate embryo quality and/or endometriosis (10). Of the patients who had previously undergone laparoscopy, 52.8% had been diagnosed with endometriosis, of which 57.1% was stage III–IV disease.

Return of $\alpha_v\beta_3$ vitronectin expression with administration of a GnRH agonist or danazol has been described in prospective but nonrandomized trials (11, 12). This may represent an explanation for the improved pregnancy rates reported in endometriosis patients administered a prolonged course of a GnRH agonist before IVF-ET (13, 14). This approach has also been shown to result in IVF cycle outcomes that were no different than in integrin-positive patients who were not administered this agent (10). However, the question of whether endometrial $\alpha_v\beta_3$ vitronectin expression can predict which subgroup of endometriosis patients will benefit from prolonged pre-IVF cycle GnRH agonist therapy has not been assessed, and is the objective of the current investigation.

MATERIALS AND METHODS

This investigation is a prospective randomized pilot trial approved by the HCA/HealthOne institutional review board. The study population consisted of patients with surgically

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documented endometriosis and no current sonographic evidence of an ovarian endometrioma >2 cm in mean diameter. All patients had evidence of normal ovarian reserve testing (day 3 serum FSH level <11 mIU/mL, E_2 level <60 pg/mL, and bilateral antral follicle count >4). A normal clomiphene challenge test was required for all women >38 years. All patients had a normal uterine cavity documented at precycle office hysteroscopy. Patients had received no depot preparations of a GnRH agonist, danazol, or other hormonal suppressive therapy for endometriosis within 6 months of study entry.

Endometrial biopsies were performed on all patients 9 to 11 days after an LH surge was documented by urinary ovulation predictor kits. Tissue was evaluated for the presence or absence of $\alpha_v\beta_3$ vitronectin by commercial assay (Adeza, Sunnyvale, CA) using previously described techniques (1). All endometrial biopsy samples were confirmed to be in phase ±2 days by standard histologic criteria.

After the biopsy results were obtained, patients were randomized by two separate computer generated randomized number tables, one for women expressing the integrin and one for women who did not express the integrin, to either receive an intramuscular preparation of the GnRH agonist leuprolide acetate (TAP Pharmaceuticals, Waukegan, IL) 3.75 mg every 28 days for three injections before initiation of controlled ovarian hyperstimulation or to proceed directly to controlled ovarian hyperstimulation and IVF. This randomization scheme was designed to allow for more equal distribution of treatment regimens for both integrin-positive and -negative patients given the presumed unequal distribution of biopsy results on previously published data (10).

Patients were randomized into one of four groups based on $\alpha_v\beta_3$ vitronectin expression and treatment protocol. Those integrin-positive patients randomized to the control group were assigned to group 1, and those who were integrin negative were assigned to group A. Patients who were randomly assigned to receive a prolonged course of the GnRH agonist who were integrin positive were assigned to group 2, whereas those who were integrin negative were assigned to group B.

All patients subsequently underwent controlled ovarian hyperstimulation employing standard GnRH agonist down-regulation or microdose flare protocols. The determination for which protocol was to be employed was based on ovarian reserve testing and response in previous cycles when appropriate. Indications for hCG administration, day 3 vs. 5 embryo transfer and numbers of embryos to transfer were based on previously published protocols and ASRM/SART guidelines (15–17). Remaining viable embryos were subsequently cryopreserved at the blastocyst stage 5 and/or 6 days after oocyte aspiration. Pregnancy tests were performed 14 days after oocyte aspiration.

Biochemical pregnancy rate was defined as the number of positive serum pregnancy tests obtained 14 days after oocyte aspiration per embryo transfer procedure. Ongoing pregnancy rate was defined as the presence of an intrauterine gestational sac with fetal cardiac activity documented by ultrasound evaluation performed 4 to 5 weeks after a positive pregnancy test per embryo transfer procedure. Implantation rate was defined as the number of intrauterine gestational sacs with fetal cardiac activity as documented by ultrasound examination per number of embryos transferred.

Data were analyzed by Student's group *t* tests and chi-square analyses as appropriate. Values of $P<.05$ were considered to be statistically significant.

RESULTS

Forty patients were initially recruited to participate in the trial. The overall incidence of absent endometrial integrin expression was 45%. Thirty-seven underwent randomization after three dropped out: two for personal reasons and one who elected to proceed with oocyte donation because of multiple failed IVF cycles with uniformly compromised embryo quality. A summary of the randomization scheme is displayed in Figure 1.

Baseline clinical characteristics are displayed in Table 1. There were no significant differences between group 1 and 2 or groups A and B with regard to age, characteristics of ovarian reserve, extent of endometriosis, or number of prior failed cycles. It is interesting to note that only 18.9% of the patients who underwent randomization had previously undergone an unsuccessful IVF cycle. Of these 7 patients, only one had failed more than one prior cycle.

Outcomes of controlled ovarian hyperstimulation are displayed in Table 2.There were no statistically significant differences between group 1 and 2 or groups and B for any of the parameters assessed. However, higher gonadotropin dose requirements that did not reach statistical significance were noted for both groups 2 and B, who received prolonged courses of the GnRH agonist. Similarly, there were no significant differences between group 1 vs. group 2 and group A vs. group B with regard to the incidence of day 3 vs. 5 embryo transfer, use of intracytoplasmic sperm injection, or assisted hatching. One group B cycle was cancelled before oocyte aspiration for poor response. One patient randomized to group A patient conceived spontaneously before initiation of therapy.

Cycle outcomes are displayed in Figures 2 and 3. There were no significant differences between groups 1 and 2 or groups A and B with regard to biochemical pregnancy, ongoing pregnancy, or implantation rates. An apparent trend toward higher ongoing pregnancy and implantation rates in group 2 vs. 1 did not achieve statistical significance (odds ratio [OR] 2.33; 95% confidence interval [CI]: 0.37–14.61 and OR 2.08; 95% CI: 0.60–7.14, respectively). Based on the small patient numbers evaluated in this study, the value of a negative integrin biopsy in predicting an ongoing pregnancy after a prolonged course of GnRH agonist was 44.4%. The value of a positive integrin biopsy in predicting