Embryo quality before and after surgical treatment of endometriosis in infertile patients

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Received: 22 September 2008 / Accepted: 12 December 2008 / Published online: 12 February 2009
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Abstract

Purpose To investigate the hypothesis that surgical treatment of endometriosis in infertile patients may improve pregnancy rates by improving embryo quality.

Methods We conducted a retrospective evaluation of 30 infertile patients treated with in vitro fertilization (IVF) before and after surgery for endometriosis. Patients served as their own controls and only cycles with similar stimulation protocols were compared.

Results Using standard visual evaluation, embryo quality on day 3 was similar before and after surgical treatment of endometriosis. Fifty seven percent of patients had stage I–II endometriosis and 43% had stage III–IV disease. No patients had a live birth after the first IVF cycle and 43% of patients had a live birth with the IVF cycle after surgery.

Conclusions Surgical treatment of endometriosis does not alter embryo quality in patients with infertility treated with IVF.

Keywords Endometriosis · Embryo quality · Infertility

Introduction

Endometriosis is often associated with infertility; however, the exact mechanism is unclear [1]. Two common treatment options for infertile patients with endometriosis are in vitro fertilization (IVF) and surgery. Despite some evidence showing lower pregnancy rates with IVF in patients with endometriosis compared to patients with other indications for IVF [2], IVF can be an effective treatment for infertility associated with endometriosis [3]. Surgical removal of endometriosis lesions and adhesions has been shown to improve monthly fecundity rates in women attempting to conceive spontaneously [4, 5]. In addition, we have found in an earlier study higher pregnancy rates in patients who had surgical treatment of endometriosis after a failed IVF attempt compared to those without surgery [6]. Without clear understanding of how endometriosis fully affects fertility, it is difficult to conclude how treatment with IVF or surgery improves pregnancy rates in patients with endometriosis-related infertility.

We hypothesize that surgical removal of endometriosis lesions may result in improved embryo quality. If this is true, it may partially explain the higher pregnancy rates observed after surgery for endometriosis in some patients. We compare embryo quality in the same patients undergoing IVF cycles before and after surgical treatment of endometriosis to examine whether or not surgery alters embryo quality in patients with endometriosis-related infertility.

Capsule Surgical treatment of endometriosis did not alter embryo quality in patients undergoing in vitro fertilization.

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Materials and methods

Patient selection

We conducted a retrospective chart review of IVF cycles in our program from January 1, 2000 to December 31, 2007. Patients with surgical treatment of endometriosis and more than one IVF cycle at our program were identified. Patients served as their own controls and embryo quality from one IVF cycle before surgical treatment of endometriosis was compared to embryo quality from one cycle after surgery. Patients were included for analysis if they had one IVF cycle followed by laparoscopic treatment of endometriosis followed by another IVF cycle. Only IVF cycles with the same ovarian stimulation protocol before and after surgery were included. Cycles with donor oocytes, preimplantation genetic diagnosis, and frozen embryo transfer were excluded. All patients had the same male partner during treatment in this time period.

Data collected include patient demographics, stimulation protocol, number of oocytes retrieved, fertilization method, fertilization rate, endometrial thickness, quantity and quality of embryos available, and pregnancy rates after the second IVF cycle.

Surgical treatment of endometriosis

Surgery involved laparoscopic treatment of endometriosis lesions by cautery, excision, or laser, and removal of endometriomas by cystectomy. All surgery was performed at our institution. Seventeen patients (56%) had peritoneal biopsies which confirmed the diagnosis of endometriosis. Thirteen patients were diagnosed with endometriosis based on visual inspection and did not have biopsies taken at the time of surgery. One patient was not included in the study because the biopsy of a presumed endometriosis lesion was not confirmed to be endometriosis. All patients had evaluation of their uterine cavity by hysteroscopy or hysterosalpingogram before the first IVF cycle and no patients had congenital uterine anomalies or submucosal fibroids. Polyps, if present, were removed by hysteroscopy.

Ovarian stimulation, oocyte retrieval, and embryo transfer

Ovarian stimulation was achieved with one of three standard protocols: agonist down regulation (long protocol), antagonist protocol, or microdose lupron (flare protocol). Details of these protocols have been described previously [7]. Ultrasound monitoring of follicular growth was performed after 4 days of gonadotropin stimulation and approximately every other day as indicated. Serum estradiol levels were measured as needed. A dose of 10,000 units of human chorionic gonadotropin (hCG) was administered when at least two follicles reached an average diameter of

17 mm. Oocyte retrieval was performed by transvaginal ultrasound guidance 35 h after hCG administration.

In most cases, fertilization of the oocytes was achieved with IVF, but intracytoplasmic sperm injection (ICSI) was used if there were poor semen parameters or other indications for ICSI. The oocytes were examined for fertilization status 16–18 h after IVF or ICSI. Embryo development was assessed on the morning of Day 3, including blastomere number, size, and regularity, and the presence and percentage of fragmentation. If there were at least three 8-cell-stage embryos at grade 1 or grade 2 on the morning of Day 3, Day 5 blastocyst transfer was recommended, and embryos were cultured in Sage Blastocyst Medium (Cooper Surgical, Inc.) with 10% synthetic serum substitute for another 2 days.

Ultrasound-guided embryo transfer was performed using a Tefcat or Echotip Softpass catheter (Cook Ob/GYN). Progesterone supplementation with vaginal suppositories was performed in all patients.

Embryo quality assessment

A single team of experienced embryologists evaluated the embryos on post-retrieval day 3, 68 to 72 h after oocyte harvest. Embryos were examined for cleavage (cell number) and grade, which includes cytoplasmic fragmentation. Embryos were graded as follows on day 3: Grade 1, blastomeres have equal size and no cytoplasmic fragmentation; Grade 2, blastomeres have equal size and minor cytoplasmic fragmentation involving < 10% of the embryo; Grade 3, blastomeres have unequal size and fragmentation involving 10–20% of the embryo; Grade 4, blastomeres have equal or unequal size, and moderate to significant cytoplasmic fragmentation covering 20–50% of the embryo; and Grade 5, few blastomeres and severe fragmentation covering ≥50% of the embryo [8].

Assessment of blastocyst quality on day 5 was based on the grading system described by Gardner et al. [9]. In this grading system, blastocysts are assessed in three ways: a numerical score from one to six describing the degree of expansion and hatching; followed by a letter A, B, or C grading the inner cell mass; followed by a letter A, B, or C grading the trophectoderm. Any expanding, expanded, and hatching blastocysts with good inner cell mass and trophectoderm epithelium (3BB or better) were frozen on day 5 or day 6 with protocols described elsewhere [10]. Embryos which either arrested or were of worse quality than 3BB were discarded or donated to research.

Outcome

A pregnancy test was considered positive if the beta-hCG level was >5 mIU/mL 13 days after retrieval. Clinical

intrauterine pregnancy (IUP) was defined as gestational sac seen on 6 week transvaginal sonogram. Spontaneous pregnancy loss (SAB) was defined as loss of pregnancy after clinical IUP. Live birth data was collected by contacting patients.

Statistical analysis

Statistical analysis was performed on contingency tables using χ^2 statistics for dichotomous variables and student *t* test for continuous variables. Statistical significance was determined at $P < 0.05$.

Institutional review board

Institutional Review Board approval was obtained for this study.

Results

A total of 30 patients met inclusion criteria for analysis. For ethnicity, obstetric history, age, cycle day 3 follicle stimulating hormone (FSH) level, and other patient characteristics see Table 1. Patients were on average 1 year older at the time of the IVF cycle after surgery compared to the IVF

Table 1 Patient characteristics

Total number of patients	30	
Race	Caucasian	40%
	Asian	40%
	Indian	13%
	Hispanic	7%
Gravida ^a	33%	
Para ^a	3%	
SAB ^a	27%	
Body mass index	22.4 (17.0–29.0)	
Age at first IVF retrieval	35.5 (28.6–39.9)	
Age at time of surgery	36.0 (31.8–41.9)	
Age at second IVF retrieval	36.7 (30.9–40.6)	
Interval between surgery and second IVF cycle	240 days (47–819)	
FSH level at first IVF cycle	6.9 (2.4–11.7)	
FSH level at second IVF cycle	7.1 (1.9–19.6)	
Endometriosis found at previous surgery (Endometriosis diagnosis known at 1st IVF cycle)	33%	
Stage of endometriosis	Stage I	40%
	Stage II	17%
	Stage III	13%
	Stage IV	30%
	Long	40%
Protocol used	Antagonist	37%
	Micro flare	23%

^a at time of first IVF retrieval

Table 2 IVF parameters in cycles before and after laparoscopic treatment of endometriosis ($N=30$)

	IVF cycle before surgery	IVF cycle after surgery
Days on OCPs	20.3±3.2	18.4±4.6
Days of stimulation	10.5±2.4	10.9±1.9
Amount of gonadotropins in IU	4,950±540	5,025±420
Endometrial lining in mm	10.0±1.2	10.1 +/1 1.8
Number of follicles	15.2±2.6	12.8±1.8
Number of oocytes	11.6±2.3	9.9±3.3
ICSI	17%	23%
Fertilization rate	65% IVF 70% ICSI	68% IVF 75% ICSI
Assisted hatching	53%	67%
Number of ET	2.8±1.1	3.3±0.9
Number of eight cell day 3 embryos	2.6±1.1	2.3±0.9
Number of day 3 embryos six cell or higher & Grade I or II	3.8±1.2	3.3±1.6
Blastocyst (day 5) transfers	13%	20%
Number of blastocysts frozen	2.1±1.3	2.8±2.1

No P -value < 0.05

cycle before surgery, but their CD3 FSH was similar. Approximately two thirds of patients (67%) had no known history of endometriosis at the time of surgery. For the remaining 33% of patients, this was their second surgery for endometriosis.

Findings at surgery included stage of endometriosis based on the revised American Society for Reproductive Medicine Classification [11]. Stage I–II endometriosis was diagnosed in 57% of patients and the remaining 43% of patients were diagnosed with stage III–IV disease. Approximately half of the patients (43%) had endometriomas removed at surgery and no patients had hydrosalpinges.

More patients had ICSI and/or assisted hatching in the second cycle (23% and 67%, respectively) compared to the first (17% and 53%, respectively) but this did not reach statistical significance (see Table 2). One patient had assisted hatching with acid in the IVF cycle before surgery and laser in the second cycle.

The embryo quality was similar before and after surgical treatment of endometriosis. The average number of eight cell embryos and good quality embryos (defined as six cell or more and grade I or II) was not significantly different (Table 2). A similar percentage of patients met criteria for and underwent blastocyst transfer in the cycles before and after surgery (Table 2). There was no significant difference in good quality (3BB or better) blastocysts transferred on day 5 in the cycles before surgery compared to those after surgery (Table 2).

The live birth rate per IVF cycle after surgical treatment of endometriosis was 43%. The average age of patients who

conceived was 34.6 compared to 36.6 for women who did not conceive ($P=0.1$). The average time between surgery and retrieval date for IVF cycle after surgery was 211 days in patients that conceived compared to 263 days in those that did not ($P=0.4$). Live birth rate was 53% for patients with Stage I–II endometriosis and 31% for those with Stage III–IV endometriosis with the second IVF cycle.

Discussion

In this descriptive study, we examine the hypothesis that surgical treatment of endometriosis may impact embryo quality, and thus may help to explain why some studies find improved pregnancy rates in women with endometriosis and infertility after surgery. We attempted to limit some factors affecting embryo quality by comparing embryo quality from IVF cycles in the same women with the same partners and same stimulation protocol before and after laparoscopic treatment of endometriosis.

There are several limitations to a study with this design. Due to our strict criteria for entry, few patients met the inclusion criteria of IVF treatment before and after surgery for endometriosis; therefore, we lack the power to see small differences. Patients were an average of 1 year older at the time of the IVF cycle after surgery compared to the cycle before surgery. A third limitation to our study is the use of visual inspection of the embryos. Although cell number and fragmentation are the standard means for evaluating embryos, they do not always correlate with implantation potential. [12] We may be missing important comparative data by only using these qualitative descriptions. Therefore, it is still possible the oocyte and embryo quality are different after surgical treatment of endometriosis, but our ability to detect and measure that quality is our limiting factor.

The design of this study is to examine the effect of surgical treatment for endometriosis on embryo quality, not to examine IVF success rates; however, the pregnancy rates after treatment are interesting. The pregnancy rates of 53% and 31% after laparoscopy for stage I–II and III–IV disease, respectively, are higher than those reported in a meta-analysis of 22 studies evaluating the impact of endometriosis on IVF outcomes (21% for stage I–II and 14% for stage III–IV) [2]. Importantly, the studies summarized in this meta-analysis did not consistently specify whether surgical treatment occurred prior to IVF, and it is possible that the lower rates reflect less pre-IVF surgical treatment. The reason for the difference in pregnancy rates is unclear but may reflect a different pathophysiology between patients with stage I–II and patients with stage III–IV disease [2]. Due to the small sample size in this study, we lack the statistical power to compare these groups.

As the embryo quality appears similar in both cycles, the mechanism for the higher pregnancy rate after surgery may be related to an improved environment for implantation. Though lower implantation rates in the setting of endometriosis have been reported in multiple clinical studies [13, 14], the relative contribution of embryo quality and endometrial receptivity to this finding can be debated. A study by Arici et al. [15] found a lower implantation rate in women with endometriosis compared to controls despite similar measures of oocyte/embryo quality, implicating an altered endometrial environment. In addition, compelling molecular evidence has shown fundamental differences in the endometrium from women with endometriosis compared to endometrium from women without endometriosis. Endometrium from women with endometriosis seems to have decreased expression of integrin [16] and HOXA10 [17], alterations in apoptosis [18], and a gene expression profile consistent with progesterone resistance [19]. We did not evaluate molecular data in this study and the only measure of endometrial receptivity we studied, endometrial thickness, was similar in patients before and after surgery (Table 2).

Conclusions

We investigated the hypothesis that the improved pregnancy rates observed in some patients after surgery for endometriosis-related infertility may be explained by improved embryo quality. We found that surgical treatment of endometriosis did not alter embryo quality in our patients. Despite an abundance of clinical and basic science research, the mechanisms for the impact of endometriosis and its treatment on fertility remain unclear. Further investigation is needed.

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