Effects of Endometriomas on Ooccyte Quality, Embryo Quality, and Pregnancy Rates in In Vitro Fertilization Cycles: A Prospective, Case-Controlled Study

ELENA H. YANUSHPOLSKY,[1,3] CRAIG L. BEST,[1,2] KATHARINE V. JACKSON,[1] ROBERT N. CLARKE,[1] ROBERT L. BARBIERI,[1] and MARK D. HORNSTEIN[1]

Submitted: September 16, 1997
Accepted: November 6, 1997

Purpose: *The effect of endometriomas on oocyte quality, embryo quality, and pregnancy rates in in vitro fertilization (IVF) cycles was evaluated.*
Methods: *Forty-five women had "chocolate" cysts aspirated at the time of oocyte retrieval, and cyst fluid CA 125 levels were measured to ascertain presence of "true" endometriomas. Fifty-seven women without any complex cysts at the time of oocyte retrieval served as controls. IVF cycle outcome parameters were compared between the two groups.*
Results: *Women with endometriomas experienced a significantly higher rate of early pregnancy loss compared to controls (47 vs 14%). There was also a trend toward fewer oocytes retrieved and fewer embryos reaching at least the four-cell stage 48 hr after retrieval in patients with true endometriomas vs controls.*
Conclusions: *The presence of endometriomas at the time of oocyte retrieval is associated with increased rates of early pregnancy losses. The number of oocytes retrieved and the embryo quality may also be affected adversely in the presence of endometriomas.*

KEY WORDS: embryos; endometriosis; in vitro fertilization; spontaneous abortion.

INTRODUCTION

Endometriosis affects 5 to 15% of asymptomatic women of reproductive age. In contrast, 30 to 40% of women with infertility have been reported to have endometriosis (1). This discrepancy in the prevalence of endometriosis between the fertile and the infertile populations suggests a possible causal relationship between endometriosis and infertility. A number of mechanisms for endometriosis-associated infertility have been suggested, but a complete understanding of how endometriosis may affect fertility is still lacking (2). A few studies suggested improved pregnancy rates after surgical treatment of mild endometriosis (3,4), whereas several other studies reported no relationship between minimal and mild endometriosis and infertility, and patients had similar pregnancy rates after treatment for endometriosis or expectant management (5). For women with advanced endometriosis, however, the prognosis for pregnancy was significantly less favorable than for those with milder disease (6), and women with ovarian endometriosis had lower pregnancy rates than women with peritoneal lesions only (7). In addition, after treatment with in vitro fertilization (IVF)–embryo transfer (ET), which overcomes the impact of pelvic adhesions, women with advanced endometriosis involving the ovaries have been reported to have significantly lower pregnancy rates than women with non-endometriosis-related pelvic disease (8–10). These results suggest that the presence of ovarian endometriosis (endometrioma) may be the most important predictor of a poor reproductive outcome.

Our hypothesis for this study was that the presence of endometriomas during an IVF cycle adversely affects oocyte production, embryo quality, pregnancy, and live birth rates. We tested our hypothesis in a prospective, case-controlled study comparing IVF cycle parameters and outcomes between women with endometriomas and controls.

[1] Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115.
[2] Center for Women's Health, Quincy Hospital, Quincy, Massachusetts 02169.
[3] To whom correspondence should be addressed at Brigham and Women's Hospital, 75 Francis Street, Boston, Massachusetts 02115.

1058-0468/98/0400-0193$15.00/0

MATERIALS AND METHODS

Patient Selection

From January 1, 1994, until August 1, 1995, 45 patients were found to have complex "chocolate" ovarian cysts during transvaginal ultrasound (US) at the time of oocyte retrieval. Chocolate cyst fluid was aspirated under US guidance and processed as described below. Clear follicular fluids from the same patients and from 57 controls (patients without any complex cysts at the time of retrieval collected in temporal proximity to cases) were also aspirated under transvaginal US guidance during the same period. Clear follicular fluids from each patient were pooled and processed as described below. Endometriomas were differentiated from other complex ovarian cysts by CA 125 cyst fluid measurements (11). Institutional Review Board approval for use of discarded materials and medical record information was obtained prior to initiation of the study.

Cyst/Follicular Fluid Processing and CA 125 Measurements

All endometriomas were aspirated separately and the collection system was flushed with culture medium at least once prior to proceeding with aspiration of other follicles.

Chocolate cyst and follicular fluids were collected into 15-ml plastic tubes (Fischer Scientific, Medford, MA), prewarmed to 37°C. Samples were transferred to large petri dishes (Falcon, Model 3003; Becton-Dickinson Co., Lincoln Park, NJ) for identification and removal of oocytes. Remaining fluid was placed into 15-ml conical test tubes (Corning Inc., Corning, NY) via sterile pipettes and centrifuged at 200*g* for 15 min. The supernatant was removed and stored in 1.8-ml cryovials (Irvine Scientific, Irvine, CA) at −70°C until all samples were ready to be assayed for CA 125. CA 125 levels were measured using the CA 125 II radioimmunoassay kit according to the manufacturer's specifications (Centocor, Inc., Malvern, PA). Intraassay and interassay coefficients of variation were less than 10%.

Defining Endometriomas and Staging of Endometriosis

Koninckx *et al.* (11) reported that only 68% of chocolate cysts were indeed endometriomas when examined histologically. CA 125 levels $>10^4$ U/ml in chocolate ovarian cysts were diagnostic of endometriomas by histology, with 100% sensitivity and 100% specificity. Therefore, in this study, only chocolate cysts with CA 125 levels $>10^4$ U/ml were considered "true" endometriomas.

All patients in our study had advanced endometriosis as evidenced by the presence of endometriomas, all of which were >1 cm in diameter. Endometriomas of that size alone would be classified as Stage III according to The American Society for Reproductive Medicine Revised Classification of Endometriosis (12). Additional lesions and/or adhesions would make the classification of Stage IV disease.

IVF Protocol

All patients, except two, underwent ovarian down-regulation with leuprolide acetate (LA; Lupron; TAP Pharmaceuticals, Inc., Deerfield, IL), 1.0 mg sc each day for 10 days, starting either in the midluteal or in the early follicular phase of the menstrual cycle. Two patients were on a "flare" protocol with LA, 1.0 mg sc, starting on cycle day 2 for 1 day only, followed by ovulation induction starting on cycle day 3. The LA dose was decreased to 0.5 mg sc per day at the beginning of ovulation induction. Ovulation induction was achieved by IM injections of Pergonal (human menopausal gonadotropin; Serono Laboratories, Inc., Randolph, MA) and/or Metrodin (follicle stimulating hormone; Serono Laboratories, Inc.) according to a previously described protocol (13). Transvaginal US follicular monitoring and serum estradiol (E_2) measurements were performed as described previously (14). Patients received IM injections of 10,000 IU human chorionic gonadotropin (hCG; Profasi, Serono Laboratories, Inc.) when two or more follicles reached a maximum diameter of 18 mm and the E_2 level was >600 pg/ml (conversion factor to SI units, 3.671). Transvaginal US-guided oocyte retrieval occurred 36 hr after hCG administration. Sperm sample preparations and in vitro culture procedures were performed as described previously (13). ETs were performed 48 to 51 hr after oocyte retrieval using Dulbecco's phosphate-buffered saline (PBS; GIBCO Chemical Co., Grand Island, NY) with 1% maternal serum. Progesterone (P), 50 mg im, supplementation was given daily starting on the evening of ET for a total of 16 days after ET. Pregnancy tests (β-hCG) were drawn 16 days after ET. Progesterone supplementation was continued if a pregnancy test was positive. Chemical pregnancy was defined as positive pregnancy test without subsequent US confirmation of a gestational sac. Spontane-

ous abortion was defined as pregnancy loss prior to 10 weeks' gestation. Ongoing pregnancy was defined as a viable gestation between 10 weeks and delivery.

Statistical Analysis

All statistical calculations were performed on an Apple Macintosh Quadra 700 computer (Apple Computers, Cupertino, CA) using either Statview (Abacus Concepts, Berkeley, CA) or JMP (SAS Institute, Cary, NC) statistical software. Significant differences were determined using either Mann–Whitney *U*, chi-square, or Fisher exact test when appropriate. Significance was assumed at $P < 0.05$.

RESULTS

Forty-five patients had chocolate cysts aspirated during oocyte retrieval. CA 125 levels were ≥10,000 U/ml (range, 10,000 to 960,000 U/ml) in 37 patients, and they were designated as having "true" endometriomas. Seven of them had bilateral endometriomas, and 30 had unilateral endometriomas. The remaining eight patients with chocolate cysts had CA 125 levels <10,000 U/ml (range, 5 to 7739 U/ml) and were excluded from the analysis. CA 125 levels in the clear follicular fluid of patients with endometriomas ranged from 16 to 6400 U/ml. One of 57 controls had a CA 125 level >10,000 U/ml and was excluded from the analysis. The remaining 56 controls had clear follicular fluid CA 125 levels ranging from 3 to 6600 U/ml.

The "true" endometrioma group ($n = 37$) and the control group ($n = 56$) were compared with respect to the age of the female partner, duration of infertility, and type of primary infertility diagnosis. Except for the expected higher prevalence of endometriosis in the "endometrioma" group, there were no significant differences between the two groups (Table I). There were also no significant differences between the two groups with respect to sperm parameters, number of days of downregulation with LA, total number of ampoules of Pergonal and/or Metrodin used for ovulation induction, duration of ovulation induction, or the peak E_2 level on the day of hCG (Table II). Twenty-four (66%) patients in the endometrioma group were nulligravid, and five (14%) had prior spontaneous pregnancy losses ranging from 1 to 3. Of the 56 controls, 27 (45%) were nulligravid, and 15 (27%) had prior pregnancy losses ranging from 1 to 4. These differences were also not significant.

Table I. Demographics of IVF Patients with and Without Endometriomas

Demographics	Endometrioma ($n = 37$)	Controls ($n = 56$)	*P*
Age (mean±SD)	36 ± 4	36 ± 4	NS[a]
Duration of infertility (mean ±SD)	3.8 ± 2.1	3.9 ± 1.9	NS
Primary diagnosis			
Male	6	9	NS
Tubal	11	20	NS
Unexplained	2	6	NS
Anovulation	0	8	NS
LPD[b]	0	1	NS
Cervical	0	2	NS
DES[c]	1	0	NS
Endometriosis	17	10	0.04

[a] Not significant.
[b] Luteal-phase defect.
[c] Diethylstilbestrol exposure in utero.

Table II. IVF Cycle Parameters of Patients with and Without Endometriomas

IVF cycle parameter (mean ± SD)	Endometrioma ($n = 37$)	Controls ($n = 56$)	*P*
Total motile sperm ($\times 10^6$)	120 ± 107	97 ± 99	0.24
Estradiol (pg/ml) on day of hCG	1635 ± 988	1857 ± 989	0.21
No. days of stimulation	11 ± 3	11 ± 2	0.90
No. days of GnRH-A	24 ± 8	22 ± 4	0.20
No. hMG ampoules	50 ± 38	41 ± 23	0.26

There was a similar distribution of patients in groups going through their first, second, third, fourth, fifth, or sixth cycle (Table III).

Of the 30 patients who had unilateral endometriomas, there were no statistically significant differences in oocytes and embryo parameters from ovaries containing endometriomas compared to the contralateral.

There were fewer oocytes retrieved from patients with endometriomas, and although fertilization rates

Table III. Distribution of Patients in Cycle Nos. 1 Through 6

Cycle No.	Control ($n = 56$)	Endometriosis ($n = 37$)	*P*
1	18 (32)[a]	9 (25)	NS
2	18 (32)	11 (30)	NS
3	11 (20)	8 (23)	NS
4	4 (8)	4 (10)	NS
5	1 (1)	3 (7)	NS
6	4 (7)	2 (5)	NS

[a] Values in parenthesis are percentages.

were similar, there were also fewer embryos reaching at least the four-cell stage 48 hr after oocyte retrieval (Table IV). Seventeen (46%) of 37 patients with endometriomas and 21 (38%) of 56 controls had a positive pregnancy test 16 days after ET ($P = 0.40$), and implantation rates (gestational sacs/embryos transferred) were 12 and 14%, respectively. However, 8 (47%) of 17 patients with endometriomas, but only 3 (14%) of 21 controls had an early pregnancy loss, i.e., chemical pregnancy or spontaneous abortion before 10 weeks' gestation ($P = 0.04$).

DISCUSSION

The results of this study support our hypothesis that the presence of endometriomas during an IVF cycle adversely affects the cycle outcome, with an increased rate of early pregnancy loss. Patients with endometriomas produced fewer oocytes, and although fertilization rates were similar between endometrioma patients and controls, there was also a lower percentage of embryos reaching the four-cell or greater cleavage stage among patients with endometriomas. Previous studies have demonstrated a greater likelihood of successful implantation for more developmentally advanced embryos (≥four cells 48 hr after insemination) (15,16). Therefore one of the reasons for the lower pregnancy rates in women with advanced endometriosis may be a lower embryo quality and greater proportion of developmentally arrested embryos that are less likely to implant. It is possible that ovarian endometriomas produce substances that are toxic to maturing oocytes and that these compounds may adversely impact cleavage of the oocytes after fertilization.

Table IV. Oocyte and Embryo Parameters and Pregnancy Rates of Patients with and Without Endometriomas

IVF cycle parameter	Endometrioma (n = 37)	Controls (n = 56)	P
No. oocytes (mean±SD)	13 ± 9	17 ± 10	0.06
% fertilization (mean±SD)	62 ± 27	62 ± 26	0.80
% embryos ≥4 cells (mean±SD)	47 ± 34	61 ± 27	0.09
No. (+)hCG	17 (46%)	21 (38%)	0.40
Implantation rate	12%	14%	0.59
No. early pregnancy losses (SAb + chemical)[a]	8 (47%)[b]	3 (14%)[c]	0.04
Live births	9 (24%)	18 (32%)	0.41

[a] SAb, spontaneous abortion.
[b] Five chemicals, three SAb.
[c] Two chemicals, one SAb.

Our data also indicate a significant increase in early pregnancy loss in patients with endometriomas. In calculating early pregnancy losses we chose not to make a distinction between chemical pregnancies and spontaneous abortions because both represent failed embryo development, only at different times in gestation. Early pregnancy loss may be due to either inherent oocyte/embryo abnormalities, or inadequate endometrial support, or both. Adverse effects of endometriosis on oocyte and embryo quality as well as on implantation rates have been reported by several investigators (17–19). An association between endometriosis and increased rates of pregnancy loss has also been noted by a number of investigators (20), although a causal relationship between endometriosis and pregnancy loss is difficult to prove. The difference in early pregnancy losses between patients with endometriomas and controls in our study was much greater than the differences in the number of oocytes retrieved or the number of developmentally advanced embryos. In addition, no significant differences in oocyte and embryo parameters were observed in oocytes/embryos derived from the endometrioma-containing ovary vs the unaffected contralateral side in the same patient. These observations suggest that, in addition to oocytes and embryos, endometrial receptivity may also be significantly impaired in patients with advanced endometriosis.

Olivennes and colleagues from The New York Hospital–Cornell Medical Center did not observe any adverse effects of endometriomas on oocyte and embryo quality or pregnancy rates and pregnancy losses in the retrospective review of their IVF data (21). Unfortunately they did not describe their method of distinguishing an endometrioma from a complex hemorrhagic cyst. Complex ovarian cysts have little or no effect on IVF outcome (22), and the distinction between endometriomas and complex cysts is very difficult to make in a retrospective analysis of data. Furthermore, they consistently observed a decreased number of oocytes retrieved and significantly lower pregnancy rates when severe mechanical infertility was associated with endometriosis. The authors chose to make a distinction between severe mechanical factors and endometriosis, but according to the American Society for Reproductive Medicine Revised Classification for Endometriosis (12), a combination of these two factors would be classified as stage IV endometriosis. With such a modification, their results would confirm lower IVF pregnancy rates and higher abortion rates for women with advanced endometriosis.

The single most important IVF outcome parameter for both patients and physicians remains the live births

per cycle. Although we observed a lower live birth rate in patients with endometriomas (24 vs 32%), the data did not reach statistical significance. This would be an important point in counseling patients with moderate–severe endometriosis regarding their chances for successful outcome through IVF. While their overall chances in IVF are good, they may be at a higher risk for early pregnancy loss than patients without endometriosis. More research is necessary to identify the etiology of this early pregnancy loss, which may involve suboptimal oocyte/embryo quality and/or a fundamental "field" defect in endometrial receptivity in women with advanced endometriosis.

ACKNOWLEDGMENTS

We appreciate the help of Patricia Griffin, B.S., embryologist; David Travassos, B.S., senior embryologist; and Xiaoying Gao, Ph.D., research associate; Department of Obstetrics and Gynecology, Brigham and Women's Hospital, in the handling of technical procedures throughout the study. We also appreciate Centocor, Inc.'s (Malvern, PA) generosity in providing CA 125 II assay kits free of charge.

REFERENCES

1. Mahmood TA, Templeton A: Prevalence and genesis of endometriosis. Hum Reprod 1991;6:544–549
2. Olive DL, Schwartz LB: Endometriosis. N Engl J Med 1993;328: 1759–1769
3. Paulson JD, Asmar P, Saffan DS: Mild and moderate endometriosis: Comparison of treatment modalities for infertile couples. J Reprod Med 1991;36:151–155
4. Marcoaux S, Maheux R, Berube S, *et al.*: Laparoscopic surgery in infertile women with minimal or mild endometriosis. N Engl J Med 1997;337:217–222
5. Mahmood TA, Templeton A: Pathophysiology of mild endometriosis: Review of literature. Hum Reprod 1990;5:765–784
6. Olive DL, Lee KL: Analysis of sequential treatment protocols for endometriosis-associated infertility. Am J Obstet Gynecol 1986;154:613–619
7. Telimaa S: Danazol and medroxyprogesterone acetate inefficatious in the treatment of infertility in endometriosis. Fertil Steril 1988;50:872–875
8. Matson PL, Yovich JL: The treatment of infertility associated with endometriosis by in vitro fertilization. Fertil Steril 1986;46:432–434
9. Yovich JL, Matson PL, Richardson PA, Hilliard C: Hormonal profiles and embryo quality in women with severe endometriosis treated by in vitro fertilization and embryo transfer. Fertil Steril 1988;50:308–313
10. Dlugi AM, Loy RA, Dieterle S, Bayer SR, Seibel MM: The effect of endometriomas on in vitro fertilization outcome. J In Vitro Fert Embryo Transfer 1989;6:338–341
11. Koninckx P, Muyldermans M, Moerman P, Mueleman C, Deprest J, Cornille F: CA 125 concentrations in ovarian "chocolate" cyst fluid can differentiate an endometriotic cyst from a cystic corpus luteum. Hum Reprod 1992;7:1314–1317
12. The American Fertility Society: Revised American Fertility Society classification of endometriosis. Fertil Steril 1985;43:351–352
13. Jackson KV, Nurredin A, Clarke RN, Hornstein MD, Rein MS, Friedman AJ: The appearance of one-pronuclear human oocytes is associated with better ovulation-induction response and successful pregnancy outcome. Fertil Steril 1992; 58:366–372
14. Stewart EA, Jackson KV, Friedman AJ, Rein MS, Fox JH, Hornstein MD: The effect of baseline complex ovarian cysts on in vitro fertilization outcome. Fertil Steril 1992;57: 1274–1278
15. Dawson KJ, Conaghan J, Ostera GR, Winston RML, Hardy K: Delaying transfer to the third day post-insemination, to select non-arrested embryos, increases development to the fetal heart stage. Hum Reprod 1995;10:177–182
16. Grillo JM, Gamerre M, Lacroix O, Noizet A, Vitry G: Influence of the morphological aspect of embryos obtained by in vitro fertilization on their implantation rate. J In Vitro Fert Embryo Transfer 1991;8:317–321
17. Arici A, Oral E, Bukulmez O, Duleba A, Olive DL, Jones EE: The effect of endometriosis on implantation: Results from the Yale University in vitro fertilization and embryo transfer program. Fertil Steril 1996;65:603–607
18. Dicker D, Goldmlan GA, Ashkenazi J, Feldberg D, Voliovitz I, Goldman JA: The value of pretreatment with gonadotrophin releasing hormone (GnRH) analogue in IVF-ET therapy of severe endometriosis. Hum Reprod 1990;5:418–420
19. Sung L, Mukherjee T, Takeshige T, Bustillo M, Copperman AB: Endometriosis is not detrimental to embryo implantation in oocyte recipients. J Assist Reprod Genet 1997;14:152–156
20. Hornstein MD: Endometriosis and spontaneous abortion. *In* Recurrent Pregnancy Loss, Infertility and Reproductive Medicine, AJ Friedman (guest editor), Clinics of North America. Philadelphia, W. B. Saunders, 1991, pp 175–185
21. Olivennes F, Feldberg D, Liu HC, Cohen J, Moy F, Rosenwaks Z: Endometriosis: A stage by stage analysis—The role of in vitro fertilization. Fertil Steril 1995;64:392–398
22. Penzias AS, Jones EE, Seifer DB, Grifo JA, Thatcher AA, DeCherney AH: Baseline ovarian cysts do not affect clinical response to controlled ovarian hyperstimulation. Fertil Steril 1992;57:1017–1021