Outcome of IVF in Patients with Endometriosis in Comparison with Tubal-Factor Infertility[1]

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Purpose: *The aim of this retrospective study was to compare the outcome of in vitro fertilization and embryo transfer in women with endometriosis and a control group with tubal-factor infertility.*
Methods: *Forty-eight patients with endometriosis underwent 65 cycles of in vitro fertilization and embryo transfer at Huddinge University Hospital. The matched control group with tubal-factor infertility consisted of 98 cycles in 98 patients. These groups were retrospectively analyzed regarding stimulation, fertilization, embryo development, implantation, and pregnancy outcome.*
Results: *The fertilization rate was significantly lower in women with endometriosis, but the cleavage, implantation, and pregnancy rates did not differ.*
Conclusions: *Our results show that women with endometriosis have a lower fertilization rate compared with women with tubal-factor infertility. However, once the oocyte is fertilized, it seems that the preembryo has a normal chance of implantation, leading to similar pregnancy rates.*

KEY WORDS: endometriosis; infertility; in vitro fertilization; implantation; fertilization.

INTRODUCTION

Endometriosis is one of the most puzzling gynecologic diseases. The relationship between endometriosis and subfertility/infertility has been in debate for many years. Several causes of infertility in women with endometriosis have been suggested, such as altered folliculogenesis, ovulatory dysfunction, and luteal-phase defect (1–3). In vitro fertilization and embryo transfer (IVF-ET) has become a common method to help women with endometriosis-associated infertility. Using IVF-ET, it is possible to bypass the suspected disturbed functions. It is discussed whether the results of IVF-ET are as good in women with endometriosis as in patients with tubal-factor infertility (4). Some investigators have reported high success rates with IVF treatment (5–7). Others report a significantly lower fertilization rate in women with endometriosis compared with women with tubal damage, resulting in lower pregnancy rates (8–11). No consensus view can be extracted from these other studies, possibly because they are less well controlled or the patient selection for the control group is different. Against this background, and to test the hypothesis that women with endometriosis have a lowered fertility compared with healthy women, it is important to evaluate the results in endometriosis patients compared with a matched control group. An analysis of different steps in IVF-ET may also give guidance for further research on the etiology of subfertility associated with endometriosis.

The aim of this retrospective study was, therefore, to compare the outcome of IVF-ET in women with endometriosis with that of a matched control group with tubal-factor infertility.

MATERIALS AND METHODS

During the period of January 1994 to July 1997, 1253 IVF-ET treatments were performed at Huddinge University Hospital. A total of 65 cycles was completed in 48 patients with endometriosis as the only apparent cause of infertility. Diagnosis had previously been made by direct laparoscopic visualization and, when convincing diagnosis was not obtained macroscopically, by biopsy of endometriotic implants. The

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mean age of the patients with endometriosis was 33.6 ± 3.5 years, and their mean duration of infertility was 5.6 ± 2.6 years. The group included 51 cycles in women with primary infertility. Five of the women were undergoing gonadotropin-releasing hormone agonist treatment when starting IVF. These women were not included when the duration of down-regulation was calculated.

The control group consisted of 98 cycles in 98 patients in whom tubal factor was the only apparent cause of infertility. To control for variability over time, we included up to two patients who underwent ovum pickup (OPU) within 1 week before or after the OPU of patients with endometriosis. The mean age of the controls was 33.8 ± 3.2 years, and their mean duration of infertility was 7.2 ± 3.1 years. Women with primary infertility contributed with 33 cycles. Women with endometriosis were included up to three times, depending on whether they became pregnant at the first or second IVF. The control women were only included once, independent of the IVF cycle number.

In both groups the cycle represented in this study varies from number 1 to number 30. All semen samples in both groups were normal. No cryopreserved embryos were used. A standard IVF protocol was used. Briefly, treatment started on cycle day 1 or 21 with daily intranasal administration of buserelin acetate (Suprecur; 900–1200 μg/day; Hoechst, Frankfurt, Germany). The duration until complete down-regulation (endometrium less than 4 mm and/or serum estradiol less than 100 p*M*) of the ovaries was 33 ± 12 for the women with endometriosis and 32 ± 9.6 for the controls. Ovarian stimulation was achieved with follicle-stimulating hormone (FSH) (Fertinorm HP; Serono, Geneva, Switzerland) given daily by subcutaneous self-administration. The starting dose was generally 150–225 IU, and the dose was adjusted according to the serum estradiol level and follicular growth as monitored by ultrasound. The criterion for administration of 10,000 IU of human chorionic gonadotropin (hCG; Profasi; Serono) was the presence of at least three follicles larger than 18 mm in diameter. OPU by transvaginal ultrasound guidance was performed 37 hr after the hCG administration. Standard IVF procedures were used. Fertilization was checked after 18–20 hr, and cleavage stages evaluated after 40 hr of culture. The embryos were graded according to their morphology and cleavage stage on the day of ET. A scale from 1 to 3 was used, with 3.0 being the best embryos. Luteal support was given as 1250 IU of hCG on days 3, 6, 9, and 12 after ET or by progesterone, 50 mg/day intramuscularly. Pregnancy was diagnosed by a rising level of serum hCG, which was assayed 14–16 days after ET. Clinical pregnancies were determined by the presence of a gestational sac during the sixth to seventh weeks of pregnancy.

Table I. Results of Stimulation in IVF-ET Cycles in Women with Endometriosis and Women with Tubal-Factor Infertility[a]

	Endometriosis	Tubal factor	*P*
No. of cycles (patients)	65 (48)	98 (98)	
Dose of FSH (IU)	2597 ± 1276	2798 ± 1091	0.2845
OPU day	12.0 ± 2.4	12.4 ± 2.3	0.1902
E_2 at hCG (p*M*)	3286 ± 2307	3532 ± 2337	0.5087
No. of follicles >10 mm	10.6 ± 4.7	11.1 ± 5.3	0.6057

[a] OPU, ovum pickup; hCG, human chorionic gonadotropin; IVF/ET, in vitro fertilization/embryo transfer; E_2, estradiol.

Statistical Analysis

Values are usually presented as mean ± standard deviation. Comparison between groups was performed by Student's *t* test or Mann–Whitney *U* test. A *P* value less than 0.05 was considered significant. The implantation rate was analyzed with Yates corrected chi-square test.

RESULTS

The results of ovarian stimulation are shown in Table I. There were no differences between the groups in duration of down-regulation, total dose of FSH administered, duration of stimulation, estradiol levels at hCG administration, number of follicles larger than 10 mm, or endometrial thickness.

The results of fertilization and embryo development are presented in Table II. While the number of eggs retrieved did not differ between the two groups, the

Table II. Results of Fertilization and Embryo Development in IVF-ET Cycles

	Endometriosis	Tubal factor	*P*
No. of eggs	10.8 ± 5.7	10.9 ± 5.5	0.9811
No. of fertilized eggs	6.0 ± 4.3	8.5 ± 4.8	0.001
Fertilization rate (%)	60.1 ± 31.7	78.3 ± 18.3	0.00001
No. of cleaved eggs	5.8 ± 3.9	7.5 ± 4.7	0.0219
Cleavage rates (%)	85.2 ± 22.1	87.9 ± 19.1	0.4327
No. of eggs to ET	2.0 ± 0.42	2.1 ± 0.43	0.4279
Morphologic score of embryo for ET	2.4 ± 0.4	2.5 ± 0.39	0.4469

fertilization rate was significantly lower in the women with endometriosis ($P < 0.00001$).

However, cleavage rate and the quality and number of the transferred embryos showed no statistically significant difference.

The IVF-ET outcome is shown in Table III. The implantation and pregnancy outcome were similar. The ongoing pregnancies have all passed the first trimester. Three women with endometriosis had implantation of two embryos, but in all three cases one embryo disappeared and the women delivered one baby each.

Five of the women with endometriosis were already undergoing gonadotropin-releasing hormone agonist treatment when starting IVF treatment. Their data on oocyte numbers and fertilization rate did not differ significantly from those of the whole group.

There was no significant difference regarding the data for women with endometriosis who were included once and those who were repeatedly included with a maximum of three cycles.

DISCUSSION

In the present well-controlled study, women with endometriosis undergoing IVF-ET had a significantly lower fertilization rate in comparison with a control group with tubal-factor infertility. Thus, a smaller number of embryos were available for implantation in women with endometriosis. In assisted reproduction with ovarian stimulation, when large numbers of oocytes are collected, this problem might be overcome, as indicated by the fact that the pregnancy rate after IVF was similar in controls and in women with endometriosis. It seems as though implantation is not affected by endometriosis, whereas oocyte quality is. This might be of significance in natural cycles, when only one oocyte is available. Indeed, the frequency of primary infertility was much higher in the endometriosis group than in the control group.

Table III. Analysis of Pregnancy Outcome in Women with Endometriosis and Women with Tubal-Factor Infertility

	Endometriosis	Tubal factor
No. of ET	57	98
No. of positive hCG per OPU(%)	22/65 (34%)	39/98 (40%)
Implantation[a] rate (%)	26/116 (22%)	47/205 (23%)
No. of spontaneous abortions	5[b]	5
No. of ectopic pregnancies	0	3
Biochemical pregnancies only	2	2
Ongoing and delivered per OPU (%)	18/65 (28%)[c]	29/98 (30%)[d]

[a] Defined as the number of embryos implanted according to ultrasound examination.
[b] Three women with two implantations but one live birth.
[c] One twin pair.
[d] Five twin pairs.

This study has its limitation in being retrospective, but the strength is that the methods are well controlled and the same during the period studied. The control patients had tubal disease–related infertility, but only a few had ever undergone pelvic surgery. However, in case of hydrosalpinges, the tubes had been surgically removed. An alternative control group might have been cases with male-factor infertility, but too few cases were available during the study period.

In patients with endometriosis, the pelvic cavity contains an elevated number of hyperactivated macrophages (12–15). These macrophages, like other lymphocytes, produce different cytokines, some of which are cytotoxic and might have an adverse effect on the follicle or the oocyte (16) or contribute to an environment unsuitable for fertilization (17). Also, the endometriotic lesions produce different cytokines such as interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-α (18,19). An increased level of these cytokines, as well as interferon-γ and transforming growth factor-β, has been shown in peritoneal fluid in women with endometriosis (20). These substances have been shown to inhibit sperm function, gamete interaction, and embryo development, both in sperm penetration tests and in in vitro models (21). It has been shown that peritoneal fluid from infertile women with endometriosis decreases both the sperm swimming capacity and the acrosome reaction, which could contribute to impaired fertilization (22–24). With IVF, on the other hand, the oocyte is not in contact with the peritoneal fluid. Steinleitner *et al.* (25) showed that fertilization, but not ovulation, was affected by transfer of hyperactivated macrophages to the peritoneal cavity. Their study also showed that women with endometriosis, receiving oocytes from nonendometriotic ovaries, had an implantation rate similar to that of recipients without endometriosis. If toxic factors from the peritoneal fluid are able to pass into the follicle, the oocyte would be affected before ovulation. A preovulatory exposure of gametotoxic substances, like cytokines, to the oocyte or the surrounding granulosa cells might influence different surface receptors. Our study gives no indication of abnormal folliculogenesis, since the response to FSH was similar in patients with endometriosis and in patients with tubal factor infertility.

The number of cases in this study was low, which is why the power of the study might be limited. However, in a later study comparing the outcome of intracytoplasmic sperm injection in women with

endometriosis and cases with male-factor infertility, there were also indications of a reduced oocyte quality, the cleavage rate being significantly lower in oocytes from women with endometriosis (Naffah *et al.*, manuscript in preparation).

Cleavage failure may be another consequence of alterations within the oocyte. A significantly lower cleavage rate was also reported by Tanbo *et al.* (26) and an increased incidence of aberrant morphological phenotypes in human embryos has been reported (27). However, we did not observe any statistically significant difference in the cleavage rate.

A further possible cause of endometriosis-associated infertility is impaired implantation of the embryo. This has been observed in a number of studies (1,28,29). Arici *et al.* (1) confirmed implantation by a vaginal ultrasound examination during the fifth week, while at our clinic the ultrasound examination was performed during the sixth to seventh week. However, the risk of missing an early spontaneous abortion is minimal, because implantation is indicated by a rising level of serum hCG. A functional disturbance of the oocyte was shown by a study based on oocyte donation, where oocytes derived from endometriotic ovaries showed significantly reduced implantation rates after IVF-ET compared with oocytes donated from women without endometriosis (30). The conclusion was that infertility in women with endometriosis may be related to alterations within the oocyte, which in turn result in embryos less likely to implant. We did not see any difference in implantation rates between the two groups.

Several previous studies have shown that the fertility of women with endometriosis is not increased by hormonal treatment. Thus we allowed women with endometriosis to be included in the study, even if they had been undergoing gonadotropin-releasing hormone agonist treatment when ovulation stimulation was initiated. Their results did not differ from those of the rest of the patients. In this study it was not possible to correlate the IVF results with the extent of endometriotic lesions. However, this might be of minor interest, as Palmisano *et al.* (31) have shown that the anatomic site and type of lesion, including endometriotic cysts, are insufficient for predicting fertility when used as sole components of a clinical staging system for endometriosis.

CONCLUSIONS

We have observed that the average fertilization rate is decreased in women with endometriosis compared with in women with tubal-factor infertility. From a clinical point of view, however, the slight decrease in the number of fertilized eggs did not impair the chances of pregnancy, which was excellent. In older patients and in those with a poor response to gonadotropins, the decreased fertilization rate might be a significant limiting factor. Ongoing studies on the outcome of intracytoplasmic sperm injection in couples with combined endometriosis and severe male factor might shed further light on the mechanism of the lower fertilization rate observed here with standard IVF. It is not possible to tell from this retrospective study if follicular fluid toxicity caused the decrease. Therefore, analysis of follicular fluid in relation to oocyte maturation and fertilization has to be performed.

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REFERENCES

1. 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
2. Hill JA: Immunologic factors in endometriosis and endometriosis-associated reproductive failure. Infert Reprod Med Clin North Am 1992;3:583–596
3. Gleicher N: Endometriosis, a new approach is needed. Hum Reprod 1992;7:821–824
4. Shushan A, Eisenberg VH, Schenker JG: Subfertility in the era of assisted reproduction: Changes and consequences. Fertil Steril 1995;64:459–469
5. Mahmood TA, Arumugam K, Templeton AA: Oocyte and follicular fluid characteristics in women with mild endometriosis. Br J Obstet Gynecol 1991;8:573–577
6. 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
7. Geber S, Paraschos T, Atkinson G, Margara R, Winston RML: Results of IVF in patients with endometriosis: The severity of the disease does not affect the outcome, or the incidence of miscarriage. Hum Reprod 1995;10:1507–1511
8. Wardle PG, McLaughlin EA, McDermot A, Mitchell JD, Ray BD, Hull MGR: Endometriosis and ovulatory disorder: reduced fertilization *in vitro* compared with tubal and unexplained infertility. Lancet 1985;2:236–239
9. Yovich JL, Yovich JM, Tuvik AI, Matson PL, Willcox DL: *In-vitro* fertilization for endometriosis. Lancet 1985;2:552 (Letter)
10. O'Shea RT, Chen C, Weiss T, Jones WR: Endometriosis and *in-vitro* fertilization. Lancet 1985;2:723.8 (Letter)

11. Wardle PG, Foster PA, Mitchell JD, McLaughlin EA, Sykes JA, Corrigan E, Hull MG, Ray BD, McDermott A: Endometriosis and IVF: Effect of prior therapy. Lancet 1986;1:276–277
12. Badaway SZA, Cuenca V, Marshall L, Munchback R, Rinas AC, Coble DA: Cellular components in peritoneal fluid in infertile patients with and without endometriosis. Fertil Steril 1984;42:704–708
13. Halme J, Becher S, Hammond M, Raj HMG, Raj S: Increased activation of pelvic macrophages in infertile women with mild endometriosis. Am J Obstet Gynecol 1983;145:333–337
14. Olive DL, Weinberg JB, Haney AF: Peritoneal macrophages and infertility: The association between cell number and pelvic pathology. Fertil Steril 1985;44:772–777
15. Dunselman GAJ, Hendrix MGR, Bouckaert PXJM, Evers JLH: Functional aspects of peritoneal macrophages in endometriosis of women. J Reprod Fertil 1988;82:707–710
16. Anderson DJ, Hill JA: Cell-mediated immunity in infertility. Am J Reprod Immunol Microbiol 1988;17:22–30
17. Sueldo CE, Lambert H, Steinleitner A, Rathwick, G, Swanson JA: The effect peritoneal fluid from patients with endometriosis on murine sperm-oocyte interaction. Fertil Steril 1987;48:697–699
18. Akoum A, Lemay A, Paradis I., Rheault N, Maheux R: Secretion of interleuikin-6 by human endometric cells and regulation by proinflammatory cytokines and sex steroids. Hum Reprod 1996;11:2269–2275
19. Bergquist A, Nejaty H, Fröjsa B, Bruce C, Sjöblom P, Söder O. Production of interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor (TNF) α in separated and cultured stromal and epithelial cells from endometriotic tissue and endometriosis. Mol Human Reprod 1998. (accepted for publication)
20. Rana N, Braun DP, House R, Gebel H, Rotman C, Dmowski WP: Basal and stimulated secretion of cytokines by peritoneal macrophages in women with endometriosis. Fertil Steril 1996;65:925–930
21. Sueldo CE, Kelly E, Montoro L, Subias E, Baccaro M, Swanson J, Steinleitner A, Lambert H: Effect of interleukin-1 on gamete interaction and mouse embryo development. J Reprod Med 1990;35:868–872
22. Tasdemir M, Tasdemir I, Kodama H, Tanaka T: Effect of peritoneal fluid from infertile women with endometriosis on ionophore-stimulated acrosome loss. Hum Reprod 1995;10:2419–2422
23. Arumugam K: Endometriosis and fertility: Raised iron concentration in the peritoneal fluid and its effect on the acrosome reaction. Hum Reprod 1994;9:1153–1157
24. Aeby TC, Huang T, Nakayama RT: The effect of peritoneal fluid from patients with endometriosis on human sperm function *in vitro*. Am J Obstet Gynecol 1996;174:1779–1783
25. Steinleitner A, Lambert H, Roy S: Immunomodulation with pentoxifylline abrogates macrophage-mediated infertility in an *in vivo* model: A paradigm for a novel approach to the treatment of endometriosis-associated subfertility. Fertil Steril 1991;55:26–31
26. Tanbo T, Omland A, Dale PO, Abyholm T: *In vitro* fertilization/embryo transfer in unexplained infertility and minimal peritoneal endometriosis. Acta Obstet Gynecol Scand 1995;74:539–543
27. Brizek CL, Schlaff S, Pellegrini VA, Frank JB, Worrilow KC: Increased incidence of aberrant morphological phenotypes in human embryogenesis—an association with endometriosis. J Assist Reprod Genet 1995;12:106–112
28. Gutiérrez A, Oliveira N, Cano F, Ruiz A, Simon C, Remohi J, Pellicer A: *In vitro* fertilization in infertile patients with endometriosis. Assist Reprod Rev 1994;4:162–171
29. Dmowski WP: Immunological aspects of endometriosis. Int J Gynecol Obstet 1995;50 (Suppl. 1):3–10
30. Simón C, Gutiérrez A, Vidal A, de los Santos MJ, Tarin JJ, Remohi J, Pellicer A: Outcome of patients with endometriosis in assisted reproduction: Results from *in-vitro* fertilization and oocyte donation. Hum Reprod 1994;9:725–729
31. Palmisano GP, Adamson GD, Lamb EJ. Can staging systems for endometriosis based on anatomic location and lesion type predict pregnancy rates? Int J Fertil 1993;38:241–249