Effects of early endometriosis on IVF-ET outcomes

Keiko Mekaru[1], Chiaki Yagi[1], Kozue Asato[1], Hitoshi Masamoto[1], Kaoru Sakumoto[1], Yoichi Aoki[1]

[1]*Department of Obstetrics and Gynecology, Graduate School of Medical Science, University of the Ryukyus, Okinawa, Japan*

TABLE OF CONTENTS

1. ABSTRACT

There have been very few reports on the outcomes of *in vitro* fertilization and embryo transfer (IVF-ET) in women with stage I/II endometriosis. The objective of this study was to investigate IVF-ET outcomes in women with early-stage endometriosis. We enrolled 35 women less than 40 years with unexplained infertility who underwent IVF-ET into the study. We compared 18 women with stage I/II endometriosis according to the revised American Society for Reproductive Medicine classification for endometriosis, who underwent 39 IVF-ET cycles [En (+) group] with 17 women without endometriosis who underwent 41 IVF-ET cycles [En (−) group]. Higher requirements of total gonadotropin, a lower percentage of high-quality embryos of all fertilized eggs (9.0% vs. 16.3%), a relatively lower pregnancy rate (33.3% vs. 41.5%), and a lower live birth rate (25.6% vs. 34.1%) were observed in the En (+) group. Although no significant effect on IVF-ET outcome was observed, ovarian response may be decreased in women with stage I/II endometriosis. Considering the decreased number of high-quality embryos in the En (+) group, stage I/II endometriosis may have detrimental effects on embryo quality.

2. INTRODUCTION

The effects of endometriosis on fertility and the mechanisms involved are unclear and frequently debated, and they have not been clarified in spite of the accumulated research over the years. Severe endometriosis is associated with pelvic adhesions and distortion of pelvic anatomy, and these factors can result in infertility. In stage I/II endometriosis, a study demonstrated that cumulative pregnancy rates (PRs) increased significantly in patients after laparoscopic resection and ablation of endometriotic lesions (1). This study suggested that mild lesions may also affect the reproductive process.

It has been proposed that intrauterine insemination (IUI) and *in vitro* fertilization and embryo transfer (IVF-ET) should be performed for women with stages I/II and III/IV endometriosis, respectively (2, 3). In cases of women with stage I/II endometriosis who fail to conceive with insemination, IVF-ET should be performed (2, 3). Several studies have compared all the stages of endometriosis and tubal infertility. These studies demonstrated that severe endometriosis tends to decrease IVF-ET success rates compared with mild endometriosis

(4). However, there have been few reports regarding women with stage I/II endometriosis.Therefore, it is unclear whether mild endometriosis affects IVF-ET outcomes.

A meta-analysis including 22 studies (2,377 IVF-ET cycles for women with endometriosis vs. 4,383 IVF-ET cycles for women without endometriosis) demonstrated an association between endometriosis and negative IVF-ET outcomes (5). It showed a 35% decrease in the likelihood of pregnancies in women with endometriosis compared with those without endometriosis (odds ratio, 0.63; 0.51–0.77) (5). The same study compared early-stage endometriosis (stage I/II) with severe endometriosis (stage III/IV) and demonstrated that women with severe endometriosis had significantly lower PRs and implantation rates (5). Furthermore, when women with early-stage endometriosis were compared with those with tubal infertility, the fertilization and implantation rates, number of oocytes retrieved, and peak estradiol (E2) levels on the day of oocyte retrieval were significantly lower in the former group; however, pregnancy rates were not significantly different. Another study showed a significant decrease in PRs in women with severe endometriosis (6). However, women with early-stage endometriosis showed comparable cumulative birth rates compared to those with tubal infertility (40.0% vs. 36.6%) (6).

Thus, the aim of the present study was to investigate the effects of stage I/II endometriosis on IVF-ET outcomes with a view to provide better counseling of patients and appropriate ovarian stimulation.

3. MATERIALS AND METHODS

3.1. Patients

We performed a retrospective analysis of patients with a primary diagnosis of endometriosis, who underwent IVF-ET. Between January 2004 and December 2008, 141 women aged less than 40 years with unexplained infertility underwent laparoscopic evaluation and resection of endometriosis. We enrolled 35 women who underwent IVF-ET after not achieving a successful pregnancy with 4–6 cycles of timed intercourse. Eighteen women with stage I/II endometriosis who underwent 39 IVF-ET cycles [En (+) group] were compared with 17 women without endometriosis who underwent 41 IVF-ET cycles [En (−) group]. Endometriosis was evaluated according to the revised American Society for Reproductive Medicine (r-ASRM) classification for endometriosis, and the lesions were cauterized as much as possible. In this classification, endometriotic lesions are scored according to their site, diameter, and depth, whereas adhesions are scored according to their site, density, and degree of enclosure. A total r-ASRM score (for both lesions and adhesions) of 1–5, 6–15, 16–40, and more than 40 corresponded to stages I, II, III, and IV, respectively. Unexplained infertility was defined as bilateral tubal patency observed on hysterosalpingography, no ovulatory disorder, no abnormal sperm analysis, normal postcoital test, and normal findings on transvaginal ultrasonography. Women with myomas or adenomyosis observed on transvaginal ultrasonography were excluded.

3.2. IVF-ET

Basal follicle-stimulating hormone (FSH) levels were measured on day 3 of the pretreatment cycle. Controlled ovarian stimulation was carried out using a gonadotropin-releasing hormone agonist. Buserelin acetate (Suprecur®; Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) was intranasally administered at a dosage of 900 µg/day from the midluteal phase of the pretreatment cycle to the day of human chorionic gonadotropin [hCG, 10,000 international units (IU); Gonatropin, Asuka Pharmaceutical Co., Ltd., Tokyo, Japan] injection. An initial dosage of 300 IU/day of human menopausal gonadotropin (HMG Teizo; Teizo, Tokyo, Japan) was administered on days 3 and 4 of the cycle. Later, 225 IU/day of HMG was administered. hCG was administered when 2 ovarian follicles were observed to be 18 mm or more in diameter. Peak E2 levels were measured before hCG administration. Transvaginal oocyte retrieval was performed 35 h after hCG administration. Oocytes were fertilized either by conventional IVF-ET or by intracytoplasmic sperm injection (ICSI) 4 h after retrieval. ICSI was performed only for couples with failed fertilization in their first cycle of conventional IVF-ET. Embryos were cultured in early cleavage medium™ (IS Japan Co., Ltd., Saitama, Japan) under an atmosphere of 5% CO_2, 5% O_2, and 89% N_2 at 37°C in an incubator (Astec, Fukuoka, Japan). Embryo quality was evaluated on the basis of Veeck's criteria. Embryos containing 7 or 8 mononucleated blastomeres that were approximately equal in size without fragmentation were classified as grade 1. Those with similar mononucleated blastomeres and less than 10% fragmentation were classified as grade 2. Embryos containing fewer than 7 or unequal blastomeres without fragmentation were classified as grade 3. Those with unequal blastomeres and more than 15% fragmentation were classified as grade 4. High-quality embryos were embryos classified as grade 1 according to Veeck's criteria. One to three embryos were transferred 3 days after oocyte retrieval using ET catheters (Kitasato Supply, Shizuoka, Japan) under ultrasound guidance. For luteal phase support, 5,000 IU of hCG was injected on the day of oocyte retrieval, then on days 3 and 7. The World Health Organization classification was used for ovarian hyperstimulation syndrome (OHSS) assessment. Cryopreservation began in July 2008, and frozen embryo transfers were excluded from the study. Clinical pregnancy was defined by the presence of a gestational sac with fetal heartbeat. The main outcome measures were the response to controlled ovarian hyperstimulation, number of oocytes retrieved, fertilization rates, PRs, and live birth rates (LBs).

3.3. Statistical analysis

Proportions were compared using Fisher's exact test or the chi-square test. Continuous variables were analyzed and compared using the Student's t-test or the Mann–Whitney U-test. A p value of <0.05 was considered statistically significant.

4. RESULTS

None of the women had hydrosalpinx. In En (+) group, 14 women (29 cycles) were stage I and 4 women (10

Table 1. Characteristics of patients in the En (+) and En (−) groups

Variable	En (+) group (39 IVF-ET cycles)	En (−) group (41 IVF-ET cycles)	*P*
Interval between laparoscopy and oocyte retrieval (months) [1]	16.7 ± 8.5 (2–36)	15.9 ± 8.6 (3–32)	0.1
Mean age (years) [1]	33.9 ± 3.5 (23–39)	32.7 ± 4.3 (24–39)	0.3
Duration of infertility (years) [1]	4.5 ± 3.4	5.0 ± 3.0	0.65
Nulligravida (%)	10 (55.6)	9 (52.9)	0.88
Basal FSH level (mIU/ml) [1]	9.7 ± 4.1 (5.5–23)	8.2 ± 1.6 (5.4–11.4)	0.19
Type of fertilization (%)			
IVF-ET	33 (84.6)	32 (78.0)	0.64
ICSI	6 (15.4)	9 (22.0)	0.64

Values are expressed as mean ± SD (range) [1]

Table 2. Cycle characteristics of patients and pregnancy outcomes in the En (+) and En (−) groups

Outcome	En (+) group (39 IVF-ET cycles)	En (−) group (41 IVF-ET cycles)	*P*
Dose of HMG administered (IU) [1]	2,208.1 ± 407.5	1,984.1 ± 338.1	0.017
No. of oocytes retrieved per patient [1]	7.9 ± 4.1	10.0 ± 5.5	0.065
No. of fertilized oocytes per patient [1]	4.5 ± 2.6	5.2 ± 4.1	0.44
No. of grade 1 embryos (% of all fertilized embryos)	14/155 (9.0)	32/196 (16.3)	0.044
No. grade 1 embryos per patient [1]	0.41 ± 0.82	0.86 ± 1.3	0.082
No. of grade 1 and grade 2 embryos (% of all fertilized embryos)	32/155 (20.6)	53/196 (27.0)	0.16
No. of embryos transferred per patient [1]	2.3 ± 0.82	2.3 ± 0.80	0.95
Serum E2 levels (pg/ml) on the day of hCG administration [1]	2,331.9 ± 1419.6	3,165.4 ± 2221.7	0.063
Pregnancy rate (%)	13/39 (33.3)	17/41 (41.5)	0.45
Live birth rate (%)	10/39 (25.6)	14/41 (34.1)	0.41
Implantation rate (%)	15/85 (17.6)	21/88 (23.9)	0.31
Implantation rate including grade 1 embryos (%)	7/33 (21.2)	9/38 (23.7)	0.97
OHSS III	5/39 (12.8%)	11/41 (26.8%)	0.12

Values are expressed as mean ± SD (range) [1]

cycles) were stage II. Of the total subject population, the mean number of IVF attempts was 2.1 ± 1.0 (range, 1–4); 1 IVF attempt for 9 women, 2 for 12 women, 3 for 9 women, and 4 for 5 women. There were no cancellation cycles because of poor follicle growth or OHSS. Characteristics of patients in both groups are shown in Table 1. In the En (+) group, 3 women (6 cycles) needed ICSI for previous fertilization failure. In the En (−) group, 5 women (9 cycles) needed ICSI for the same reason. No significant differences were observed between the groups with regard to age, duration of infertility, and whether IVF-ET or ICSI was undertaken. FSH levels were not significantly different.

Significantly higher requirements of total gonadotropin (2,208.1 ± 407 IU vs. 1,984.1 ± 338.1 IU, p = 0.017) and a lower percentage of high-quality embryos of all fertilized eggs were found (9.0% vs. 16.3%, p = 0.044) in the En (+) group. In En (+) group, there were 12 cycles of embryo transfers including at least 1 or more grade 1 embryo. Thirty-three embryos were transferred in the 12 cycles, the implantation rate with grade 1 embryos in En (+) group was 21.2% (7/33). In the same way, there were 15 cycles of embryo transfers including at least 1 or more grade 1 embryo in the En (−) group. The implantation rate with grade 1 embryos in the En (−) group was 23.7% (9/38), and the difference between the groups was not significant. Although a lower percentage of grade 1 embryos was observed in En (+) group, if embryo transfer was performed with at least 1 or more grade 1 embryo, there was no significant difference in implantation rates between the groups.

The En (+) group showed relatively lower PRs and lower LBs compared with the En (−) group (33.3% vs. 41.5% and 25.6% vs. 34.1%, respectively; difference not significant) (Table 2). The number of cycles required to achieve pregnancy in the En (+) and En (−) groups were 1.8 ± 0.87 cycles and 2.2 ± 1.2 cycles, respectively; the difference was not significant (p = 0.18). PR per patient was 61.1% (11/18) in the En (+) group and 82.4% (14/17) in the En (−) group; the difference was not significant (p = 0.26). LB per patient was lower in the En (+) group (55.6%: 10/18) than in the En (−) group (82.4%: 14/17) (not significant, p = 0.18).

When patients in the En (+) group were divided into 2 groups on the basis of the interval between the most recent surgical intervention and oocyte retrieval [12 months or less (14 cycles) and more than 12 months (25 cycles)], no significant difference in PRs was found (33% vs. 27%).

5. DISCUSSION

Compared with the En (−) group, dose of HMG was significantly higher and the number of grade 1 embryos was significantly lower in the En (+) group. Hence, stage I/II endometriosis may affect ovarian response and embryo quality. Several studies have found that women with endometriomas or stage III/IV endometriosis required higher HMG doses and had poorer ovarian responses (7–10). Previous surgery for severe endometriosis or ovarian endometriosis was considered to cause this (8). In a comparison between cases of stage I/II endometriosis that mainly consist of peritoneal endometriotic lesions and cases of tubal infertility, Bergendal *et al.* reported no differences in the total dose of FSH administered, peak E2 levels on the day of oocyte retrieval, and number of oocytes retrieved (11). Preliminary data indicated that FSH receptor levels were lower in poor responders and in patients with stage III/IV endometriosis, suggesting a similar mechanism for the poor response. But patients with stage I/II endometriosis had FSH receptor levels similar to those of

patients with normal responses. The dose of gonadotropin required was also similar. The investigators concluded that the different signaling pathways that are activated through the FSH receptors in normal ovaries are disrupted in poor responders and in patients with severe endometriosis (12). Further studies are required to assess the effect of ovarian response and embryo quality in women with stage I/II endometriosis.

There is conflicting data on the results of IVF-ET for patients with stage I/II endometriosis. Tanbo *et al*. found that women with stage I endometriosis had lower cleavage rates compared with those with tubal infertility; however, no differences in PRs were observed. They concluded that gamete defects may cause infertility (13). Simon *et al*. compared 14 cycles of women with stage I/II endometriosis and 96 cycles of women with tubal infertility, and there were no differences in PRs and implantation rates (14). Another study found that only the implantation rate was lower in women with stage I/II endometriosis when compared to women with unexplained infertility (15). Although a lower percentage of grade 1 embryos of all fertilized embryos was found in the En (+) group, PRs and LBs were not significantly different. Because total number of grade 1 and grade 2 embryos were similar with both groups, and 1–3 embryos were transferred in this study, relatively-good-quality embryos like grade 1 and grade 2 embryos were transferred at the same time. It may be one of the reasons that no significant difference was observed in implantation rate including grade 1 embryos, PRs and LBs. Therefore, stage I/II endometriosis may affect oocyte quality without significantly affecting IVF-ET outcomes. Defective embryos or altered endometrium may cause a failure in implantation. But the mechanisms of infertility associated with minimal to mild endometriosis are poorly understood. Garrido *et al*. reviewed literature focusing on the differences in the quality of endometrium and embryos in endometriosis-related infertility (16). They concluded that an altered follicular microenvironment or an intrinsic ovarian problem was associated with poor-quality embryos, and that mixed causes (defects of both embryos and endometrium) cannot be ruled out.

A large meta-analysis examined 22 studies published from 1983 to 1998 included 2,602 IVF-ET cycles of women with stage I/II endometriosis and tubal infertility (5). This report showed that women with mild endometriosis had significantly lower fertilization and implantation rates, lower numbers of oocytes retrieved, and lower peak E2 levels on the day of oocyte retrieval; however, PRs were not significantly different. Another study conducted between 1996 and 2003 demonstrated that women with stage I/II endometriosis and those with tubal infertility did not show significant differences in the mean number of oocytes retrieved, fertilization rates, or embryo quality. In addition, cumulative LBs resulting from 4 IVF-ET treatments, including frozen embryo transfers, showed no significant difference compared with cases of tubal infertility (55.8% vs. 43.7%) (17). Because IVF-ET success rates have rapidly increased in recent years, the period

when each study was conducted must be considered. In addition, the presence of hydrosalpinx should be taken into account when tubal factors considered. None of the women in our study had hydrosalpinx, which is known to have a detrimental effect on the outcomes of IVF-ET (18).

The limitations of this retrospective study were the small number of patients and IVF-ET cycles. Moreover, factors associated with ovarian reserve, such as antral follicle count, serum inhibin B levels, and serum anti-Müllerian hormone levels before IVF-ET, were not measured in this study. In addition, the effect of laparoscopic resection in our study group on IVF-ET outcomes was uncertain. Shahine *et al*. hypothesized that surgical treatment of endometriosis might result in improved embryo quality (19). This study included 17 patients with stage I/II endometriosis who underwent IVF-ET before and after surgical treatment for endometriosis (19). There were no significant differences in the number of follicles, number of oocytes retrieved, and embryo quality before and after laparoscopic removal of endometriosis.

Because endometriosis is a progressive disease, a gradual decline in the IVF-ET success rates with increasing time after the last endometriosis removal may be seen. However, our study showed no difference in PRs when patients were divided into 2 groups on the basis of the interval between the most recent surgical intervention and oocyte retrieval (12 months or less vs. more than 12 months).

In conclusion, although stage I/II endometriosis was found to have no obvious effects on IVF-ET outcomes, it may have some detrimental effects on embryo quality. Because endometriosis is a progressive disease, IVF-ET treatment should be suggested as early as possible after unsuccessful superovulation and IUI before it progresses to advanced stages. A larger study is necessary to draw a definite conclusion.

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Abbreviations: IVF-ET: *In vitro* fertilization and embryo transfer; r-ASRM : rev:ised American Society for Reproductive Medicine; PR: pregnancy rate; BR: birth rate; HMG human menopausal gonadotropin; hCG: human chorionic gonadotropin; ICSI: intracytoplasmic sperm injection; OHSS: ovarian hyperstimulation syndrome; FSH: follicle-stimulating hormone

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Send correspondence to: Keiko Mekaru, 207 Uehara Nishihara, Okinawa 903-0215, Japan, Tel: 81-098-895-1177, Fax: 81-098-895-1426, E-mail: f041252@eve.u-ryukyu.ac.jp