Effect of endometriosis on implantation rates when compared to tubal factor in fresh non donor *in vitro* fertilization cycles

Neeta Singh, Kusum Lata, Moumita Naha, Neena Malhotra, Abhinash Tiwari, Perumal Vanamail
Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, New Delhi, India

Address for correspondence:
Dr. Neeta Singh, Department of Obstetrics and Gynecology, Room No. 3090A, Third Floor, All India Institute of Medical Sciences, Ansari Nagar, New Delhi - 110 029, India.
E-mail: drneetasingh@yahoo.com

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ABSTRACT

OBJECTIVE: The objective of the following study is to compare the outcome of *in vitro* fertilization and embryo transfer (IVF-ET) in women with endometriosis and tubal-factor infertility. **DESIGN:** Retrospective study. **SETTING:** Tertiary referral hospital, assisted reproductive technologies unit. **MATERIALS AND METHOD:** The study group consisted of 78 women diagnosed with advanced stage endometriosis. The control group included 100 women with tubal-factor infertility. These groups were retrospectively analyzed regarding stimulation, fertilization, embryo development, implantation and pregnancy outcome. **INTERVENTION (S):** Controlled ovarian hyperstimulation and IVF-ET. **RESULTS:** Lower oocyte yield with lower fertilization rate were found in women with endometriosis compared with tubal-factor control subjects. However, no differences were found in cleavage, implantation and clinical pregnancy rates between the endometriosis and tubal-factor groups. **CONCLUSIONS:** Our results showed that women with endometriosis have a lower oocyte yield and lower fertilization rate compared with women with tubal-factor infertility. However, once the oocyte is fertilized, it seems that the embryo has a normal chance of implantation, leading to similar pregnancy rates and adequately treated women with endometriosis have equal chances of conception as seen with tubal-factor infertility.

KEY WORDS: Endometriosis, implantation, infertility, *in vitro* fertilization-embryo transfer, ovarian reserve, pregnancy

INTRODUCTION

Endometriosis is a chronic disease, characterized by the presence of the endometrium like tissue outside the uterus, most commonly on the ovary and peritoneum and the main clinical features are chronic pelvic pain, dyspareunia and infertility. Endometriosis affects 10-15% of all women of reproductive age. On the contrary, 30-40% of women with infertility have been reported to have endometriosis[1] and the infertile women are 6-8 times more likely to have endometriosis than fertile women.[2]

Endometriosis affects natural fertility through various mechanisms. Various factors contributing to reduced fertility are impaired utero-tubal transport of sperm[3] due to distorted anatomy, disturbed ovulation,[4] subtle impairment of oocyte and embryo quality,[5-7] implantation defects[8-10] and antiendometrial antibodies.[11]

A meta-analysis on the effects of endometriosis on *in vitro* fertilization (IVF) outcomes concluded that endometriosis interferes with all aspects of the reproductive process and success rate among women with endometriosis was almost half when compared to women undergoing IVF for other indications.[12]

Thus, the possible impact of endometriosis on assisted reproductive technologies results remain a controversial issue. The aim of the present study was to compare IVF-embryo transfer (ET) outcomes in women with advanced stage (Grades 3 and 4)

endometriosis to women with tubal infertility who underwent IVF during the same time period.

MAERIALS AND METHOD

A retrospective, database-searched study was conducted. Data were extracted from the database of the IVF center of a tertiary referral hospital from July 2009 to March 2013. The data collected included age, factor for infertility, type of infertility, dosage of gonadotropin, baseline follicle-stimulating hormone (b-FSH) levels, luteinizing hormone (LH) levels, anti-müllerian hormone (AMH) ovarian stimulation protocol, days of stimulation, oocytes retrieved number, fertilized oocyte number, embryo number, high-quality embryo number, numbers of the embryo and high-quality embryo for transplantation and the clinical pregnancy outcome.

Inclusion criteria

A total of 178 women who had undergone IVF-ET treatment from July 2009 to March 2013 were retrospectively analyzed. The study group comprised of 78 women with stages III-IV endometriosis having no other known infertility factor besides endometriosis while the control group consisted of 100 women with tubal-factor infertility. Women in endometriosis group were diagnosed by laparoscopy or laparotomy and all were treated surgically. None of the patients was having any endometrioma before starting the cycle. All patients in both groups underwent a routine infertility work-up. The patients in endometriosis group were scored according to the revised classification of the American Fertility Society (1997) after laparoscopy or laparotomy.

Exclusion criteria

The exclusion criteria in this study were (1) patients older than 42 years at the onset of the controlled ovarian hyperstimulation (COH) cycle, (2) poor ovarian reserve with a day 3 b-FSH concentration of more than 12 IU/L and serum AMH of <0.7 ng/ml (3) inadequate data for analysis.

COH

During ovarian stimulation two types of the protocol were used namely long gonadotropin-releasing hormone (GnRH) agonist and GnRH antagonist protocol depending upon the patient age and ovarian reserve.

The patients in agonist group were given 1 mg injection of leuprolide acetate (injection Lupride, Sun Pharmaceutical Ind. Ltd., Mumbai) starting from day 21 of menstruation for 14 days. Down-regulation was confirmed by biochemical markers (LH <5 IU/ml, E2 <50 pg/ml and progesterone <1 ng/ml) and transvaginal ultrasound (TVS) assessment of endometrial thickness (ET) and ovarian status (ET <3 mm, no ovarian cyst >2 cm). After down-regulation, dose of leuprolide was reduced to 0.5 mg/day and patients were started on recombinant FSH (injection Gonal-f, Merck Serono Specialities Pvt. Ltd., Italy). The starting dose was between 150 IU/day and 225 IU/day depending upon patient's characteristics and was adjusted according to follicular growth as monitored by ultrasound. In antagonist group, patients were scanned for any ovarian cyst on 1st day of the menstrual cycle and were started on injection gonal-f (150 IU to 300 IU) from day 2.

In GnRH antagonist protocol cetrorelix acetate (Injection Cetrotide, AEterna Zentaris, Canada) 0.25 mg was added on 6th day of the menstrual cycle (fixed dose regime). Follicular monitoring was done in both groups using TVS and dose of gonadotropin was adjusted accordingly. The cycles were cancelled in patients with no follicle more than 10 mm after 10 days of gonadotropin stimulation. Ovulation was triggered when three or more follicles reached 16 mm using 250 mg of recombinant human chorionic gonadotropin (HCG) (Injection Ovitrelle, Marck Serono, UK). Serum estrogen (E2) and ET were measured on the day of HCG trigger. Oocyte retrieval was done under general anesthesia after 32-35 h. ET was done between day 2 and day 5 depending upon the number of good quality embryo. All patients were given luteal phase support by intramuscular injection of progesterone 100 mg/day. On 16th day of ET, pregnancy was assessed by serum beta HCG assay and confirmed by the presence of the gestational sac with fetal pole and fetal cardiac activity on transvaginal ultrasound after another 4 weeks. Biochemical pregnancies were not included in our analysis.

Oocytes were inseminated either by IVF with about 50,000 motile spermatozoa or by intracytoplasmic sperm injection (ICSI). Embryo quality assessment was based on morphology and rate of development in culture on day 3. Four grades of embryos were defined: Grade 1, embryos had blastomeres of equal size and no cytoplasmic fragmentation; Grade 2, embryos had blastomeres of equal or unequal size and cytoplasmic fragmentation of less than 20% of the embryo surface; Grade 3, embryos had blastomeres of equal or unequal size and 20-50% overall cytoplasmic fragmentation; and Grade 4, embryos had blastomeres of equal or unequal size and cytoplasmic fragmentation of more than 50% of the embryo surface.

Statistical analysis

Data were computerized and analyzed using the statistical product and service solutions (SPSS) version 16.0. Descriptive statistics were computed for base-line characteristics of patients, ovarian stimulation factors, hormonal profile and endometrial thickness on the day of HCG trigger and embryological variables for each study group. After determining whether the data met

the normality assumption, student's *t*-test independent two-tailed test was conducted to test whether the means of continuous variables were significantly different between the two study groups. Nominal or frequency data were analyzed using Chi-square test or Fishers's exact test as appropriate. For the entire statistical tests a $P < 0.05$ was considered to be statistically significant.

RESULTS

The baseline characteristics of patients with ovarian endometriosis and tubal infertility are shown in Table 1. There was no significant difference in mean age, body mass index and percentage of patient with primary infertility, day 2 FSH, LH, AMH, antral follicle count, percentage of patients with pre-menstrual proliferative or hyperplastic endometrium between two groups. However, the combined ovarian volume was significantly higher in endometriosis group.

Most of the patients in both the groups underwent agonist protocol. A total of 18 women in tubal group while 10 women in endometriosis group were stimulated by antagonist protocol and there was no statistical difference between type of protocol used and pregnancy outcome.

IVF was done in the majority of the females while 17 women in tubal group and 10 in endometriosis group underwent ICSI due to male factor infertility.

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 >10 mm or endometrial thickness. As depicted in Table 2 in endometroisis group 2 cycles were cancelled because no oocyte were retrieved while fertilization failure was seen in 10 patients while in tubal group 5 cycles were cancelled due to failure of oocyte development and four cases had failed fertilization. Thus, cancellation rates were 15.4% and 11% in endometriosis and tubal groups respectively.

The number of oocyte retrieved and fertilization rate were significantly lower in endometriosis group when compared to tubal group, but we did not find any significant difference in percentage of metaphase II (M2) oocyte, cleavage rate, percentage of Grade 1 embryo formed between two groups [Table 3]. Mean number of embryo transfered was also not different in two groups. The clinical pregnancy rate between the two groups was comparable.

Table 1: Baseline characteristics in women with endometriosis compared with women with tubal infertility. Where appropriate, data are expressed as mean±SD

Parameter	Endometroisis (*N*=78)	Tubal (*N*=100)	*P* value
Age	32.7±3.5	31.9±3.7	0.166
BMI	24.3±3.5	25.1±4.2	0.178
D2 FSH	6.9±2.1	6.3±1.9	0.652
D2 LH	5.3±2.4	5.02±2.4	0.442
AMH	2.9±1.4	2.7±1.5	0.382
AFC	11.1±5.1	10.9±3.8	0.722
Combined ovarian volume	11.8±5.8	10.3±4.3	0.043

SD=Standard deviation; BMI=Body mass index; FSH=Follicle-stimulating hormone; LH=Luteinizing hormone; AMH=Anti-müllerian hormone; AFC=Antral follicle count

Table 2: Ovarian stimulation characteristics in women with endometriosis compared with women with tubal infertility. Where appropriate, data are expressed as mean±SD

Parameter	Endometriosis (*N*=78)	Tubal (*N*=100)	*P* value
Total dose of gonadotrophin	3453±982	3480±1213	0.874
Days of stimulation	10.1±1.6	9.9±1.9	0.466
No of follicles on day of HCG	8.09±4.3	10.04±5.4	0.117
E2 on day of HCG	2600.2±2061	2304.8±1524	0.408
ET on day of HCG	8.44±1.65	8.72±2.015	0.315
Cycle cancellation due to arrest of follicular growth	12 (12/78)	11 (11/100)	0.084

SD=Standard deviation; HCG=Human chorionic gonadotropin

Table 3: IVF laboratory parameters in women with endometriosis compared with women with tubal infertility. Where appropriate, data are expressed as mean±SD

	Endometroisis group (*N*=78)	Tubal group (*N*=100)	*P* value
Oocyte retrieved	6.2±3.6	7.9±5.5	0.016
M2 oocyte[a]	69.5	69.3	0.944
Fertilization rate[a]	64.8	70.2	0.044
Cleavage rate[a]	94.9	96.4	0.298
Grade 1 embryo[a]	49.6	50.4	0.767
No of embryo transfered	2.4±1.1	2.68±1.2	0.276
Clinical pregnancy	19 (19/78)[b]	34 (34/100)[c]	0.222

[a]percentage. [b]Embryo transfer not done in 12 cases. [c]Embryo transfer not done in 11 cases

DISCUSSION

IVF-ET outcomes in women with endometriosis have been examined by various retrospective studies. A reduced response to gonadotropins, lower oocyte yield and poor clinical pregnancy rates per cycle have all been described.[13]

We observed similar IVF outcomes with GnRH-a and GnRH antagonist protocols in mild-to-moderate endometriosis suggesting that GnRH antagonist is an alternative to GnRH-a. A recent study has stated that outcomes of COH with both GnRH-ant and GnRH-a were similar in patients with stages III-IV endometriosis. The number of retrieved oocytes, the number of obtained embryos, the implantation rates and the clinical pregnancy rates were similar with GnRH-ant and GnRH-a protocols.[14]

Among two recent meta-analyses, Ludwig *et al*. also do not report any significant difference in the pregnancy rates between GnRH antagonist and GnRH-a protocols.[15]

In the present well-controlled study, women with endometriosis undergoing IVF-ET had a significantly lower oocyte yield and lower fertilization rate in comparison with tubal-factor infertility. Many authors have reported that endometriosis can reduce the ovarian reserve to decrease the number of oocytes retrieved.

A meta-analysis by Barnhart *et al*. proposed that the chance of achieving pregnancy was lower for endometriosis patients compared to those with tubal-factor infertility (odds ratio: 0.56; 95% confidence interval, 0.44-0.70). The inferior IVF/ICSI outcomes of endometriosis women may result from decreasing number of retrieved oocytes.[12]

On the other hand, several studies presented that the endometriosis patients who underwent IVF/ICSI achieved comparable outcomes to infertile patients with tubal-factors.[16-19]

Ho *et al*.[20] in their study have reported significantly fewer follicles and significantly fewer oocytes in patients who had undergone unilateral cystectomy for an endometrioma. Kumbak *et al*.[21] compared ovarian endometriosis and simple ovarian cysts to evaluate the space-occupying effect on the ovary and found a decline of the ovarian reverse and the poor response in patients with ovarian endometriosis.

Increased apoptosis, alterations in the cell cycle and higher incidence of oxidative stress have l been observed in granulosa cells derived from women with all stages of endometriosis, including endometriomas, when compared with the granulosa cells of women with other causes of infertility.[22] Therefore it was suggested that oocyte quality is severely affected in endometriosis.

Endometriosis has a detrimental effect on fertilization also. 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.[23-25] In a meta-analysis, decreased fertilization rates after IVF in endometriosis-associated infertility compared with other infertility categories have been documented. In the present study, there were ten cases of failed fertilization in endometroisis group which were statistically significant.

Tanbo *et al*. have reported[26] a significantly lower cleavage rate in patients with endometriosis. However, we did not observe any statistically significant difference in the cleavage rate between the two groups.

A further possible cause of endometriosis-associated infertility is impaired implantation of the embryo. It has been postulated that embryos derived from women with endometriosis appear to develop more slowly compared to those embryos derived from women with tubal disease.[27] Women with moderate to severe endometriosis who receive oocytes from disease-free women in oocyte donation cycle appear to have normal endometrial receptivity and pregnancy rates conversely when donor oocytes from women with endometriosis are transferred into women without endometriosis, implantation rates are lower and quality of the embryo is reduced.[28]

Based on the data of our study, we observed that endometriosis adversely affects average fertilization rate and oocyte yield. From a clinical point of view, however, this decrease in the number of fertilized eggs did not impair the chances of pregnancy in endometriosis group.

CONCLUSIONS

Despite less well ovarian response, reduced embryo quality and impaired implantation in moderate/severe cases, endometriosis patients obtain comparable IVF/ICSI success to patients with tubal-factors infertility. Combination effect of aggressive COH, appropriate pituitary suppression and efficient surgery before IVF seemed to be crucial in IVF/ICSI success of patients with endometriosis. This study has its limitation in being retrospective, but the methods are well controlled and studied during the same period. This is the only study of IVF outcomes in endometriosis patients from a developing country depicting that adequately treated women with endometriosis have equal chances of conception as seen with tubal-factor infertility.

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