GYNECOLOGY

Exposure of Human Oocytes to Endometrioma Fluid Does Not Alter Fertilization or Early Embryo Development*

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Background: Extensive endometriosis causes a mechanical disturbance in the pelvis leading to obstructive-type infertility. However, minimal or mild endometriosis is suspected to cause infertility, possibly through a humoral agent. Previous studies reported the presence of a factor in the serum of patients with endometriosis which reduced fertilization and early embryo formation in a rat IVF model.

Methods: In the present article, we report a comparison of oocytes exposed to endometrioma fluid and oocytes not exposed (controls) in the context of a human IVF setting. We have been in the practice of aspirating oocytes into prewarmed 60-ml syringes containing culture medium. We have shown previously that this technique reduces the length of oocyte retrieval without compromising success. In 14 women undergoing oocyte retrieval, we inadvertently entered an endometrioma. This resulted in retrieved oocytes that were either exposed or not exposed to endometrioma fluid.

Results: In contrast to previous reports, we found no difference in fertilization or early embryo development between the two groups. The fertilization rate for oocytes exposed to

an endometrioma was 60%, versus 56% for controls. The good-quality embryo formation rate for oocytes exposed to an endometrioma was 45%, versus 46% for controls.

KEY WORDS: Embryo; endometrioma; fertilization; in vitro fertilization; oocyte; endometriosis.

INTRODUCTION

A study by Miller *et al.* (1995) has been frequently cited as an experimental backing for the hypothesis of a factor present in patients with endometriosis which renders them infertile. They studied the effects of the addition of serum from infertile women with endometriosis (to the culture medium) on fertilization and embryonic development in a murine in vitro fertilization (IVF) model. They obtained serum samples from women before and after their endometriosis was treated and used control serum samples from women without endometriosis. They reported that, compared with serum from fertile women, serum from infertile women with endometriosis reduced the fertilization rate (51 versus 81%) and subsequent embryonic development rate (46 versus 79%). The inhibitory effect was greater as the stage of endometriosis increased. Treatment of endometriosis by laser laparoscopy improved both fertilization (56 versus 51%) and early embryonic development (58 versus 46%).

In this article, we report a similar setting in human IVF where, in some patients, all of their retrieved oocytes and, in some patients, a proportion of their retrieved oocytes were inadvertently exposed to their own endometrioma fluid.

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MATERIALS AND METHODS

Patients

Fourteen women who were enrolled in our IVF program had their endometrioma inadvertently aspirated into syringes during oocyte retrieval. Their ages ranged from 31 to 41 years, with an average of 36.4 ± 3.52 years (mean $\pm$ SD) and a median of 36.5 years. The cause of infertility was diagnosed to be tubal ($n = 11$), tubal and male factor ($n = 1$), uterine fibroids ($n = 1$), and absence of one ovary (infertility of undetermined cause; $n = 1$). The length of infertility ranged from 3 to 14 years, with an average of 6.3 ± 3.70 years. Twelve patients had primary and two patients had secondary causes of infertility. The husband's semen characteristics for volume of ejaculate, motility, sperm concentration, and number of total motile sperm in ejaculate were 3.8 ± 1.33 ml, $49.2 \pm 19.35\%$, $66.7 \pm 43.03 \times 10^6/\text{ml}$, and $150 \pm 152.6 \times 10^6$, respectively.

Ovarian Stimulation and Oocyte Retrieval

A standard protocol was utilized, with long-term Buserelin treatment from day 20 of the menstrual cycle (Khamisi *et al.*, 1998).

At our institute, we have had an ongoing commitment to simplification of IVF, with the mandate of improving patient safety and reducing costs (Khamisi *et al.*, 1998). Oocytes are retrieved by insertion of an oocyte collection needle (Casmed UK, Surrey, England) attached to a 60-ml syringe filled with 8 ml of culture medium (prewarmed at 37°C). First, the patient is given 50 to 100 μg fentanyl and 5 to 10 mg Diazemuls (according to her weight). After she is calmed in this manner and assured repeatedly, a number of oocytes are aspirated one after another into the same syringe. Serious attention is paid not to enter any cystic structure except follicles. However, in the last 2 years, we have unknowingly entered an endometrioma during aspiration. When this happened, we pulled out the needle and sent the syringe to the laboratory for oocyte identification, and we either used a new needle or carefully washed the previous needle before further use.

Oocyte Culture, IVF, ICSI, and Embryo Transfer

The retrieved oocyte-cumulus complexes were washed two or three times in culture medium (IVF Science Scandinavia, Gothenburg, Sweden), trans-

ferred individually into 100- μl microdrops of culture medium overlaid by oil, and then put in an incubator (37°C , 5% CO_2). In all 14 patients, the endometrioma was aspirated inadvertently into a syringe along with the retrieved oocytes. In nine patients, some retrieved oocytes were exposed to endometrioma fluid and the remaining retrieved oocytes were not, whereas in the other five patients, all the retrieved oocytes were exposed to endometrioma fluid. The endometrioma fluid had a turbid dark-brown appearance and contained many fine black particles when examined microscopically. The length of exposure of the oocytes to the endometrioma fluid was 2 to 30 min. The cumulus of such oocytes developed a yellowish-greenish discoloration with many endometriotric particles. Washing of the oocyte-cumulus complexes in culture medium several times did not remove the stain or particles from the cumulus. Standard IVF insemination and ICSI protocols were followed as described previously (Khamisi *et al.*, 1998, 1999). The fertilization rate was the ratio of the number of fertilized oocytes to the total number of oocytes allotted. Good-quality embryo formation was the ratio of the number of good-quality embryos to the total number of oocytes allotted.

Statistical Analysis

Proportions of oocytes that fertilized and proportions of oocytes that resulted in good-quality embryo formation in treatment groups were analyzed by chi-square analysis using SAS (1988). Significance was assumed at $P < 0.05$.

RESULTS

The details of patients' profiles and data are shown in Table I. Fertilization rates and formation of good-quality embryos are shown in Table II. Since the fertilization rate and good-quality embryo formation did not differ between IVF and ICSI ($P = 0.35$ and $P = 0.58$, respectively), data were pooled to compare the effect of exposure of oocytes to endometrioma fluid. Oocytes not exposed to endometrioma fluid (controls) and those exposed to endometrioma fluid resulted in fertilization rates of 56.00% (28/50) and 60.00% (36/60), respectively ($P = 0.67$). Oocytes not exposed to endometrioma fluid and those exposed to endometrioma fluid resulted in good-quality embryo formation rates of 46.00% (23/50) and 45.00% (27/60), respectively ($P = 0.92$).

Table I. Details of Patients' Profiles and Husbands' Semen Characteristics: Endometrioma Study, 1999

No.	Name	Age	Retrieval date	Cause	Type of infertility	Years of infertility	Fertilization rate (%)		Good embryos (%)		Sperm ^a		
							Endometrioma	Control	Endometrioma	Control	Volume (ml)	Motility (%)	Count (M/ml)
1	KA	36	12-3-98	Uterine fibroids	Primary	3.5	0/2	1/10	0/2	1/10	3	50	50
2	SD	32	5-8-98	Tubal	Primary	9	7/8	8/8	3/8	5/8	2.5	55	71
3	YT	36	28-10-98	Tubal	Secondary	9	1/2	—	1/2	—	4.5	75	88
4	BS	39	11-11-98	Tubal	Primary	3	0/1	0/3	0/1	0/3	—	—	—
5	EI	32	21-1-99	Tubal	Primary	4	0/2	—	0/2	—	5	55	58
6	SR	41	24-1-99	Tubal	Primary	7	3/7	—	3/7	—	4.5	80	155
7	BS	39	5-4-99	Tubal	Primary	3	3/5	—	3/5	—	3.5	40	38.5
8	NB	39	5-4-99	Tubal	Primary	7	2/2	—	2/2	—	6	50	40
9	EI	32	20-4-99	Tubal	Primary	4	3/4	2/5	2/4	2/5	5.5	60	73
10	BIA	40	29-5-99	Tubal	Secondary	5	8/14	1/1	4/14	1/1	2.5	65	140
11	BS	39	16-6-99	Tubal	Primary	3	1/2	1/1	1/2	1/1	4	30	31
12	MC	34	15-7-99	♂, Tubal	Primary	13	1/3	10/13	1/3	8/13	4.5	10	9
13	PM	31	8-9-99	1 ovx	Primary	4	6/7	5/7	6/7	5/7	2	40	87
14	KO	39	6-10-99	Tubal	Primary	14	1/1	0/2	1/1	0/2	2	30	26

^a M, millions.

Table II. Fertilization Rate and Good-Quality Embryo Formation in Oocytes Previously Exposed to Endometrioma Fluid

Exposure	Control	Endometrioma	P
Fertilization rate (%)	56.00 (28/50) ^a	60.00 (36/60)	0.67
Good embryo formation (%)	46.00 (23/50)	45.00 (27/60)	0.92

^a Proportions in parentheses are based on the numbers of oocytes in treatment groups.

DISCUSSION

The results show that previous exposure of retrieved oocytes to endometrioma fluid has no detrimental effect on fertilization rate or good-quality embryo development. This is the mechanism of infertility related to severe endometriosis, mechanically interfering with oocyte pickup and transportation. Severe endometriosis results in a mechanical problem with the natural process of oocyte and embryo flow through the fallopian tube. It is much more difficult to arrive at a logical conclusion as to how minimal or mild endometriosis may be the cause of, rather than an innocent association with, infertility. Some search has been made for this possible factor, which is produced by the endometriotic tissue and influences infertility. The subject has been reviewed from time to time (Webster *et al.*, 1987; Schmidt, 1988) with no conclusive answer, implementing prostanoids (particularly PGF_{2α}), macrophages, folliculogenesis, ovulation, and the luteal phase. The studies testing human serum from patients with endometriosis in a murine IVF experiment, referred to in the Introduction, was one of the strongest pieces of evidence in favor of the existence of an endometriosis factor. Therefore, we were very surprised to find that, in the

setting of human IVF, exposure to actual endometrioma fluid had no detrimental effect on the fertilization or embryo development of retrieved oocytes. This study encourages clinicians to have an open mind with respect to the relationship of minimal–mild endometriosis and infertility.

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