The impact of endometriosis in couples undergoing intracytoplasmic sperm injection because of male infertility

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To assess the impact of endometriosis on intracytoplasmic sperm injection (ICSI) outcome, we have retrospectively evaluated 980 ICSI cycles, comparing the results of women with and without endometriosis. A total of 101 cycles was identified in which various degrees of endometriosis were involved, and in the remaining 879 cycles, male infertility was the only cause of infertility. Ejaculated spermatozoa were microinjected in all cycles. There was a significant reduction ($P = 0.004$) in the number of oocytes retrieved from women with endometriosis as compared to those without endometriosis. However, there were no significant differences in either fertilization or pregnancy and implantation rates between women with or without endometriosis. We conclude that the presence of endometriosis in patients undergoing ICSI because of severe male infertility does not affect fertilization, pregnancy and implantation rates, although significantly fewer oocytes are retrieved from patients with endometriosis.

Key words: endometriosis/intracytoplasmic sperm injection (ICSI)/ICSI outcome/male infertility

Introduction

Intracytoplasmic sperm injection (ICSI) has become the most widely applied method of assisted fertilization for the treatment of male infertility (Palermo et al., 1992). The results of ICSI are independent of the severity of the male factor, and even patients with secretory azoospermia with elevated follicle-stimulating hormone (FSH) concentrations (Gil-Salom et al., 1995b) or absence of mature spermatozoa (Tesarik et al., 1995, 1996) are also candidates for fatherhood. A trend towards reduced fertilization rates is observed, however, in ICSI patients when testicular or epididymal spermatozoa are injected as compared to ejaculated spermatozoa (Gil-Salom et al., 1995a; Silber et al., 1995; Devroey et al., 1996). Moreover, female factors such as age and oocyte quality seem to be crucial parameters affecting the results of ICSI in terms of pregnancy and implantation rates (Oehninger et al., 1995; Sherins et al., 1995; Abdelmassih et al., 1996).

Endometriosis is a frequent and enigmatic disease that often is associated with marked subfertility (Hull, 1992). In spite of the fact that the relationship between endometriosis and infertility is still controversial (Gleicher, 1992), several authors have suggested various hypotheses to explain how endometriosis can cause infertility (Pellicer et al., 1995a). Our studies of in-vitro fertilization (IVF) and oocyte donation programmes (Simón et al., 1994) showed that the oocyte quality in endometriosis patients may be impaired, resulting in embryos of reduced ability to implant. This has been confirmed by morphological analysis of human embryos using video recording during the first developmental stages (Brizek et al., 1995) and the results of IVF in other programmes (Arici et al., 1996). Furthermore, an altered follicular environment has been described in women with endometriosis (Cahill et al., 1995) which is the consequence of an altered function of the granulosa cells (Harlow et al., 1996), and may perfectly explain reduced quality of the oocytes retrieved in endometriosis patients.

Based on the above mentioned information, we were concerned about the possibility that the presence of endometriosis in the female and the stage of the disease could impair the overall results of the ICSI process in a couple. To assess the impact of endometriosis on ICSI outcome, we have retrospectively evaluated our ICSI files comparing the results of women with and without endometriosis.

Materials and methods

Patients

In the present study a total of 980 cycles, corresponding to 800 patients included in our ICSI programme during the period January 1, 1995 and October 31, 1996, was analysed. In all cases, the indication for ICSI was male infertility, and ejaculated spermatozoa were microinjected in all the cycles. A total of 101 cycles was identified as having various degrees of endometriosis and in the remaining 879 cycles there was no sign of the disease. All patients included in the study had a laparoscopy/laparotomy during the infertility work-up, no longer than 2 years before IVF. The diagnosis of ovarian endometriomas by ultrasound was employed to classify patients for all degrees (I–IV) of endometriosis (American Fertility Society, 1985), but we always required the persistence of the ultrasonic image for at least 90 days plus the aspiration of dense chocolate-like material during the process of ovum retrieval to use ultrasound as a diagnostic tool. As a result, 41 (40.5%) women were classified as having degree I–II of endometriosis and 60 (59.5%) degree III–IV.

Ovarian stimulation

The protocol for ovarian stimulation was started by pituitary desensitization with daily administration of 1 mg leuprolide acetate s.c.
(Procrin®; Abbot SA, Madrid, Spain) and began in the luteal phase of the menstrual cycle. Serum oestradiol <60 pg/ml and negative vaginal ultrasonographic scans were used to define ovarian quiescence. At days 1 and 2 of ovarian stimulation, 2 ampoules/day of human menopausal gonadotrophin (HMG, Pergonal®; Serono, Madrid, Spain) were administered together with 2 ampoules of FSH (Fertinorm®; Serono). At days 3, 4 and 5 of ovarian stimulation, 1 ampoule/day of FSH and HMG was administered to each patient. Beginning on day 6, FSH and HMG were administered on an individual basis according to serum oestradiol and transvaginal ultrasound scans. The criteria for human chorionic gonadotrophin (HCG) administration (10 000 IU, Profasi®; Serono) were the presence of two or more follicles >19 mm in greatest diameter and serum oestradiol >800 pg/ml. Leuprolide acetate and gonadotrophins were discontinued from the day of HCG administration. Oocyte retrieval was scheduled 36–38 h after HCG administration. The luteal phase was supported with 400 mg/day of intravaginal micronized progesterone (Progeffik®; Laboratorio Effik, Madrid, Spain).

**Oocyte preparation and ICSI procedure**

The cumulus–corona cells were initially removed by exposure to Flushing’s medium (Medicult, Copenhagen, Denmark) and 80 IU hyaluronidase (Sigma Chemical Company, St Louis, MO, USA) for up to 1 min. After removing the corona cells, only metaphase II oocytes were injected.

The standard ICSI procedure has been previously described (Gil-Salom et al., 1995a). Ejaculated spermatozoa were microinjected in all the cycles. For injection, a motile and morphologically normal spermatozoon was immobilized and aspirated tail first into the tip of the microinjection pipette. The metaphase II oocyte was held by the holding pipette with the polar body at the 12 or 6 o’clock position. The injection pipette was pushed through the zona pellucida and into the ooplasm at the 3 o’clock position. A single spermatozoon was injected. The injection pipette was withdrawn and the oocyte was released from the holding pipette. After microinjection the oocytes were incubated in 20 µl microdrops of IVF medium under lightweight mineral oil.

Fertilization was assessed 18 h after injection by examining for pronuclei. Embryo cleavage was assessed 24 h later and embryo quality was analysed under the dissecting microscope before transfer according to the method of Conaghan et al. (1993). Briefly, embryos were graded as follows: grade 1, containing intact and symmetrical blastomeres with no extracellular fragmentation; grade 2, extracellular fragments; grade 3, at least one degenerated cell; grade 4, only one blastomere intact; and grade 5, completely fragmented embryo with fragments; grade 3, at least one degenerated cell; grade 4, only one blastomeres with no extracellular fragmentation; grade 2, extracellular fragmentation (10 000 IU, Profasi®; Serono) were the presence of two or more follicles >19 mm in greatest diameter and serum oestradiol >800 pg/ml. Leuprolide acetate and gonadotrophins were discontinued from the day of HCG administration. Oocyte retrieval was scheduled 36–38 h after HCG administration. The luteal phase was supported with 400 mg/day of intravaginal micronized progesterone (Progeffik®; Laboratorio Effik, Madrid, Spain).

**Statistical methods**

The Student’s t-test was used to compare the average age of female partner and the mean number of oocytes retrieved. Fertilization and cleavage rates, mean number of blastomeres and mean degree of fragmentation were expressed as the mean of the values of the variables within each cycle ± SD and were compared using the Mann-Whitney U test. The mean number of embryos per transfer was also compared using the Mann-Whitney U test. Pregnancy, implantation and miscarriage rates were compared using $\chi^2$ test. A $P$ value <0.05 was considered statistically significant.

**Results**

As shown in Table I, patient’s age was similar in women with and without endometriosis (32.9 ± 3.0 and 32.3 ± 3.8 years, respectively). Similarly, the percentage of microinjected metaphase II oocytes and degenerated oocytes after microinjection was not significantly different. However, there was a significant reduction ($P = 0.004$) in the mean number of oocytes obtained in endometriosis patients as compared to controls.

The results of the ICSI procedure are shown in Table II. Fertilization rates were not different between patients with (77.9 ± 22.6) and without endometriosis (76.9 ± 19.7) (Table I). Furthermore, Table II shows that the mean cleavage rate per cycle and the number of transferred embryos were not significantly different between groups, and neither were the percentages of good quality embryos, the mean number of blastomeres per embryo and the degree of embryo fragmentation.

A total of 26 pregnancies was achieved in endometriosis patients and 251 in the controls. This represented a pregnancy rate per started cycle and per transfer of 25.7% and 28.3% respectively in the women with endometriosis. All cycles started in patients without endometriosis reached embryo transfer, resulting in a pregnancy rate of 28.6%. Thus pregnancy rates were similar in women with and without endometriosis. Similarly, no significant differences were found in implantation and miscarriage rates between the groups established.


**Discussion**

Since the introduction of ICSI (Palermo et al., 1992) it has been accepted that the quality of injected spermatozoa does not seem to influence the outcome of ICSI (Palermo et al., 1993). In keeping with this concept, several groups have analysed the results of ICSI based on female factors, such as age and ovarian response (Sherins et al., 1995; Oehninger et al., 1995; Abdelmassih et al., 1996). There is a consensus that both factors affect pregnancy and implantation rates, and this may be related to the fact that both age and ovarian response have been linked to oocyte quality (Pellicer et al., 1995b). Thus, the quality of the oocytes microinjected might have relevance for the cases in which ICSI is indicated because of male infertility.

The results of IVF in endometriosis patients in the literature are contradictory. Some recent reports have shown decreased pregnancy rates in women affected with endometriosis because of a reduced fertilization (Mills et al., 1992) and impaired implantation (Simón et al., 1994; Arici et al., 1996). Others, however, have found similar pregnancy rates (Geber et al., 1995; Olivennes et al., 1995), even considering the different stages of the disease (Olivennes et al., 1995). Our previous experience using the ovum donation model (Simón et al., 1994), the results of IVF culturing of embryos up to 72 h (Pellicer et al., 1995a), and the observations of other groups in terms of morphological appearance of the embryos (Brizke et al., 1995) and implantation rates (Arici et al., 1996), support the notion that oocyte quality may be defective in endometriosis patients. This hypothesis is reinforced by endocrinological studies on the follicular compartment (Cahill et al., 1995; Harlow et al., 1996). Therefore, we anticipated in the present study that the results of ICSI may be affected by the presence of endometriosis in the female partner because of lower oocyte quality. However, we did not observe any significant difference in fertilization, pregnancy or implantation rates after ICSI in endometriosis patients compared with women without endometriosis.

This observation raises the question of whether ICSI can overcome an apparent defect which may be present in oocytes derived from endometriosis patients and which may result in embryos of lower quality. In all animal species, the process of fertilization initiates a cascade of events in the oocyte, termed oocyte activation, which launches the egg on a path leading to DNA synthesis and further embryonic development (Epel, 1990). ICSI has been shown to participate directly in the activation of the oocyte (Tesarak et al., 1994; Tesarik and Sousa, 1995), and although the presence of a spermatozoon is sufficient to activate the egg (Dorzhotev et al., 1995), the mechanical aspiration which occurs during the ICSI procedure seems also to play a role (Tesarik and Sousa, 1995). Thus, it is tempting to speculate that some of the factor(s) involved in the process of oocyte activation may be altered in endometriosis patients, resulting in embryos of abnormal morphology (Brizke et al., 1995), reduced cleavage potential (Pellicer et al., 1995a), and lower ability to implant (Simón et al., 1994; Arici et al., 1996). When ICSI is employed, these defects are overcome, resulting in normal development and implantation. These findings need to be confirmed in prospective studies.

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**References**


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