Conventional IVF versus ICSI in sibling oocytes from couples with endometriosis and normozoospermic semen

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Abstract

Purpose This study compares the fertilization rate and embryonic development of oocytes randomly inseminated by conventional IVF or ICSI in patients with endometriosis and normozoospermic semen during IVF cycles.

Methods Sibling oocytes were randomized to be inseminated either by ICSI or IVF. Rates of fertilization, cleavage, blastulation and embryonic morphology were assessed.

Results A total of 786 sibling cumulus-oocyte complexes (COC) were randomized between insemination by conventional IVF (387 COC) or ICSI (399 COC). A significantly higher fertilization rate was found in the ICSI group (ICSI versus IVF, $73.3 \pm 23\%$ versus $54.7 \pm 31.9\%$ respectively; $P=0.003$), yielding a higher mean number of day 2 embryos (5.2 ± 3.4 versus 3.6 ± 2.9 respectively; $P=0.002$). Triploid fertilization rate (3PN/COC) was significantly higher in the IVF group compared to the ICSI group ($3.9 \pm 8.7\%$ versus $0.9 \pm 3.1\%$ respectively; $P=0.02$). The morphology score and rate of development of day 2 and 3 embryos were not different between the two groups. Comparison of embryo transfer cycles in which either IVF or ICSI only embryos were transferred did not reveal any statistically significant differences in pregnancy or implantation rates.

Conclusion ICSI appears to be a better treatment option than conventional IVF in endometriosis-associated infertility, since it offers the advantages of higher fertilization rate and mean number of embryos and lower rate of total fertilization failure and triploid fertilization.

Keywords Endometriosis · Fertilization procedure · ICSI · IVF · Sibling oocytes

Introduction

Endometriosis is characterized by the presence of endometrial tissue outside the uterus, most commonly on the peritoneum and ovary. It is associated with dysmenorrhea, chronic pelvic pain, and infertility. It affects approximately 10 % of the women in their reproductive age and up to 50 % of the women with infertility [1–4]. The pathophysiology of the infertility in endometriosis is still unknown, although different mechanisms have been suggested [5]. Severe endometriosis is associated with pelvic adhesions and a distortion of pelvic anatomy leading to a possible mechanical or anatomical disturbance [6]. Since minimal foci of endometriosis may be associated with marked subfertility [7], even mild stages of the disease may have a direct negative effect on oocyte quality, embryogenesis and implantation [6]. Indeed, patients with endometriosis-associated infertility undergoing IVF respond with significantly decreased levels of markers of reproductive success, resulting in almost one half of the pregnancy rate found among women with other indications for IVF [6].

There are conflicting data regarding the relationship between endometriosis and impaired fertilization. Several studies reported a reduced fertilization rate with IVF in women with endometriosis [2, 8–14]. Pal et al. (1998) reported a significant reduction in fertilization rates in stage III and IV of endometriosis compared with stage I and II. Other studies

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were unable to identify a significant effect on the fertilization rate [15, 16]. Notably, it has been demonstrated that peritoneal fluid from women with endometriosis inhibits the binding of spermatozoa to the zona-pellucida [17, 18]. It has been suggested that intracytoplasmic sperm injection (ICSI), which was initially developed for severe male factor infertility, can be applied in other non-male factor infertile patients such as previous complete fertilization failure or a low fertilization rate in conventional IVF cycles. Split insemination of oocytes by IVF and ICSI was suggested in patients with unexplained infertility undergoing IVF treatment, to eliminate this risk of fertilization failure after IVF [19].

The present study was conducted in patients with endometriosis and normozoospermic spouses undergoing IVF treatment by insemination of sibling oocytes using randomly conventional IVF or ICSI from the same semen sample. The primary outcome measure was fertilization rate. The secondary outcome measures were embryonic morphology, cleavage rate, blastocyst formation and pregnancy rate.

Materials and methods

Patients

In the last decade our policy in cases with endometriosis consists of inseminating their sibling oocytes randomly, either with conventional IVF or ICSI from the same semen sample. This randomization is performed in couples where the male is normozoospermic and the woman has at least six cumulus–oocyte complexes (COC) retrieved. The rationale behind this policy was to overcome the suspected egg factor associated with endometriosis and lack of data in the literature about the preferable insemination method in these women. When no or few (less than 20 %) fertilizations were observed with conventional insemination, the following trial would be with ICSI only. In these cases, the ensuing cycles were not included in the data presented in the current study, since there was no randomization between IVF and ICSI.

We reviewed the medical records of all patients with endometriosis who underwent IVF procedures in our program between January 2000 and December 2011. The study included 35 patients with stages III and IV endometriosis, categorized according to the revised classification of the American Fertility Society [20]. Diagnosis was made by laparoscopic observation and biopsy of endometriotic implants.

All male partners had performed their semen analysis in our andrology laboratory. Their semen parameters were: count of $>20 \times 10^6$ sperm cells/ml, >50 % progressively motile sperm cells and ≥ 6 % sperm cells with normal forms (Kruger's strict criteria). This level of normal forms of spermatozoa coincides in our program with over 40 % rate of fertilization with conventional insemination. The

conventional insemination is performed in our place in microdroplets of 25 μ l where the oocyte is placed and 100,000 motile sperm are put inside. The exclusion criteria of these collected cases were: (i) age >40 years; (ii) day 3 FSH levels >12 mIU/ml; (iii) less than six COC retrieved.

Ovarian stimulation

Patients included in the study were treated by routine controlled ovarian hyperstimulation (COH) for IVF using the long GnRH agonist protocol. This protocol consisted of mid-luteal pituitary down-regulation, using a daily dose of GnRH agonist (nafarelin acetate, 200 mg three times a day, via intranasal spray; or triptorelin, 0.1 mg/day, via subcutaneous injection), followed by an individually adjusted urinary or recombinant gonadotrophins administration.

Oocytes were retrieved 35–38 h after administration of 5,000 IU of hCG, which was administered when at least two leading follicles were of ≥ 18 mm diameter). Routine oocyte pick-up was performed using a 17 G oocyte aspiration needle. Follicles were aspirated using 150 mmHg pressure.

Sperm preparation, oocyte retrieval, insemination or injection, embryo evaluations

The culture medium used was G-1 $\hat{\text{O}}$ v5 (Vitrolife, CT, USA) supplemented with human serum albumin (Vitrolife, CT, USA). After completion of oocyte retrieval, the COCs were randomly allocated to ICSI or IVF. The oocytes for ICSI were stripped of the cumulus cells and only the MII oocytes were injected. The oocytes randomized to the conventional insemination were partially stripped prior to culture, in order to ensure the maturation state. Both procedures were done simultaneously. In order to obtain a certain number of MII oocytes in each group, additional oocytes were stripped as needed. Immature oocytes (including GV and MI oocytes) were not included in the study. The COC were pre-incubated for 3–6 h before insemination or injection. Both procedures were done simultaneously. The semen sample on the day of oocyte retrieval was verified to be normal. Sperm were prepared using swim-up technique, and were inseminated at a concentration of 100,000 motile sperm/ml. The cumulus cells were removed in the group of COC treated by ICSI, and only metaphase II (MII) oocytes were injected as described by Van Steirteghem et al. [21]. Embryos were checked for pronuclei 16–18 h later. The fertilization rate was expressed as numbers of oocytes with two pronuclei (2PN) per numbers of inseminated or injected oocytes. The embryos were evaluated 24 h later (42 h after insemination or injection) by another embryologist. Embryo classification was modified from the system described by Cummins et al. [22]. Grade 1 embryos consisted of symmetrical blastomeres of without anucleated fragments. Grade 2 embryos had blastomeres of even or

uneven with < 10 % of the volume of embryos filled with anucleated fragments. Grade 3 embryos had anucleated fragments occupying between 10 and 50 % of the volume of the embryos. Grade 4 embryos had anucleated fragments >50 % of the volume of the embryos.

Embryo transfer and pregnancy

Embryo transfer was performed on days 2 or 3 after insemination or injection. The best embryos were selected for transfer. Supernumerary top quality embryos were frozen and the rest were followed for blastocyst formation. Luteal phase was supported by 600 mg of vaginally administered micronized progesterone (Utrogestan; Laboratories Piette International S.A., Belgium). Clinical pregnancy was defined as a visible sac on the 5th gestational week.

Statistical analysis

The mean values of the parameters studied within each cycle were compared using the Wilcoxon signed-rank test. Chi-square test and Fisher's exact test were used for the statistical analysis where appropriate. Statistical significance was reached when $P < 0.05$. Analysis was performed using the SPSS statistical package for Windows 10.0 (SPSS Inc., USA). In order to detect a clinically significant 10 % difference (between 55 and 65 %) in the fertilization rate between IVF and ICSI with 95 % power and a double-sided α of 5 %, 270 oocytes were needed in each group.

Results

A total of 35 patients, which were eligible for the current study, underwent 79 IVF cycles, 55 of them were fresh and 24 were frozen/thawed. The mean age of the study group ($\pm$ SD) was 30.9 ± 3.5 years (range: 25–40 years).

The fertilization outcome of the sibling oocytes inseminated by conventional IVF or ICSI is shown in Table 1. In the 55 fresh

IVF cycles, a total of 893 COC were collected (mean of 16.2 ± 8.4 per cycle); of these 786 MII sibling COC were randomized between insemination by conventional IVF (387 COC) or ICSI (399 COC). The fertilization rate was significantly higher in the ICSI group, compared with the group inseminated by IVF (73.3 ± 23 % versus 54.7 ± 31.9 % respectively, $P = 0.003$). Total fertilization failure occurred in a significantly higher rate of inseminated eggs by IVF as compared with ICSI (12.7 % versus 1.8 % respectively, $P = 0.03$). While there was no significant difference in the rate of 1PN zygotes between the two groups, a significantly higher rate of triploid fertilization (three pronuclei) occurred in the inseminated oocytes than the injected ones (3.9 ± 8.7 % versus 0.9 ± 3.1 % respectively, $P = 0.02$).

Sub-analysis of the data presented in Table 1, which includes only the first time cycles, revealed the same statistical trend (Table 2). A total of 427 MII sibling COC were randomized between insemination by conventional IVF (209 COC) or ICSI (218 COC). As in the data of the total study group, the fertilization rate was significantly higher in the ICSI group, compared with the group inseminated by IVF (73.5 ± 20.3 % versus 47.9 ± 28.7 % respectively, $P = 0.002$). Total fertilization failure occurred in a significantly higher rate of inseminated eggs by IVF as compared with ICSI (10.7 % versus 0 % respectively, $P = 0.0001$).

Cleavage rate and development following fertilization by conventional IVF or ICSI are shown in Table 3. The significantly higher rate of normal fertilizations in the ICSI group yielded a significantly higher mean number of day 2 embryos as compared to the IVF group (5.2 ± 3.4 versus 3.6 ± 3.0 respectively; $P = 0.002$). A significantly higher mean number of embryos were cryopreserved in the ICSI group compared to the IVF group (3 ± 3.5 versus 1.5 ± 1.6 respectively; $P = 0.009$). There were no significant differences in the mean number of blastomeres or the morphology of day 2 and day 3 embryos between the two study groups. The blastocyst developmental rate was similar in the two study groups.

The best embryos were selected for transfer independently of their mode of insemination, preferably involving only one type of insemination. The outcome of embryo transfer cycles

Table 1 Fertilization in sibling oocytes inseminated by conventional IVF or ICSI of the total study group

	IVF	ICSI	<i>P</i>
Total no. of oocytes	387	399	
Mean no. of oocytes/cycle	7 ± 4.2	7.3 ± 4.1	NS
Total no. of 2PN oocytes	206	295	
% 2PN (per oocytes)	54.7 ± 31.9	73.3 ± 23	0.003
% 1PN (per oocytes)	2.4 ± 7	4.2 ± 8.6	NS
% 3PN (per oocytes)	3.9 ± 8.7	0.9 ± 3.1	0.02
% Total fertilization failure	12.7	1.8	0.03

Table 2 Fertilization in sibling oocytes inseminated by conventional IVF or ICSI –first time cycles

	IVF	ICSI	<i>P</i>
Total no. of oocytes	209	218	
Mean no. of oocytes/cycle	7.5 ± 4.1	7.8 ± 4.8	NS
Total no. of 2PN oocytes	103	163	
% 2PN (per oocytes)	47.9 ± 28.7	73.5 ± 20.3	0.002
% 1PN (per oocytes)	4.2 ± 9.2	5.5 ± 10.1	NS
% 3PN (per oocytes)	1.7 ± 4.8	0.9 ± 3.0	NS
% Total fertilization failure	10.7	0	0.0001

in which either ICSI only or IVF only embryos were transferred are presented in Table 4. There were no statistically significant differences in the rate of frozen embryo transfer cycles as well as in the mean number of embryos transferred between the two groups. There was no statistically significant difference in the pregnancy rate (per embryo transfer cycle) between the IVF and the ICSI group (26.1 % versus 21.8 % respectively). Likewise, there was no statistically significant difference in the implantation rate between the IVF and the ICSI group (16.7 % versus 7.9 % respectively). Finally, there were no statistically significant differences in the chemical pregnancy, clinical abortion and ongoing pregnancy rates between the two groups. Sub-analysis of the data presented in Table 4, which includes either only fresh cycles (Table 5) or frozen-thawed cycles (Table 6) revealed the same statistical trend, namely no significant differences in pregnancy outcome between the two groups.

Discussion

In the present study we have reviewed the cases of patients with severe endometriosis and normozoospermic semen, in whom sibling oocytes were randomly inseminated either by conventional IVF or ICSI. We found that the oocytes inseminated by ICSI had a significantly higher fertilization rate than those inseminated by conventional IVF, yielding a significantly higher mean number of day 2 embryos, as well as higher mean number of embryos suitable for cryopreservation. Despite the insemination method, the developmental potential of day 2 and 3 embryonic morphology and cleavage rate were similar. Complete fertilization failure rate was significantly higher following conventional IVF as compared to insemination by ICSI. Of note, a significantly

Table 4 Comparison of pregnancy outcome in women with fertilization after conventional IVF or ICSI

	IVF	ICSI	P
No. of embryo transfers (ET)	23	32	
Total embryos transferred	54	84	
Frozen-thawed cycles (%)	30.4	53.1	NS
Embryos/transfer (mean $\pm$ SD)	2.3 $\pm$ 0.5	2.6 $\pm$ 0.8	NS
Pregnancy rate per ET	26.1	21.8	NS
Clinical pregnancy rate per ET	21.7	21.9	NS
Implantation rate per transferred embryo	16.7	7.9	NS
Pregnancy outcome			
Chemical pregnancy (%)	4.3	0	NS
Missed abortion (%)	8.7	6.3	NS
Ongoing pregnancy $\geq$ 12 weeks (%)	13	15.6	NS

higher rate of triploid fertilization occurred in oocytes inseminated by IVF as compared to those treated by ICSI. Finally, a comparison between embryo transfer cycles in which either ICSI only or IVF only embryos were transferred revealed no statistically significant differences in pregnancy rate, implantation rate or pregnancy outcome.

Previous studies found similar fertilization rates of sibling oocytes inseminated by either ICSI or conventional IVF in couples with tubal infertility and normozoospermic semen [23–25]. In contrast, in the current study we found a significantly higher fertilization rate of oocytes inseminated by ICSI as compared to conventional IVF. These results might be explained by the different source of the oocytes. In patients with tubal infertility it is primarily a mechanical barrier to sperm-oocyte contact that causes their infertility. Since exposure of the oocytes to the spermatozoa was

Table 3 Comparison of embryonic cleavage and development in women with fertilization after conventional IVF or ICSI

	IVF	ICSI	P
Total no. of embryos	199	288	
Mean no. of day 2 embryos per cycle	3.6 $\pm$ 2.9	5.2 $\pm$ 3.4	0.002
Day 2 embryos			
Mean no. of blastomeres	3.6 $\pm$ 0.7	3.8 $\pm$ 0.7	NS
Mean morphology score ^a	2 $\pm$ 0.6	1.9 $\pm$ 0.4	NS
Day 3 embryos			
Mean no. of blastomeres	6.5 $\pm$ 1.6	7.2 $\pm$ 1.1	NS
Mean morphology score ^a	2 $\pm$ 0.5	2 $\pm$ 0.5	NS
Blastocysts			
% blastocysts (per cultured embryos)	33.6 $\pm$ 41.3	43.6 $\pm$ 35.2	NS
Mean no. of blastocysts/cycle	1.1 $\pm$ 1.6	1.5 $\pm$ 1.3	NS
Embryo Cryopreservation			
Mean no. of cryopreserved embryos	1.5 $\pm$ 1.6	3 $\pm$ 3.5	0.009
Mean no. of cryopreserved blastocysts	0.6 $\pm$ 1.3	0.5 $\pm$ 0.9	NS

^aMorphology score 1- best, 4- worst

Table 5 Comparison of pregnancy outcome in women with fertilization after conventional IVF or ICSI – Fresh cycles

	IVF	ICSI	<i>P</i>
No. of embryo transfers (ET)	16	15	
Total embryos transferred	36	33	
Embryos/transfer (mean $\pm$ SD)	2.3 $\pm$ 0.5	2.2 $\pm$ 0.6	NS
Pregnancy rate per ET	31.3	20	NS
Clinical pregnancy rate per ET	25	20	NS
Implantation rate per transferred embryo	17.7	8.9	NS
Pregnancy outcome			
Chemical pregnancy (%)	6.3	0	NS
Missed abortion (%)	6.3	6.7	NS
Ongoing pregnancy $\geq$ 12 weeks (%)	25	13.3	NS

impossible, proof of the fecundity of the apparently normal spermatozoa is absent. During the IVF procedure, the mechanical barrier is removed and the fecundity of the spermatozoa can be proven in vitro [23]. In contrast, in women with endometriosis one possible mechanism for reduced fertilization rates is compromised oocyte quality [3, 26–28]. This is consistent with results of a study that evaluated pregnancy rates of patients undergoing IVF with donated oocytes, which showed that recipients who had endometriosis conceived with no difference than those with other causes of infertility. However, when the results were classified by donor factors, the eggs from women with endometriosis led to a statistically significant decrease in pregnancy rate regardless of recipient status [14]. This is indicative of a possible detrimental effect in oocyte development even before the oocyte is released into the peritoneum, where it may come in contact with the endometriosis lesion or its secretory products. Indeed, a previous study found microtubule and chromosomal changes in poor

Table 6 Comparison of pregnancy outcome in women with fertilization after conventional IVF or ICSI – Frozen cycles

	IVF	ICSI	<i>P</i>
No. of embryo transfers (ET)	7	17	
Total embryos transferred	18	51	
Embryos/transfer (mean $\pm$ SD)	2.6 $\pm$ 0.5	3.0 $\pm$ 0.8	NS
Pregnancy rate per ET	14.3	23.5	NS
Clinical pregnancy rate per ET	14.3	23.5	NS
Implantation rate per transferred embryo	14.3	7.1	NS
Pregnancy outcome			
Chemical pregnancy (%)	0	0	NS
Missed abortion (%)	14.3	5.9	NS
Ongoing pregnancy $\geq$ 12 weeks (%)	14.3	17.6	NS

quality oocytes, which could potentially explain the reduced fertilization rates, as well as reduced implantation rates [2]. Furthermore, a direct correlation between the disease and prevalence of nuclear and cytoplasmic aberrations was observed in pre-implantation embryos obtained from women undergoing IVF cycles for endometriosis-associated infertility [29]. Another possible mechanism involves the effect of follicular fluid (FF) on gamete interaction. Normal FF induces the acrosome reaction and enhances sperm binding to the zona-pellucida. Qiao et al. [30] reported that FF from patients with endometriosis undergoing IVF resulted in significantly decreased binding of spermatozoa to the zona compared with patients with tubal factor. In a mouse model, exposure of oocytes to endometriotic peritoneal fluid was found to attenuate oocyte fertilization [31].

Interestingly, a previous study which compared the fertilization rate and embryonic development of oocytes randomly inseminated by conventional IVF or ICSI in patients with polycystic ovarian syndrome (PCOS) and normozoospermic semen during IVF cycles found a significantly higher fertilization rate in the ICSI group. The authors concluded that this difference could be explained by poor oocyte quality resulting from the abnormal hormonal milieu in PCOS patients as well as abnormality in the zona pellucida [32]. Taken together, it seems that in several egg factor cases (such as in PCOS or endometriosis) ICSI may have favorable effect on fertilization and embryo quality over conventional IVF.

For most IVF programs, the incidence of fertilization failure occurs in 5–10 % of IVF cycles and 2–3 % in ICSI cycles [33]. Since the couples enrolled in our study had normal semen quality, a good fertilization rate and low incidence of complete fertilization failure was expected following [33]. However, the complete fertilization failure rate by IVF was as high as 12.7 % in contrast to significantly lower rate of fertilization failure occurring in the oocytes inseminated by ICSI (1.8 %). A significantly higher rate of total fertilization failure occurred in sibling oocytes which were inseminated by conventional IVF as compared to ICSI also in PCOS patients [32]. Because ICSI bypasses the sperm–zona pellucida interaction, our results imply that some abnormality in the zona pellucida might be present in patients with endometriosis. It is therefore suggested that ICSI could overcome the high complete fertilization failure rate in patients with endometriosis during their IVF cycles. Another evidence of zona pellucida malfunction in women with endometriosis could be demonstrated by the significantly higher triploid fertilization rate which was found in oocytes inseminated by conventional IVF compared to ICSI. Among the several underlying causes for triploidy, dispermy is recognized to be the most common [34].

Immediately after egg retrieval, the cortical granules are thought still to be migrating toward the oocyte surface.

Thus, after fertilization, there may be a delay in the release of cortical granule contents, which subsequently may affect the zona reaction to block further sperm entry [35]. It is noteworthy that 100,000 sperm/mL are the optimum concentration for insemination in our culture system. We routinely perform inseminations between 2 and 4 h after retrieval. Taken together these findings it seems unlikely that oocytes in our study population were prone to an abnormal kinetics of cortical granule exocytosis that, in turn, resulted in dispermy. Instead, the significantly higher rate of triploidy following conventional IVF suggests zona pellucida malfunction. In this manner, ICSI was found to overcome a high incidence of trippronucleate zygotes resulting from standard insemination in a previous cycle, without affecting overall embryo quality [36].

The quality of embryos obtained in terms of days 2 and 3 embryonic morphology and rate of cellular development were comparable, irrespective the method of insemination. Similarly, day 2 embryonic morphology and rate of development were not different regardless of the insemination methods in sibling oocyte model in PCOS patients [32]. Hence, micromanipulation technique appears to have no negative effect on the embryonic development. These results are in view of previous studies, which found no differences in the embryo development in sibling oocytes regardless of the fertilization procedure [23, 37].

The present study involves patients that were treated over a period of a decade. Yet, we believe that this study group can be analyzed as a single group, since throughout the whole study period the outcome of the ICSI as well as IVF procedures used in our laboratory are fairly consistent.

From the results of this study we may conclude that ICSI appears to be a better treatment option than conventional IVF in endometriosis-associated infertility, since it offers the advantages of higher fertilization rate, lower rate of total fertilization failure, lower rate of triploid fertilization and higher mean number of embryos. Whether ICSI has really an impact on pregnancy rate should be examined in a larger study, in order to see whether ICSI should be performed in all, or at least part of oocytes of patients with endometriosis undergoing IVF cycles.

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