Human Reproduction Vol.17, No.2 pp. 362–369, 2002

Outcome of conventional IVF and ICSI on sibling oocytes in mild male factor infertility

Michelle Plachot[1], Joëlle Belaisch-Allart, Jean-Marc Mayenga, Albert Chouraqui, Laurent Tesquier and Alain Michel Serkine

C.H.I. Jean Rostand, 141 Grande Rue, 92310 Sèvres, France

[1]To whom correspondence should be addressed. E-mail: mplachot@maildoc.com

BACKGROUND: Decisions concerning the treatment choice for assisted reproduction (IVF or ICSI) are usually made after the evaluation of male fertility factors, or after taking into account the results of previous IVF attempts. There are no widely accepted criteria, so decisions for couples with male subfertility are often empirical and may lead to complete fertilization failure after IVF, or to the unnecessary use of ICSI. METHODS: A study was conducted in which half the oocytes from each of 58 couples with moderate oligo ± astheno ± teratozoospermia were inseminated (conventional IVF) and the other half microinjected (ICSI). The technique used for subsequent cycles depended on the results of the first cycle. RESULTS: Nineteen of the 58 IVF/ICSI attempts resulted in fertilization after ICSI only (32.8%) and 39 in fertilization after IVF and ICSI (67.2%). For patients with oocyte fertilization only after ICSI, 61.5% of the oocytes microinjected were fertilized. A mean of 2.2 embryos per patient were transferred, leading to eight clinical pregnancies (42.1%).The implantation rate was 21.4%. All subsequent cycles were carried out with ICSI. Couples with oocyte fertilization after both IVF and ICSI had slightly better semen characteristics than those with oocyte fertilization only after ICSI, but this difference was not significant. Overall, no statistically significant difference was observed between IVF and ICSI in sibling oocytes for any of the variables studied: fertilization rate, embryo morphology and rates of development, pregnancy and implantation. Although only small numbers of oocytes or embryos were available for each couple, six couples had lower fertilization rates after IVF and eight had lower embryo quality after IVF. Eight patients had lower sperm quality in the second cycle, and only seven couples underwent subsequent IVF cycles. CONCLUSIONS: This strategy enabled us to avoid 32.8% of complete fertilization failures after IVF, but not to decrease significantly the number of ICSI attempts in subsequent cycles. However, the uncertainties concerning the safety of ICSI suggest that ICSI should be used cautiously and judiciously.

Key words: embryo development/failed fertilization/ICSI/IVF

Introduction

In assisted reproduction programmes, decisions concerning the treatment technique (IVF or ICSI) are usually made after the evaluation of male fertility factors, or taking into account the results of previous IVF attempts. There are no widely accepted criteria, so decisions for couples with male subfertility (i.e. at least one sperm characteristic abnormal) are often empirical and may lead to complete fertilization failure after IVF, or to the unnecessary use of ICSI.

As ICSI is now a common treatment for patients with severely impaired spermatogenesis (Palermo *et al*., 1992), it is tempting to propose ICSI to couples with borderline semen characteristics (moderate oligo ± astheno ± teratozoospermia) as a means of decreasing the risk of complete fertilization failure (Fishel *et al*., 2000). However, the unnecessary use of ICSI is time-consuming, costly, and unethical, because this technique, although very successful, is still undergoing safety evaluation. Indeed, 5–10% of oocytes are damaged after rupture of the oolemma. The risks of disturbing the spindle during introduction of the pipette (Asada *et al*., 1995; Blake *et al*., 2000; Dumoulin *et al*., 2001), the possible asynchronized decondensation of sperm chromosomes (Terada *et al*., 2000), the reduced capacity for blastocyst formation *in vitro* particularly in cases of poor sperm motility and morphology (Griffiths *et al*., 2000; Miller and Smith, 2001), the lower survival (Schnorr *et al*., 2001) and implantation rates of frozen–thawed embryos originating from ICSI than of embryos obtained by IVF (Macas *et al*., 1998), the malformations and chromosome abnormalities observed in the fetus (Bonduelle *et al*., 1999; Wennerholm *et al*., 2000) and the risk of transmission of infertility and other genetic defects to the offspring are still open to debate (Chang *et al*., 1999).

Attempts have been made to avoid fertilization failure using simple and less expensive procedures. One study (Hall *et al*.,

1995) compared high insemination concentration (1×10^6 to 1×10^7/ml motile spermatozoa) and ICSI on sibling oocytes in cases of very severe teratozoospermia (a mean of 2.9% normal morphology using Kruger strict criteria) and demonstrated similar fertilization rates, embryo quality and implantation rates with both techniques. Conversely, others (Kastrop *et al*., 1999; Fishel *et al*., 2000) found that ICSI was much more effective than IVF if high sperm concentrations were used for insemination.

Attempts to rescue failed IVF cycles by sperm microinjection 20–24 h later have resulted in normally fertilized oocytes, but frequently with a low pregnancy rate and a high incidence of abnormal fertilization (Nagy *et al*., 1995; Morton *et al*., 1997). However, one study (Yuzpe *et al*., 2000) reported a 20.7% pregnancy rate after ICSI rescue.

Due to these conflicting results, several groups have suggested that IVF and ICSI should be performed on sibling oocytes in patients with male subfertility (Calderon *et al*., 1995; Aboulghar *et al*., 1998; Pisarska *et al*., 1999; Verheyen *et al*., 1999) and in couples with normozoospermic semen (Nagy *et al*., 1998; Staessen *et al*., 1999; Khamsi *et al*., 2001). In each study, the risk of complete fertilization failure was lower after ICSI than after IVF.

We therefore conducted a study in which half the oocytes from couples with male subfertility and moderate oligoasthenoteratozoospermia were inseminated (IVF) and the other half microinjected (ICSI) in the first cycle. The results obtained determined the technique used for subsequent attempts, IVF or ICSI. We now report the results of cycles in which we compared the fertilization and embryo development of sibling oocytes treated by IVF and ICSI and the results of subsequent cycles.

Materials and methods

Patients

Fifty-eight couples undergoing their first IVF/ICSI cycle and for whom at least six oocytes were recovered were included in the study between February 1997 and July 2000. All patients were counselled for IVF treatment, and were informed that due to the possibility of total fertilization failure, ICSI and IVF would be performed on sibling oocytes. Informed consent was obtained from all couples involved in the study. The mean age of the women was 32.2 ± 3.3 years and that of the men was 34.3 ± 4.2 years. We performed a total of 102 cycles: one cycle only in 24 couples, two in 25 couples, three in eight couples and four cycles in one couple. Forty-four couples had purely male infertility and 14 couples had male infertility associated with female infertility (tubal infertility, endometriosis, dysovulation, polycystic ovarian syndrome).

Male infertility was classified according to WHO criteria (World Health Organization, 1992): oligozoospermia, $<20\times10^6$ sperm/ml; asthenozoospermia, <50% of sperm with grades a/b motility; teratozoospermia, <40% normal morphology. We also scored sperm movement from 0 to 5: 0, immotile sperm; 1, moving without progression; 2, slow non-linear progression; 3, forward linear progression; 4, rapid progression; 5, hyperactivation (capacitated sperm). In our series, 21 men had oligoasthenoteratozoospermia, 12 teratozoospermia, 12 oligoteratozoospermia, 10 asthenoteratozoospermia, two oligoasthenozoospermia and one oligozoospermia.

Patients with <500 000 morphologically normal and progressive motile sperm in ejaculate and patients with obstructive or non-obstructive azoospermia were included in our routine ICSI programme.

Ovarian stimulation

All patients underwent ovarian stimulation by a long desensitization protocol using GnRH agonist in association with recombinant FSH (rFSH). Patients were given triptoreline on the first or second day of the cycle (0.1 mg Decapeptyl; Ipsen–Biotech, Paris, France). rFSH (Gonal-F; Serono, Boulogne, France; or Puregon; Organon, Puteaux, France) was started on day 10 if down-regulation was demonstrated by a low estrogen level (<50 pg/ml) and the absence of cysts on ultrasound scans. rFSH (150 or 225 U) was administered for 5 days, and the dose was adjusted thereafter according to the ovarian response, as assessed by ultrasound scan and estrogen levels. HCG (5000 IU) was injected when at least three follicles had reached a diameter of ⩾18 mm. Oocyte retrieval was scheduled for 36 h after HCG injection (day 0).

Insemination and sperm microinjection procedure

Oocyte–cumulus complexes (OCC) were identified with a dissecting microscope, and assigned alternatively in order of retrieval into two groups. For both groups, the OCC were placed in 1 ml of IVF medium (Medicult, Lyon, France) in tubes, and incubated in a humidified 37°C incubator in 5% CO_2 in air. Semen was prepared by a 45–90% discontinuous gradient method using PureSperm (Nidacon International AB, Gothenburg, Sweden). After preparation, the same semen sample was used for both conventional IVF and ICSI. All the oocytes in one group were treated by conventional IVF and were inseminated ~4 h after retrieval with 60 000 motile sperm in 1 ml of IVF medium. The other group of oocytes was treated by ICSI. Immediately before micromanipulation, cumulus and corona cells were removed enzymatically by incubating the oocytes in 1 ml of IVF medium containing 80 IU/ml hyaluronidase (Medicult, Lyon, France) for 2–3 min. The denuded oocytes were examined to assess integrity and maturity. Only those oocytes that had extruded the first polar body (metaphase II oocytes) were microinjected. Immediately before injection, the sperm suspension was added to a 50 µl droplet of polyvinylpyrrolidone (PVP; Medicult). Oocytes were microinjected ~5 h after retrieval in microdroplets of IVF medium covered with lightweight paraffin oil. A single motile spermatozoon with apparently normal morphology was immobilized by touching its tail with the injection pipette and aspirated tail-first into the injection pipette. The sperm was microinjected into the ooplasm at the 3 o'clock position, the polar body being oriented at the 6 or 12 o'clock position.

Assessment of fertilization and embryo quality

Fertilization was assessed 15–18 h after insemination or microinjection. For conventional insemination, the cumulus and corona cells surrounding the oocytes were removed by dissection using a thin pipette in an organ culture dish. The injected oocytes were observed for any sign of damage and for the presence of pronuclei. Oocytes were classed as fertilized if two pronuclei (2PN) were present and the second polar body had been extruded. Abnormally fertilized oocytes (1PN or 3PN) were excluded. Normally fertilized oocytes were left in culture for a further 24 h. Embryos were classified according to a simplified system based on morphological criteria: (i) type A embryos had equal-sized blastomeres and anucleate fragments, if present, accounted for <10% of the volume of the embryos, (ii) type B embryos had blastomeres unequal in size and/or 10–30% fragmentation and (iii) type C embryos had >30% fragmentation.

Embryo transfer

Embryos were transferred ~48 h after oocyte retrieval. Priority was given to the transfer of IVF embryos if both ICSI and IVF embryos

were available and of the same quality. Embryo quality was assessed according to the usual criteria: number of blastomeres (four blastomeres on day 2 if available) and percentage fragmentation (<10% fragmentation if possible). A mixture of IVF and ICSI embryos were transferred if insufficient high quality embryos were available from a single technique. To reduce the risk of high-rank multiple pregnancies, the number of embryos replaced was mostly limited to two or three. All embryos were transferred using a Frydman catheter (CDD, Paris, France). Utrogestan (600 mg per day) was administered to all patients for 14 days beginning 2 days after oocyte retrieval. Clinical pregnancies were detected by a serum β-HCG level of >1000 IU/l and the presence of a gestational sac on ultrasound scans performed 6 weeks after embryo transfer. The implantation rate was defined as (the number of gestational sacs divided by the number of embryos transferred)×100.

Cryopreservation

Excess embryos with <30% fragmentation were frozen (Mandelbaum *et al*., 1998).

Statistical analysis

Student's *t*-test and the χ^2-test were used as appropriate; $P < 0.05$ was considered statistically significant.

Results

Nineteen of the 58 first IVF/ICSI cycles (32.8%) resulted in fertilization after ICSI only and 39 in fertilization with both IVF and ICSI (67.2%).

Fertilization after ICSI only

For 19 couples, fertilization occurred only after ICSI. The characteristics of the semen samples of the men used for both IVF and ICSI are shown in Table I.

According to WHO criteria (World Health Organization, 1992), two of the men had teratozoospermia, one had oligozoospermia, four had asthenoteratozoospermia, four oligoteratozoospermia, two oligoasthenozoospermia and six oligoasthenoteratozoospermia. Fourteen couples had purely male infertility and five couples had male and female infertility (one case of endometriosis and four of tubal infertility).

Eleven couples who experienced implantation failure or spontaneous abortion after the transfer of fresh or frozen–thawed embryos obtained in the first cycle underwent a total of 13 additional ICSI cycles.

The results of the first and subsequent cycles are shown in Table I. There was no difference in the mean number of oocytes recovered, in the percentage of immature (prophase or metaphase I) or atretic oocytes or in sperm quality between the first and subsequent cycles. The mean number of oocytes allocated to ICSI (5.6) and microinjected (4.8) was lower in the first cycle than in the other cycles (10.7 and 9.1) because half the oocytes were used for IVF and half for ICSI in the first cycle whereas all oocytes were used for ICSI in the other cycles. A total of 107 oocytes were allocated to ICSI, 91 were microinjected, and 56 were fertilized (61.5%); 106 oocytes were inseminated, none of which were fertilized. Fertilization rate, embryo development and embryo morphology were similar in the first and subsequent cycles. The clinical pregnancy rate was 42.1% after transfer of a mean of 2.21 embryos in the first cycle. In subsequent cycles, the number of embryos transferred was increased slightly to a mean of 2.92 per transfer, leading to a clinical pregnancy rate of 23.1%. Of the 11 pregnancies obtained in this group of patients, one was ectopic, one spontaneously aborted and nine led to the birth of seven singletons and two sets of twins. The overall implantation rate was 21.4, 10.0 and 12.5% at the first, second and third cycles respectively.

Four couples had frozen–thawed embryos transferred: no pregnancy occurred. Four couples still have frozen embryos.

Fertilization in IVF and ICSI

For 39 couples (67.2%), fertilization was achieved by both IVF and ICSI. The semen characteristics of the men are shown in Table II.

The number of sperm and sperm motility were slightly higher in this group than in the group with fertilization only after ICSI, but this difference was not significant. According to WHO criteria, 11 patients had teratozoospermia, six asthenoteratozoospermia, 8 oligoteratozoospermia and 14 oligoasthenoteratozoospermia. Thirty couples had male infertility only and nine couples had both male and female infertility (five cases of tubal infertility, three of dysovulation and one of PCOS).

Sixteen couples underwent only one cycle and 14 of these couples achieved a pregnancy. Twenty-three couples who experienced implantation failure or spontaneous abortion after the transfer of fresh or frozen–thawed embryos obtained in the first cycle underwent a total of 31 additional cycles. The choice of technique for subsequent cycles depended on the results of the first cycle: seven couples underwent a second conventional IVF cycle, and 16 couples underwent a total of 24 subsequent ICSI cycles because fertilization rate (six cycles) or embryo development (eight cycles) were better with ICSI in the first cycle or because sperm quality decreased (eight cycles). In six cycles, these factors were combined.

The outcomes of insemination and microinjection in the first and subsequent cycles are shown in Table II. Thirty-four of the 228 oocytes allocated to ICSI in the first cycle (14.9%) were found to be immature or atretic after cumulus cell removal, a frequency similar to that observed in patients with fertilization after ICSI only (15.0%) and during subsequent ICSI cycles (11.8%). No statistically significant difference in sperm characteristics was noted between patients who continued IVF and those who underwent ICSI treatment in the first and subsequent cycles, but some sperm characteristics appeared to be slightly better in those patients who continued IVF. Of the 194 mature healthy microinjected oocytes obtained in the first cycle, 118 were fertilized (51.8% of the oocytes allocated to ICSI, 60.8% of the oocytes actually microinjected). This rate of fertilization was similar to that of oocytes allocated to IVF and inseminated (53.7%). No difference was found during subsequent cycles. All seven couples who underwent a second IVF cycle also underwent embryo transfer. When pooling all ICSI or IVF cycles for this group of patients, the mean fertilization rate for oocytes inseminated by conventional IVF was 54.9%, and that for oocytes allocated to ICSI was

Table I. Comparison of sperm quality, fertilization rate and embryo development in 19 first IVF/ICSI cycles and 13 subsequent ICSI cycles in couples for whom fertilization was achieved by ICSI only

	First IVF/ICSI cycle	Subsequent ICSI cycles	*P*
Cycles	19	13	
Mean no. oocytes/cycle	11.2	10.7	NS
Immature or atretic oocytes (%)	15.0	15.0	NS
Sperm quality (mean ± SD)			
Sperm count ($\times 10^6$/ml)	21.4 ± 39.1	21.1 ± 32.2	NS
Motile spermatozoa (%)	29.7 ± 14.5	37.3 ± 17.6	NS
Motility score	2.35 ± 0.2	2.42 ± 0.2	NS
Abnormal forms (%)	71.2 ± 18.1	71.0 ± 20.0	NS
Oocytes allocated to ICSI (per patient)	107 (5.6)	140 (10.7)	<0.05
Microinjected oocytes (per patient)	91 (4.8)	119 (9.1)	<0.05
Microinjected fertilized oocytes (%)	56 (61.5)	56 (51.8)	NS
Oocytes allocated to IVF (per patient)	106 (5.6)	–	
Inseminated oocytes (per patient)	106 (5.6)	–	
Inseminated fertilized oocytes	0	–	
1PN oocytes (%)	0	2 (1.7)	NS
3PN oocytes (%)	2 (2.2)	1 (0.8)	NS
Embryos	56	56	
Mean no. of blastomeres on day 2	3.53	3.36	NS
Morphology of embryos[a]			
Good embryos (grade A) (%)	77	61	NS
Fair embryos (grade B) (%)	18	27	NS
Poor embryos (grade C) (%)	5	12	NS
Embryo transfers	19	13	
Mean no. of embryos transferred	2.21	2.92	NS
Clinical pregnancies (%)	8 (42.1)	3 (23.1)	NS
Implantations (%)	9 (21.4)	4 (10.5)	NS

[a]Assessed 42 h after insemination.
PN = pronuclei.

Table II. Comparison of sperm quality, fertilization rate and embryo development in 39 first cycles and 31 subsequent cycles in couples for whom fertilization was achieved after both IVF and ICSI

	First IVF/ICSI cycle		Subsequent cycles		*P*
	IVF	ICSI	IVF	ICSI	
Cycles	39		7	24	
Oocytes (mean no./cycle)	218 (5.6)	228 (5.8)	75 (10.7)	237 (9.9)	<0.05
Immature or atretic oocytes (%)	–	34 (14.9)	–	28 (11.8)	NS
Sperm quality (mean ± SD)					
Sperm count ($\times 10^6$/ml)	33.8 ± 45.0		48.9 ± 38.0	30.3 ± 33.4	NS
Motile spermatozoa (%)	35.1 ± 15.0		37.9 ± 17.7	30.4 ± 19.2	NS
Motility score	2.37 ± 0.4		2.35 ± 0.3	2.36 ± 0.3	NS
Abnormal forms (%)	75.6 ± 21.5		68.5 ± 19.4	73.9 ± 20.1	NS
Microinjected oocytes (per patient)		194 (5.0)		209 (8.7)	<0.05
Microinjected fertilized oocytes		118		137	
per oocytes allocated to ICSI (%)		51.8		57.8	NS
per microinjected oocytes (%)		60.8		65.6	NS
Inseminated fertilized oocytes	117 (53.7)		44 (58.7)		NS
1PN oocytes (%)	2 (0.9)	1 (0.5)	3 (4)	1 (0.5)	NS
3PN oocytes (%)	2 (0.9)	5 (2.6)	3 (4)	2 (1.0)	NS
Embryos	117	118	40	131	
Morphology of embryos					
Good embryos (grade A) (%)	76 (65.0)[a]	85 (72.0)[e]	17 (42.5)[b]	76 (58.0)[f]	
Fair embryos (grade B) (%)	31 (26.5)	25 (21.2)	13 (32.5)	37 (28.2)	
Poor embryos (grade C) (%)	10 (8.5)[c]	8 (6.8)	10 (25.0)[d]	18 (13.7)	
Mean no. of blastomeres on day 2	3.12	3.57	2.76[g]	3.58[h]	

[a,b]$P < 0.02$; [c,d]$P < 0.01$; [e,f]$P = 0.02$; [g,h]$P < 0.001$.
PN = pronuclei.

54.7% (i.e. 63.4% of those actually microinjected), which is not significantly different.

The rate of abnormal fertilization, defined as the presence of one or three pronuclei, was between 0.5 and 4%, which was not statistically significant. Indeed, if all ICSI or IVF cycles were pooled, 1.7% of inseminated oocytes and 0.5% of

Table III. Outcome of embryo transfer cycles with fertilization by both IVF and ICSI in the first cycle

	ICSI	IVF	Mixed	Total	*P*
No. of embryo transfers	36	14	19	69	
First cycle	13	7	19	39	
Subsequent cycles	23	7	0	30	
No. of embryos transferred	99	36	50	185	
First cycle	32	18	50	100	
Subsequent cycles	67	18	0	85	
Embryos/transfer	2.8	2.6	2.6	2.7	NS
First cycle	2.5	2.6	2.6	2.6	
Subsequent cycles	2.9	2.6	–	2.8	
No. of clinical pregnancies (% per embryo transfer)	12 (33.3)	6 (42.8)	6 (31.6)	24 (34.8)	NS
First cycle	6 (46.2)	4 (57.1)	6 (31.6)	16 (41.0)	
Subsequent cycles	6 (26.1)	2 (28.6)	–	8 (26.7)	
Implantation rate (%)	13 (13.1)	7 (19.4)	10 (20.0)	30 (16.2)	NS
First cycle	6 (18.8)	5 (27.8)	10 (20.0)	21 (21.0)	
Subsequent cycles	7 (10.4)	2 (11.1)	–	9 (10.6)	

Values in parentheses are percentages.

microinjected oocytes showed a single pronucleus and 1.7% of inseminated or microinjected oocytes showed three pronuclei.

Most of the embryos were morphologically normal. Indeed, in the first cycle, 65.0% of the embryos resulting from IVF and 72.0% of those resulting from ICSI belonged to group A (not significant). Embryo morphology seemed to be better in the first cycle than in subsequent cycles as shown by the higher frequency of grade A embryos in the first IVF cycle (65.0 versus 42.5%, $P < 0.02$) and the lower rate of grade C embryos (8.5 versus 25.0%, $P < 0.01$). A similar trend was observed in ICSI, with a statistically significant higher rate of grade A embryos in the first cycle (72.0%), than in subsequent cycles (58.0%, $P = 0.02$). However, if all IVF or ICSI cycles were pooled, the rates of grade A embryos resulting from IVF and ICSI were 59.2 and 64.6% respectively, and these two values are not significantly different.

We observed a trend (which, however, was not statistically significant) toward the number of blastomeres on day 2 being higher after ICSI (3.57) than after IVF (3.12). The same trend was observed in subsequent cycles and reached statistical significance. However, if all IVF or ICSI cycles were pooled, the number of blastomeres in embryos resulting from IVF and ICSI was 3.04 and 3.55 respectively, and these two values are not significantly different.

For the 39 couples as a whole, the mean number of embryos transferred per cycle was 2.7 (Table III). Ninety-nine ICSI embryos were transferred in 36 cycles (2.8/cycle), resulting in 12 clinical pregnancies (33.3%). Transfer was cancelled for one cycle because of fertilization failure due to oocyte quality: for nine oocytes, six were immature in prophase I and three were atretic.

Thirty-six IVF embryos were transferred in 14 cycles (2.6/cycle), resulting in six clinical pregnancies (42.8%). In 19 cycles, a mixed transfer of IVF and ICSI embryos (23 IVF and 27 ICSI in total) was performed (2.6/cycle), resulting in six clinical pregnancies (31.6%). The implantation rate was 13.1, 19.4 and 20% in the three groups of transfers, and these values are not significantly different.

The pregnancy and implantation rates were higher in the first cycle (41.0 and 21.0%, respectively) than in other cycles (26.7 and 10.6% respectively). Twenty-seven couples (69.2%) had extra embryos, which were cryopreserved in the course of one or several cycles. Twenty-five transfers of frozen–thawed embryos were performed, resulting in three clinical pregnancies. Thirteen couples still have frozen embryos.

Discussion

This study confirms that performing conventional IVF and ICSI in sibling oocytes in the first cycle for couples with borderline semen quality decreases the risk of transfer cancellation over that for conventional IVF alone. It is also an excellent test of sperm fertilizing ability, to be used as a guideline for the management of possible future cycles.

Similar studies comparing IVF and ICSI in sibling oocytes from couples with borderline semen quality have been reported (Calderon *et al*., 1995; Aboulghar *et al*., 1998; Pisarska *et al*., 1999; Verheyen *et al*., 1999). All concluded that in a large proportion of patients (up to 49%) this practice prevented the cancellation of embryo transfer due to complete fertilization failure with conventional IVF. Indeed, the overall fertilization rate was higher after ICSI (50–63% depending on the study) than after IVF (18–23%). The fertilization rate depended on the number of sperm defects in conventional IVF: 28.1, 16.6 and 5% in the presence of single, double and triple defects, respectively; it was similar for ICSI (45–53%) (Calderon *et al*., 1995).

In 19 cycles (32.8%), fertilization occurred only after ICSI. The mean number of spermatozoa for these men (21.4×10^6/ml) was above the threshold of WHO criteria (20×10^6/ml) and was not limiting for IVF. However, motility (29.7% motile sperm) and morphology (<30% with normal morphology) were below the threshold of normal semen characteristics according to WHO criteria and were the limiting factors. All patients had abnormal semen according to WHO criteria. This observation, along with the absence of dysmorphic

oocytes or a large number of immature oocytes, is consistent with a pure sperm defect. One study (Chocat *et al*., 2001) determined retrospectively the predictive value of semen analysis for IVF outcome in 114 couples. A tight relationship between morphology and cleavage rate was observed. Using receiver operating characteristics analysis, for >82% abnormal sperm morphology, cleavage failure was noted in 71% of couples undergoing IVF. Abnormal sperm morphology may also decrease the fertilization rate after ICSI (Gomez *et al*., 2000). However, in neither study could complete failure of fertilization be predicted.

The fertilization rate (61.5%) and clinical pregnancy rate per transfer (42.1%) were similar to those reported in the literature for ICSI (Aboulghar *et al*., 1996; Benadiva *et al*., 1999; Staessen *et al*., 1999). This led us to suggest to patients who failed to become pregnant that they should continue treatment but with oocyte microinjection only. Subsequent ICSI cycles showed characteristics similar to the first cycle in terms of fertilization rate, embryo development and morphology. The mean number of transferred embryos increased from 2.21 for the first cycle to 2.92 thereafter. Although not statistically significant, the pregnancy and implantation rates of subsequent cycles (23.1 and 10.5%) tended to be lower than those of the first cycle (42.1 and 21.4% respectively). Ten of the 14 couples suffering from isolated male subfertility succeeded in achieving a pregnancy during the study period (71.4%) whereas only one couple (of five) with both male and female infertility achieved a pregnancy. ICSI is the method of choice in cases of male infertility. Female infertility seems, in many cases, to be the limiting factor for embryo implantation and further development.

In 39 cycles, fertilization was achieved with both the IVF and ICSI technique. We compared the mean semen characteristics of these patients with those of patients for whom fertilization succeeded only after ICSI. We found that the total number of sperm and percentage motile sperm were slightly higher—although not significantly so—in patients for whom fertilization was achieved by both IVF and ICSI. However, according to WHO criteria, all these patients had semen abnormalities. No difference was observed in the incidence of immature or atretic oocytes between these two groups of patients, indicating that oocyte quality and maturity was not the limiting factor.

We compared the fertilization rate and incidence of abnormal fertilization for IVF and ICSI in sibling oocytes and found no difference. Our results are consistent with those of others (Yang *et al*., 1996; Nagy *et al*., 1998; Staessen *et al*., 1999; Bukulmez *et al*., 2000). No specific criteria were identified for the semen of these patients that could predict the occurrence of fertilization with conventional IVF. However, according to one study (Pisarska *et al*., 1999), ICSI results in higher fertilization rates (66%) than does conventional IVF (48%) in cases of severe teratozoospermia; for the subfertile male population, ICSI tends to give higher rates of fertilization, with lower rates of fertilization failure (6 versus 28%).

As observed here, most studies have shown that embryo morphology is similar in patients undergoing ICSI and IVF (Pisarska *et al*., 1999; Staessen *et al*., 1999; Verheyen *et al*., 1999; Bukulmez *et al*., 2000; Fishel *et al*., 2000; Griffiths *et al*., 2000). One study (Yang *et al*., 1996) found a higher percentage of embryos displaying equally sized blastomeres and no fragmentation for ICSI-treated oocytes than for oocytes treated by IVF, but only considered a small series. It has been claimed (Bar-Hava *et al*., 1997; Hsu *et al*., 1999) that embryos obtained by IVF are superior to those obtained by ICSI in terms of embryo morphology. However, pregnancy and spontaneous abortion rates were similar in both groups, confirming that the cleavage status of day 3 embryos is a limited indicator of implantation outcome. It is also generally accepted that the timing of pronucleus development differs after ICSI and IVF, with more 4-cell embryos on day 2 for ICSI. Indeed, pronuclear development and first cleavage generally take place 4 h earlier after ICSI than after IVF. This difference occurs because sperm must pass through the oocyte investment (cumulus and corona cells) and zona pellucida in IVF (Nagy *et al*., 1998; Staessen *et al*., 1999). The mean number of blastomeres was slightly higher after ICSI than after IVF in our study but this difference was not statistically significant. Although morphologically similar on days 2 and 3, after prolonged embryo culture, the ICSI procedure results in a reduced capacity for blastocyst formation (Griffiths *et al*., 2000; Miller and Smith, 2001). Indeed, if sibling oocytes were randomly subjected to ICSI or IVF with sperm from the same semen sample, 20% of ICSI embryos and 50% of IVF embryos formed blastocysts ($P < 0.01$). It is not clear whether this difference is due to damage incurred during the injection process or to a negative effect of overriding or altering the physiological events of normal spermatozoon–oocyte fusion (Griffiths *et al*., 2000).

The technique used for subsequent cycles was selected according to the results of the first cycle, taking into account the usual criteria: the number of fertilized oocytes, embryo morphology and the number of blastomeres, and sperm quality on the day of oocyte retrieval. Only seven of the 23 couples for whom fertilization was achieved with both techniques in the first cycle, and who underwent further cycles, undertook further IVF cycles. Only a small number of oocytes or embryos was analysed for each couple, but six couples had lower fertilization rates after IVF than after ICSI, eight had lower embryo quality after IVF (the mean number of blastomeres was often higher in ICSI) and eight had lower sperm quality in the second cycle; in six cases these factors were combined. The semen characteristics in this group of patients with mild factor infertility varied in different tests. It would have been scientifically interesting to perform IVF in the second cycle for all patients who obtained at least one fertilized oocyte after conventional IVF in the first cycle, regardless of other criteria. However, we considered it unethical not to take into account all the information obtained during previous cycles.

Embryo quality was higher in the first than in subsequent cycles. The cause of this difference is unclear because oocyte quality (frequency of immature and atretic oocytes), sperm quality and fertilization rate were similar. It may be due to the small sample size, or to different time period corresponding to different culture and environmental conditions. Moreover, couples who achieved a pregnancy in the first cycle were

probably good cases, with better embryo quality than those who failed to become pregnant and needed subsequent cycles.

Priority was given to the transfer of IVF embryos if both ICSI and IVF embryos were available and of the same quality. A mixture of IVF and ICSI embryos were transferred if not enough high quality embryos were available from a single technique. There was no difference, either in clinical pregnancy rate per transfer or in implantation rate, between the three groups. The overall rate of clinical pregnancy was 34.8% per transfer and that of implantation 16.2% per embryo transferred. These results are consistent with those of another study (Aboulghar *et al*., 1998) which showed in a prospective randomized study that, in cases of tubal factor infertility with normal semen, there was no significant difference in implantation and pregnancy rates between IVF and ICSI.

In conclusion, this strategy enabled us to avoid 32.8% of complete fertilization failures after IVF, but not to decrease significantly the number of ICSI attempts in subsequent cycles. No threshold for sperm characteristics defining sperm not capable of fertilization in conventional IVF was identified for this group of patients. As the safety of ICSI is still under evaluation and the rates of blastocyst development and implantation of frozen–thawed embryos following conventional IVF are significantly higher than those following ICSI, we suggest that ICSI should be used cautiously and judiciously.

Acknowledgements

The authors wish to thank the laboratory staff (E.Darnis, B.Bassery, C.Gaboriaud) for their major contribution.

References

Aboulghar, M.A., Mansour, R.T., Serour, G.I., Amin, Y.M. and Kamal, A. (1996) Prospective controlled randomized study of *in vitro* fertilization versus intracytoplasmic sperm injection in the treatment of tubal factor infertility with normal semen parameters. *Fertil. Steril*., **66**, 753–756.

Aboulghar, M.A., Mansour, R.T. and Serour, G.I. (1998) Intracytoplasmic sperm injection in nonmale factor patients. In Kempers, R.D., Cohen, J., Haney, A.F. and Younger, J.B. (eds), *Fertility and Reproductive Medicine*. Elsevier, Amsterdam, pp. 475–481.

Asada, Y., Baka, S.G., Hodgen, G.D. and Lanzendorf, S.E. (1995) Evaluation of the meiotic spindle apparatus in oocytes undergoing intracytoplasmic sperm injection. *Fertil. Steril*., **64**, 376–381.

Bar-Hava, I., Ashkenazi, J., Shelef, M., Schwartz, A., Brengauz M., Feldberg, D., Orvieto, R. and Ben-Rafael, Z. (1997) Morphology and clinical outcomes of embryos after in-vitro fertilization are superior to those after intracytoplasmic sperm injection. *Fertil. Steril*., **68**, 653–657.

Benadiva, C.A., Nulsen, J., Siano, L., Jennings, J., Bet Givargis, H. and Maier, D. (1999) Intracytoplasmic sperm injection overcomes previous fertilization failure with conventional *in vitro* fertilization. *Fertil. Steril*., **72**, 1041–1044.

Blake, M., Garrisi, J., Tomkin, G. and Cohen, J. (2000) Sperm deposition site during ICSI affects fertilization and development. *Fertil. Steril*., **73**, 31–37.

Bonduelle, M., Camus, M., De Vos, A., Staessen C., Tournaye H., Van Assche, E., Verheyen, G., Devroey, P., Liebaers, I. and Van Steiteghem, A. (1999) Seven years of intracytoplasmic sperm injection and follow-up of 1987 subsequent children. *Hum. Reprod*., **14** (Suppl. 1), 243–264.

Bukulmez, O., Yarali, H., Yucel, A., Sari, T. and Gurgan, T. (2000) Intracytoplasmic sperm injection versus *in vitro* fertilization for patients with a tubal factor as their sole cause of infertility: a prospective, randomized trial. *Fertil. Steril*., **73**, 38–42.

Calderon, G., Belil, I., Aran, B., Veiga, A., Gil, Y., Boada, M., Martinez, F., Parera, N., Coroleu, B., Penella, J. *et al*. (1995) Intracytoplasmic sperm injection versus conventional *in vitro* fertilization: first results. *Hum. Reprod*., **10**, 2835–2839.

Chang, P.L., Sauer, M.V. and Brown, S. (1999) Y chromosome microdeletion in a father and his four infertile sons. *Hum. Reprod*., **14**, 2689–2694.

Chocat A., Creveuil, C. and Galeraud-Denis, I. (2001) Valeur prédictive des paramètres spermatiques non automatisés et des paramètres cinétiques automatisés sur les taux de clivage en fécondation *in vitro*. *Gynecol. Obstet. Fertil*., **29**, 301–307.

Dumoulin, J.C.M., Coonen, E., Bras, M., Bergers-Janssen, J.M., Ignoul-Vanvuchelen, R.C.M., van Wissen, L.C.P., Geraedts, J.P.M. and Evers, J.L.H. (2001) Embryo development and chromosomal anomalies after ICSI: effect of the injection procedure. *Hum. Reprod*., **16**, 306–312.

Fishel, S., Aslam, I., Lisi, F., Rinaldi, L., Timson, J., Jacobson, M., Gobetz, L., Green, S., Campbell, A. and Lisi, R. (2000) Should ICSI be the treatment of choice for all cases of in-vitro conception? *Hum. Reprod*., **15**, 1278–1283.

Gomez, E., Perez-Cano, I., Amorocho, B., Landeras, J., Ballesteros, A. and Pellicer, A. (2000) Effect of injected spermatozoa morphology on the outcome of intracytoplasmic sperm injection in humans. *Fertil. Steril*., **74**, 842–843.

Griffiths, T.A., Murdoch, A.P. and Herbert, M. (2000) Embryonic development *in vitro* is compromised by the ICSI procedure. *Hum. Reprod*., **15**, 1592–1596.

Hall, J., Fishel, S., Green, S., Fleming, S., Hunter, A., Stoddart, N., Dowell, K. and Thorton, S. (1995) Intracytoplasmic sperm injection versus high insemination concentration *in vitro* fertilization in cases of very severe teratozoospermia. *Hum. Reprod*., **10**, 493–496.

Hsu, M.I., Mayer, J., Aronshon, M., Lanzendorf, S., Muasher, S., Kolm, P. and Oehninger, S. (1999) Embryo implantation in *in vitro* fertilization and intracytoplasmic sperm injection: impact of cleavage status, morphology grade, and number of embryos transferred. *Fertil. Steril*., **72**, 679–685.

Kastrop, P.M.M., Weima, S.M., Van Kooij, R.J. and Te Velde, E.R. (1999) Comparison between intracytoplasmic sperm injection and in-vitro fertilization (IVF) with high insemination concentration after total fertilization failure in a previous IVF attempt. *Hum. Reprod*., **14**, 65–69.

Khamsi, F., Yavas, Y., Roberge, S., Wong, J.C., Lacanna, I.C. and Endman, M. (2001) Intracytoplasmic sperm injection increased fertilization and good-quality embryo formation in patients with non-male factor indications for *in vitro* fertilization: a prospective randomized study. *Fertil. Steril*., **75**, 342–347.

Macas, E., Imthurn, B., Borsos, M., Rosselli, M., Maurer-Major, E. and Keller, P.J. (1998) Impairement of the developmental potential of frozen-thawed human zygotes obtained after intracytoplasmic sperm injection. *Fertil. Steril*., **69**, 630–635.

Mandelbaum, J., Belaisch-Allart, J., Junca, A.M., Antoine, J.M., Plachot, M., Alvarez, S., Alnot, M.O. and Salat-Baroux, J. (1998) Cryopreservation in human assisted reproduction is now routine for embryos but remains a research procedure for oocytes. *Hum. Reprod*., **13** (Suppl. 3), 161–174.

Miller, J.E. and Smith, T. (2001) The effect of intracytoplasmic sperm injection and semen parameters on blastocyst development *in vitro*. *Hum. Reprod*., **16**, 918–924.

Morton, P.C., Yoder, C.S., Tucker, M.J., Wright, G., Brockman, W.D.W. and Kort, H.I. (1997) Reinsemination by intracytoplasmic sperm injection of 1-day-old oocytes after complete conventional fertilization failure. *Fertil. Steril*., **68**, 488–491.

Nagy, Z.P., Staessen, C., Liu, J., Joris, H., Devroey, P. and Van Steirteghem, A. (1995) Prospective, auto-controlled study on reinsemination of failed-fertilized oocytes by intracytoplasmic sperm injection. *Fertil. Steril*., **64**, 1130–1135.

Nagy, Z.P., Janssenswillen, C., Janssens, R., De Vos, A., Staessen, C., Van de Velde, H. and Van Steirteghem, A.C. (1998) Timing of oocyte activation, pronucleus formation and cleavage in humans after intracytoplasmic sperm injection (ICSI) with testicular spermatozoa and after ICSI or in-vitro fertilization on sibling oocytes with ejaculated spermatozoa. *Hum. Reprod*., **13**, 1606–1612.

Palermo, G., Joris, H., Devroey, P. and Van Steirteghem, A. (1992) Pregnancies after intracytoplasmic sperm injection of single spermatozoon into an oocyte. *Lancet*, **340**, 17–18.

Pisarska, M.D., Casson, P.R., Cisneros, P.L., Lamb, D.J., Lipshultz, L.I., Buster, J.E. and Carson, S.A. (1999) Fertilization after standard *in vitro* fertilization versus intracytoplasmic sperm injection in subfertile males using sibling oocytes. *Fertil. Steril*., **71**, 627–632.

Schnorr, J., Brown, S., Oeninger, S., Mayer, J., Muasher, S. and Lanzendorf, S. (2001) Impact of intracytoplasmic sperm injection on embryo cryopreservation and clinical outcome. *Fertil. Steril*., **75**, 636–637.

Staessen, C., Camus, M., Clasen, K., De Vos, A. and Van Steirteghem, A. (1999) Conventional in-vitro fertilization versus intracytoplasmic sperm injection in sibling oocytes from couples with tubal infertility and normozoospermic semen. *Hum. Reprod*., **14**, 2474–2479.

Terada, Y., Luetjens, C.M., Sutovsky, P. and Schatten, G. (2000) Atypical decondensation of the sperm nucleus, delayed replication of the male genome, and sex chromosome positioning following intracyto_plasmic sperm injection (ICSI) into golden hamster eggs: does ICSI itself introduce chromosomal anomalies? *Fertil. Steril*., **74**, 454–460.

Verheyen, G., Tournaye, H., Staessen, C., De Vos, A., Vandervorst, M. and Van Steirteghem, A. (1999) Controlled comparison of conventional in-vitro fertilization and intracytoplasmic sperm injection in patients with asthenozoospermia. *Hum. Reprod*., **14**, 2313–2319.

Wennerholm, U.-B., Bergh, C., Hamberger, L., Lundin, K., Nilsson, L., Wikland, M. and Kallen, B. (2000) Incidence of congenital malformations in children born after ICSI. *Hum. Reprod*., **15**, 944–948.

World Health Organization (1992) *WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction*. Cambridge University Press, Cambridge.

Yang, D., Shahata, M.A., Al-Bader, M., Al-Natsha, S.D., Al-Flamerzia, M. and Al-Shawaf, T. (1996) Intracytoplasmic sperm injection improving embryo quality: comparison of the sibling oocytes of non-male-factor couples. *J. Assist. Reprod. Genet*., **13**, 351–355.

Yuzpe, AA., Liu, Z. and Fluker, M.R. (2000) Rescue intracytoplasmic sperm injection (ICSI)-salvaging in-vitro fertilization (IVF) cycles after total or near-total fertilization failure. *Fertil. Steril*., **73**, 1115–1119.

Submitted on February 3, 2000; resubmitted on July 13, 2001; accepted on October 2, 2001