Fertilization, Embryo Quality, and Cryosurvival in In Vitro Fertilization and Intracytoplasmic Sperm Injection Cycles

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Purpose: Our purpose was to investigate the influence of semen quality on fertilization, embryo morphology, cleavage, and cryosurvival in conventional in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) programs.

Methods: A retrospective analysis of 513 couples undergoing IVF and 255 couples undergoing ICSI was done.

Results: Semen quality influenced fertilization in IVF and abnormal fertilization in IVF and ICSI, but no effects on the development, morphology, implantation capacity, or cryosurvival of embryos were found. Fertilization, embryo quality, and cryosurvival rates were similar after IVF and ICSI. The fertilization rate of mature oocytes in IVF was lower when cytoplasmic immaturity in the oocyte population was frequent. The speed of development of embryos was 2 hr faster after ICSI than after IVF. Two-cell-stage embryos survived best after cryopreservation with propanediol and sucrose on day 2.

Conclusions: After fertilization, semen parameters had no effect on the quality or cryosurvival of embryos in either IVF or ICSI.

KEY WORDS: cryopreservation; embryo quality; fertilization; in vitro fertilization; intracytoplasmic sperm injection.

INTRODUCTION

During in vitro fertilization (IVF), fertilization is impaired if the concentration, motility, or morphology of spermatozoa is poor or if there are antisperm antibodies in the semen (1,2). The duration of stimulation and the size of the aspirated follicles affect the fertilization rate of oocytes (3). In intracytoplasmic sperm injection (ICSI), fertilization is unaffected by the qual-

ity of semen, at least if motile spermatozoa with normal morphology can be found (4). A small number of collected oocytes or the presence of abnormal oocytes (5) is related to diminished fertilization rates after ICSI. The experience of the ICSI performer, and the handling of spermatozoa and the oolemma during injection are also important (6). ICSI cycles do not show improved fertilization rates compared with IVF cycles when using good-quality semen (7–9).

In both IVF and ICSI, the potential for implantation of the transferred embryos can be predicted in part by the morphology and speed of development of the embryos and by the age of the female. Prolonged contact of oocytes with a large number of spermatozoa in IVF (10) and poor seminal parameters in both IVF (11–13) and ICSI (14,15) have been suspected to influence embryo morphology, developmental capacity, and implantation rate. In IVF (3) and ICSI cycles (16), the size of the aspirated follicles has been suggested to be associated with embryo morphology. There are contradictory results on the influence of microinjection regarding embryo morphology (8,17–19).

Cryopreservation of excessive embryos increases the cumulative pregnancy rates after both IVF and ICSI. After freezing and thawing, fully intact embryos have the best implantation potential (20). Embryo cleavage stage and morphology before freezing influence the survival rates (21). The survival of embryos appears to be similar after IVF and ICSI after pronuclear-stage freezing (22), or with dimethylsulfoxide as the cryoprotectant (23). Survival rates have not been compared between IVF and ICSI when propanediol has been used for cleaved embryos.

We investigated the influence of seminal parameters on fertilization of oocytes, and morphology, cleavage, implantation, and cryosurvival of embryos in IVF and ICSI programs. We also compared these results between couples assigned to conventional IVF or ICSI.

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MATERIALS AND METHODS

The material included all the IVF ($n = 710$) and ICSI ($n = 361$) cycles using ejaculated semen and without donated gametes, carried out in the Infertility Clinic of the Family Federation of Finland between January 1, 1994, and December 31, 1995. Indications for IVF included tubal pathology, severe endometriosis, and failed intrauterine inseminations with ovulation induction. ICSI was recommended if there were fewer than 1 million motile spermatozoa after semen preparation in a Percoll (Pharmacia, Uppsala, Sweden) gradient, fewer than 2% spermatozoa with normal morphology according to World Health Organization (WHO) criteria (24), or less than 25% fertilization in a previous conventional IVF treatment. Cycles in which the oocytes were divided between IVF and ICSI were omitted, and each couple was included only once. The analysis finally covered 513 couples undergoing conventional IVF and 255 couples undergoing ICSI.

Ovarian stimulation was carried out using human menopausal gonadotropin (Humegon, Organon, Oss, The Netherlands; Pergonal, Serono, Aubonne, Switzerland) or follicle-stimulating hormone (Follegon, Organon; Fertinorm and Fertinorm HP, Serono) after pituitary down-regulation with a gonadotropin-releasing hormone agonist (Suprecur, Hoechst AG, Frankfurt am Main, Germany; Synarela, Searle, Morpeth, UK) started in the midluteal phase. Human chorionic gonadotropin (hCG) (Pregnyl, Organon; Profasi, Serono; 5000 IU) was administered when the three largest follicles exceeded 18 mm in diameter. The follicles were aspirated transvaginally with ultrasound guidance, and the oocytes cultured in Medi-Cult Universal IVF Medium (Medi-Cult A/S, Copenhagen, Denmark).

The concentration and motility of spermatozoa were analyzed in a Makler chamber according to WHO guidelines (24). The results of mixed antiglobulin reaction tests (MAR; information available with regard to 479 IVF and 211 ICSI cycles) and morphology assessment (information available with regard to 485 IVF and 242 ICSI cycles) in a previous semen analysis were recorded. All samples were processed by centrifugation in a discontinuous Percoll gradient (40 and 90%). In the conventional IVF cycles, the collected oocytes were incubated at 37°C in 5% CO₂ for 3–6 hr before insemination with 100,000 to 1,000,000 spermatozoa per ml (mean, 375,000/ml; range, 100,000 to 4,200,000). The presence of pronuclei (PN) and polar bodies was assessed 17–20 hr postinsemination. The meiotic stage of all unfertilized oocytes was assessed in the IVF cycles performed in 1995, and the rate of immaturity of oocytes is reported in those cycles only.

In ICSI cycles, cumulus cells were removed from the oocytes by brief exposure to hyaluronidase (80 IU/ml; Sigma Chemical Co., St. Louis, MO), and the corona cells mechanically using a mouth-controlled pipette. The meiotic maturation stage and morphology of the oocytes were recorded. A droplet of medium with prepared spermatozoa was mixed with up to 5% polyvinylpyrrolidone (Sigma). Metaphase-II oocytes were placed in a HEPES-buffered (Sigma) droplet and injected with one spermatozoon each after gentle aspiration of the cytoplasm. The oocytes were assessed for pronuclei formation 17–19 hr after the injection, and the oocytes with two PN were cultured further.

The cleavage status of the normally fertilized oocytes was assessed 48 hr after oocyte collection. The developmental stages of embryos were classified as two-cell (two or three blastomeres), four-cell (four or five blastomeres), or six-cell (six to eight blastomeres) stages. Embryo morphology was assessed according to Veeck (25): grade 1—no anucleate fragments, symmetrical blastomeres; grade 2—fragmentation <10% of volume; grade 3—10 to 20% fragmentation; grade 4—20 to 60% fragmentation; and grade 5—more than 60% fragmentation or total asymmetry. Approximately 48 hr after oocyte collection two embryos were transferred, except to women older than 38 years of age with previous treatments, when a maximum of three embryos was transferred if available. The remaining embryos of grades 1–4 were frozen on day 2 with 1.5 M 1,2-propanediol and 0.1 M sucrose (Sigma) as cryoprotectants (21) in a programmable cryomachine (Kryo10 series II; Planer Products Ltd., Sundbury-on-Thames, UK) with manual seeding at –8°C. For luteal support, vaginal progesterone (Lugesteron; Leiras, Turku, Finland) was started (600 mg daily) the day after embryo transfer (ET), and pregnancy was verified using a serum β -hCG assay 14 days after ET. A clinical pregnancy was recorded if an intrauterine gestational sac was present in ultrasound scan 4–5 weeks after ET, or if serum β -hCG concentrations exceeded 1000 IU/L. The embryos were thawed by warming and gradual removal of the propanediol and sucrose (21).

The results are expressed as means and medians. As assumptions for normality were not met after transformations, the Mann–Whitney *U* test, Spearman's correlation coefficient, and the chi-square test were used, as appropriate. Linear regression and general linear model analysis of variance were used to test independent effects of multiple parameters. A *P* value less than 0.05 was considered significant. SPSS for

Windows software was used for statistical analysis (SPSS Inc., Chicago, IL).

RESULTS

Causes of infertility and a description of the IVF and ICSI couples are presented in Table I. The men in the ICSI group had a lower concentration and motility of spermatozoa than the men in the IVF group, and there were fewer men in the IVF group with teratozoospermia (less than 30% normal forms; 61.2% in IVF and 86.8% in ICSI; $P < 0.00001$) and antisperm antibodies (greater than 10% positive; 13.4% in IVF and 27.0% in ICSI; $P = 0.00001$). There were no significant differences between the IVF and the ICSI groups in the numbers and maturity of collected oocytes and the ages of the male and female partners. Details of the cycles are presented in Table II.

Fertilization

There were no overall differences between IVF and ICSI cycles in the rates of formation of two PN (Table II). However, there were more couples without fertilization in the IVF group (12.9% in IVF and 0.8% in ICSI; $P < 0.00001$). The concentration ($\rho = -0.26$, $P < 0.00001$), motility ($\rho = -0.25$, $P < 0.00001$), and morphology of spermatozoa ($\rho = -0.22$, $P < 0.00001$) and the presence of antisperm antibodies

($\rho = -0.10$, $P = 0.025$) were inversely correlated with the fertilization rate in IVF. In the IVF group, the proportion of immature oocytes in the collected oocyte population compromised fertilization of the metaphase-II oocytes ($\rho = -0.38$, $P < 0.00001$). In ICSI cycles, the proportion of immature oocytes at retrieval, seminal parameters and poor fertilization in a previous IVF cycle had no correlation with the fertilization rate.

Three-PN formation was detected in 4.5% of the inseminated oocytes after IVF and in 5.9% of the injected oocytes after ICSI. In both IVF and ICSI cycles, the rate of 3-PN formation was positively correlated with the number of collected oocytes ($P = 0.00001$ in IVF and $P = 0.003$ in ICSI) and the motility of spermatozoa ($P = 0.0001$ in IVF and $P = 0.003$ in ICSI). After ICSI, 1-PN formation was seen more frequently than after IVF (in 32.5% of the ICSI cycles and 20.7% of the IVF cycles; $P = 0.0003$). The rate of 1-PN formation was correlated with the number of collected oocytes in both IVF and ICSI cycles (in IVF, $\rho = 0.24$, $P < 0.00001$; in ICSI, $\rho = 0.18$, $P = 0.004$). There were more degenerated oocytes after ICSI than after IVF (5.0% in ICSI and 1.5% in IVF; $P < 0.00001$).

Embryo Cleavage and Morphology

Details of cleavage and embryo morphology assessed on day 2 are presented in Table III. After

Table I. Clinical Characteristics and Semen Quality in the IVF and ICSI Groups^a

	IVF			ICSI			P
	Median	Range	n	Median	Range	n	
Age of the female partner (years)	34.0	21–43	513	34.0	23–44	255	ns
Duration of infertility (years)	5.0	1–21	507	5.0	1–20	254	0.012
Type of infertility in the wife							
Primary infertility			259			155	
Secondary infertility			254			100	0.007
Cause of infertility							
Tubal factor			46			1	
Other or combined female factors ^b			83			3	
Male factor ^c			85			104	
Combined female and male factors			278			146	
Unexplained			21			1	
Ampoules (75 IU) hMG or FSH used	30.0	14–152	513	33.0	18–120	255	0.003
Number of previous retrievals	0.0	0–6	513	1.0	0–7	255	<0.000001
Age of the male partner (years)	35.0	24–58	513	36.0	23–51	255	ns
Concentration of spermatozoa in the ejaculate ($\times 10^6/\text{ml}$)	80.0	0.9–304.0	513	14.3	0.1–216.0	255	<0.000001
Progressively motile spermatozoa in native semen (%)	52.0	1–90	513	29.0	0–86	254	<0.000001

^a ns, not significant; hMG, human menopausal gonadotropin; FSH, follicle-stimulating hormone.

^b Includes ovulatory and uterine disorders and endometriosis and/or tubal occlusion.

^c Abnormal findings according to World Health Organization criteria.

Table II. Oocyte, Fertilization, and Embryo Parameters in IVF and ICSI^a

	IVF			ICSI			<i>P</i>
	Median	Range	<i>n</i>	Median	Range	<i>n</i>	
Oocytes collected/cycle	11.0	1–50	513	12.0	1–34	255	ns
Immature oocytes (%) ^b	12.5	0–70.0	247	15.4	0–73.7	199	ns
Fertilization rate of all oocytes (%)	52.4	0–100	513	50.0	0–100	255	ns
Fertilization rate of metaphase II oocytes (IVF) or injected oocytes (ICSI) (%) ^b	66.7	0–100	247	66.7	4.5–100	199	ns
Embryos/patient ^c	5.0	0–24	461	6.0	0–15	217	0.006
Embryos/transfer ^c	2.0	1–4	380	2.0	1–3	212	ns
Cryopreserved embryos/cycle ^d	5.5	1–23	262	5.5	1–14	156	ns
Thawed embryos/cycle ^d	3.0	0–16	262	3.0	0–14	156	ns

^a Only cycles with all collected oocytes allocated to either IVF or ICSI. PN, pronucleus; ns, not significant.

^b Only the cycles in 1995. (In ICSI in 1994–1995, mean 19.9% and median 16.7% of oocytes immature, and mean 66.8% and median 66.7% of injected oocytes fertilized.)

^c Only cycles with planned second-day transfer and/or freezing.

^d Only cycles with freezing.

ICSI, the 2-PN zygotes cleaved more frequently than after IVF ($P = 0.003$). In both IVF and ICSI cycles, the cleavage rate was inversely correlated with the number of collected oocytes ($\rho = -0.30$, $P < 0.00001$ in IVF and $\rho = -0.21$, $P = 0.002$ in ICSI).

The two-cell- and four-cell-stage embryos were less often of poor morphology than the further cleaved embryos in both IVF and ICSI (proportion of grade-4 to 5 embryos of two-cell-, four-cell-, six-cell-stage embryos: 19.9, 25.0, and 52.9% in IVF and 15.5, 29.0, and 63.0% in ICSI; all comparisons significant within IVF and within ICSI, $P \leq 0.003$).

Because the microinjections were often performed late in the afternoon, there was an increased proportion

of two-cell-stage embryos at 48 hr after oocyte recovery in ICSI compared with IVF (Table III). Oocyte insemination was performed at 13.30–15.30 hr in 328 conventional IVF cycles, and microinjection at 15.30–17.30 hr in 95 ICSI cycles. Between these cycles, there were no significant differences in the numbers of cells in embryos or their morphology on day 2.

In addition to the association between embryo quality and the cell stage of the embryos, there were more good-quality embryos in IVF cycles with a smaller number of collected oocytes ($P = 0.012$). After either IVF or ICSI, the cleavage rates of zygotes and embryos and the morphology of embryos were not affected by the rate of oocyte nuclear immaturity, seminal paramete-

Table III. Embryo Parameters in IVF and ICSI^a

	IVF			ICSI			<i>P</i>
	Mean	Median	<i>n</i>	Mean	Median	<i>n</i>	
Cleavage rate of 2 PN zygotes (%)	94.4	100	404	96.0	100	218	0.003
Grade 1 embryos of all embryos (%)	15.2	0	404	16.0	0	218	ns
Grade 1–2 embryos (%)	48.3	50.0	404	48.7	50.0	218	ns
Grade 4–5 embryos (%)	20.8	12.5	404	21.3	11.8	218	ns
2-cell-stage embryos (%)	55.2	54.6	404	66.2	66.7	218	0.00006
Microinjection 2 hr later than insemination ^b	57.7	57.1	328	62.0	66.7	95	ns
Microinjection 3 hr later than insemination ^c	55.6	57.1	328	66.1	66.7	87	0.006
Microinjection 4 hr later than insemination ^d	55.6	57.1	328	70.3	75.0	65	0.0008
4-cell-stage embryos (%)	39.6	39.6	404	30.4	25.0	218	0.0002
6-cell-stage embryos (%)	5.1	0	404	3.4	0	218	0.016
Intact embryos after thawing (%)	56.9	55.6	205	62.4	66.7	121	ns
Totally degenerated embryos after thawing (%)	13.7	0	205	13.9	0	121	ns

^a Only cycles with all collected oocytes allocated to either IVF or ICSI. Embryos cultured 48 hr after oocyte collection. PN, pronucleus; ns, not significant.

^b Inseminations at 1330–1530 and microinjections at 1530–1730 hr.

^c Inseminations at 1300–1500 and microinjections at 1600–1800 hr.

^d Inseminations at 1300–1500 and microinjections at 1700–1900 hr.

ters, the number of inseminated spermatozoa (in IVF cycles), or the previous IVF fertilization rate (in ICSI cycles).

The implantation rate in ICSI cycles was similar to that in IVF cycles (18.2% in IVF and 19.5% in ICSI, second-day transfers to women younger than 40 years of age). In addition, the implantation rate in transfers with only two-cell-stage embryos was similar in IVF and ICSI (17.7% in IVF and 16.2% ICSI; transfers to women younger than 40 years of age). In both IVF and ICSI, the implantation rate was higher when only four-cell-stage embryos were transferred (24.5% in IVF and 30.9% in ICSI) than in transfers with only two-cell-stage embryos. The difference between implantation rates of two-cell- and four-cell-stage embryo transfers was only significant in ICSI ($P = 0.006$). The indications for ICSI did not influence the implantation rates, nor did any seminal parameter in IVF or ICSI.

Embryo Survival After Freezing and Thawing

Conventional IVF and ICSI did not differ in regard to the rates of partial or total degeneration of embryos after thawing (Table III). In both IVF and ICSI, the proportion of intact two-cell-stage embryos after thawing was higher (64.8 and 66.5%) than that of the four-cell (45.2 and 51.0%; $P < 0.00001$ and $P = 0.0005$ in IVF and ICSI, respectively), or six-cell-stage embryos (39.3 and 40.7%; $P = 0.0001$ and $P = 0.007$ in IVF and ICSI, respectively). In IVF, the rate of survival showed an inverse correlation with the proportion of poor-quality embryos frozen ($\rho = -0.26$, $P = 0.0002$). In ICSI, the rate of survival was lower in the cycles with poor seminal parameters as the indication for ICSI (mean 58.7%; $n = 69$), compared with the cycles with previous poor fertilization in IVF as the indication (mean, 72.9%; $n = 39$; $P = 0.008$). Otherwise, seminal parameters had no significant effect on embryo survival.

DISCUSSION

In the present study, fertilization, embryo quality, and postthaw survival were studied in detail in simultaneous IVF and ICSI programs. Seminal parameters were important for normal fertilization in IVF, and they were also associated with abnormal fertilization after both IVF and ICSI. Otherwise, the influence of seminal quality on the cleavage of zygotes and embryos, and embryo morphology, implantation, and

cryosurvival rates was negligible. These issues have been controversial, but our results agree with previous ones showing unimpaired embryo quality in patients with poor semen in IVF (26,27) and ICSI (28,29). Despite the poorer seminal quality in ICSI, no differences were noted between IVF and ICSI as regards embryo quality or cryosurvival.

After ICSI, fertilization and implantation have been better when the indication for ICSI has been poor quality of spermatozoa rather than previous poor fertilization in IVF (30,31). We did not find such a difference, which agrees with an earlier report on implantation (32). However, the cryosurvival of embryos was better in the ICSI cycles with previous poor fertilization in IVF than in those with poor semen.

There were no differences in the numbers of oocytes or those of mature oocytes collected or in the mean fertilization rates between IVF and ICSI. In the IVF cycles, there were fewer abnormally fertilized and degenerated oocytes, but more couples with unexpected poor fertilization and a lower overall cleavage rate of normally fertilized oocytes. Thus, more embryos were obtained after ICSI than after IVF (Table II).

In this study, the fertilization rate of mature metaphase-II oocytes in IVF was diminished when the proportion of metaphase-I oocytes in the collected oocyte population was high. Nuclear immaturity is more likely in oocytes originating from small follicles and if the number of collected oocytes is large (33). In these conditions, signs of cytoplasmic immaturity such as premature chromosome condensation (34) are more frequent (35). This may explain the lower fertilization ability of metaphase-II oocytes in the cycles with a high proportion of nuclear immaturity.

In clinical practice, prolonged ovarian stimulation may not lead to a continuous rise in the rate of success, as the quality of oocytes originating from very large follicles may be compromised (16). This may also be the case with excessive ovarian stimulation and a large number of collected oocytes. In our study, there was an increased incidence of 1-PN and 3-PN formation and an impaired zygote cleavage rate in the cycles with large numbers of collected oocytes in both the IVF and the ICSI groups. In addition, embryo morphology was poorer in IVF. The adverse effects of a high ovarian response to stimulation have been shown earlier in conventional IVF (36,37).

After ICSI, the development and disappearance of PN and the first cell division have been reported to take place 2–4 hr earlier than after insemination in IVF (38). The present results show that when later

cleavage stages are considered, the time difference is not longer than approximately 2 hr (Table III). As the microinjections in our program were often performed late, there were more two-cell-stage embryos on day 2 in the ICSI cycles compared with the IVF cycles.

On day 2, the two-cell-stage embryos had better morphology, poorer implantation capacity, and an increased cryosurvival rate compared with more advanced embryos after both IVF and ICSI. However, the overall embryo morphology, implantation capacity and post-thaw survival of the IVF and ICSI embryos were similar, which is in agreement with previous reports (17,22,23). In clinical practice, it may be advantageous to freeze embryos in the two-cell stage, if propanediol and sucrose are used for cryoprotection, as demonstrated in this study as well as in a previous report on IVF (39). However, the faster cleaving embryos that appear at the four-cell stage on day 2 should be preferred in fresh transfer.

CONCLUSIONS

Embryo development, implantation rate, and cryosurvival were not affected by semen quality. Similar fertilization rates, embryo morphology, implantation rates, and postthaw survival of embryos were observed after IVF and ICSI. Fertilization rates in IVF were influenced by the immaturity of oocytes in the collected oocyte population and the quality of spermatozoa. A high response to ovarian stimulation resulted in a higher abnormal fertilization rate and a lower cleavage rate of zygotes in both IVF and ICSI and impaired embryo morphology in IVF. At 48 hr after oocyte collection, the rate of development of the ICSI embryos was 2 hr faster than that of the IVF embryos. Two-cell-stage embryos survived better than four- to eight-cell-stage embryos after cryopreservation with propanediol and sucrose on day 2.

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