ANDROLOGY

Impact of Fertilization History and Semen Parameters on ICSI Outcome

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Purpose: The objectives of this study were (1) to investigate intracytoplasmic sperm injection (ICSI) outcome according to its indications, i.e., a history of failed or poor fertilization and unsuitable sperm parameters for conventional IVF, and (2) to examine the impact of a female's age, sperm concentration, motility, morphology, presence of antisperm antibodies, and hemizona assay (HZA) results on overall outcome.

Methods: Two hundred seventy-nine ICSI cycles performed in 207 couples were retrospectively evaluated.

Results: Clinical pregnancy and delivery rates were 36.8 and 29.8% for patients with prior failed fertilization, 23.2 and 17.8% for patients who had prior poor fertilization, and 28.6 and 21.3% for patients with unsuitable sperm parameters. The differences among all groups were found to be insignificant. There was a significant, negative correlation between a female's age and pregnancy results. No difference was found in the three basic sperm parameters between those patients who produced and those who did not produce a pregnancy, but the fertilization rate was significantly higher in patients with more adequate sperm parameters. Although there was a trend toward a better fertilization rate in patients with a hemizona index (HZI) greater than 30 (indicative of a superior sperm-zona pellucida binding capacity) than in those with a HZI less than 30, the difference was not significant. There were no differences in fertilization rate according to the presence or absence of antisperm antibodies.

Conclusions: Fertilization history in a conventional IVF cycle has no effect on success rates following ICSI, and there is no correlation among the basic sperm parameters, the presence of antisperm antibodies, and pregnancy rates.

KEY WORDS: fertilization; intracytoplasmic sperm injection; pregnancy rates; semen parameters.

INTRODUCTION

Since the first successful pregnancy reported by Palermo and co-workers (1), intracytoplasmic sperm injection (ICSI) has become the treatment of choice for severe male infertility and for couples with prior failed or poor fertilization rates in conventional IVF. Many cases of severe male infertility are refractory to IVF even after using Percoll density-gradient centrifugation (2); a high concentration of spermatozoa at insemination (3), stimulation of sperm motility with pentotifylline (4), or removal of cumulus cells before insemination (5), therefore requiring techniques for assisting fertilization. Initial results with partial zona dissection (PZD) (6) and subzonal insertion (SUZI) (7,8) showed relatively low fertilization rates, high polyspermy rates, and the inability to treat severe oligospermia. Consequently, ICSI has been adopted as the only microassisted fertilization technique at most IVF centers. This technique also provides the opportunity to treat patients with congenital absence of the vas deferens after microsurgical epididymal aspiration (9) and those in whom only testicular sperm can be retrieved due to obstructive and nonobstructive azoospermia (10,11).

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Fertilization rates of 60 to 70% and clinical pregnancy rates of 25 to 35% have been reported with ICSI (12–15). Age of the female partner and gamete quality influence success rates in IVF as well as in ICSI (13,16,17). Although no correlation has been found between sperm parameters and success rates in most previous reports (13,18,19), the data regarding treatment outcome following ICSI in patients with prior failed or poor fertilization in conventional IVF are scant.

Van Steirteghem *et al.* (8) reported acceptable rates of fertilization in patients with normal semen parameters with prior failed or poor fertilization outcome in standard IVF. Nevertheless, patients with fertilization failure following standard IVF may have a fertilization defect that may also persist through ICSI cycles, particularly if associated with severe sperm dysfunctions. Therefore it could be hypothesized that success rates with ICSI might be lower in patients with prior failed or poor fertilization with conventional IVF. The aim of this study was to investigate if the treatment outcome differs between patients with a history of failed or poor fertilization in previous IVF attempts and patients who underwent ICSI with other indications. We also evaluated the impact of the female's age, the three basic sperm parameters (i.e., concentration, motility, and morphology), the presence of antisperm antibodies, and hemizona assay scores (an evaluation of sperm zona–pellucida binding capacity) on fertilization and pregnancy results following ICSI.

MATERIALS AND METHODS

Patient Selection

A total of 207 couples undergoing 279 consecutive ICSI cycles between April 1994 and March 1996 was evaluated retrospectively (results from the first 92 couples subjected to ICSI treatment have been published previously) (13). The ages of the women ranged from 24 to 44 years (average, 34.9 ± 3.9 years). Indications for ICSI were (i) previous total fertilization failure of mature oocytes ($n = 57$); (ii) poor fertilization ($n = 56$), defined as a fertilization rate of mature oocytes less than 50% during one or more prior IVF attempts (fertilization values less than 50% are considered abnormal in our IVF program and therefore assigned to the category of male factor in assisted reproduction) (20); (iii) poor sperm parameters unsuitable for conventional IVF ($n = 136$) [defined as less than 1 million total motile sperm after separation and/or poor sperm–

zona pellucida binding capacity under hemizona assay (HZA) conditions with a hemizona index (HXI) less than 30 (21)] ; and (iv) other indications ($n = 30$, including 1 patient with retrograde ejaculation, 4 cases of epididymal aspiration due to congenital absence of the vas deferens, 5 patients in whom testicular sperm extraction was performed due to nonobstructive azoospermia, and 20 patients with normal semen parameters who had undergone previous IVF cycles with repeated failure to achieve a pregnancy). Patients were enrolled in the ICSI program after written consent was obtained following approval of the Institutional Review Board of Eastern Virginia Medical School.

Ovarian Stimulation, Oocyte Retrieval, and Embryo Transfer (ET) Procedures

Ovarian stimulation was performed using a combination of a GnRHa and gonadotropins in patients 38 years of age or younger or with a normal day 3 serum FSH level (IMX; Abbott Laboratories, Abbott Park, IL). Leuprolide acetate (LA; Lupron; Tap Pharmaceuticals, Abbott Park, IL) was begun in the midluteal phase of the preceding cycle at a dose of 1 mg/day sc; the dose was reduced to 0.5 mg/day on the first day of the menses and continued until the day of hCG administration. Follicle stimulating hormone (Metrodin, Serono, Randolph, MA) or human menopausal gonadotropin (Pergonal; Serono, MA) was started on day 3 of the menstrual cycle with a total of four ampoules daily and then individualized in a step-down fashion. Female patients older than 38 years or with basal serum FSH levels greater than 10 mIU/ml were stimulated with either pure gonadotropins beginning on the third day of the cycle or a combination of GnRHa started in the preceding luteal phase and discontinued at the time of menses (stop Lupron protocol). In these couples the initial gonadotropin dose was 6–8 ampoules; this dose was individualized thereafter in a step-down fashion. For both protocols, 10,000 IU hCG (Steris Laboratories, Phoenix, AZ) was administered when three or more follicles reached 16 mm in diameter. Transvaginal follicular aspiration was performed 35–36 hr later, and embryo transfer 2 to 3 days after aspiration. Progesterone supplementation (50 mg/day im) was begun on the day of transfer and was continued until the pregnancy test was performed and, if positive, given until the tenth week of pregnancy. All patients underwent an initial serum test for β -hCG 12–14 days after embryo transfer. A clinical pregnancy was defined as the presence of a gestational sac by ultrasound at 5 weeks.

Gamete Preparation and ICSI Procedures

Sperm concentration and motility were assessed by computer-assisted semen analysis (Hamilton-Thorne Research, Beverly, Massachusetts); manual analysis was performed in patients with severe oligoasthenospermia. Sperm morphology was evaluated using strict criteria after Diff-Quick staining (Baxter Scientifics, Columbia, Maryland) (22). A swim-up (23) procedure was performed for the selection of superior motile sperm for the ICSI procedure.

Cumulus cells were removed from the oocytes by placing them in 0.1% hyaluronidase (H-3506; Sigma Chemical Co) in Ham's F-10 medium (GIBCO Laboratories, Grand Island, New York) supplemented with 15% human fetal cord serum for 10 to 30 sec. Oocytes then were transferred to fresh medium and repeatedly pipetted through a 27-gauge needle attached to a 1-ml syringe for removal of any adhering corona cells.

Egg-holding and injection micropipettes were produced using a Sutter pipette puller (P-87; Sutter Instrument Co., Navato, California) and a Narishige microforge (MF-9; Narishige USA, Inc., Greenvale, New York). Egg-holding and injection pipettes were prepared with inner diameters of 20 to 35 and 5 to 7 μm , respectively. A sharp beveled point was produced on the injection pipet by grinding the tip to 45° angle with a Narishige beveler (EG-4; Narishige USA, Inc.). Control of the fine movements within the injection pipette was provided by a picoinjector (Medical Systems, Inc., New York). Narishige micromanipulators were used in conjunction with a heated microscope stage (Mini-tub; TS Scientific, Perkasi, Pennsylvania) on a Nikon Inverted Microscope (Nikon Inc., Garden City, New York).

For the procedure, a dish was prepared by adding a 100- μl drop of 10% polyvinylpyrrolidone (PVP; MW = 360,000; ICN Biochemicals, Cleveland, Ohio) in medium to the center. To the center of this drop, 1 to 10 μl of the sperm sample was added. Drops of medium, one for each oocyte to be injected, were added around the central PVP drop, and the entire dish was covered with washed mineral oil that had been equilibrated at 37°C in 5% CO₂ overnight. The medium for injection consisted of Earle's balanced salt solution (GIBCO) supplemented with 15% human fetal cord serum, also equilibrated overnight before use.

Immediately after dish preparation, oocytes were added to the medium droplets and the dish was placed on the heated stage (37°C) of the inverted microscope. After alignment of the pipets within the dish, a motile or "twitching" sperm was identified and rendered non-

motile by striking the sperm at right angle in the area of the midpiece with the edge of the injection pipette. The nonmotile sperm then was aspirated tail first into the pipette. With the oocyte held in place by the egg-holding pipette with the polar body at the 12-o'clock position, the injection pipette was pushed firmly through the zona pellucida, deep into the cytoplasm. A small amount of cytoplasm was aspirated into the injection pipette and then was expelled along the spermatozoon. This was done to ensure that the plasma membrane was broken and that the oocyte was activated, a step necessary for continued development. Injected oocytes were washed in fresh medium and incubated in a standard fashion until evaluated for fertilization.

Only metaphase II polar body-bearing oocytes underwent the injection procedure. Metaphase I oocytes remained in culture until extrusion of the first polar body, at which time they underwent the injection procedure. Three or four embryos were replaced in patients younger than 34 years of age, four or five embryos were replaced in patients 35 to 39 years of age, and five or six embryos were replaced in patients 40 years of age or older. Extra embryos were cryopreserved at either the pronuclear or the cleavage stage and transferred in estrone/estradiol-supplemented cycles.

Hemizone Assay (HZA)

Tight binding of sperm to the human zona pellucida was assessed using the HZA during the initial workup as detailed previously (24). Briefly, salt-stored human oocytes were microbisected into matching hemizonae using Narishige micromanipulators mounted on a phase-contrast inverted microscope (Nikon Diaphot, Garden City, New York). One hemizona was coincubated with patient's swim-up processed semen, whereas the matching hemizonae was incubated with donor's sperm. Four hours after gamete coincubation, the hemizonae were washed to remove loosely attached sperm using a finally drawn glass pipette and the number of sperm tightly bound to the outer zona surface was counted using a phase-contrast microscope (magnification, $\times 200$). Results are expressed as HZI calculated as follows: number of sperm bound for patient/control $\times 100$ (24). Failed or poor fertilization in IVF is associated with a HZI less than 30 (21,24).

Antisperm Antibodies

MAR test (Fertility Technologies Inc., Natick, Massachusetts) and direct immunobead tests (Irvine Scien-

tific, Santa Anna, California) were used for detection of IgG and IgA antisperm antibodies. A positive test was defined as 30% or more of sperm showing binding in IgG or IgA.

Statistical Evaluation

Clinical pregnancy, delivery, and fertilization rates were compared using contingency tables. Chi-square analysis was used to compare categorical parameters (e.g., poor vs good sperm parameters). Fertilization and pregnancy rates were correlated with a female's age using Pearson's product-moment and Spearman rank-order correlation. Data are presented as mean (X) $\pm$ standard error (SE); P levels less than 0.05 were considered significant.

RESULTS

Overall Results

Retrospective analysis of the 279 ICSI cycles revealed an overall clinical pregnancy rate of 29% and a delivery rate per cycle of 23%. The normal diploid fertilization rate was 64%, and the egg degeneration rate following ICSI was 10% (Table I). Seven pregnancies were established following the transfer of cryopreserved, and all seven pregnancies have been delivered. Thus, so far (some patients still have cryopreserved embryos) the augmented clinical pregnancy and delivery rates per cycle including pregnancies from cryopreservation are 32 and 25%, respectively.

One hundred fifty-one patients underwent a single stimulated cycle, 42 patients underwent two stimulation cycles, 13 patients underwent three cycles, and 1 patient underwent four cycles. Twenty-nine percent (60/207) achieved a pregnancy on their first attempt, 31% (18/56) achieved a pregnancy on their second attempt, and 21% (3/14) achieved a pregnancy on their third attempt.

Table I. Overall ICSI Results in Norfolk (April 1994 to March 1996)

No. of patients	207
No. of cycles	279
No. of oocytes injected	2944
No. of oocytes fertilized	1878 (64%)
No. of oocytes degenerated	286 (10%)
Clinical pregnancy per cycle	29%
Clinical pregnancy per patient	39%
Delivery per cycle	23%
Delivery per patient	31%

Age

Evaluation of the data with respect to the age of the female partner revealed a significant inverse correlation between obtaining a clinical ($P = 0.008$) or delivered pregnancy ($P = 0.005$) and age. For women age 34 years or younger, the clinical pregnancy and delivery rates were 35% (44/127) and 28% (35/127), respectively. For women age 35 to 38 years, these rates were 30% (27/90) and 24% (22/90), respectively; and for women 39 years or older, these rates were 16% (10/62) and 11% (7/62), respectively.

Indications for ICSI

As shown in Table II there were no significant differences among all groups with respect to clinical pregnancy and delivery rates ($P = 0.4$ and 0.4), but there were significant differences in fertilization rates ($P = 0.007$). Failed fertilization and poor sperm parameters groups had higher fertilization rates (68 and 65%, respectively) than poor fertilization and other indications (61 and 58%, respectively).

Sperm Parameters

Semen parameters (count, motility, and strict morphology) recorded prior to initiation of the stimulation cycle, as well as those obtained on the day of the aspiration procedure, were evaluated to determine if semen quality had an impact on ICSI outcome. No significant differences were noted between the three parameters and a patient's ability to achieve a pregnancy and give birth following ICSI (Table III). Patients were also categorized into those presenting with the following: count, less than 20 million per ml; forward progression, less than 50%; and strict morphology, less than 4% normal forms—samples were defined as "poor quality." Samples that did not meet all three of these criteria were considered "good quality." No significant differences were noted in clinical pregnancy and delivery rates between good- and poor-quality samples; however, the fertilization rate was significantly higher in patients with good-quality samples (Table IV) ($P < 0.01$).

Hemizoma Assay (HZA)

A HZA was performed in 39 cases (19% of couples) before the performance of the ICSI procedure. The fertilization, clinical pregnancy, and delivery rates were 64% (237/371), 12% (4/32), and 12% (4/32),

Table II. Clinical Pregnancy and Delivery Rates Based on Indications for Performing ICSI

	Prior failed fertilization	Prior poor fertilization	Poor sperm parameters	Others
Fertilization*	386/567 (68%)	340/55 (61%)	969/1502 (65%)	183/317 (58%)
Clinical pregnancy	21/57 (36.8%)	13/56 (23.2%)	39/136 (28.6%)	8/30 (26.6%)
Delivery	17/57 (29.8%)	10/56 (17.8%)	29/136 (21.3%)	8/30 (26.6%)

* $P = 0.007$.**Table III.** Semen Parameters According to Pregnancy Outcome Following ICSI

Value	Clinical pregnancy			Delivery		
	Yes	No	<i>P</i>	Yes	No	<i>P</i>
Count ($\times 10^6/\text{ml}$)						
Mean	24.0	38.1		27.3	36.0	
SD	24.8	63.8		26.1	61.6	
Median	20.1	19.1	0.3	22.6	18.0	0.7
Motility (%)						
Mean	32.5	33.1		32.3	33.1	
SD	20.9	20.5		20.8	19.6	
Median	32.0	30.1	0.7	32.0	30.0	0.7
Morphology (%)						
Mean	3.7	4.1		4.1	4.0	
SD	2.8	2.9		2.9	2.9	
Median	3.5	4.0	0.3	4.0	3.9	0.8

respectively, for those with an abnormal hemizona index (HZI) under 30 (an index that is predictive of poor IVF outcome). There were no husbands with a HZI between 30 and 35 (borderline index). The same rates for those with a normal HZI (over 35) were 72% (123/171), 33% (7/21), and 29% (6/21), respectively. There was a clear trend toward better fertilization and clinical pregnancy rates for patients with a higher HZI. These differences were not significant ($P = 0.06$ and $P = 0.06$).

Antisperm Antibodies

The fertilization, clinical pregnancy, and delivery rates were 70% (145/207), 36% (8/22), and 36% (8/22) for patients with positive antisperm antibodies, whereas these rates were 63% (784/1235), 39% (73/

185), and 30% (56/185) for patients without antisperm antibodies, respectively. The fertilization rate was higher in patients with positive antisperm antibodies ($P = 0.06$); however there was no difference regarding clinical pregnancy and delivery rates ($P = 0.9$ and $P = 0.4$).

DISCUSSION

Although high fertilization and pregnancy rates have been achieved with ICSI, there is still a group of patients who have poor fertilization even after ICSI. This fertilization failure may be due to oocyte or sperm factors such as total necrospemia, round-head sperm syndrome, isolated defects of sperm chromatin, lack or deficiency of egg activation, few oocytes for injection, intrinsic oocyte abnormalities, and oocyte damage during insemination or it may be unexplained (25,26). Because most of these factors are also responsible for fertilization failure in conventional IVF, it can be hypothesized that fertilization and pregnancy rates might be lower in patients with prior poor or failed fertilization than in other patients. However, because ICSI bypasses many potential barriers to normal fertilization (27), it can overcome at least some of these problems, particularly those related to the presence of abnormal sperm parameters.

In a study evaluating 20 patients with previous total fertilization failure after IVF and 121 patients with a significant male infertility, Tucker and co-workers (28) found that fertilization, clinical pregnancy, and birth rates following ICSI were significantly lower in patients with previous total fertilization failure. In another study, McLachlan and coinvestigators (29) evaluated 65 patients with male infertility (defined as oligoasthenospermia, sperm autoimmunity and epididymal sperm, electroejaculated sperm, or sperm recovered after retrograde ejaculation) and 70 patients with previous poor fertilization (less than 35%) and found that implantation and clinical pregnancy rates per transfer were not significantly different between the two groups. Bourne *et al.* (18) also could not demon-

Table IV. Comparison of Semen Parameters and Success Rates Following ICSI

Rate	Poor quality	Good quality	<i>P</i>
Fertilization	60% (429/716)	65% (1449/2228)	0.01
Clinical pregnancy	27% (17/62)	29% (64/217)	0.7
Delivery	18% (11/62)	24% (53/217)	0.2

strate a difference in fertilization, implantation, and pregnancy rates between patients with previous low fertilization and those with male infertility. In the present study, no significant difference was noted in clinical and ongoing pregnancy rates between patients with prior failed fertilization or prior poor fertilization and those presenting with poor semen parameters. However, failed fertilization and poor semen parameters groups had significantly higher rates of fertilization compared with groups with prior poor fertilization. Sperm from individuals with prior failed fertilization or poor semen analysis may be deficient in their ability to bind and penetrate the zona pellucida and/or oolema, a defect that can be corrected with injection directly into the ooplasm. However, samples exhibiting poor fertilization rates following standard IVF may contain a population of sperm that are incapable of participating in early fertilization events even when some steps (zona and oolema penetration) are bypassed with ICSI. Parrington and coinvestigators (30) described a soluble sperm protein that exhibits Ca^{2+} oscillation-inducing ("oscillogen") activity, an event that triggers egg activation and early development in the embryo. It may be that the sperm of some individuals are deficient in a sperm factor(s) necessary for critical early fertilization events. Fertilization rates also differed significantly between patients with poor and good sperm parameters, but again, we did not find a correlation between semen parameters and clinical pregnancy and delivery rates. These differences in fertilization rate, although statistically significant, may not have a major clinical impact, particularly if a high number of mature oocytes is recovered. In any event, ICSI appears to overcome most of the factors related to sperm parameters that cause fertilization failure in conventional IVF.

It is well-known that female fertility decreases with age and this decline is due primarily to aging of the oocyte. It has been demonstrated that the success rates in IVF as well as ICSI decrease with advanced female age (16,13). In the present study, the clinical pregnancy rate was 35% for patients age 34 or younger, while it was only 13% for patients age 40 or older. These results are in agreement with those of previously reported series.

Although fertilization rates differed significantly between patients with poor and those with good sperm parameters, we did not find a correlation between semen parameters and clinical pregnancy and delivery rates. These findings are in agreement with those of previously reported studies (13,18,19)

We have reported an excellent correlation between HZA and fertilization rate in conventional IVF (24,31).

The predictive role of the HZA in ICSI is not known. In this study, fertilization and clinical pregnancy rates were lower in those patients with a HZI less than 30 (indicative of poor sperm–zona binding capacity) than in those with a normal HZI (greater than 35). These results support the use of the HZA as a sperm function test in the decision making process in male infertility. We are presently using the HZA as a sperm function test to identify prospectively patients at risk for failed fertilization to IVF therapy (21). Patients with an HZI less than 30 are directed to ICSI irrespective of other sperm parameters. The HZA assesses tight sperm binding to the zona pellucida, a step that reflects multiple sperm functions. However, if these dysfunctions are bypassed by ICSI, then we should not expect the test to be predictive of ICSI success or failure.

The role of antisperm antibodies in routine IVF is controversial. Acosta *et al.* (32) reported significantly lower fertilization rates in patients with positive antisperm antibodies on their sperm surface in IVF and GIFT cycles. Although the pregnancy rate was also lower than in controls, the difference was found to be insignificant. In contrast, Pagidas *et al.* (33) found that antisperm antibodies either on the sperm surface or in the female serum had no adverse effect on fertilization and pregnancy rates, but their sample size was too small to make a conclusion. There are few data about the effect of antisperm antibodies in ICSI cycles. Nagy *et al.* (34) obtained significantly higher fertilization rates in patients with positive antisperm antibodies than in the negative group in ICSI cycles. Although they obtained a comparable embryonic development, a higher proportion of poor-quality embryos was obtained with antibody positive samples. They concluded that fertilization, embryonic development, and pregnancy rates after ICSI are not influenced by antisperm antibodies. Similarly, Hamberger *et al.* (35) reported 7 pregnancies of 18 cycles in patients with severe antisperm antibodies, and they suggested that ICSI should be the first choice of treatment for patients with positive antisperm antibodies. In the present study, we obtained similar clinical pregnancy and delivery rates after ICSI in patients with positive and negative antibodies, but the fertilization rate was slightly better in the positive group.

It seems reasonable to conclude that the pregnancy outcome of ICSI in patients with prior poor or failed fertilization is not different from that of patients with poor sperm parameters. Also, the three basic sperm parameters do not influence pregnancy success rates. The female age is one of the most important prognostic factors affecting fertilization and pregnancy results.

Further studies are required to clarify the effect of antisperm antibodies on fertilization and pregnancy rates on ICSI.

REFERENCES

- Palermo G, Joris H, Devroey P, *et al.*: Pregnancies after intracytoplasmic injection of a single spermatozoon into an oocyte. *Lancet* 1992;340:17-18
- Ord T, Patrizio P, Mareello E, *et al.*: Mini-Percoll: A new method of semen preparation for IVF in severe male factor infertility. *Hum Reprod* 1990;5:987-989
- Oehninger S, Acosta AA, Morshedi M, *et al.*: Corrective measures and pregnancy outcome in *in vitro* fertilization in patients with severe sperm morphology abnormalities. *Fertil Steril* 1988;50:283-287
- Yovich JL: Pentoxifylline: actions and applications in assisted reproduction. *Hum Reprod* 1993;8:1786-1791
- Ord T, Patrizio P, Balmaceda JP, *et al.*: Can severe male factor infertility be treated without micromanipulation? *Fertil Steril* 1993;60:110-115
- Cohen J, Malter H, Fehilly C, *et al.*: Implantation of embryos after partial opening of oocyte zona pellucida to facilitate sperm penetration. *Lancet* 1988;2:162
- Fishel S, Jackson P, Antinori S, *et al.*: Subzonal insemination for the alleviation of infertility. *Fertil Steril* 1990;54:828-835
- Van Steirteghem AC, Liu J, Joris H, *et al.*: Higher success rates by intracytoplasmic injection than by subzonal insemination. Report of second series of 300 consecutive treatment cycles. *Hum Reprod* 1993;8:1055-1060
- Tournaye H, Devroey P, Liu I, *et al.*: Microsurgical epididymal sperm aspiration and ICSI: a new effective approach to infertility as a result of congenital bilateral absence of the vas deferens. *Fertil Steril* 1994;62:644-647
- Schoysman R, Vanderzwalmen P, Nijs M, *et al.*: Pregnancy after fertilization with human testicular spermatozoa. *Lancet* 1993;342:1237
- Silber SJ: What forms of male infertility are left to be cured? *Hum Reprod* 1995;10:503-504
- Van Steirteghem AC, Nagy Z, Joris H, *et al.*: High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum Reprod* 1993;8:1061-1066
- Oehninger S, Veeck L, Lanzendorf S, *et al.*: Intracytoplasmic sperm injection: achievement of high pregnancy rates in couples with severe male factor infertility is dependent primarily upon female and not male factors. *Fertil Steril* 1995;64:977-981
- Sherins RJ, Thorsell LP, Dorfmann A, *et al.*: Intracytoplasmic sperm injection facilitates fertilization even in the most severe forms of male infertility: Pregnancy outcome correlates with maternal age and number of eggs available. *Fertil Steril* 1995;64:369-375
- Tournaye H, Liu J, Nagy Z, *et al.*: Intracytoplasmic sperm injection (ICSI): Brussels Experience *Reprod Fertil Dev* 1995;7:269-279
- Tan SL, Royston P, Campbell S, *et al.*: Cumulative conception and live birth rates after *in vitro* fertilization. *Lancet* 1992;339:1390-1394
- Abdelmassih R, Solli S, Moretto M, *et al.*: Female age is an important parameter to predict treatment outcome in intracytoplasmic sperm injection. *Fertil Steril* 1996;65:573-577
- Bourne H, Richings N, Harari O, *et al.*: The use of intracytoplasmic injection for the treatment of severe and extreme male infertility. *Reprod Fert Dev* 1995;7:237-245
- Nagy Z, Liu J, Joris H, *et al.*: The results of intracytoplasmic sperm injection are not related to any of the three basic sperm parameters. *Hum Reprod* 1995;10:1123-1129
- Acosta A. Male factor in assisted reproduction. *Infertil Reprod Med Clin North Am* 1992;3:487-503
- Oehninger S, Franken D, Kruger T: Approaching the next millennium: how should we manage andrology diagnosis in the intracytoplasmic sperm injection era? *Fertil Steril* 1997;67:434-436
- Kruger TF, Acosta AA, Simmons KF, *et al.*: Predictive value of abnormal sperm morphology in *in vitro* fertilization. *Fertil Steril* 1988;49:112-117
- Oehninger S, Acosta R, Morshedi M, *et al.*: Relationship between morphology and motion characteristics of human spermatozoa in semen and in the swim-up sperm fractions. *J Androl* 1990;11446-11452
- Oehninger S, Acosta AA, Veeck L, *et al.*: Recurrent failure of fertilization *in vitro*: Role of the hemizona assay in the sequential diagnosis of specific sperm-oocyte defects. *Am J Obstet Gynecol* 1991;164:1210-1215
- Flaherty SP, Payne D, Swann NJ, *et al.*: Aetiology of failed and abnormal fertilization after intracytoplasmic sperm injection. *Hum Reprod* 1995;10:2623-2629
- Liu J, Nagy Z, Joris H, *et al.*: Analysis of 76 total fertilization failure cycles out of 2732 intracytoplasmic sperm injection cycles. *Hum Reprod* 1995;10:2630-2636
- Uehara T, Yanagimachi R: Microsurgical injection of spermatozoa into hamster eggs with subsequent transformation of sperm nuclei into male pronuclei. *Biol Reprod* 1976;15:467-470
- Tucker MJ, Morton PC, Wright PE, *et al.*: Factors affecting success with intracyto-plasmic sperm injection. *Reprod Fert Dev* 1995;7:229-236
- McLachlan RI, Fuscaldo G, Rho H, *et al.*: Clinical results from intracytoplasmic sperm injection at Monash IVF. *Reprod Fert Dev* 1995;7:247-253
- Parrington J, Swann K, Shevchenko VI, *et al.*: Calcium oscillation in mammalian eggs triggered by a soluble sperm protein. *Nature* 1996;379:364-368
- Franken DR, Acosta AA, Kruger TF, *et al.*: The hemizona assay: its role in identifying male factor infertility in assisted reproduction. *Fertil Steril* 1993;59:1075-1080
- Acosta AA, Van der Merwe JP, Doncel G, *et al.*: Fertilization efficiency of morphologically abnormal spermatozoa in assisted reproduction is further impaired by antisperm antibodies on the male partner's sperm. *Fertil Steril* 1994;62:826-833
- Pagidas K, Hemings R, Falcone T, *et al.*: The effect of antisperm antibodies in male or female partners undergoing *in vitro* fertilization and embryo transfer. *Fertil Steril* 1994;62:363-369
- Nagy ZP, Verheyen G, Liu J, *et al.*: Results of 55 intracytoplasmic sperm injection cycles in the treatment of male-immunological infertility. *Hum Reprod* 1995;10(7):1775-1780
- Hamberger L, Sjogren A, Lundin K, *et al.*: Microfertilization techniques—The Swedish experience. *Reprod Fert Dev* 1995;7:263-268