Assessment of the Dominant Abnormal Form Is Useful for Predicting the Outcome of Intracytoplasmic Sperm Injection in the Case of Severe Teratozoospermia

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Submitted: November 23, 1998
Accepted: February 15, 1999

Purpose: *Our purpose was to investigate the relation between the dominant sperm anomaly and sperm morphology and the outcome of intracytoplasmic sperm injection (ICSI).*
Methods: *Two hundred ninety-five patients who underwent a total of 181 cycles of in vitro fertilization (n = 168) and/or 217 cycles of ICSI (n = 177) between July 1995 and May 1997 at Keio University Hospital were investigated.*
Results: *The rates of fertilization and pregnancy were 63.3 and 27.8%, respectively, in ICSI cycles with ≤4% normal forms. When the percentage of strictly normal morphology was ≤4, the fertilization rate was lower in the case of severely tapered head (13.0%; n = 4) than in the cases of other deformities in ICSI. The acrosomal defect made no difference in the fertilization rate with ICSI.*
Conclusions: *The predominant abnormal form affects the ICSI outcome in the case of ≤4% normal forms.*

KEY WORDS: intracytoplasmic sperm injection; morphology; outcome; teratozoospermia.

INTRODUCTION

The so-called "strict criteria" for evaluation of sperm morphology make it possible to identify male-factor patients and increase the predictive value of sperm morphology for the outcomes of in vitro fertilization (IVF) (1–5), gamete intrafallopian transfer (GIFT) (6), and intrauterine insemination (IUI) (7). Ejaculate with ≤4% normal forms is associated with decreased rates of fertilization, pregnancy, and ongoing pregnancy in IVF (2,3). Reports of successful pregnancies and births after subzonal insemination (SUZI) (8,9) and ICSI (10) have led to an increase in the clinical use of these procedures for patients who failed to achieve fertilization after IVF and those with too few spermatozoa for insemination (11). ICSI is far superior to SUZI and routine IVF for treating severe male infertility (11,12). Individual sperm parameters, such as concentration, progressive motility, and morphology, are not correlated with the outcome of ICSI (11,13,14). Furthermore, a high pregnancy rate has been reported in patients with 0% normal forms (13). However, ICSI is not successful in all patients. Establishment of a useful method of predicting ICSI outcome is an urgent requirement.

Although the percentage of normal morphology is not useful for predicting ICSI outcome, the characteristics of the sperm morphology may be the significant factor. The incidence of structural chromosomal aberrations is about four times higher in spermatozoa with amorphous, round, and elongated heads than in those with morphologically normal heads (15). Severely tapered sperm show a prolonged and incomplete decondensation pattern in the in vitro decondensation condition (16). The present study investigated the effect of sperm morphology on the fertilization rate and pregnancy outcome.

MATERIALS AND METHODS

Patients

We studied 168 patients who underwent a total of 181 cycles of IVF and 177 patients who underwent a total of 217 cycles of ICSI between July 1995 and May 1997 at Keio University Hospital. Couples who

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1058-0468/99/0900-0436$16.00/0

underwent ICSI had previously failed standard IVF and/or had extremely low sperm parameters (≤500,000 progressive motile spermatozoa/ml). One hundred forty-four of 177 ICSI patients had previously undergone IVF and failed. The total number of patients was 295 because 50 patients underwent both IVF and ICSI between July 1995 and May 1997 at Keio University. The mean age of patients was 33.9 ± 0.3 years (range: 20 to 40 years) in the IVF group and 34.1 ± 0.2 years (range: 25 to 40 years) in the ICSI group. Patients were divided into three groups according to the percentage strictly normal morphology (SNM) group I (n = 74), ≤4% normal forms; group II (n = 135), >4 to ≤14% normal forms; and group III (n = 170), ≤14% normal forms.

Follicular Stimulation and Oocyte Retrieval

Follicular stimulation was performed by administration of a gonadotropin releasing hormone (GnRH) agonist, buserelin (Suprecure; Hoechst, Brussels, Belgium), follicle stimulating hormone (FSH; Fertinome; Serono, Brussels, Belgium) or human menopausal gonadotropin [hMG; Pergonal (Teikoku Hormone, Tokyo) or Humegon (Organon, Oss, The Netherlands)], and human chorionic gonadotropin (hCG; Gonatropin; Teikoku Hormone). Buserelin was administered through the nasal mucous membrane in the midluteal phase of the preceding cycle at a dose of 900 μg/day and continued until the day of hCG administration. FSH or hMG was initiated on day 3 of menstruation at an initial dose of 300 IU/day for ≥2 days, which was then adjusted in an individualized fashion using a stepdown protocol. When the leading follicles were >18 mm in diameter, 10,000 IU of hCG was administered to induce ovulation. Transvaginal follicular aspiration was performed 34 to 36 hr after injection of hCG.

Semen Analysis and Selection of Spermatozoa

At the time of oocyte retrieval, semen samples obtained by masturbation after a minimum of 3 days of abstinence were collected and allowed to liquefy for at least 20 min before analysis. The same ejaculates were used for IVF and ICSI. Semen was considered abnormal at sperm densities $<20 \times 10^4$/ml or at a progressive motility <50% (16) or when <14% of the spermatozoa revealed normal morphology (1). The sperm morphology was evaluated by spreading 5 μl of semen on alcohol-cleaned slides. The slides were air-dried for 3 min, fixed for 15 sec in Diff-Quik fixative (0.002 g/L of fast green in methyl alcohol; International Reagents Corporation, Hyogo, Japan), and then stained with Diff-Quik solution 1 (1.22 g/L of eosin G in phosphate buffer) for 10 sec, followed by Diff-Quik solution 2 (1.1 g/L of thiazine dye in phosphate buffer) for 5 sec. A total of 200 spermatozoa per patient was evaluated under oil immersion at ×1000 magnification. The percentage SNM was assessed according to a modification of strict criteria (4). Spermatozoa were considered normal when the head had a smooth oval configuration with a well-defined acrosome involving about 40 to 70% of the sperm head, as well as an absence of neck, midpiece, or tail defects. No cytoplasmic droplets more than half the size of the sperm head should be present. The length of a normal sperm head was 4 to 6 μm, and the diameter 2.4 to 3.5 μm. A micrometer in the eyepiece of the microscope was used to do the routine measurements. We also investigated the predictive value of dominant abnormal forms.

The semen was washed twice by centrifugation at 300g for 10 min in human tubal fluid medium (HTF; GIBCO, Grand Island, NY) with 10% of the patient's serum. The spermatozoa from the pellet were allowed to swim up in 1.0 to 2.0 ml of HTF with 10% of the patient's serum. The concentration of motile spermatozoa in the insemination medium was adjusted to between 100 and 500 $\times 10^3$/ml.

Intracytoplasmic Sperm Injection

For ICSI, oocytes were placed in 0.1% hyaluronidase (H-3506; Sigma, St. Louis, MO) in HTF medium supplemented with 10% of the patient's serum for 10 to 30 sec to remove cumulus cells. An egg-holding micropipette was manually made to adjust an inner diameter of 30 μm using a pipette puller (PN-3; Narishige, Tokyo) and a microforge (MF-79; Narishige). The microinjection pipettes used for ICSI had a 35° angle and an outer diameter of 7 μm (Cook, Queensland, Australia). Microinjection was performed on the heat stage (37°C) of an inverted microscope (IX-70; Olympus, Tokyo). A 3- to 5-μl drop of 10% polyvinylpyrrolidone (PVP; Irvine, Santa Ana, CA) was placed on a dish in medium, and a sperm suspension drop (50×10^3/ml of spermatozoa) was placed below the PVP drop. One dish for each oocyte to be injected was prepared with 3- to 5-μl drops of medium. The dishes were covered with mineral oil. A motile and apparently morphologically normal spermatozoon was chosen from the sperm suspension drop and stunned into immobility in the PVP drop. The tail of

the nonmotile spermatozoon was first aspirated into the pipette. Suction was used to fix the oocyte onto the holding pipette with the polar body at the 12 or 6 o'clock position. The injection pipette was then pushed through the zona pellucida and the oolemma deep into the cytoplasm, and the spermatozoon was injected into the ooplasm. Only metaphase II polar body-bearing oocytes were used for the injection procedure. The fertilization rate in ICSI referred to all injected oocytes. The fertilization rate includes only diploid fertilization (two pronuclei).

Embryo Transfer

Uterine transfer of four- to eight-cell embryos was performed 2 days after oocyte retrieval. The number of embryos transferred was or two less in patients <35 years of age and three in patients ≥35 years of age. Excess embryos were cryopreserved at the cleavage stage. Luteal-phase supplementation was performed with 30 mg/day of retroprogesterone (Duphaston; Daiichi, Tokyo). A patient with a positive hCG was defined when the hCG level on day 14 was more than double that on day 10.

Statistical Analysis

The data are presented as the mean ± SE. Differences in the mean sperm concentration and sperm motility among morphological groups were analyzed by ANOVA because the data were distributed normally. The relation of morphology to the fertilization rate was analyzed by the chi-square test.

RESULTS

Sperm concentration and motility in IVF and ICSI cycles were significantly lower in groups I and II than in group III (Tables I and II) (ANOVA analysis, $P < 0.01$). There were no differences in the maternal age, the number of retrieved oocytes, and the number of embryos per transfer among morphological groups. The overall rates of fertilization and pregnancy per embryo transfer were 63.2 and 35.5%, respectively, in IVF cycles and 63.3 and 23.0%, respectively, in ICSI cycles (Tables I and II). The fertilization rate was significantly lower in groups I and II than in group III in IVF cycles (Table I) (χ^2, $P < 0.01$). The fertilization rate was not different among the three morphological groups in ICSI. The pregnancy rate tended to be higher and the rate of pregnancy loss tended to be lower in group I than in the other two ICSI groups (Table II). The rates of fertilization, pregnancy, and delivery in group III tended to be higher in IVF (67.0, 37.4, and 26.2, respectively) than those in ICSI (56.5, 17.4, and 6.5, respectively) (Tables I and II).

Whereas the data are not shown in tables, the predominant abnormal form made no difference in the IVF and ICSI outcomes when the SNM was >4%. The predominant abnormal form made a difference in the IVF and ICSI outcomes when the SNM was ≤4%. In the four cases whose predominant abnormal form was severely tapered head, tapered heads represented more than 30% of the total population. When the other abnormal forms were predominant, severely tapered head spermatozoa represented less than 10% of the total population. The IVF group included 13 patients

Table I. IVF Outcomes According to the Percentage Strictly Normal Morphology[a,*]

% strictly normal morphology	Total	Group I ≤4	Group II >4 to ≤14	Group III >14
Sperm concentration (×10⁶/ml)	63.6 ± 2.2	41.3 ± 6.9[a]	45.6 ± 3.9[b]	69.8 ± 2.5[c]
Sperm motility (%)	58.5 ± 1.5	46.5 ± 6.5[d]	47.5 ± 3.0[e]	62.7 ± 1.6[f]
Maternal age	33.9 ± 0.3	34.4 ± 1.0	35.1 ± 0.6	33.5 ± 0.4
Retrieved oocytes	8.0 ± 0.3	8.4 ± 1.2	8.0 ± 0.6	8.0 ± 0.3
No. of cycles	181	13	36	132
No. of retrieved oocytes	1453	109	288	1056
No. of embryos per transfer	2.07 ± 0.22	2.22 ± 0.32	2.19 ± 0.28	2.01 ± 0.25
% normal fertilization (2PN)	63.2 (919/1453)	35.8 (39/109)[g]	51.4 (148/288)[h]	67.0 (736/1056)[i]
% ET	77.9 (141/181)	69.2 (9/13)	72.2 (26/36)	81.1 (107/132)
% positive hCG per ET	35.5 (50/141)	22.2 (2/9)	30.8 (8/26)	37.4 (40/107)
% clinical pregnancy per ET	33.3 (47/141)	22.2 (2/9)	26.9 (7/26)	35.5 (38/107)
% delivery per ET	24.1 (34/141)	11.1 (1/9)	23.1 (6/26)	26.2 (28/107)
% pregnancy loss per positive hCG	32.0 (16/50)	50.0 (1/2)	25.0 (2/8)	30.0 (12/40)[b]

[a] Mean ± SE. ET, embryo transfer; PN, pronuclei; hCG, human chorionic gonadotropin.
[b] Including two ectopic pregnancies with a fetal heartbeat confirmed by ultrasonography.
* a vs c, b vs c, d vs f, and e vs f: $P < 0.01$ (ANOVA). g vs h, g vs i, and h vs i: $P < 0.01$ (χ^2 test).

Table II. ICSI Outcomes According to the Percentage Strictly Normal Morphology[a,*]

% strictly normal morphology	Total	Group I ≤4	Group II >4 to ≤14	Group III >14
Sperm concentration ($\times 10^6$/ml)	24.6 ± 1.6	12.8 ± 2.5[a]	25.9 ± 2.2[b]	34.7 ± 2.9[c]
Sperm motility (%)	27.9 ± 1.3	22.4 ± 2.4[d]	27.1 ± 1.7[e]	34.9 ± 2.6[f]
Maternal age	34.1 ± 0.2	33.7 ± 0.4	34.5 ± 0.4	34.0 ± 0.5
Retrieved oocytes	68.1 ± 0.4	8.9 ± 0.6	8.1 ± 0.5	8.1 ± 0.6
No. of cycles	217	62	94	61
No. of injected oocytes	1244	417	521	306
No. of embryos per transfer	2.09 ± 0.21	2.16 ± 0.25	2.12 ± 0.23	1.93 ± 0.28
% normal fertilization (2PN)	63.3 (788/1244)	63.1 (263/417)	67.6 (352/521)	56.5 (173/306)
% ET	84.3 (182/217)	85.5 (53/62)	88.3 (83/94)	75.4 (46/61)
% positive hCG per ET	23.0 (42/182)	30.2 (16/53)	22.9 (19/83)	17.4 (8/46)
% clinical pregnancy per ET	19.1 (35/182)	24.5 (13/53)	18.1 (15/83)	15.2 (7/46)
% delivery per ET	14.2 (26/182)	20.8 (11/53)	14.5 (12/83)	6.5 (3/46)
% pregnancy loss per positive hCG	38.1 (16/42)	31.3 (5/16)	36.8 (7/19)	62.5 (5/8)

[a] Mean ± SE. ET, embryo transfer; PN, pronuclei; hCG, human chorionic gonadotropin.
* a vs b, a vs c, and d vs f: $P < 0.01$. b vs c and e vs f: $P < 0.05$ (ANOVA).

with ≤4% normal forms, whereas the ICSI group included 62 such patients. The fertilization rates in IVF with ≤4% normal forms were lower in the cases of amorphous head (24.3%; $n = 3$) and coiled tail (0%; $n = 1$) than in the cases of other deformities (Table III). The fertilization rates in ICSI with ≤4% normal forms were lower in the case of severely tapered head (13.0%; $n = 4$) and neck deformity (14.3%; $n = 1$) than in the cases of other deformities (Table IV). The fertilization rate of globozoospermia did not differ from those of other deformities. One of six cases of globozoospermia was conceived but resulted in chemical abortion. In the case of acrosomal defect (oval head with small acrosome) with 0% normal forms ($n = 6$), the rates of fertilization and pregnancy were 79.7 and 57.1%, respectively.

DISCUSSION

Although various seminal parameters are correlated with the fertilization rate after IVF (18–20), sperm morphology evaluated according to strict criteria is the most useful parameter for predicting the sperm fertilization capacity in IVF (1–3). Sperm concentration and motility in IVF and ICSI cycles were significantly lower in groups I and II than in group III, showing that sperm morphology is dependent on sperm concentration and motility. The fertilization rate was significantly lower in groups I and II than in group III in IVF cycles, whereas the fertilization rate was not different among three morphological groups in ICSI cycles. The predominant abnormal form made differences to the IVF and ICSI outcomes when the SNM was ≤4%. These findings indicate that the characterization of the sperm morphology is useful for predicting the ICSI outcome.

ICSI has resulted in successful fertilization in patients with previous IVF failure and in those with extremely impaired semen parameters. Individual sperm parameters, such as the concentration, motility, and morphology, are not correlated with the fertilization rate or the pregnancy rate after ICSI (11,13,14). Several studies have shown that the pregnancy rate

Table III. IVF Outcomes According to the Most Frequently Detected Morphology in the Case of ≤4% SNM[a]

Morphology	n	Mean (range) Age	Concentration (10^6/ml)	Mortility (%)	FR (%)	PR (%)	CR (%)	DR (%)
Acrosomal defect	7	35.9 (33–38)	60.0 (30–80)	44.3 (10–80)	45.8 (27/59)	33.3 (2/6)	33.3 (2/6)	16.7 (1/6)
Elongated head	2	35.5 (33–38)	36.0 (2–70)	47.5 (25–70)	60.0 (3/5)	0 (0/1)	0 (0/1)	0 (0/1)
Amorphous head	3	29.3 (27–32)	45.0 (20–75)	46.7 (20–80)	24.3 (9/37)	0 (0/2)	0 (0/2)	0 (0/2)
Coiled tail	1	37	40	60	0 (0/8)	0 (0/0)	0 (0/0)	0 (0/0)
Total	13	34.3 (27–38)	39.7 (2–80)	44.1 (10–80)	35.8 (39/109)	22.2 (2/9)	22.2 (2/9)	11.1 (1/9)

[a] FR, fertilization rate (2PN) of oocytes; PR, pregnancy rate per embryo transfer; CR, clinical pregnancy rate per embryo transfer; DR, delivery rate per embryo transfer.

Table IV. ICSI Outcomes According to the Most Frequently Detected Morphology in the Case of ≤4% SNM[a]

Morphology	*n*	Mean (range) Age	Concentration (10^6/ml)	Mortility (%)	FR (%)	PR (%)	CR (%)	DR (%)
Acrosomal defect	21	32.6 (27–38)	11.5 (0–85)	24.0 (0–70)	65.7 (94/143)	44.4 (8/18)	38.9 (7/18)	33.3 (6/18)
Elongated head	13	34.2 (31–38)	12.0 (0.1–50)	27.7 (5–70)	63.0 (51/81)	30.8 (4/13)	30.8 (4/13)	23.1 (3/13)
Amorphous head	12	33.8 (31–39)	10.8 (0–70)	14.6 (0–50)	73.0 (65/89)	20.0 (2/10)	10.0 (1/10)	10.0 (1/10)
Rounded head	6	33.7 (30–39)	9.9 (0.1–30)	12.7 (6–20)	56.4 (22/39)	16.4 (1/6)	0 (0/6)	0 (0/6)
Severely tapered head	4	36.3 (35–38)	14.0 (0.01–40)	17.5 (0–30)	13.0 (3/23)	0 (0/1)	0 (0/1)	0 (0/1)
Small head	2	33.0 (30–36)	5.0 (0.06–10)	4.5 (3–6)	100 (9/9)	0 (0/1)	0 (0/1)	0 (0/1)
Cytoplasmic droplet	2	36.5 (36–37)	57.5 (25–90)	11.0 (6–16)	71.4 (15/21)	0 (0/2)	0 (0/2)	0 (0/2)
Coiled tail	1	34	0.05	50	60.0 (3/5)	100 (1/1)	100 (1/1)	100 (1/1)
Neck deformity	1	31	15.0	10	14.3 (1/7)	0 (0/1)	0 (0/1)	0 (0/1)
Total	62	33.7 (27–39)	12.8 (0–90)	22.4 (0–70)	63.1 (263/417)	30.2 (16/53)	24.5 (13/53)	20.8 (11/53)

[a] FR, fertilization rate (2PN) of oocytes; PR, pregnancy rate per embryo transfer; CR, clinical pregnancy rate per embryo transfer; DR, delivery rate per embryo transfer.

after ICSI tends to be higher in patients with a lower percentage SNM than in patients with a higher percentage SNM (13,21,22). In the present study, the pregnancy rate tended to be higher in group I than in groups II and III after ICSI, although the difference among groups was not significant. The maternal age, the number of retrieved oocytes, and the number of embryos per transfer were not different among three morphological groups. The rates of fertilization, pregnancy, and delivery in group III tended to be higher in IVF than those in ICSI. Most of the ICSI cases (144 of 177) had had the treatment of IVF and resulted unsuccessfully in fertilization. The pregnancy rates of the patients with >14% normal forms were 6.5% in ICSI, whereas 26.2% in IVF. Patients with >14% normal forms who failed to conceive with IVF hardly conceived with ICSI. There was no remarkable evidence of sperm morphology in the patients with >14% normal forms who failed to conceive with both IVF and ICSI. The other undetermined male or female factors other than the maternal age, number of retrieved oocytes, and sperm morphology may exist to be involved in the establishment of pregnancy.

Strict criteria are characterized by the accurate definition of normal acrosomal morphology (1). The 1987 WHO criterion is also correlated with the IVF outcome combined with acrosomal criteria (23), and acrosomal morphology alone can predict the fertilization potential of IVF (24–26). In the present study, when an acrosomal defect, such as an oval head with a small acrosome, was predominantly abnormal in form in severe teratozoospermia, the fertilization rate and pregnancy rate were 65.7 and 44.4%, respectively, in ICSI. In ICSI which does not require the process of the acrosome reaction, it is unlikely that acrosomal defect affects the rates of fertilization and pregnancy.

The relationship between the implantation rate with ICSI and sperm morphology is controversial. Palermo *et al.* (11) have reported that the implantation rate is lower in patients with ≤4% normal forms than in patients with 5 to 14% normal forms. In contrast, other studies have found higher rates of implantation and clinical pregnancy in patients with <4% normal forms (13,21). The rate of pregnancy loss also has controversial relations with the percentage SNM. In the present study, the rates of clinical pregnancy and delivery tended to be higher and the pregnancy loss rate tended to be lower in patients with ≤4% normal forms compared with those of patients with higher percentages of normal forms. These findings suggest that the female factor may be more dominant than the male factor in the groups of higher percentage of normal forms in ICSI patients.

Although Nagy *et al.* (13) observed high fertilization and pregnancy rates in patients with 100% abnormal forms, the characteristics of the sperm abnormalities have not been described. Poor outcomes have been reported in patients with 100% abnormal heads (27,28), but the rates of fertilization and pregnancy are high in patients with 100% midpiece deformities (28). Lundin *et al.* (29) described the first successful delivery in a patient with 100% acrosomeless sperm (rounded heads). We also found that even patients with 0% normal forms in the presence of an acrosomal defect (oval heads) had high pregnancy and delivery rates after ICSI. In contrast, the fertilization rate was particularly low in cases of severely tapered sperm. Severely tapered sperm show a prolonged and incom-

plete decondensation pattern in the in vitro decondensation condition (16). When the dominant abnormality of the specimen is the severe tapering form, ICSI may not be effective to increase fertilizability compared with conventional IVF, possibly by the defective decondensation potential of the sperm nucleus. Further investigations including chromosomal analysis of different types of abnormal sperm forms are needed to clarify the relationship between sperm morphology and ICSI outcome.

The rate of chromosomal aberrations differs with different sperm abnormalities. Small- or large-headed spermatozoa are not associated with sperm chromosomal abnormalities (15), whereas the incidence of structural chromosomal aberrations is higher in spermatozoa with amorphous and elongated heads than in those with morphologically normal heads (15). There are contradictory reports about the incidence of chromosomal aberrations associated with round-headed spermatozoa (15,30). The rate of malformations of newborn infants conceived after ICSI is somewhat higher than the expected rate in the normal population (31). In this study, the rates of fertilization, pregnancy, and clinical pregnancy in ICSI were high in patients with ≤4% normal forms. Although the reason was uncertain, one possibility is that apparently morphologically normal spermatozoa were selected in ICSI. The rates of fertilization, pregnancy, and clinical pregnancy may not be affected by the percentages of normal morphology when morphologically normal spermatozoa were chosen.

In conclusion, the most important male factor for predicting ICSI outcome is considered to be sperm nuclear morphology which is related to the nuclear-decondensing capability. Evaluation of the percentage SNM and assessment of the predominant type of abnormal morphology are useful for predicting the ICSI outcome. Although ICSI is superior to IVF for treating patients with morphological abnormalities, the selection of spermatozoa is an important issue, especially in patients with 0% normal forms.

ACKNOWLEDGMENTS

The authors would like to thank Kimiko Tokizawa, Masako Horii, Emi Saito, Kaori Amemiya, Akiko Hayakawa, and Hiromi Yoshida for their assistance.

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