Effect of abnormal sperm head morphology on the outcome of intracytoplasmic sperm injection in humans

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The present study was designed to determine the efficacy of intracytoplasmic sperm injection (ICSI) using spermatozoa with abnormal head morphology in 17 cases with total teratozoospermia. A total of 160 oocytes were retrieved and 144 metaphase II oocytes were injected. The fertilization and cleavage rates were 50.7 and 93.2% respectively. Fertilization failure occurred in two couples. A total of 54 embryos were transferred and pregnancy rates per initiated and per embryo transfer cycle were 17.6 and 20.0% respectively, while the clinical pregnancy rates per initiated and embryo transfer cycle were 11.8 and 13.3%. The implantation rate was 3.7% (2/54). Out of two pregnancies achieved, one resulted in abortion in the first trimester. The ongoing pregnancy rates per initiated and embryo transfer cycle were 5.88% (1/17) and 6.6% (1/15) respectively. Although the implantation and ongoing pregnancy rates are very low, ICSI seems to be the only treatment modality in cases where teratozoospermia was total with 100% abnormal head morphology.
Key words: intracytoplasmic sperm injection/pregnancy rate/sperm head morphology/teratozoospermia

Introduction

Sperm morphology as assessed by strict criteria is an excellent biomarker of sperm dysfunction(s) that assists the clinician in determining the source of male infertility and in predicting the outcome of assisted reproductive technologies (Acosta *et al.*, 1989; Franken *et al.*, 1990; Oehninger *et al.*, 1990, 1992). Severely impaired sperm morphology is strongly correlated with fertilization failures *in vitro* (Kruger *et al.*, 1987, 1988). With the successful application of intracytoplasmic sperm injection (ICSI) to clinical practice, high fertilization and pregnancy rates, no different from men with normal sperm morphology, have been obtained in patients with severe teratozoospermia (Cohen *et al.*, 1994; Mansour *et al.*, 1995). In our previous study in cases with absolute teratozoospermia (100% morphologically abnormal spermatozoa), although we achieved high fertilization and cleavage rates, the implantation and ongoing pregnancy rates were rather low, with a high incidence of early pregnancy loss (Taşdemir and Taşdemir, 1997). The sperm head defects may be markers for other sperm defects that significantly impair fertility. Although sperm nucleus defects have been associated with infertility (Zamboni, 1987), data about the effect of head morphology on the outcome of ICSI are lacking in the literature. Therefore in this study we sought to determine the success of ICSI using spermatozoa with abnormal head morphology.

Materials and methods

Patients

A total of 17 couples with totally morphologically abnormal spermatozoa diagnosed on two different occasions were referred for ICSI treatment. Three patients had a history of failed fertilization in a previous in-vitro fertilization (IVF) attempt. All the spermatozoa in the samples had morphologically abnormal heads. The male patients underwent a complete infertility work-up. In four patients the follicle-stimulating hormone (FSH) concentrations were elevated to 17, 18, 21 and 23 mIU/ml. Of the 17 male patients, 11 (64.70%) had had a varicocelectomy operation. The duration of infertility was 6.9 ± 3.4 (mean $\pm$ SD) years. The (mean $\pm$ SD) ages of the male and female patients were 34.8 ± 5.3 (range 24–44) and 29.8 ± 5.1 (range 21–38) respectively.

Sperm sampling and examination

Semen samples were collected by masturbation after 3–5 days of sexual abstinence and were allowed to liquefy for at least 30 min at 37°C before analysis. Sperm concentration and motility were evaluated according to the recommendations of the World Health Organization (WHO, 1992). The concentration and the motility of the samples obtained were 5×10^6 spermatozoa/ml (median with range $0.5 \times 10^6 - 30 \times 10^6$) and 15% (5–45%) respectively. The incidence of progressively motile spermatozoa was 5% (range 0–25%). For morphology assessment, on the day of oocyte retrieval two slides were prepared for each patient and were stained with Spermac as previously described (Tyler *et al.*, 1981; Cohen *et al.*, 1982). At least 100 spermatozoa were evaluated using oil-immersion optics ($\times 1000$) by the same observer for all samples according to strict criteria, described by Kruger *et al.* (1988). In our laboratory, the within-observer correlation coefficient was 0.925. All patients had 0% normal forms by strict criteria; there were no slightly abnormal spermatozoa, all being severely amorphous or bizarre.

Semen was washed in Earle's medium by centrifuging for 5 min at 1.800 *g* after a 30 min liquefaction period. The pellet was put on a three-layer Percoll gradient (90% to 70% to 50%) and then was centrifuged at 300 *g* for 20 min. The 90% Percoll fraction was washed again with Earle's medium for 5 min at 1.800 *g* and the pellet was centrifuged in Earle's medium just before microinjection.

Ovarian stimulation

Pituitary down-regulation was achieved using gonadotrophin-releasing hormone analogue (GnRH-a; Suprefact®; Hoechst, Germany), given as nasal spray starting in the luteal phase and was continued for 14 days. Multiple follicular development was then induced with the injection of gonadotrophins [follicle-stimulating hormone (FSH) and human menopausal gonadotrophin (HMG); Metrodine®; Serono, Istanbul, Turkey and Humegon®; Organon, Istanbul, Turkey]. Follicular development was monitored by serial hormone measurements and ultrasound. Follicles were aspirated under transvaginal ultrasound guidance 34–36 h after injection of 10 000 IU human chorionic gonadotrophin (HCG; Pregnyl®; Organon).

Oocyte preparation

Cumulus cells of the aspirated oocytes were removed by placing the oocytes in a solution of HEPES-buffered Earle's medium with up to 80 IU/ml hyaluronidase (type VIII, specific activity 320 IU/mg; Sigma, St Louis, MO, USA) for about 30 s. The removal of the cells was completed by repeatedly pipetting through a hand-drawn glass pipette. The oocytes then were assessed for maturity using an inverted microscope equipped with a Hoffman modulation contrast system. Maturation stage was recorded as prophase I, metaphase I or metaphase II according to the presence or absence of a germinal vesicle or the first polar body. Oocytes were incubated in Earle's balanced salt solution (EBSS) medium at 37°C in an atmosphere of 5% CO_2 with air covered by mineral oil. Injection was performed only on metaphase II polar body-bearing oocytes.

Intracytoplasmic sperm injection procedure

Egg-holding and injection pipettes were produced from 30 μl borosilicate glass capillary tubes (Drummond Scientific Company, Broomall, PA, USA) with 0.97 mm external and 0.69 mm internal diameter. After washing several times, the pipettes were pulled on a horizontal microelectrode puller (PB-7; Narishige Co. Ltd, Tokyo, Japan). The holding pipette was also cut and fire-polished on a microforge to obtain a 20 μm inner and 80 μm outer diameter. To prepare the injection pipette, the pulled capillary was opened on a microgrinder (EG-4; Narishige) to an inner diameter of 5 μm and an outer diameter of 7 μm; the bevel angle was 45°C, bent by a microforge (MF-9; Narishige).

The oocytes were placed one by one in HEPES-buffered Earle's medium droplets, and 5 μl of resuspended sperm pellet was put in 10% polyvinylpyrrolidone (PVP, P5288; Sigma). The ICSI procedure was done on the heated stage of an inverted microscope (IMT-2, Olympus Corporation, Tokyo, Japan). The microscope was equipped with two coarse positioning manipulators (MM-188 and MO-109, Narishige). A single living spermatozoon was injected into an oocyte as described previously (Kahraman et al., 1996). The injected oocytes were incubated for fertilization and further cleavage.

Further evaluation of injected oocytes and establishment of pregnancy

Oocytes were observed for 16–18 h after the injection procedure. Fertilization was assessed as normal when two clearly distinct pronuclei containing nucleoli were present. At 24 h after fertilization, cleavage of the fertilized oocytes was assessed. The embryos were evaluated according to the blastomere size equality and the relative proportion of anucleate fragments. A maximum of four embryos was transferred in exceptional cases when all the available embryos had >50% fragmentation.

All patients underwent an initial test for serum β-HCG at 12–14 days after embryo transfer. The pregnancy was confirmed when serum β-HCG concentrations were rising on at least two separate occasions

Table I. Outcome of intracytoplasmic sperm injection

No. of cycles	17
No. of retrieved oocytes	160
No. of injected oocytes	144
No. of fertilized oocytes (2PN)	73
Fertilization rate per injected oocyte (%)	50.7
Cleavage rate (%)	93.2
No. of cycles with embryo transfer	15
Total no. of embryos transferred	54
Mean no. embryos per transfer	3.6
No. of β-HCG positive patients	3
No. of clinical pregnancies	2
No. of gestational sacs	2
Implantation rate (%)	3.7
No. of clinical abortions	1
Clinical pregnancy rate per initiated cycle (%)	11.8
Clinical pregnancy rate per embryo transfer (%)	13.3
Ongoing pregnancy rate per initiated cycle (%)	6.7

HCG = human chorionic gonadotrophin.
PN = pronuclei.

at 3 day intervals. Cycles where the β-HCG concentrations remained elevated for a minimum of three consecutive tests at 5 day intervals but failed to progress to a full pregnancy were reported as biochemical pregnancies. Clinical pregnancy was diagnosed by ultrasonography at 7 weeks gestation.

Results

All 17 sperm samples examined on the day of oocyte retrieval were totally teratozoospermic, with 100% abnormal head morphology. The predominant types of morphological abnormality diagnosed for the 17 patients were as follows: amorphous head (n = 12), elongated head (n = 2), small head (n = 2) and large head (n = 1). The outcome of ICSI is shown in Table I. Of the 160 retrieved oocytes, 144 were metaphase II and were injected. Of these, 73 fertilized normally with two distinct pronuclei (2PN), of which 68 achieved cleavage. The fertilization and cleavage rates were 50.7 and 93.2% respectively. Fertilization failure occurred in two couples. A total of 54 embryos was transferred with an average of 3.6 embryos per transfer. The pregnancy test result was positive in three patients and clinical pregnancy was confirmed in two patients. Pregnancy rates per initiated and per embryo transfer cycle were 17.6 and 20.0% respectively, while the clinical pregnancy rates per initiated and embryo transfer cycle were 11.8 and 13.3%. The implantation rate was 3.7% (2/54). Out of two pregnancies achieved, one resulted in abortion in the first trimester. Early pregnancy loss, biochemical pregnancy plus first trimester clinical abortion occurred at the rate of 66.6% (2/3), while the clinical pregnancy loss was 50.0% (1/2). The ongoing pregnancy rates per initiated and embryo transfer cycles were 5.88% (1/17) and 6.6% (1/15) respectively.

Discussion

It is well known that certain seminal parameters such as sperm morphology (Kruger et al., 1988), some aspects of sperm motility (Mahadevan and Trounson, 1984; Liu et al., 1991), and the ability of spermatozoa to undergo acrosome reaction

(Cummins *et al.*, 1991; Taşdemir *et al.*, 1993) are strongly correlated with the fertilization rate *in vitro*. Moreover, several researchers reported that the morphology, evaluated with strict criteria, is the most valuable parameter in predicting the sperm fertilizing capacity *in vitro* (Rogers *et al.*, 1983; Kruger *et al.*, 1986, 1987, 1988). In using strict criteria, a clear threshold of 14% normal sperm morphology was observed (Kruger *et al.*, 1986). There was a drastic drop in the fertilization rate in couples with <14% normal morphology. Furthermore, a severe impairment of fertilization takes place in couples with <4% normal morphology (Kruger *et al.*, 1987, 1988) and the incidence of fertilization failure is significantly high in cases with severe teratozoospermia.

With the introduction of ICSI a new era commenced of successful fertilization in cases of previous IVF failure and in cases of extremely impaired semen parameters. The effect of different sperm parameters on the fertilization and pregnancy rates after ICSI was studied by different researchers. Palermo *et al.* (1993) reported that none of the single sperm parameters, such as concentration, progressive motility or morphology, was correlated with the outcome of ICSI. On the other hand, they found a correlation between the number of progressively motile spermatozoa with normal morphology in the whole ejaculate and the fertilization and pregnancy rates. They also demonstrated that embryos resulting from ICSI with spermatozoa obtained from men with extensive teratozoospermia implanted at a significantly lower rate than those resulting from spermatozoa obtained from men with less severe teratozoospermia; however, they commented that these findings required confirmation on a larger number of pregnancies. Furthermore, a retrospective cohort approach revealed that severe teratozoospermia yields a lower implantation and ongoing pregnancy rate when compared with results from similar couples with normal sperm morphology (Menkveld *et al.*, 1996). It has been reported that in couples with severe teratozoospermia spontaneous term pregnancy rate is very low, whereas the miscarriage rate is higher than in patients with normal sperm morphology (Oehninger *et al.*, 1988). On the contrary Cohen *et al.* (1994) reported that in ICSI there was no significant correlation between the percentage of normal sperm forms, and fertilization and implantation rates. Moreover, Mansour *et al.* (1995) showed that there was no significant difference in fertilization and pregnancy rates after ICSI in patients with severe teratozoospermia as compared with less severe forms, as they were able to inject morphologically well-shaped live spermatozoa in all but eight patients with 100% teratozoospermia. In two patients with 100% teratozoospermia and amorphous sperm head, there was complete failure of fertilization. On the other hand there were excellent fertilization and pregnancy rates in patients with mid-piece deformities.

The sperm head defects may be markers for other sperm defects that significantly impair fertility. Sperm nucleus defects have been associated with infertility (Zamboni, 1987), and the association of teratozoospermia, chromosomal aberrations and male infertility has been documented (Abramsson *et al.*, 1982; Zamboni, 1987). On the other hand, Lee *et al.* (1996) found no increase in chromosome aberrations in spermatozoa with small or large heads, but the incidence of structural chromo-

somal aberrations was about four times higher in spermatozoa with amorphous heads than in those with morphologically normal heads. Furthermore, Rybouchkin *et al.* (1996) demonstrated that globozoospermia was not associated with sperm karyotype abnormalities. It is possible that abnormal sperm head morphology reflects abnormality in spermatogenesis that is manifested by embryos with a low potential for establishing a normal pregnancy. In spermatozoa with morphologically abnormal heads, whether sperm head decondensation defects and DNA abnormalities are also present still needs to be resolved. Human gene expression first occurs between the 4- and 8-cell stages of preimplantation development (Brande *et al.*, 1988). This might explain why the embryonic dysfunction associated with poor morphology pattern expressed itself as poor implantation and not decreased early cleavage. As long as there is a morphologically normal spermatozoon available for injection, it seems that the outcome of ICSI is not related to the incidence of morphologically abnormal spermatozoa in the sample (Svalander *et al.*, 1996).

In the present study, although we achieved high fertilization and cleavage rates, the implantation and the ongoing pregnancy rates were very low with a high incidence of early pregnancy loss. Although in the present study we did not have any control group, during the same period our ongoing pregnancy rate was 27% for ICSI cycles initiated, using morphologically normal sperm injection. These results support the contention of the previous reports showing the association of sperm nucleus defects/chromosomal aberrations with male infertility and early embryogenesis (Abramsson *et al.*, 1982; Zamboni, 1987).

Whatever the cause of abnormal sperm morphology, a common consequence of total teratozoospermia is the failure of fertilization. Although the implantation and ongoing pregnancy rates are low, ICSI seems to be the only treatment modality in cases with totally morphologically abnormal spermatozoa.

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