

# Sperm quality may adversely affect the chromosome constitution of embryos that result from intracytoplasmic sperm injection

Michael Obasaju, Ph.D.,\* Arjun Kadam, Ph.D.,\* Khalid Sultan, M.D.,\* Majid Fateh, M.D.,\* and Santiago Munné, Ph.D.†

New York Fertility Institute, New York, New York; and Saint Barnabas Medical Center, Livingston, New Jersey

**Objective:** To determine whether the high rate of chromosomal abnormalities in the embryos of an infertile couple were caused by a paternal factor that may have involved the sperm centriole.

**Design:** Case report.

**Setting:** Private IVF program.

**Patient(s):** An infertile couple who underwent IVF-ET because of severe male factor infertility and endometriosis.

**Intervention(s):** Preimplantation genetic diagnosis of chromosomal abnormalities in embryos derived from two cycles of ICSI in which the husband's sperm was used and one in which donor sperm was used.

**Main Outcome Measure(s):** Preimplantation genetic diagnosis with fluorescence in situ hybridization using probes for chromosomes X, Y, 13, 16, 18, and 21, and determination of hCG levels.

**Result(s):** Most of the embryos derived from the cycles in which the husband's sperm was used were chromosomally abnormal (82%), whereas all the embryos derived from the cycle in which donor sperm was used were chromosomally normal. The cycle in which donor sperm was used resulted in an ongoing pregnancy.

**Conclusion(s):** Paternal factors, which most likely derive from the centrosome, can contribute to numerical chromosomal abnormalities, which in turn may predispose to implantation failure. (*Fertil Steril*® 1999;72:1113-5. ©1999 by American Society for Reproductive Medicine.)

**Key Words:** Embryo biopsy, PGD, mosaicism, embryo quality

A probable adverse role of the male gamete in preimplantation embryo development has been ascertained indirectly from observations of poor embryo quality, decreased implantation rates, and increased spontaneous abortion rates in the female partners of men with oligoasthenozoospermia who undergo IVF-ET (1). Fertilization rates are not compromised irrespective of the severity of the sperm abnormality. To date, only the nuclear material and the centrosome have been confirmed to participate in zygote formation; the rest of the spermatozoon is degraded by the third mitotic division (2). Therefore, although very low rates of chromosomal abnormalities have been detected in human spermatozoa compared with human oocytes (3), the centrosome could be another source of abnormal embryo development.

With the use of fluorescence in situ hybridization (FISH) techniques, numerical abnormalities can be diagnosed with high efficiency for the chromosomes studied in single cells obtained through the biopsy of preimplantation embryos (4). The paternal origin of numerical chromosomal anomalies can be determined in the sex chromosomes but not in the autosomes with the FISH technique used in this study. A direct link between oligoasthenozoospermia and chromosomal abnormalities in embryos can be inferred through the biopsy of embryos that result from cycles in which normozoospermic donor specimens are used for comparison.

We describe herein a patient who underwent several unsuccessful cycles of IVF-ET with her husband's sperm, including two cycles that in-

Received January 19, 1999; revised and accepted June 24, 1999.  
Reprint requests: Michael Obasaju, Ph.D., New York Fertility Institute, 1016 Fifth Avenue, New York, New York 10028 (FAX: 212-734-6059; E-mail: obasaju@msn.com).

\* New York Fertility Institute.

† The Institute for Reproductive Medicine and Science, Saint Barnabas Medical Center.

0015-0282/99/\$20.00  
PII S0015-0282(99)00391-X

cluded embryo biopsy. She then underwent another cycle with donor sperm that also included embryo biopsy. This management strategy was aimed at determining the possible contribution of paternal factors to the failure of the IVF-ET attempts with her husband's sperm. The study was approved by the internal review board of the New York Fertility Institute.

## CASE REPORT

A 29-year-old karyotypically normal woman (46,XX) with a 4-year history of infertility presented to the New York Fertility Institute for clinical evaluation and treatment. Stage III endometriosis was diagnosed at laparoscopy and the patient was referred for IVF after she failed to conceive in several IUI cycles. Her husband was a 30-year-old karyotypically normal man (46,XY) with a history of varicocele and oligoasthenozoospermia. IVF with intracytoplasmic sperm injection (ICSI) was recommended because of his poor semen characteristics.

The patient underwent ovulation induction in all cycles using a "short" protocol that included a GnRH agonist (Lupron; TAP Pharmaceuticals, Deerfield, IL) and hMG (Humegon; Organon, West Orange, NJ) or FSH (Metrodin; Serono, Randolph, MA). hCG (Profasi; Serono) was administered when 2–≥3 follicles that measured 17 mm were observed. Oocytes were collected vaginally under ultrasound guidance 35 hours later.

The husband's semen had a volume of 3 mL, a count of  $11.5 \times 10^6$ /mL, 5% motility, and 30% normal morphology by World Health Organization criteria. Comparable values for the donor sperm sample were 1.5 mL,  $106 \times 10^6$ /mL, 50%, and 50%, respectively. The semen samples were allowed to liquefy (husband's samples) or were thawed at 37°C (donor sample), and motile sperm were recovered by a modified swim-up procedure. Intracytoplasmic sperm injection was performed on all mature oocytes after removal of the cumulus–corona complex with hyaluronidase 5–6 hours after oocyte retrieval. Intracytoplasmic sperm injection was used in all cycles to eliminate any compounding effect of the method of oocyte insemination on the observed results.

The injected oocytes were assessed for fertilization 16–18 hours later. Normally fertilized zygotes (two polar bodies) were noted, transferred to fresh culture medium under oil, and cultured for an additional 48 hours. Cleaved embryos were assessed for blastomere number and scored for degree of fragmentation. Embryo biopsy was performed in two cycles in which the husband's sperm was used and in one cycle in which donor sperm was used.

Embryos were replaced into the uterus 72 hours after retrieval, except in cycles that included embryo biopsy; in these cycles, transfers were delayed another 6–8 hours to allow for FISH analysis. In such cases, only chromosomally normal embryos were transferred.

Luteal phase support was provided in the form of progesterone in oil (50 mg IM daily); therapy was begun in the evening after oocyte retrieval and continued until either a pregnancy test result was negative or a viable embryo was observed on ultrasound examination. Pregnancy was defined as the detection on ultrasound examination of a gestational sac with an embryo that had cardiac activity.

The patient's first four unsuccessful IVF-ET cycles were unremarkable except for the poor quality of the embryos. The next cycle resulted in a pregnancy that ended in a missed abortion because of a chromosomal abnormality (XXY) after 12 weeks. The cycle after that resulted in a biochemical pregnancy (highest hCG level, 352.2 mIU/mL; baseline hCG level, 63 mIU/mL). After a review of all six cycles, preimplantation genetic diagnosis for numerical chromosomal abnormalities was recommended. The patient underwent two cycles in which embryo biopsy was performed and the cells obtained were analyzed with probes for chromosomes X, Y, 13, 16, 18, and 21 (4). The efficiency rates for the detection of numerical abnormalities in the intended chromosomes is approximately 85% (the error rate is  $\geq 10\%$ ) (4).

In the first cycle, two of five embryos biopsied had a normal chromosomal constitution and were transferred. No pregnancy ensued in this cycle. In the next cycle, all six embryos biopsied were genetically abnormal and no transfers were performed. The patient then was offered IVF with ICSI using donor sperm, followed by biopsy of the resultant embryos in an attempt to shed further light on her problem. All six embryos biopsied from the donor sperm cycle had a normal genotype for the chromosomes analyzed (Table 1). Embryo transfer was performed and a pregnancy resulted.

In the three cycles that included preimplantation genetic diagnosis (Table 1), 82% of the embryos were abnormal when the husband's sperm was used compared with 0 when donor sperm was used. The abnormalities observed, although difficult to assess in a single cell, included haploidy, polyploidy, and (primarily) chaotic mosaicism.

## DISCUSSION

There is indirect evidence linking oligoasthenozoospermia with poor embryo quality and an increased incidence of spontaneous abortions in patients who undergo IVF-ET. In this report, a direct link between oligoasthenozoospermia and an increased incidence of chromosomally abnormal embryos was established through the combined use of ICSI with donor sperm and embryo biopsy with FISH analysis.

It appears that paternally derived factors played an important role in the observed chromosomal abnormalities because karyotypically normal embryos were obtained when sperm from a normozoospermic donor was used for ICSI, whereas most of the embryos derived from the husband's sperm were chromosomally abnormal. The involvement of the oocyte in the poor quality of the embryos observed

**TABLE 1**

The results of fluorescence in situ hybridization analysis in the three IVF-ET cycles in which preimplantation genetic diagnosis was performed.

Embryo no.	Chromosome analyzed					Diagnosis
	XY	13	16	18	21	
First cycle						
1	4X	6	2	6	3	Abnormal
2	X	1	3	1	2	Abnormal
3	XX	2	2	2	2	Normal
4	XX	2	2	2	2	Normal
5	0	0	1	1	0	Abnormal
Second cycle						
1	XX	2	3	3	2	Abnormal
2	XXY	2	3	2	2	Abnormal
4	X	0	1	0	1	Abnormal
5	X	NR	1	1	1	Abnormal
6	0	1	2	2	0	Abnormal
Third cycle						
1	XX	2	2	2	2	Normal
2	XX	2	2	2	2	Normal
3	XY	2	2	2	2	Normal
4	XX	2	NR	2	2	Normal*
5	XY	2	2	2	2	Normal
6	XX	2	2	2	2	Normal

Note: NR = no result.

\* Normal for the chromosomes for which results were available.

Obasaju. Sperm quality ICSI. Fertil Steril 1999.

cannot be ruled out because the patient had stage III endometriosis, which can compromise embryo quality.

The ICSI procedure itself did not influence our findings because we used this technique in all the cycles, including the donor sperm cycle that produced all karyotypically normal embryos. In addition, this technique recently was shown to have no teratogenic effects on chromosomes (5). The influence of the fresh sperm versus the frozen-thawed sperm on the observed results could not be determined.

Although the husband was shown to be karyotypically normal, his sperm cells were not analyzed for chromosomal abnormalities; this makes it difficult to establish the incidence of de novo chromosomal abnormalities. The high incidence of chromosomal abnormalities in these embryos suggests the presence of factors other than paternal aneuploidy, the incidence of which is <10% even in karyotypically normal infertile men (3). Further, the abnormalities found in the embryos from cycles that included preimplantation genetic diagnosis and in which the husband's sperm was used were not consistent with paternally inherited an-

euploidies. In fact, the abnormalities involved at least two chromosome types with an abnormal count, which is consistent with chaotic mosaicism (5) and not with aneuploidy.

Chaotic mosaics are embryos that undergo chaotic chromosome segregations, in which most cells are abnormal and there are diverse and abnormal counts in each cell. Because double aneuploidies are rare, the present results are more consistent with chaotic mosaicism than with aneuploidy (5). This may indicate abnormal centriole function rather than sperm chromosomal abnormalities. A few embryos were haploid, but since they developed from two-pronuclear zygotes, they were probably produced by syngamy failure, again probably related to spindle dysfunction.

Centrosomes are the microtubule-organizing centers of the cell; therefore, abnormal microtubule configurations may lead to chromosomal abnormalities such as the mosaicism observed here. For instance, excessive duplication of centrosomes or ectopic assembly of microtubule nucleating proteins could lead to the formation of spindles with multiple poles. Multipolar spindles could segregate the replicated sets of chromosomes into more than two daughter cells (6). Several factors have been involved in the abnormal behavior, number, or control of centrosomes. For example, in cancer, an abnormal number of centrioles leads to aneuploidy in somatic cell lines (i.e., mosaicism) (6).

Further studies are under way to determine the incidence of de novo chromosomal abnormalities in both the husband's sperm and the donor sperm, as well as to elucidate the influence of the sperm centriole on the observed abnormalities. Regardless of the mechanism involved, our findings provide the first direct evidence linking numerical chromosomal abnormalities (mosaicism) in embryos with oligoasthenozoospermia. The management strategy in this group of patients should include preimplantation genetic diagnosis of embryos for numerical chromosome abnormalities coupled with an early recommendation for the use of donor sperm in IVF-ET cycles.

**References**

1. Keifer D, Check JH, Katsoff D. Evidence that oligoasthenozoospermia may be an etiologic factor for spontaneous abortion after in vitro fertilization-embryo transfer. *Fertil Steril* 1997;68:545-8.
2. Sutoyky P, Navara CS, Schatten G. Fate of the sperm mitochondria, and the incorporation, conversion, and disassembly of the sperm tail structures during bovine fertilization. *Biol Reprod* 1996;55:1195-205.
3. Martin RH, Spring E, Ko E, Rademaker AW. The relationship between paternal age, sex ratios, and aneuploidy frequencies in human sperm, as assessed by multicolor FISH. *Am J Hum Genet* 1995;57:1395-9.
4. Munné S, Márquez C, Magli C, Morton P, Morrison L. Scoring criteria for preimplantation genetic diagnosis of numerical abnormalities for chromosomes X, Y, 13, 16, 18 and 21. *Hum Reprod* 1998;9:863-70.
5. Munné S, Cohen J. Chromosome abnormalities in human embryos. *Hum Reprod Update* 1998;4:842-55.
6. Doxsey S. The centrosome—a tiny organelle with big potential. *Nat Genet* 1998;20:104-6.