Assisted reproductive technology may increase clinical mutation detection in male offspring

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Objective: To investigate the risks of chromosome mutation after ART for couples with comparable genetic backgrounds.
Design: Prospective clinical observational study.
Setting: In vitro fertilization center at a tertiary-care, university-affiliated teaching hospital.
Patient(s): Ninety-seven male children whose fathers have normal spermatogenesis were recruited, including 19 babies conceived through IVF, 18 babies conceived through intracytoplasmic sperm injection (ICSI), and 60 naturally conceived babies, as well as the babies' fathers.
Intervention(s): Collection of peripheral and umbilical cord blood samples.
Main Outcome Measure(s): The Yq genetic status of the babies and fathers according to 13 Y-specific markers covering four azoospermia factor (AZF) subregions, the karyotype, and the neonatal examination.
Result(s): We found that all children had a normal 46, XY karyotype, but de novo Y-chromosome microdeletions were identified in 1 (5.3%) of 19 IVF offspring and in 3 (16.7%) of 18 ICSI offspring. The incidence of de novo Y-chromosome microdeletion in male children conceived through ICSI or IVF was statistically significantly higher than that in those conceived naturally (10.8% vs. 0). In four babies with microdeletion, one was complicated, with hypospadias.
Conclusion(s): Our results, for the first time, indicate that risks of gene mutation may increase in the ART offspring, even though their fathers have normal spermatogenesis and genetic background. Hence, intense attention should be placed on genetic safety in the ART children, and the benefits and risks of adopting ART should be balanced gingerly. (Fertil Steril® 2008;90:92–6. ©2008 by American Society for Reproductive Medicine.)

Key Words: Assisted reproductive technology, de novo, Y-chromosome microdeletion, mutation, hypospadias

Assisted reproductive technology (ART) has provided great benefit for the millions of couples who struggle with an infertility disorder; meanwhile, the growing cohort of children conceived through ART also underscores the importance of considering potential risks. There is evidence of greater risks of low birth weight, preterm delivery (1, 2), cerebral palsy (3), and major birth defects (4) after ART, although the cause remains unknown. Some researchers have questioned the genetic implications for offspring of ART and suggested higher incidences of fetal sex-chromosomal aberrations (5), de novo chromosomal anomalies (6), and spermatozoal aneuploidy (7) after intracytoplasmic sperm injection (ICSI) procedures. Then, on the basis of these investigations, a hypothesis was formed that ART may lead to the mutation of chromosomes. The main purpose of our study was to check this hypothesis and to evaluate the risks of chromosome mutation in ART offspring whose parents have a normal genetic background.

The existence of an essential spermatogenesis factor called azoospermia factor (AZF) was first suspected in 1976 by Tiepolo and Zuffardi (8). By using polymerase chain reaction (PCR) analysis of Y-specific sequence-tagged sites (STSs), numerous studies involving thousands of infertile and fertile men have demonstrated that various regions of the Y chromosome are deleted in azoospermic and severely oligospermic men, and four nonoverlapping AZF subregions, AZFa, AZFb, AZFc, and AZFd (the proximal AZFc) have been identified (9). There has been a series of reports of Y-chromosome microdeletions that are transmitted vertically from father to son via ICSI and that arise de novo during ICSI (10–16). These studies mainly focused on the genetic defects among ICSI-conceived children by inherited transmission of male sterility. However, the absolute risk of a genetic defect after ART for

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couples with comparable genetic backgrounds has never been investigated.

Polymerase chain reaction analysis of STSs detects chromosome deletions that cannot be detected by karyotyping. Thus, Y-chromosome microdeletion was selected in this study to evaluate the risks of chromosomal mutation after ART procedure. Here, we report the first genetic screening for microdeletions on the Y chromosome in a population of ART children whose fathers had normal spermatogenesis and genetic background.

MATERIALS AND METHODS

Patients

Ethical approval for this project was granted by the institutional review board of the School of Medicine at Zhejiang University. Ninety-seven male children and their fathers were recruited in this study, including 37 ART-conceived babies (19 IVF, 18 ICSI) as the study group and 60 naturally conceived babies as the control group. The infertility factor was female tubal infertility. The child was excluded if the parents had the following situations: abnormal seminal parameters, pesticide exposure, radiation exposure, and living in newly decorated rooms. There were no differences in the sperm parameters and paternal age between the study and control groups ($P>.05$): the mean (±SD) values for semen volumes were 3.0 ± 0.8 and 3.6 ± 1.0 mL, respectively; corresponding values for sperm concentrations were 91.5 ± 71.7 and 94.3 ± 65.5 million/mL, mean progressive motility was $54.3\% \pm 10.9\%$ and $51.8\% \pm 11.8\%$, mean percentages of normal sperm morphology were $57.9\% \pm 5.1\%$ and $56.5\% \pm 6.9\%$, and mean paternal ages were 33.8 ± 4.8 and 32.5 ± 3.0 years. Umbilical cord blood of the babies and peripheral blood of the fathers were collected during the cesarean section. General pediatric examinations were performed at birth to identify any obvious somatic abnormalities of the children.

Chromosome Analysis

Chromosome analysis in blood was performed routinely in all specimens by using a standard protocol.

Y-Chromosomal Microdeletion Assay

Isolation of genomic DNA Lymphocytes were isolated from blood by using lymphocyte separation medium (Hengxin Cop., Shanghai, China), and then genomic DNA was extracted according to the protocol provided with the QIAamp DNA Blood Mini Kit (Promega, Carlsbad, CA).

Primers for PCR All the children were screened for Yq microdeletions by using 13 STSs located in the four AZF regions: SY82, SY84, SY86, SY109, SY130, SY131, SY143, Y-chromosome RNA recognition motif 1, SY152, SY254, SY255, SY158, and SY153. The sizes of the PCR-amplified fragments were 264, 326, 320, 233, 173, 143, 311, 800, 125, 350, 126, 231, and 139 bp. The sex-determining region of Y chromosome gene was used as a positive control with an amplified fragment of 472 bp. The primer sequences of the STSs were derived from the map of STSs that has been published (17–20) and synthesized by Shanghai Sangon Corporation (Shanghai, China).

Polymerase chain reaction amplification and electrophoretic detection Deoxyribonucleic acid was amplified in a 25-μL reaction system consisting of 50–80 ng of genomic DNA, 2 mmol/L $MgCl_2$, PCR buffer, 0.05 mmol/L each deoxyribonucleotide triphosphate (Takara, Dalian, China), 0.2 μmol/L each oligonucleotide primer (Sangon), and 1 IU Taq DNA polymerase (Takara). The cycling reaction was performed in a thermal cycler (PE9600; Perkin-Elmer, Norwalk, CT). Thermocycling conditions consisted of an initial 5-minute denaturation at 94°C, followed by 35 cycles of 94°C for 45 seconds, 50°C to 63°C for 45 seconds, and 72°C for 45 seconds, and a 10-minute extension at 72°C. Polymerase chain reaction products were electrophoresed on a 2% agarose gel, stained with ethidium bromide, and visualized under ultraviolet transillumination.

Statistical Analysis

We used SPSS (version 13.0 for Windows; SPSS, Chicago, IL) to analyze the data. Fisher's exact test, Student's t test, and χ^2 test were performed. A P value of $<.05$ was considered significant.

RESULTS

Neonatal Examination and Chromosome Analysis

All children derived from both ART-conceived and naturally conceived groups had a normal 46,XY karyotype. Two children in the study (ART-conceived) group were found to have birth defects, one congenital heart malformation without de novo Y-chromosome microdeletion and one hypospadias with de novo Y-chromosome microdeletion, whereas no congenital malformation was found in the control (naturally conceived) group.

Y-Chromosome Microdeletion Screening

As shown in Figure 1 and Table 1, Y-chromosome microdeletions were detected in 4 (10.8%) of 37 ART babies, among which there were 2 cases with microdeletions in AZFa (SY82) and 2 cases with microdeletions in AZFb (Y-chromosome RNA recognition motif 1). In the control group, no Y-chromosome microdeletion was found. However, Y-chromosome microdeletions were examined in fathers of the babies, but no microdeletion was detected. The incidence of de novo Y-chromosome microdeletions in the study group was significantly higher than that in the control group (10.8% vs. 0, $P<.05$). The incidence of microdeletion was higher in children conceived via ICSI than in those conceived via IVF (3/18 vs. 1/19) but revealed no statistical significance ($P>.05$).