

Cystic fibrosis gene mutations do not affect sperm function during in vitro fertilization with micromanipulation for men with bilateral congenital absence of vas deferens

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Objective: To assess the effects of cystic fibrosis transmembrane-conductance regulator (CFTR) gene mutations on sperm function and fertility in men with bilateral congenital absence of the vas deferens.

Design: Prospective.

Setting: Division of urologic microsurgery and associated hospital-based IVF unit.

Main Outcome Measures: Fertilization and pregnancy rates.

Patients: Men referred to our fertility unit for treatment of bilateral congenital absence of the vas deferens, using sperm surgically retrieved from the epididymis with IVF and micromanipulation.

Results: Of 45 men with bilateral congenital absence of the vas, 54% (19/35) tested were found to be carriers of CFTR gene mutations, with one compound heterozygote. Epididymal sperm from men affected with CFTR mutations fertilized 19% (29/152) of oocytes, whereas men without mutations fertilized 22% (44/204) of oocytes. Pregnancy rates (PRs) were 36% (5/14) for cycles involving men with CFTR mutations and 33% (5/15) for other patients with congenital absence of the vas deferens but without detectable CFTR mutations.

Conclusions: The presence of detectable CFTR mutations does not affect fertilization rates or PRs for men with bilateral congenital absence of the vas deferens when IVF and micromanipulation are applied. Fertil Steril 1995;64:421-6

Key Words: Cystic fibrosis, congenital absence of vas, IVF, micromanipulation

Bilateral congenital absence of the vas deferens recently has been found to be associated with mutations of the cystic fibrosis transmembrane-conductance regulator (CFTR) gene (1-3). Previous studies

have found that 42% to 64% of men with congenital absence of the vas deferens have detectable CFTR mutations (1-3). The presence of $\Delta F508$ CFTR mutations in men with congenital absence of the vas undergoing sperm retrieval in conjunction with IVF has been reported to affect adversely sperm function, resulting in low fertilization and pregnancy rates (PRs) (4). The common $\Delta F508$ CFTR is associated with severe pulmonary and digestive secretory abnormalities in its homozygous condition.

We previously have reported our results using sperm retrieved from the epididymis of men with congenital absence of the vas deferens and other surgically unreconstructable causes of reproductive tract obstruction in conjunction with IVF and micromanipulation (5). For those 51 sperm and egg retrieval cycles, there was no difference in fertilization

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rates or PRs based on the etiology of reproductive tract obstruction. For men with congenital absence of the vas, fertilization was achieved in 66% of cycles, with clinical pregnancy in 25%. For other men with chronic reproductive tract obstruction, epididymal sperm retrieval yielded fertilization for 68% of cycles and clinical pregnancy in 32%.

Many men with bilateral congenital absence of the vas deferens have detectable CFTR mutations, and men with acquired obstruction rarely carry CFTR mutations. If CFTR mutations were important for the fertilizing capacity of sperm, then one would have expected different fertilization rates and PRs for these different patient groups. Given our observations of the apparent lack of effect of congenital absence of the vas on sperm function, we have analyzed the effects of CFTR gene mutations on sperm function and fertility during IVF with micromanipulation.

MATERIALS AND METHODS

All patients were referred for evaluation of obstructive azoospermia. Physical examination confirmed the absence of vasa deferentia. All men with bilateral congenital absence of the vas deferens were counseled regarding the risks of CFTR gene mutations and the risks of transmission of those mutations to offspring. All couples were recommended to have CFTR gene mutation testing.

For those couples who consented to CFTR gene testing, somatic DNA was extracted from peripheral leukocytes using high salt precipitation of proteins. Polymerase chain reaction (PCR) amplification of genomic DNA was used to amplify CFTR exons 4, 10, 11, 20, and 21, followed by allele specific oligonucleotide probe analysis for the 12 most common CFTR gene mutations (Δ F508, G551D, R553X, W1282X, R117H, G542X, 1717-1, N1303K, R560T, I507, 621+1, and S549N). Previously described primer sequences for the PCRs were used (6-10). The five separate genomic regions within the CFTR gene were amplified simultaneously in a single amplification reaction. Amplified samples were hybridized with allele-specific oligonucleotides (11, 12).

Men undergoing sperm retrieval from the epididymis were explored through a midline scrotal incision where operative confirmation of congenital absence of the vas deferens was provided. Microsurgical epididymal sperm aspiration was performed on the day of the partner's oocyte retrieval as described previously (5). Optimal sperm quality was determined empirically after micropuncture aspiration of epididymal sperm from both epididymides during these retrieval attempts. Excess retrieved sperm were cryopreserved to minimize the need for future aspi-

Table 1 Frequency of CFTR Gene Mutations Detected in 19 Men With Congenital Absence of the Vas Deferens*

Δ F508	10*
W1282X	4
G542X	2
R117H	2*
1717-1	1
N1303K	1

* One patient was a compound heterozygote (R117H/ Δ F508).

rations. Aspirated sperm quality was assessed in the operating room by an embryologist member of the IVF team after dilution of the epididymal specimens in 0.5 mL of human tubal fluid medium (Irvine Scientific, Irvine, CA) (13, 14). The decision to use standard insemination or micromanipulation was based on sperm quality at the time of retrieval (15, 16). In general, standard insemination was used for patients when >10% of the sperm demonstrated progressive motility. Micromanipulation procedures included partial zona dissection, subzonal insertion (SUZI), and intracytoplasmic sperm injection. The details for application of these techniques have been described previously (15, 16). For the last six cycles of sperm and oocyte retrieval in six couples, only intracytoplasmic sperm injection was applied because of the improved results using this technique with very poor sperm quality (17, 18).

Up to four fertilized embryos were transferred to the female partner's uterus and sequential determinations of serum β -hCG levels were obtained. The presence of a clinical pregnancy was detected by pelvic ultrasound demonstration of a fetal heart beat. Statistical evaluation was performed using χ^2 analysis for pregnancy and fertilization rates. Student's *t*-test was used for analysis of semen parameters.

RESULTS

Overall Results

Of the 45 patients with bilateral congenital absence of the vas deferens, 35 agreed to undergo CFTR gene mutation analysis. Nineteen of 35 (54%) men with bilateral congenital absence of the vas deferens were found to have at least one CFTR gene mutation. One man was found to be a compound heterozygote, positive for both R117H and Δ F508 mutations (5%). The most common gene mutation detected was Δ F508, found in 10 of 19 patients (53%) with detectable mutations (Table 1). A total of 40 cycles of egg and surgical sperm retrieval in conjunction with IVF were performed for 33 couples. Overall, 13 clinical pregnancies were achieved in those 40 cycles (32.5%). Eighteen children have been born to 12 couples, and 1 couple experienced a spontane-

Table 2 Fertilization Rate for Oocytes Incubated With Epididymal Sperm From Men With Congenital Absence of the Vas and Documented CFTR Gene Mutations Versus No CFTR Mutations

	Known CFTR mutations	No mutations
IVF alone	2/15 (13)	1/18 (6)
Partial zona dissection	0/2 (0)	2/15 (13)
SZI	15/107 (14)	33/152 (22)
Intracytoplasmic sperm injection	12/28 (43)	8/19 (42)
Total oocytes	29/152 (19)	44/204 (22)

* Values in parentheses are percentages.

ous miscarriage in the first trimester. Overall, 88 of 474 (19%) of all oocytes were fertilized, including 11 of 62 (18%) of oocytes treated with standard insemination, 4 of 41 (10%) oocytes undergoing partial zona dissection, 53 of 324 (16%) oocytes treated with SUZI, and 20 of 47 (43%) oocytes manipulated with intracytoplasmic sperm injection. These fertilization results cannot be compared directly for different micromanipulation procedures because eggs were selected for treatment based on sperm quality. Overall, $79 \pm 61 \times 10^6$ (mean \pm SD) sperm were retrieved per man, with $10.0\% \pm 15.7\%$ sperm motility and $3.6\% \pm 2.9\%$ normal forms using strict criteria sperm morphology evaluation (9).

Men With Documented CFTR Gene Mutations

For 29 of 40 cycles of sperm retrieval, the CFTR gene mutation status of the male partner was known. For 15 of the cycles performed on 12 men, there were no CFTR mutations detectable. Fourteen cycles were performed on 11 men with known CFTR gene mutations. Five of 14 cycles (36%) resulted in a clinical pregnancy. Five couples have delivered six normal healthy infants, and one boy with a perinatally detected ureterocele has been born. (The boy with the ureterocele had an ipsilateral vas deferens palpable.) Overall, 29 of 152 (19%) eggs were fertilized, despite the presence of documented CFTR gene mutations in these men (Table 2).

We separated men with $\Delta F508$ mutations from all other men with known CFTR mutations for analysis, based on prior reports of abnormal fertilization and low PRs for the $\Delta F508$ -affected men. Retrieved epididymal sperm parameters were essentially identical for men with $\Delta F508$ versus other mutations. Mean number of sperm retrieved was $67 \pm 54 \times 10^6$ sperm for men with $\Delta F508$ and $65 \pm 62 \times 10^6$ sperm for other mutations. Mean motility of $11.3\% \pm 5.1\%$ and $11.5\% \pm 6.2\%$ and strict criteria mean normal forms of $3.2\% \pm 3.7\%$ and $4.0\% \pm 3.6\%$ were observed for men with $\Delta F508$ and other mutations, respec-

tively. More importantly, 12 of 64 eggs (19%) were fertilized from men with $\Delta F508$ mutations, resulting in clinical pregnancies in 3 of 7 (43%) cycles. The seven cycles were performed on five men. These results did not differ from the 19% (17/88) fertilization rate and 29% (2/7) PR for men with other known CFTR mutations ($P > 0.5$). There was no difference in fertilization rates or PRs for cycles in which men had documented CFTR gene mutations compared with cycles for men who had evaluation for the most common mutations and no abnormalities were found (Table 2).

Men With No Detectable CFTR Mutations

For 15 cycles performed on 12 men, no documented CFTR gene mutations were identified despite intensive evaluation of all CFTR exons. Retrieval of an average of $89 \pm 61 \times 10^6$ sperm was obtained with $9.6\% \pm 7.8\%$ motility and $3.9\% \pm 3.2\%$ normal forms. Overall, 44 of 204 (22%) oocytes fertilized in these 15 cycles. Within this group, 5 of 15 (33%) cycles resulted in clinical pregnancy. Seven children have been born to five couples (two sets of twins).

Evaluation of Female Partners

For 30 of 33 couples who underwent the 40 cycles of IVF with epididymal sperm, CFTR gene mutation analysis was performed on the female partner of the couple. All 30 women had no detectable CFTR gene mutations. Two women did not have analysis early in our experience because their husbands had no detectable mutations. One woman refused testing and accepted responsibility for any risk of having a child with cystic fibrosis (CF). She also refused embryo biopsy for analysis of CFTR mutations in embryos to be transferred.

Untested Men

Ten men who were not tested for CFTR gene mutations underwent 11 cycles of sperm and egg retrieval. A mean of $89 \pm 61 \times 10^6$ sperm were retrieved with $9.6\% \pm 6.1\%$ motility and $3.9\% \pm 2.7\%$ normal forms with strict criteria evaluation. Fertilization was obtained for 15 of 118 (13%) oocytes. Three of 11 cycles (27%) resulted in clinical pregnancies. The three couples have delivered four healthy infants. Fertilization rates and PRs were similar for cycles in which men had CFTR mutational analysis versus those who did not have evaluation ($P > 0.6$).

DISCUSSION

Previous findings of impaired fertilization using sperm from men with $\Delta F508$ CFTR gene defects (4) are in contrast to our experience. Animal data have

demonstrated CFTR gene expression in the seminiferous tubules of the adult rat testis (19), supporting the possible effect of CFTR gene mutations on spermatogenesis. However, CFTR gene expression is not detectable in the human testis by *in situ* hybridization (20) or Northern blot hybridization, only PCR analysis (Schlegel PN, Mielnik A, unpublished observations).

We found no evidence of effect of the etiology of obstruction (congenital absence of the vas deferens or secondary surgical obstruction) on fertilization rates or PRs in a cohort of couples undergoing 51 cycles of micropuncture retrieval of epididymal sperm in conjunction with IVF and micromanipulation (5). Our further experience, reported in this study, documents three clinical pregnancies using sperm retrieved from men with bilateral congenital absence of the vas deferens who were carriers of $\Delta F508$ gene mutations using IVF and micromanipulation. The fertilization rates and PRs were no different for men with $\Delta F508$ mutations than for men with other mutations or when compared with men with congenital absence of the vas but no detected CFTR mutations. In addition, there was no difference in sperm production, reflected by the number of sperm retrieved from the epididymides, or sperm quality, reflected by percent motility and percent normal forms, between men with $\Delta F508$ or other CFTR mutations. Therefore, our results suggest that the $\Delta F508$ CFTR mutation does not affect adversely spermatogenesis or the fertilizing capacity of spermatozoa compared with men with other CFTR mutations or no detectable mutations.

The frequency of CFTR gene mutations in men with congenital absence of the vas deferens has been documented to be much higher (42% to 64%) than is expected in the general population of white North American men (4%). This association suggests a role of the abnormal CFTR allele in causing the condition of bilateral congenital absence of the vas deferens. The mechanism by which the vas deferens and epididymis develop from Wolffian and mesonephric duct precursors is not well established. Men with congenital absence of the vas rarely have skip lesions of epididymal or vasal development. The epididymis and vas that is present is typically in direct continuity with the testis. These findings are more consistent with an abnormality in secretion and recanalization of the epididymis, not a primary abnormality of the vas deferens or mesonephric duct. If recanalization of the epididymal and vasal lumen is a factor in the normal development of these structures, then a secretory defect of the proximal epididymis, efferent ducts, or rete testis may result in a lack of development of the vas and distal epididymis. Secretory epithelial abnormalities are common in

men with homozygous defects in the CFTR gene. The presence of a secretory abnormality of the testicular "collecting system" (rete testis, efferent ducts, and proximal epididymis) with homozygous CFTR gene mutations is suggested by the nearly uniform prevalence of azoospermia and congenital absence of the vas deferens in men with CF (21). The importance of epididymal epithelial secretion in development of the epididymis and vas is further supported by the finding of CFTR gene expression in human fetal epididymal tissue (22). The heterozygous carrier may demonstrate a more mutable expression, with variable failure or only partial recanalization of the tubular lumen of the epididymis or vas deferens. This embryological defect could result in the wide variations in the amount of epididymis and vas deferens seen in men with bilateral congenital absence of the vas deferens. Because other secretory abnormalities of the rete testis or efferent ducts may prevent recanalization of the epididymis or vas, it is expected that some men with congenital absence of the vas deferens will carry no CFTR gene abnormalities. The role of an abnormality in epididymal secretion preventing recanalization of the developing Wolffian duct structures is supported by the observation that any epididymis and vas present is typically in continuity with the testis. In addition, the presence of normal spermatogenesis with documented fertilizing capacity in men with CFTR mutations suggests that the CFTR defects act distal to the testis in most cases.

It is remarkable that three different investigators, working in three different areas of the United States have found very similar CFTR gene mutation defect rates in men with congenital absence of the vas deferens. Anguiano et al. (1) found 64% of 25 patients were otherwise asymptomatic carriers, with 12% being compound heterozygotes. Patrizio et al. (3) found 59% of their 44 patients positive for at least one CFTR mutation with 9% compound heterozygotes, and we report 54% of our patients with CFTR gene mutations, with 5% of patients being compound heterozygotes. Because the vast majority of CF carriers (4% of the North American population) do not have bilateral congenital absence of the vas deferens, the presence of a CFTR gene mutation is not sufficient to prevent vasal development. The consistent absence of detectable CFTR mutations in up to 40% of men with congenital absence of the vas suggests that other secretory abnormalities may prevent development of the vasa deferentia. However, not all functionally important mutations of the CFTR gene have been described for patients with CF (23).

Our model of epididymal and vasal recanalization during development suggests that other as yet unidentified genes or factors may be involved with se-

cretion by the epithelium of the "testicular collecting duct." However, the presence of CFTR mutations in a consistent subset of men with congenital absence of the vas supports contribution of abnormal CFTR function to maldevelopment of the vas in many patients. Therefore, men with congenital absence of the vas interested in fertility should be screened carefully for CFTR mutations. More importantly, screening of the man's partner for an incidental CFTR carrier condition is critical to predict the risk of a couple to have a child affected by CF. For couples with known CFTR gene mutations undergoing IVF with epididymal sperm, the use of embryo biopsy can allow detection of some homozygous CFTR gene mutations before ET (24, 25). However, differential amplification of the two copies of DNA from the CFTR gene of a single cell may be highly variable, affecting the reliability of single-cell PCR results. Therefore, until additional clinical experience is available with the reliability of these evaluations, the results of these embryo biopsy procedures for pretransfer diagnosis of CF may be considered experimental.

The ability to provide pregnancies for couples in whom the man carries CF gene mutations will result in persistence of these abnormalities in the population as a whole. In addition, there is a minute risk of transmission of CF to the offspring because not all CFTR mutations can be identified before the IVF procedure. The risk of CF transmission must be discussed openly with the couple before the epididymal sperm retrieval procedure.

Patrizio et al. (4), who previously reported poor fertilization rates for sperm from men with $\Delta F508$ mutations, did not use micromanipulation during IVF cycles for these patients. Data from our center (15, 16) and another (17, 18) have suggested that fertilization rates and PRs with micromanipulation are relatively independent of sperm quality. Sperm retrieved from the epididymis have been well documented to have poor motility and abnormal morphology. We previously have shown that the application of micromanipulation is critical to achieve optimal results with IVF using epididymal sperm (5). Therefore, the favorable results presented in this manuscript reflect what we believe are results with the standard therapy for men with congenital absence of the vas deferens. The lack of effect of CFTR mutations with micromanipulation is reflected by the nearly identical fertilization rates and PRs for men with or without CFTR mutations. Even with standard insemination there was no significant difference between fertilization rates for men with known CFTR mutations and those without CFTR mutations, as shown in Table 2. Our data suggest that the mere presence of the CFTR gene defect does not

affect the fertilizing ability of sperm during IVF with micromanipulation.

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