Association of cystic fibrosis transmembrane-conductance regulator gene mutation with negative outcome of intracytoplasmic sperm injection pregnancy in cases of congenital bilateral absence of vas deferens

Shaoming Lu, M.D.,[a] Yanyi Cui, M.Sc.,[b] Xiao Li, M.B.,[a] Haobo Zhang, M.D.,[a] Jiaolong Liu, M.B.,[a] Bin Kong, M.B.,[a] Feifei Cai, M.B.,[a] and Zi-Jiang Chen, M.D., Ph.D.[a]

[a] Center for Reproductive Medicine, Provincial Hospital Affiliated to Shandong University; and [b] Department of Urology, The Fourth People's Hospital of Jinan, Jinan, Shandong, People's Republic of China

Objective: To evaluate intracytoplasmic sperm injection (ICSI) results with regard to congenital bilateral absence of vas deferens (CBAVD) versus non-CBAVD obstruction, cystic fibrosis transmembrane-conductance regulator (CFTR) mutations versus non-CFTR mutations, and miscarriages or stillbirths versus live births per embryo transferred.
Design: Retrospective study with detailed chart review.
Setting: Center for reproductive medicine.
Patient(s): Nine hundred forty-five men with obstructive azoospermia.
Intervention(s): One thousand four hundred fourteen ICSI cycles classified as CBAVD versus non-CBAVD obstruction, CFTR mutations versus non-CFTR mutations, and miscarriages/stillbirths versus live births per embryo transferred.
Main Outcome Measure(s): Frequency of CFTR mutations and rates of fertilization, good embryos, clinical pregnancy, miscarriages and stillbirths, ectopic pregnancy, and live births.
Result(s): CFTR mutations were more prevalent in men with CBAVD than in those with non-CBAVD obstruction. The rate of miscarriages and stillbirths per embryo transferred was higher in men with CBAVD than in those with non-CBAVD obstruction, whereas the rate of live births per embryo transferred was lower in men with CBAVD than in those with non-CBAVD obstruction. The rate of miscarriages and stillbirths per embryo transferred was higher in men with CFTR mutations than in those with non-CFTR mutations. The frequency of CFTR mutations was higher in patients who experienced miscarriages/stillbirths than in those with live births.
Conclusion(s): The frequency of CFTR mutations was higher in cases of CBAVD versus non-CBAVD obstruction. Possibly as a result of CFTR mutations, patients with CBAVD had a significantly increased risk of miscarriage and stillbirth and a reduced rate of live birth compared with patients with non-CBAVD. (Fertil Steril® 2014;101:1255–60. ©2014 by American Society for Reproductive Medicine.)
Key Words: Intracytoplasmic sperm injection, obstructive azoospermia, congenital bilateral absence of vas deferens, cystic fibrosis transmembrane-conductance regulator gene, pregnancy

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/lus-cystic-fibrosis-gene-mutation-icsi-pregnancy/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Received August 12, 2013; revised December 23, 2013; accepted January 21, 2014; published online February 19, 2014.
S.L. has nothing to disclose. Y.C. has nothing to disclose. X.L. has nothing to disclose. H.Z. has nothing to disclose. J.L. has nothing to disclose. B.K. has nothing to disclose. F.C. has nothing to disclose. Z.-J.C. has nothing to disclose.
This work was performed under the auspices of the Provincial Hospital Affiliated to Shandong University, China.
Reprint requests: Zi-Jiang Chen, M.D., Ph.D., Center for Reproductive Medicine, Provincial Hospital Affiliated to Shandong University, Jinan, Shandong, People's Republic of China (E-mail: sdsznk@yahoo.com).

Fertility and Sterility® Vol. 101, No. 5, May 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2014.01.033

Azoospermia occurs in approximately 1% of all men and 10%–15% of infertile men. Most cases stem from an obstruction and show normal spermatogenesis, testes of normal volume, and a normal serum level of FSH (1, 2). Obstructive azoospermia (OA) results from mechanical blockage, which can occur anywhere along the reproductive tract, including the vas deferens, epididymis, and ejaculatory duct (1–3). Common causes of OA include congenital bilateral absence of vas deferens (CBAVD), previous vasectomy, failed vasoepididymostomy, postinfective epididymitis, and other irreparable obstructions (2, 3). The most common congenital form of OA is CBAVD with anomalies in Wolffian duct–derived structures, where the body and tail of the epididymis, vas deferens, seminal vesicles, and ejaculatory ducts are atrophic or absent. CBAVD has been associated with mutations in the cystic fibrosis transmembrane-conductance regulator gene (CFTR); 60%–70% of patients with CBAVD have mutations in this gene (4, 5).

Using the intracytoplasmic sperm injection (ICSI) procedure, men with OA can father their own offspring by percutaneous epididymal sperm aspiration (PESA) (6, 7). The literature contains many studies of the outcomes of sperm acquisition and ICSI (8–10). However, few studies have evaluated the role of CFTR gene mutations on the outcome of ICSI in men with OA CBAVD. Moreover, data on live births after sperm injection in CBAVD with CFTR gene mutations are sparse and also lacking in detail regarding the effect of CFTR mutations on ICSI pregnancy.

The aims of the present study were to compare percutaneous sperm retrieval and ICSI outcomes in men with OA between CBAVD and non-CBAVD groups and to assess the effect of CFTR mutations on ICSI cycle outcome in azoospermic men with CBAVD.

MATERIALS AND METHODS

Patient Population

This retrospective analysis evaluated 1,414 ICSI cycles in 945 OA patients that were performed from January 2007 to December 2011. The study was approved by the Institutional Review Board of the Provincial Hospital Affiliated to Shandong University. All patients were counseled and signed a consent form approved by the local ethics committee. All patients underwent a complete physical examination by the same physician and a scrotal ultrasound exam, with particular attention paid to the testes, seminal vesicle, vas deferens, epididymis, and prostate gland. The diagnosis of CBAVD was based on the following criteria: a small ejaculate volume, the complete absence of spermatozoa, increased semen acidity (pH <7), the absence or decreased concentration of fructose, a decreased concentration of L-carnitine, a normal concentration of plasma FSH, impalpable scrotal vas on physical examination, and rectal/scrotal ultrasound showing absence of the seminal vesicle and a normal testicular volume. All patients included in this study were subsequently confirmed for successful sperm retrieval by percutaneous epididymis sperm aspiration. We identified the patients as CBAVD and non-CBAVD before ICSI treatment. Karyotype and genetic screening for CFTR gene mutations were performed in all men before ICSI treatment, and none of the patients with CBAVD had any other classic symptoms of cystic fibrosis (CF). Only ICSI cycles using fresh sperm obtained by PESA were included in the analysis. Of the 945 OA patients included, 352 were diagnosed as having CBAVD, 148 were diagnosed as having infective/inflammatory obstruction, 42 were diagnosed as having vasectomy obstruction, and 403 were diagnosed as having idiopathic epididymal obstruction. The 1,414 ICSI cycles were classified into [1] 531 with CBAVD versus 883 with non-CBAVD obstruction, [2] 105 with CFTR gene mutations versus 1,309 with non-CFTR mutations, and [3] 117 with miscarriages and stillbirths versus 552 with live births.

CFTR Mutations

Genomic DNA was isolated from 2 mL of peripheral blood lymphocytes using a commercially available genomic DNA extraction kit. The sequences of primers targeting exons 10 and 11 of the CFTR gene have been described elsewhere (11). Each DNA sample was subjected to four multiplex polymerase chain reaction (PCR) amplification reactions. PCR was performed using an ABI 9700 PCR system and 50-μL reaction volumes containing 50–100 ng of DNA, 1$\times$ PCR buffer, each dNTP at 200 μM, 1.25 U of HotStartTaq polymerase, and primers at 0.5 μM. PCR amplification was performed with an initial step of 94°C for 5 minutes followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 30 seconds, followed by a final 7-minute extension at 72°C. Each amplified PCR sample (5 μL) was subjected to acrylamide gel electrophoresis for confirmation.

PCR products were purified using a PCR purification kit (Fermentas). Sequencing of the purified PCR products was performed using an ABI BigDye, version 3.1, sequencing kit and an ABI 3100-Avant device (Applied Biosystems) according to the manufacturer's protocols. The sequencing data were analyzed using ABI DNA sequencing analysis software (ver. 3.7) and compared with the control sequence.

PESA

A single senior urologist performed all sperm retrieval. Retrieval was performed on an outpatient basis under local anesthesia together with an IV bolus infusion of propofol. The epididymis was held between the surgeon's thumb and index finger. The needle was introduced into the head of the epididymis (caput) as close as possible to the testicle. Only two to three punctures were performed, and syringes and needles were always changed for each puncture.

Aspirated epididymal fluid was flushed into a tube containing sperm medium and sent to the embryology laboratory for examination. Successful retrieval was defined as the presence of motile sperm. Spermatozoa were not counted because of the small quantity available. The sperm were stored at 37°C until the ICSI procedure was performed.

ICSI Procedure and Assessment of Fertilization and Embryonic Development

Ovarian stimulation, oocyte retrieval, sperm processing, sperm injection, and ET were performed as described elsewhere (12).