Female cystic fibrosis mutation carriers and assisted reproductive technology: does carrier status affect reproductive outcomes?

Teresa A. VanWort, B.A.,[a,b] Joseph A. Lee, B.A.,[a] Hrishikesh Karvir, Ph.D.,[c] Michael C. Whitehouse, B.A.,[a] Piraye Yurttas Beim, Ph.D.,[c] and Alan B. Copperman, M.D.[a,b]

[a] Reproductive Medicine Associates of New York; [b] Obstetrics, Gynecology and Reproductive Science, Mount Sinai School of Medicine; and [c] Celmatix Inc., New York, New York

Objective: To evaluate the association between female cystic fibrosis (CF) carrier status and in vitro fertilization (IVF) response and outcomes. The presence of cystic fibrosis transmembrane conductance regulator *(CFTR)* gene mutations in male carriers has been associated with infertility, yet possible adverse effects on the ovarian function and reproductive outcomes of female carriers have not been studied to date.
Design: Retrospective cohort study.
Setting: Private academic, clinical reproductive center.
Patient(s): Females <40 years of age who were screened for *CFTR* mutations and received IVF treatment between July 2002 and March 2013.
Intervention(s): Patients initiated controlled ovarian hyperstimulation with frequent monitoring, vaginal oocyte retrieval, fertilization, embryo transfer, and a pregnancy test. Various measures of IVF stimulation response and cycle outcome were evaluated for both carriers and noncarriers.
Main Outcome Measure(s): Analysis was performed by logistic regression and Poisson regression.
Result(s): IVF cycles (n = 199) from *CFTR* mutation carrier patients (n = 112) were analyzed. No significant differences in outcome were noted when carriers of different mutation loci were compared in aggregate with the noncarrier group (n = 6,420 cycles from 3,555 patients). Significant differences were noted for some metrics when the carriers were grouped by mutation loci.
Conclusion(s): Overall, no significant differences in stimulation response and cycle outcome were noted between female *CFTR* mutation carriers and noncarriers. Further research is needed to investigate whether the differences noted between specific *CFTR* mutation loci are clinically relevant and whether *CFTR* mutations may impact reproductive outcomes outside the context of assisted reproductive technologies. (Fertil Steril® 2014;102:1324–30. ©2014 by American Society for Reproductive Medicine.)
Key Words: Cystic fibrosis, in vitro fertilization, ovarian function, heterozygote, female fertility

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/vanwortt-female-cystic-fibrosis-mutation-carriers-art/

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.

Cystic fibrosis (CF) is an autosomal recessive genetic disease caused by mutations to the CF transmembrane conductance regulator (*CTFR*) gene, which is located on the long arm of chromosome 7 at position q31.2 (1–3). Its protein product codes for a transmembrane protein found in epithelial cells and functions as a cAMP-regulated ion channel that transports chloride and bicarbonate ions down their electrochemical gradients (4–7). When the channel is not functioning correctly, osmosis is disrupted and the movement of water slows, causing debilitating mucus to accumulate in many important organs. This results in several forms of morbidity and mortality, which are most commonly associated with disease of the lungs, pancreas, and gastrointestinal tract (8–11). However, the reproductive tract can also be negatively impacted, resulting in infertility. Ninety-seven percent of men affected with CF have congenital bilateral absence of the vas deferens (CBAVD), and women with CF are often infertile owing to thickened cervical mucus, disruption of the uterine environment, delayed puberty, and ovulatory dysfunction (12–22).

Received May 1, 2014; revised July 8, 2014; accepted July 14, 2014; published online September 11, 2014.
T.A.V.W. has nothing to disclose. J.A.L. has nothing to disclose. H.K. has nothing to disclose. M.C.W. has nothing to disclose. P.Y.B. has nothing to disclose. A.B.C. has nothing to disclose.
Reprint requests: Teresa A. VanWort, B.A., Reproductive Medicine Associates of New York, 635 Madison Avenue, 10th Floor, New York, New York 10022 (E-mail: tvanwort@rmany.com).

Fertility and Sterility® Vol. 102, No. 5, November 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2014.07.1234

There are over 1,900 documented *CFTR* mutations (23). The most common mutation is ΔF508, which is present in 70% of cases. The ΔF508 mutation results from the deletion of three nucleotides and, subsequently, the loss of the amino acid phenylalanine. This loss results in the improper folding of the CFTR protein, leading to it being tagged for degradation in the endoplasmic reticulum rather than being transported to the cell's surface (1, 24). The frequency of other *CFTR* mutations varies by ethnicity, but the most common mutations worldwide (frequency $\geq$1%) are W1282X, G542X, N1303K, and G551D (25). Another notable *CFTR* mutation is R117H, which typically results in milder phenotypic disruptions than other mutations and is the second most common *CFTR* mutation in the United States (26).

Interestingly, despite the recessive nature of *CFTR* mutations, a number of clinical phenotypes have been identified in CF carriers. For example, a high prevalence of single *CFTR* mutations has been observed among patients with chronic sinusitis, chronic pancreatitis, asthma, and chronic obstructive pulmonary disorder (27–31). In addition, male CF carriers have been shown to be at higher risk for fertility issues. Twenty-five percent of men with CBAVD only have one *CFTR* mutation, and there is an increased *CFTR* mutation frequency in groups of men with non-CBAVD infertility such as those with nonobstructive azoospermia, oligospermia, and asthenospermia (16, 17, 32, 33). Most recently, a study by Lu et al. in 2014 demonstrated an increase in the frequency of miscarriages/still births and prevalence of CBAVD in male CF carriers (34). Furthermore, research suggests that the CFTR protein plays a critical role in spermatogenesis. During spermatogenesis, CFTR controls HCO_3- entry into the Sertoli cells, activating soluble adenylyl cyclase (sAC) and the cAMP/PKA/CREB pathway—a pathway crucial to the process of sperm production (35). CFTR also regulates junctional complexes and BTB in the testis and mediates HCO_3- entry into sperm during capacitation (36).

A role for the CFTR protein in female factor fertility has also been proposed. In 2011, Chen et al. studied *CFTR* expression in mouse ovaries and found that CFTR indirectly regulates FSH-stimulated estrogen production by controlling HCO_3- entry into ovarian and granulosa cells, subsequently activating sAC and the cAMP/PKA/CREB pathway (37). A similar study in 2008 conducted by Jin and Tang indicated that CFTR played a role in the accumulation of follicular fluid during oocyte maturation (38). It has long been known that females affected with CF have ovulatory dysfunction, and this has previously been attributed to malnutrition and the physical stress of disease. The mouse studies suggest a more direct involvement of CFTR in ovarian function and hormone production; however, it is currently unknown whether CFTR plays a similar role in humans. A previous study of *CFTR* expression in male and female reproductive tissue did not find any *CFTR* expression in adult and newborn ovaries (39). It is possible that with more sensitive detection methods, expression could be detected.

Despite advances in understanding the effects of *CFTR* mutations on the fertility of both males and females affected with CF and of male CF carriers, the relationship between female CF carrier status and infertility has been minimally explored. In 2011, Brunoro et al. observed a higher percentage of CF carriers than expected among the 24 women with altered fertility in their study cohort (40). Another study in 2011 by Tomaiuolo et al. showed an increased frequency of a specific CF mutation—the 5T haplotype—among women with tubal disease (32). However, neither study looked at ovarian function in depth or in the context of assisted reproductive technologies (ART). To evaluate the ovarian function and reproductive outcomes of female CF carriers more thoroughly, this study seeks to evaluate the in vitro fertilization (IVF) stimulation response and treatment cycle outcomes of CF carriers in comparison with noncarriers.

MATERIAL AND METHODS

Study Population

IVF cycles (n = 199) from female patients <40 years of age (n = 112) who tested positive for a single CF mutation and underwent treatment at a private, academic reproductive center between July 2002 and March 2013 were included. The control group consisted of female patients <40 years of age who tested negative for CF mutations and underwent IVF treatment during the same time period (n = 6,420 cycles from 3,555 patients).

CF Carrier Testing

Prevalent *CFTR* mutations (between 23 and 97 of the most common mutations depending on the assay) were evaluated by external laboratories as part of standard care unrelated to this study: Quest Diagnostics (Cystic Fibrosis Screen), Genzyme/Integrated Genetics (CF*plus*), and Mount Sinai Genetics (Cystic Fibrosis Carrier Screening).

IVF Procedure Overview

Baseline hormone levels and follicle count were evaluated on day 2 or day 3 of the patients' menstrual cycles, followed by an 8- to 14-day regimen of daily gonadotropin injections to stimulate follicle development. Cycle monitoring consisted of a transvaginal ultrasound and testing of estradiol and progesterone levels by peripheral blood approximately every other day. Premature ovulation was avoided through the use of an antagonist or an agonist, depending on the specific needs of the patient.

Once optimal follicle size (17–19 mm) was achieved, the patients were administered human chorionic gonadotropin (hCG), and 36 hours later the oocytes were harvested through vaginal oocyte retrieval aspiration of the ovarian follicles. The eggs were inseminated either by intracytoplasmic sperm injection or conventional insemination, depending on clinical indications. Resulting fertilized embryos were cultured and then evaluated 3 days postretrieval for cleavage-stage formation. Those that met certain embryological quality criteria were cultured in the lab for 2 more days to achieve blastocyst stage maturation. Embryo transfer to the uterus was conducted at either the cleavage (day 3) or blastocyst (day 5/6) stage. In some cases, embryos were cryopreserved and then thawed/transferred during one of the patient's following cycles. Success rates are comparable between fresh and frozen blastocyst