ORIGINAL ARTICLE: ASSISTED REPRODUCTION

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

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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;■:■–■. ©2014 by American Society for Reproductive Medicine.)
Key Words: Cystic fibrosis, in vitro fertilization, ovarian function, heterozygote, female fertility

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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).

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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.

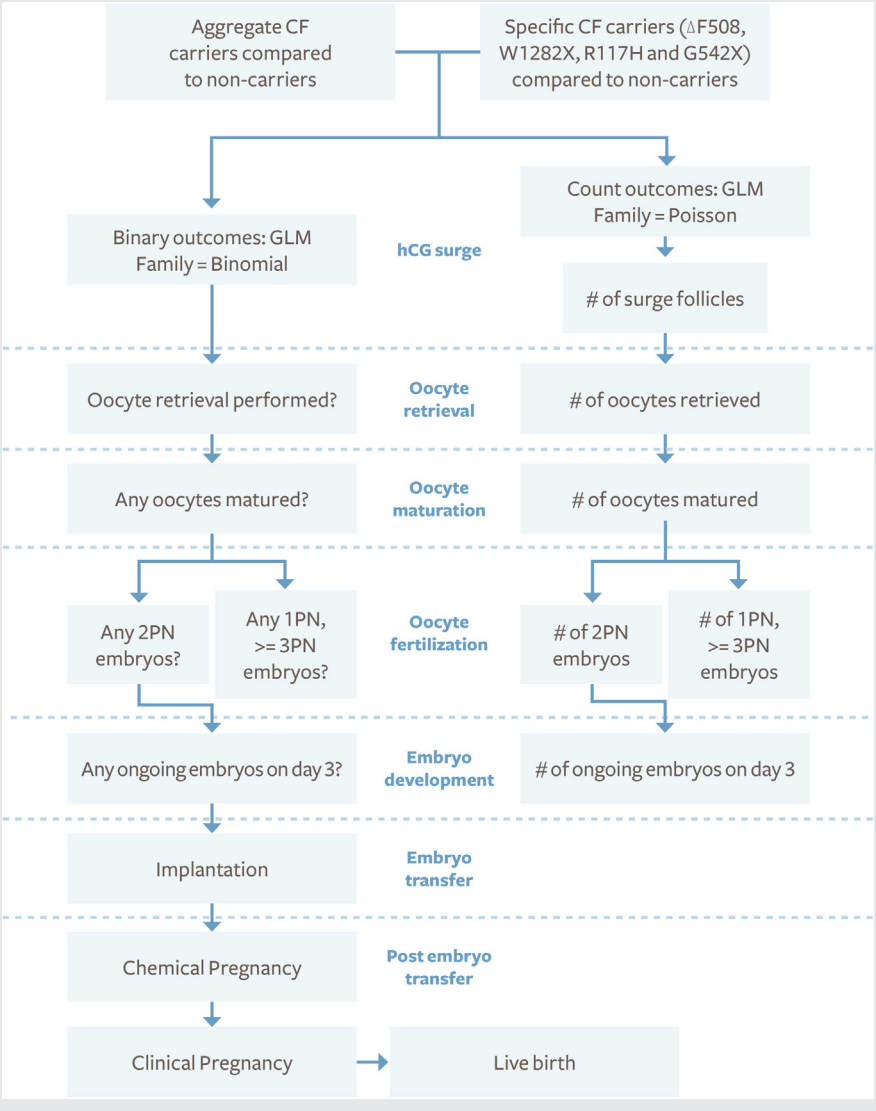
Once optimal follicle size (17–19 mm) was achieved, the patients were administered human chorionic gonadotropin (hCG), and 36 hours later the oocytes were harvested through vaginal oocyte retrieval aspiration of the ovarian follicles. The eggs were inseminated either by intracytoplasmic sperm injection 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

transfer cycles in our data set; therefore, we included both in our analyses. Fourteen days after the date of the oocyte retrieval, a pregnancy test evaluating βhCG was conducted through the collection of peripheral blood. In the event of a positive test, a repeat blood test was performed 48 hours later to ensure that the βHCG level was rising at the appropriate rate. Finally, ultrasounds and blood work were performed weekly between weeks 5 and 8 of the pregnancy to confirm the appearance of early milestones (fetal sac, yolk sac, fetal heartbeat, etc.), after which the patient was discharged into the care of her primary obstetrician. Delivery information was obtained through follow-up with the patient 9 months later.

Statistical Methods

CF carrier patients were compared with noncarrier patients with respect to multiple measures of IVF response and clinical outcomes (Fig. 1; Table 2). The most prevalent mutation loci in our patient population (ΔF508, n = 84, cycles from 59 patients; W1282X, n = 17, cycles from 12 patients; R117H, n = 14, cycles from 10 patients, and G542X, n = 9, cycles from 6 patients) were also each individually compared with the noncarrier patients. For binary outcomes, logistic regression was used to determine the effect of CF carrier status. For count outcomes, Poisson regression was used. All models controlled for age and basal antral follicle counts (BAFC), accounting for multiple patient cycles through prior weights. Missing values for BAFC were imputed using conditional mean imputation. For the clinical outcomes occurring past oocyte retrieval, the outcomes were analyzed conditional on retrieval having occurred. All analysis was conducted using the biostatistics toolbox in Matlab R2012b. The study was powered to detect moderate to large effects on reproductive outcomes—80% power to detect an odds ratio = 0.6 effect

FIGURE 1



Flow chart representing the different outcomes analyzed at different stages of infertility treatment.

VanWort. Female CF carriers: IVF response and outcome. Fertil Steril 2014.

on clinical pregnancy or live birth and 80% power to detect a shift of approximately two surge follicles (follicles >14 mm the day of surge) or number of oocytes. Smaller effects owing to CF carrier status could not be ruled out.

This research was approved by the Western Institutional Review Board.

RESULTS

We observed no significant association between CF carrier status and rates of embryo implantation, chemical pregnancy, clinical pregnancy, and live-birth (Table 2). No effect was observed on the number of retrieved oocytes, although the number of follicles >14 mm at surge and mature oocytes were significantly higher in the CF carrier patients (n = 199 cycles from 112 patients) versus in the noncarriers (n = 6,420 cycles from 3,555 patients; $P=.034$ and .022, respectively), and the number of abnormally fertilized embryos was significantly lower ($P=.0015$).

Some significant associations in parameters were noted when the CF carriers were grouped by specific mutation loci (Table 2). Particularly, patients with the R117H mutation (n = 14 cycles from 10 patients) indicated a significant reduction in the number of retrieved oocytes ($P=.032$) and the number of 2 pronuclei (2PN) embryos ($P=.032$; Table 2). On the contrary, patients with the W1282X mutation (n = 17 cycles from 12 patients) indicated a significant increase in the number of retrieved oocytes ($P<.001$) and the number of 2PN embryos ($P<.001$). For the most prevalent mutation, ΔF508 (n = 84 cycles from 59 patients), a significant increase in the number of 2PN embryos ($P=.003$) was observed along with a reduction in the number of abnormally fertilized embryos ($P=.006$).

DISCUSSION

With the cost of genomic sequencing plummeting, the age of personalized medicine has arrived. Genotype is the most personalized metric that can be assayed for a given individual. On the bench and in the clinic, the spectrum of genetic determinants underlying the variation in subfertility and infertility clinical phenotypes is becoming elucidated. In parallel, women are now also routinely genetically screened to determine whether they are carriers of recessive mutations that could put their offspring at risk for an inherited disorder. CF is an example of a disease with well-known deleterious effects on reproductive function in both sexes and, even in the carrier state, it can cause subfertility in males. We demonstrate in a large study of female patients that CF carrier status does not appear to affect reproductive outcome in the context of ART.

While no differences in implantation rates or pregnancy outcome were noted between female CF carriers and noncarriers, some significant associations in parameters were noted when the CF carriers were grouped by specific mutation loci. While these mutation loci appear to impact oocyte and/or embryo development, the contradictory associations and small sample size of each subgroup in our existing cohort (ΔF508, n = 84 cycles; W1282X, n = 17 cycles; R117H, n = 14 cycles; and G542X, n = 9 cycles) require that these observations be prospectively validated in additional patients. Also, it is worth noting that the patients in the R117H group had a higher mean age and FSH (Table 1), which could explain the lower numbers of normally fertilized embryos and oocytes retrieved in this group.

It has been suggested that CF carrier status could modulate reproductive function in females through the cellular and molecular functioning of CFTR, which could potentially explain the associations noted between specific *CFTR* mutations and oocyte and/or embryo development. CFTR mRNA has been detected in areas of the rat hypothalamus associated with reproduction and sexual maturation, providing evidence for the potential involvement of CFTR in hormone production through the hypothalamic-pituitary-gonadal (HPG) axis (20). Also, CFTR functions in regulating other channels and transporters such as the outwardly rectifying Cl-channel, the renal outer medullary potassium channel, and the epithelial sodium channel through protein-protein interactions (41–43). In addition, many genes associated with fertility and primary ovarian insufficiency, including *LEP*, *NOBOX*, *DLX5/DLX6*, and *CYP51* are in close proximity to *CFTR* on chromosome 7. *LEP*, the gene encoding the protein leptin, is located approximately 10 cM from *CFTR* and has been previously shown to be linked to *CFTR* (44–49). Leptin plays an important role in pubertal timing and in the overall function of the HPG axis, therefore, a potential link between CFTR and ovarian function through leptin might also warrant further investigation (50–53).

The contradictory nature of the associations (the fact that some mutations appear beneficial and others deleterious) could stem from the fact that different *CFTR* mutations affect the protein product in different ways. *CFTR* mutations are classified into five types based on impact: type I mutations, which result

TABLE 1

Patient demographics.

	Noncarriers (n = 3,555)	*CFTR* Mutation Carriers (n = 112)				
		ΔF508 (n = 59)	W1282X (n = 12)	R117H (n = 10)	G542X (n = 6)	Other (n = 25)
Age (y)	34.8 ± 3.6	35.0 ± 3.2	34.3 ± 4.2	37.1 ± 3.1	34.2 ± 3.2	34.8 ± 3.6
Day 3 FSH (mIU/mL)	8.5 ± 4.0	8.0 ± 4.5	7.1 ± 2.2	12.8 ± 3.6	9.0 ± 1.6	8.5 ± 2.7
Peak E_2 level (pg/mL)	2,066 ± 1,182	2,007 ± 1191	2,741 ± 1,555	1,350 ± 633	2,147 ± 960	2,138 ± 997
BAFC	10.5 ± 4.8	10.4 ± 4.9	12.5 ± 6.1	8.3 ± 3.1	9.9 ± 3.9	10.6 ± 4.5
No. of retrieved oocytes	12.7 ± 9.5	12.5 ± 10.4	16.5 ± 9.5	9.6 ± 5.2	12.4 ± 8.0	12.8 ± 8.3

VanWort. Female CF carriers: IVF response and outcome. Fertil Steril 2014.

TABLE 2

Summary of the effects of aggregate CF mutations and the four most prevalent mutations on different clinical outcomes.

				Specific mutation loci							
		Aggregate mutations		ΔF508		W1282X		R117H		G542X	
Outcome	**Type**	**Effect**	***P* value**	**Effect**	***P* value**	**Effect**	***P* value**	**Effect**	***P* value**	**Effect**	***P* value**
No. of surge follicles >14 mm	Counts	1.06 [1.00–1.12]	.034	1.07 [0.99–1.16]	.076	1.07 [0.91–1.25]	.410	0.81 [0.65–1.01]	.064	1.00 [0.79–1.28]	.956
Retrieved oocytes	Binary	0.87 [0.50–1.53]	.645	0.62 [0.31–1.23]	.175	0.78 [0.14–4.39]	.777	2.61 [0.16–41.33]	.497	0.95 [0.08–10.73]	.965
No. of retrieved oocytes	Counts	1.03 [0.98–1.08]	.208	1.07 [0.99–1.15]	.080	1.27 [1.11–1.45]	<.001	0.81 [0.66–0.98]	.032	0.89 [0.69–1.13]	.324
Mature oocytes	Binary	0.87 [0.57–1.34]	.530	0.70 [0.39–1.26]	.237	1.31 [0.30–5.72]	.716	2.26 [0.44–11.56]	.326	1.49 [0.19–11.26]	.696
No. of mature oocytes	Counts	1.17 [1.02–1.33]	.022	1.07 [0.87–1.31]	.534	1.16 [0.81–1.66]	.410	1.33 [0.91–1.95]	.135	0.49 [0.21–1.13]	.093
Normally fertilized oocytes	Binary	–	–	–	–	–	–	–	–	–	–
No. of normally fertilized oocytes	Counts	1.05 [0.98–1.13]	.137	1.15 [1.05–1.26]	.003	1.39 [1.18–1.65]	<.001	0.74 [0.56–0.97]	.032	0.99 [0.74–1.36]	.996
Abnormally fertilized oocytes	Binary	0.46 [0.18–1.16]	.098	0.34 [0.07–1.54]	.161	0.76 [0.09–6.11]	.798	–	–	–	–
No. of abnormally fertilized oocytes	Counts	0.43 [0.26–0.73]	.002	0.25 [0.09–0.68]	.006	0.89 [0.33–2.39]	.824	–	–	–	–
Day 3 ongoing embryos	Binary	1.05 [0.67–1.67]	.821	1.19 [0.62–2.32]	.600	–	–	0.27 [0.06–1.13]	.074	1.43 [0.19–10.70]	.730
No. of day 3 ongoing embryos	Counts	1.02 [0.93–1.11]	.673	1.10 [0.98–1.24]	.098	1.38 [1.15–1.65]	.001	0.74 [0.43–1.25]	.259	0.68 [0.43–1.08]	.103
Embryo implantation	Binary	1.24 [0.81–1.91]	.317	1.30 [0.75–2.25]	.350	0.46 [0.14–1.45]	.185	2.15 [0.52–8.87]	.291	1.12 [0.21–5.97]	.896
Chemical pregnancy	Binary	1.30 [0.81–2.10]	.279	1.83 [0.87–3.84]	.111	0.57 [0.16–2.05]	.389	1.57 [0.36–6.83]	.551	0.74 [0.12–4.47]	.746
Clinical pregnancy	Binary	1.26 [0.81–1.96]	.299	1.55 [0.81–2.96]	.184	0.55 [0.16–1.93]	.352	2.07 [0.49–8.68]	.322	1.07 [0.18–6.42]	.945
Live birth	Binary	1.16 [0.76–1.76]	.487	1.19 [0.66–2.15]	.563	0.65 [0.18–2.27]	.495	2.55 [0.62–10.40]	.192	1.09 [0.19–6.28]	.924

Note: CF carrier patients as an aggregate (n = 199 cycles from 112 patients) and by specific mutation loci (ΔF508, n = 84 cycles from 59 patients; W1282X, n = 17 cycles from 12 patients; R117H, n = 14 cycles from 10 patients; and G542X, n = 9 cycles from 6 patients) compared with noncarrier patients (n = 6,420 cycles from 3,555 patients) with respect to multiple clinical outcomes. Numbers greater than 1.00 represent a positive effect, while numbers less than 1.00 represent a negative effect, with significance at $P < .05$.

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in early termination of translation; type II mutations, which result in a misfolded protein product that is not transported to the cell surface; type III mutations, which affect the regulation of CFTR protein activity; type IV mutations, which affect the conductance of the CFTR protein channel; and type V mutations, which affect splice sites and result in reduced splicing efficiency (24, 54–59). Type I, II, and III mutations cause a loss in protein function and increased disease severity, while type IV and V mutations only reduce protein function, resulting in milder disease states (60). Many combinations of different types of mutations are possible, causing a wide heterogeneity in the symptoms of affected patients (59). In follow-up studies, it will be of interest to note whether reproductive outcomes and metrics are impacted to greater or lesser degrees in carriers of different mutation classes.

Lastly, the precise pathophysiology remains to be elucidated through which a single-allele CF mutation could result in a phenotypic alteration. One possibility is that CF phenotypes are dosage dependent and that reproductive tissues are perhaps more sensitive to the partial loss of CFTR than tissues in other parts of the body. The possibility that symptomatic CF carriers are actually affected with a mild CF phenotype owing to the presence of an undiscovered and/or rare CF mutation on the sister allele is also worth investigation. In support of this possibility is a 2010 study in which six out of 15 male CF carriers affected with CBAVD who had been originally screened for the 23 basic *CFTR* mutations and the 5T polymorphism were found to have a second *CFTR* mutation upon further investigation (61). Therefore, full genetic sequencing of the sister allele should be considered, particularly for CF carriers who are symptomatic.

It has long been known that *CFTR* mutations impact the fertility of males and females affected with CF and the fertility of male CF carriers, but potential effects on the fertility of female CF carriers have not been thoroughly investigated. Women undergoing ART routinely undergo carrier screening, which provided us an opportunity to begin to explore this possible link. We demonstrated that, overall, no significant differences in ART outcomes were noted between female CF carriers and noncarriers. Therefore, women should not be concerned that their CF carrier status might have a significant negative impact on their chances of achieving pregnancy with ART. Further research is now needed to investigate whether certain specific CF mutations in females may impact oocyte and/or embryo development and whether positive CF carrier status affects fertility in a non-ART context.

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