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CASE REPORT

PREVENTABLE HAZARDS FROM IN VITRO FERTILIZATION – A CASE SERIES OF CF PATIENTS FROM BULGARIA

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ABSTRACT

Pre-implantation genetic diagnosis (PGD) is not often performed when donor gametes are used, due to its high cost. This is with the presumption that the donors are healthy.

We report on five cases of babies with confirmed cystic fibrosis (CF), being the result from in vitro fertilization (IVF) with donor (4 cases) or own gametes (one case). There has been no family history for CF in any of the families affected. The clinical presentation in the children ranged from meconium ileus to recurrent respiratory infections and severe nasal polyposis. The age of diagnosis also varied from birth until 9 years.

Since one of the presented cases was discovered in a very renowned private IVF clinic, the clinic changed their own protocol, and currently they test every donor for CF carriership. The percentage of CF carriers in the donor population is roughly the same as the one predicted in the general population of Bulgaria – 1/33.

Although PGD is costly, the costs for proper care for a CF patient are currently much higher. The more economical option would to screen every donor for CF carriership.

IVF requires a lot of physical and psychological stamina. The couples that go through this procedure also require a great deal of hope. It is essential to be more preconscious for possible congenital diseases.

We advocate every IVF center to test the donors for CF carriership or to provide PGD for their clients.

Key words: donor oocytes, preimplantation diagnosis, cystic fibrosis, carrier screening

List of Abbreviations: CF – cystic fibrosis/ PGD – Pre-implantation genetic diagnosis / IVF - In vitro fertilization / pwCF – patients with cystic fibrosis / BG pwCF – Bulgarian patients with CF / SSCP - single-strand conformation polymorphism / MLPA - multiplex ligation dependent probe amplification analysis / NGS - next-generation sequencing / cystic fibrosis transmembrane regulator (CFTR)/ FTT – failure to thrive/ NP – nasal polyposis/ SA- *Staphylococcus aureus* / MI – meconium ileus / RI – respiratory insufficiency / PA – *Pseudomonas aeruginosa* / NBS - newborn screening

INTRODUCTION

Cystic fibrosis (CF) is a rare, progressive, genetic, multi-organ disease with a chronic clinical course and commonly premature death. The disease is inherited in an autosomal recessive manner through mutations in the cystic fibrosis transmembrane regulator gene (CFTR) [1, 2]. The gene encodes a protein of the same name that serves as an ion chloride channel. Even mildly impaired function of the CFTR protein can affect the body's organs. The vas deferens appears to be one of the most vulnerable structures in patients with CF (pwCF) – leading to very high male infertility as a consequence [3]. In vitro fertilization (IVF) and all assisted reproduction techniques enabled pwCF to become parents. Although, success rates for IVF are around 20 to 40%, this may be the only option for most CF men and many CF women. Worldwide, it is routine to test one's partner, whether he/she is a CFTR mutation carrier, and if the result is positive, pre-implantation genetic diagnosis (PGD) is offered. Some pwCF even choose to

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use donor gametes in hope of not passing the CFTR mutation to their child. However due to the high cost, PGD is not commonly performed when donor gametes are used, provided the donors are healthy [4]. On the other hand, people not affected by CF may also have reproductive problems and the need for IVF. In such cases, PGD is often not offered and there are numerous possible consequences if the child is born affected. Undergoing an IVF-treatment is an expensive (physical, emotional, and financial) burden for the prospective parents [5]. The long and hard road, in many cases with an uncertain outcome, could be sometimes endured purely on the hope for a healthy child. However, one of the unforeseen risks is that the longed-for child suffers from a fatal disease.

MATERIALS, METHODS AND RESULTS

In Bulgaria there are currently 248 living confirmed pwCF (BGpwCF) [author unpublished data]. All patients have genetic confirmation of the disease, with more than 95% carrying two disease causing mutations. Genetic tests include evaluating the most common CF causing mutations using "in house" methods. The methodology for mutation detection includes single-strand conformation polymorphism (SSCP), Sanger sequencing, multiplex ligation dependent probe amplification (MLPA) analysis and next-generation sequencing (NGS) of the entire CFTR coding region and splice site junctions (Applied Biosystems, USA) if none or only one mutation was found [6]. Since newborn screening (NBS) for CF is not yet performed in our country, the diagnosis depends solely on clinical presentation. The median age at diagnosis in Bulgaria is currently 1.2 years (minimum 0 years – maximum 64 years)

In the group of 248 BGpwCF, 5 were conceived by IVF and born in a family not previously affected by CF. In 4 families, donor oocytes had to be used, while in the last case the parent's own gametes were used. Two IVF pregnancies involved twins – in both cases the sibling is a healthy CF carrier. All five children were diagnosed within the last 5 years. The age of diagnosis ranged from 0 (for two children with meconium ileus) to 9 years (Table 1). There was a significant delay in diagnosis in one of the patients (9 years), despite classical failure to thrive and severe nasal polyposis. The case was peculiar as the parents didn't want to accept the diagnosis for almost 4 years and only recently agreed to proper therapy for CF. Due to severe polyposis the parents were aiming for biological therapy, but after appropriate explanation of the disease agreed for surgery. The turning point for accepting diagnosis in this family was the approval of CFTR modulator therapies in Bulgaria.

All patients but one are carriers of at least one c.1521_1523 delCTT, the most common mutation described in 80.65% of BGpwCF. One female patient is homozygous for the same mutation, and this combination occurs in 35.58% of BGpwCF. After birth, she suffered from diarrhea and failure to thrive. She also contracted COVID19 at three months of age. The infection resulted in severe respiratory insufficiency and CF was confirmed by sweat test and genetic analysis. It took three months in the hospital and 4 more months on home oxygen before the patient reached the current, stable condition. She also had positive sputum cultures for *Pseudomonas aeruginosa* and is on an inhaled antibiotic.

The second girl in the group also has a healthy twin carrier of c.1521_1523 delCTT. The mutation is inherited from the father, while the donor oocyte carried the c.828C>A mutation. The patient suffered recurrent airway obstructions, but due to good weight gain, the diagnosis was confirmed when she was 11 months old. At the time of diagnosis, positive sputum cultures for *Pseudomonas aeruginosa* led to aggressive therapy with intravenous and inhaled antibiotic. However, eradication of the bacterium was not achieved. Currently, her condition is stable and expecting to begin CFTR modulator therapy next month.

The c.828C>A mutation is found in approximately 1.6% of BGpwCF. There is another patient with this mutation in our group (the only case where donor gametes were not used). He had inherited c.828C>A from his father, while his mother's oocyte carried c.3909C>G. The boy was born with meconium ileus, and had survived two surgeries (an initial one, at diagnosis, and later a reconstructive surgery). He is currently in a stable condition with no infectious bacteria from respiratory samples. He has a good weight/height curve. The c.3909C>G is the second most common allele in BGpwCF found in 5.6% and is also confirmed in another child in this study. The patient had c.1739_1740insT and underwent surgery for meconium ileus at birth and is currently in a stable overall condition. (*Table 1)*

DISCUSSION

CF is an inherited, multi-organ disease, characterized mainly by progressive chronic lung and pancreas involvement leading to premature death [1]. Standard CF therapy has been purely symptomatic and aimed primarily at maximizing symptom relief and prevention of complications. Over the last decade, drugs aimed at correcting the defective CFTR protein (CFTR-modulators) have become available for pwCF with specific CFTR mutations [2]. Introducing better standards of care, including CFTR modulators, earlier diagnosis with NBS, and specialized

Table 1. Genetic characteristics of the patients with age of diagnosis, follow up and clinical summary.

gender	genotype	oocyte mutation (origin of oocyte)	mutation of the father	age at diagnosis	current age at follow up	clinical summary
boy	c.1521_1523delCTT/ c.1739_1740insT	c.1521_1523 delCTT (donor)	c.1739_1740insT	9 years	14 years	FTT, NP, SA positive
boy	c.1521_1523delCTT/ c.3909C>G	c.1521_1523 delCTT (donor)	c.3909C>G	birth	3 months	MI
girl*	c.1521_1523delCTT/ c.1521_1523delCTT	c.1521_1523 delCTT (donor)	c.1521_1523 delCTT	3 months	13 months	RI, PA positive
girl*	c.828C>A / c.1521_1523delCTT	c.828C>A (donor)	c.1521_1523 delCTT	11 months	6 yrs 1 month	RI, PA positive
boy	c.3909C>G /c.828C>A	c.3909C>G (mother)	c.828C>A	birth	12 months	MI

FTT – failure to thrive, NP – nasal polyposis, SA- *Staphylococcus aureus*, MI – meconium ileus, RI – respiratory insufficiency, PA – *pseudomonas aeruginosa;* * the patient has twin brother – carrier of c.1521_1523 delCTT

center care have greatly improved the prognosis of the disease. However, this therapy is associated with high costs and there are still certain age limits for the medications. Caring for a child with CF has a major psychological impact on the caregivers such as depression, anxiety and even delusions [7].

Assisted reproductive techniques and especially IVF are significant breakthrough technologies that contribute to the treatment of infertility (both female and male). Since more than 90% of male pwCF have atresia of vas deferens, IVF is the most logical choice for them. Female pwCF may also have fertility problems due to the thicker secretions of the genital tracts. From 248 BGpwCF, three males and two females have undergone IVF in the last decade, (two of them are still in the process). The other three patients are currently proud parents of healthy CF-carrier children. According to recent publications, the introduction of therapy with CFTR modulators leads to increased fertility in women with CF, but these drugs cannot restore the missing vas deferens in men [8]. Testing the partner for CFTR mutations is essential in IVF procedures with a parent with CF. More than 30 years have passed since the NEJM publication of a successful pregnancy after IVF and PGD testing in both carrier parents of child with CF, and today such genetic concealing and IVF with PGD (in the case of a carrier partner) are offered to families with CF history [9].

Reproductive problems can also occur in families without a history of CF. The outcome of IVF cycles depends on several factors, such as maternal age, ovarian reserve, endocrine status of patients, body mass index, gynecological diseases such as endometriosis, pelvic inflammatory disease, immunology and others [10]. Assuming there are no risk factors, IVF could lead to positive emotions and a favorable outcome. In a study of 5600 couples who needed IVF, a higher prevalence of CF mutation carrier rate of one in 21.5 was found [11]. The authors conclude that screening prenatal/pre-conceptional patients to identify CFTR mutation carriers may reduce the incidence of CF-affected babies at birth, and that the use of preconceptional screening allows carrier couples to choose between prenatal screening and PGD [11]. On the other hand, two large Italian cohorts found no difference in carrier rates - CFTR mutations were detected in 6.2% of the tested subjects, a percentage similar to that reported in the general population [12] when carrier screening is performed in the general population. The same studies confirmed the significantly higher prevalence of CFTR-carrier status in couples who required IVF - 1 in every 22 compared with 1 in 32 for couples who did not require IVF [13]. It could be speculated that even CFTR carrier status may affect fertility without other health issues.

However, sometimes donor gametes must be used due to a very low or absent ovarian reserve or azoospermia in the male. This gives arise to a whole new legal and social side of IVF, such as paternity rights, confidentiality and financial benefits. And since we assume the donors are healthy, PGD is not often performed because of the high cost. In such situations there is a high probability of genetic diseases in the offspring, as in 5 cases described in our study. On the other hand, donors might conceal some medical facts, such as a hereditary disease in the family without considering possible legal and moral consequences.

IVF-treatment is an expensive and exhausting (physically, emotionally, and financially) procedure for the prospective parents [5]. The uncertainty of success can plunge the couple into a vicious cycle of high hopes and depression if they do not receive a positive result. This long and bumpy road (hormone treatments, daily laboratory and ultrasound checks, waiting anxiously), for an uncertain outcome is sometimes endured by the couples only because of the hope of having a healthy child. However,

one of the unforeseen risks is that the longed-for child suffers from a lethal medical condition, which leads to new disappointments.

Carrier screening could exclude the possibility that a donor gamete has a CFTR mutation. Some authors even suggested nationwide screening as a replacement for NBS, claiming the cost would be similar to screening for Down syndrome [14]. There has been controversy because a successful carrier testing programme with 90–95% coverage would indeed likely make newborn screening redundant, but this would be possible if it were universal and not just national level [15]. CF newborn and carrier screening have complementary roles and neither can replace the other.

There is a widely accepted consensus on the benefits of NBS for CF with improved quality of life, reduced complications and severity of disease, and earlier appropriate therapy [16]. NBS can also provide better insights into the prevalence of CF and genetic origin in different countries. For example, NBS in North Macedonia confirmed the most common CFTR mutation, c.1521_1523delCTT (F508del) with an overall incidence of 70.6%; the next most common mutations are c.1624G>T (G542X) (11.8%) and c.3909C>G (N1303K) (5.9%). These data are very similar to data for mutations found in BGpwCF [6, 16] which is expected for two neighbor countries with a shared history. These data could serve for the future NBS programme in Bulgaria or for testing protocol to discover which mutations the couples who need IVF have as well as any mutations among the gamete donors. The genetic results of the 5 patients described above are consistent with the aforementioned numbers.

In countries without NBS, genetic screening for newborns with respiratory distress has been proposed, but the study found 20% CFTR heterozygous newborns with respiratory issues versus 30% CFTR heterozygous newborns without respiratory problems [18]. In any case, if CFTR carrier status is confirmed, these children must be monitored closely for CFTR-related disease or possible future CF diagnosis (with more extensive NGS testing finding new mutations not currently described).

In contrast to NBS, carrier screening allows for informed reproductive decision-making before conception. Couples with positive screening result can either decide not to have children or to adopt, or they can use donor gametes or PGD [19].

One of the presented cases was in a very prestigious private IVF clinic. The clinic changed its own protocol and now tests every donor for CFTR mutations [20]. The CFTR carrier rate in the donor population is similar to that predicted in the general Bulgarian population (1/33 persons) [17].

CONCLUSION

The incidence of CF in Bulgaria was estimated more than 20 years ago using epidemiological approaches (1/3600 live births) [17]. However, the prevalence among IVF pregnancies should be much lower. IVF requires a lot of physical and psychological endurance from any couple undergoing such treatment, and it is important to be aware of possible congenital diseases. These pregnancies are followed more extensively and with more frequent visits. Given the many ethical and social issues that may arise, we strongly recommend that every IVF center test their donors for CF carrier status or offer PGD to their clients.

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Consent to participate and for publication: Informed consent was obtained from all parents of children included in this study

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REFERENCES

1. O'Sullivan BP, Freedman SD Cystic fibrosis. *Lancet,* 2009*,* 373: 1891–19 04. doi:10.1016/S0140-6736(09)60327-5
2. De Boeck K, Amaral MD Progress in therapies for cystic fibrosis. *The Lancet. Respiratory medicine*, 2016, *4*(8), 662–674. https://doi.org/10.1016/S2213-2600(16)00023-0
3. Sousa M, Servidoni MF, Vinagre AM, et al. Measurements of CFTR-mediated Cl- secretion in human rectal biopsies constitute a robust biomarker for Cystic Fibrosis diagnosis and prognosis. *PLoS One*. 2012; 7(10): e47708. doi:10.1371/journal.pone.0047708
4. Tur-Kaspa I, Aljadeff G, Rechitsky S, Grotjan HE, Verlinsky Y. PGD for all cystic fibrosis carrier couples: novel strategy for preventive medicine and cost analysis. *Reprod Biomed Online*. 2010; 21(2): 186-195. doi:10.1016/j.rbmo.2010.04.031
5. Heitman E. Social and ethical aspects of in vitro fertilization. *International Journal of Technology Assessment in Health Care*. 1999; 15(1): 22-35. doi:10.1017/S0266462399015184
6. Petrova G, Yaneva N, Hrbková J, Libik M, Savov A, Macek M Jr. Identification of 99% of CFTR gene mutations in Bulgarian-, Bulgarian Turk-, and Roma cystic fibrosis patients. *Mol Genet Genomic Med*. 2019; 7(8): e696. doi:10.1002/mgg3.696
7. Cruz I, Marciel KK, Quittner AL, Schechter MS. Anxiety and depression in cystic fibrosis. *Semin Respir Crit Care Med*. 2009 Oct; 30(5): 569-78. doi: 10.1055/s-0029-1238915.
8. Taylor-Cousar JL. CFTR Modulators: Impact on Fertility, Pregnancy, and Lactation in Women with Cystic Fibrosis. *J Clin Med*. 2020 Aug 21; 9(9): 2706. doi: 10.3390/jcm9092706. PMID: 32825766; PMCID: PMC7563981.
9. Handyside AH, Lesko JG, Tarin JJ, Winston RM, Hughes MR Birth of a normal girl after in vitro fertilization and preimplantation diagnostic testing for cystic fibrosis. *N Engl J Med.* 1992; 327: 905-909
10. Petanovska Kostova E. The Interaction of Female Age and Active Male Smoking has Negative Influence on Success Rates of the in Vitro Fertilization Treatments. *Balkan J Med Genet*. 2020; 23(1): 57-62. Published 2020 Aug 26. doi:10.2478/bjmg-2020-0005
11. Field PD, Martin NJ. CFTR mutation screening in an assisted reproductive clinic. *Aust N Z J Obstet Gynaecol*. 2011; 51(6): 536-539. doi:10.1111/j.1479-828X.2011.01348.x
12. Rongioletti M., Papa F, Majolini MB, Vaccarella C, Simonelli I, Luciano A et al. 15 Frequency of mutations of the cystic fibrosis transmembrane regulator *(CFTR)* gene in a cohort of consecutive patients candidate for assisted reproductive techniques *J Cyst Fibros*, 2012 Volume 11, S59
13. Picci L, Cameran M, Marangon O, et al. A 10-year large-scale cystic fibrosis carrier screening in the Italian population. *J Cyst Fibros*. 2010; 9(1): 29-35. doi:10.1016/j.jcf.2009.10.003
14. Massie J, Forbes R, Dusart D, Bankier A, Delatycki MB. Community-wide screening for cystic fibrosis carriers could replace newborn screening for the diagnosis of cystic fibrosis. *J Paediatr Child Health*. 2007; 43(11): 721-723. doi:10.1111/j.1440-1754.2007.01224.x
15. Wilcken B. Community-wide screening for cystic fibrosis carriers could replace newborn screening for the diagnosis of cystic fibrosis. *J Paediatr Child Health*. 2008; 44(4): 232-233. doi:10.1111/j.1440-1754.2008.01293.x
16. Fustik S, Anastasovska V, Plaseska-Karanfilska D, et al. Two Years of Newborn Screening for Cystic Fibrosis in North Macedonia: First Experience. *Balkan J Med Genet*. 2021; 24(1): 41-46. Published 2021 Jul 27. doi:10.2478/bjmg-2021-0015
17. Savov A, Genetic defects in Bulgarian cystic fibrosis patients, *Inspiro* 2011, 15(3) (Bul.)
18. Tanriverdi S, Polat M, Onay H. Determination of Cystic Fibrosis Mutation Frequency in Preterm and Term Neonates with Respiratory Tract Problems. *Balkan J Med Genet*. 2022; 24(2): 25-31. Published 2022 Jun 5. doi:10.2478/bjmg-2021-0023
19. Castellani C, Massie J. Newborn screening and carrier screening for cystic fibrosis: alternative or complementary?. *Eur Respir J*. 2014; 43(1): 20-23. doi:10.1183/09031936.00125613
20. S. Yaneva, S. Staikova, R. Staneva, G. Stamenov, D. Toncheva, S. Hadgidekova. Preimplantation genetic tests in in-vitro embryos *Akush. i ginekol*., 57, 2018, N 3, ISSN 0324-0959, 16-24 (Bul)