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## Cystic Fibrosis and Infertility

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### 1. Introduction

Cystic fibrosis (CF) - or mucoviscidosis - is a common autosomal recessive inherited disease, affecting the whole body and causing progressive deficiencies. The name 'cystic fibrosis' refers to the characteristic scarring (fibrosis) and formation of cysts in the pancreas. The first anatomical and pathological description of the disorder was done by Landsteiner in 1905. In 1936, Fanconi et al. identified it as an autonomous illness, and in 1994 Farber et al. named it mucoviscidosis, due to the thick and viscous mucus secreted by the exocrine glands. It is accounted for by several clinical manifestations. Respiratory impairment is the most serious symptom (resulting from chronic infection in the lungs) and, though it is treatable, it is resistant to antibiotics and other medication. The large number of other symptoms include sinusitis, inadequate growth, diarrhoea and infertility, each of which is an effect of CF in other parts of the body. The mutated gene is transmitted by father and mother - although it may be that neither of them manifest the disease - and it is accounted for by the change in carrying ions through the membranes of the cells (Quinton et al., 1983). This compromises the function of the exocrine glands that produce thicker and hard-eliminating substances (mucus, sweat or pancreatic enzymes).

In 1953, Di Sant'Agnese et al. noted an increased sodium chloride rate eliminated through the sweat of patients with CF. Gibson and Cooke (1958) standardised the sweat test, which has become an important tool in the diagnosis of the disease. In 1958, Shwachman and Kulczucki designed a disease-severity assessment system. In 1985, the position of the CFTR gene was determined on the long arm of chromosome 7, q31 band, by restriction fragment linked polymorphism (Knowlton et al., 1985), and, in 1989, the full-length gene was sequenced (Collins, 1992).

The clinical manifestations of CF are primarily due to the obstruction of the ducts of organs (such as the lung and the pancreas) by thick, viscous secretions, changes in electrolytic concentrations, and the presence of abnormal contents. The primary cellular defect consists of a decreased or else absent expression of the CF transmembrane conductance regulator (CFTR) protein, which causes changes in chloride secretion. This protein is present in all endodermal and mesodermal cells, and it has been found in sweat glands, organs of the digestive system, and the airways' epithelium layer (Bargon et al., 1999). The primary defect causes dehydration of the airways, leading to an increased viscosity of mucus in the intercellular environment, and it predisposes the body to chronic bacterial infections

(Tummler et al., 1999). At birth, the lung is histologically normal, and the pathophysiological changes evolve with aging (Shwachman et al., 1970). CF leads to pulmonary bronchiectasis and atelectasis, compromising the bronchi and bronchioles and so causing pulmonary emphysema. The pancreas is the organ that presents the largest functional and structural changes (Rozov et al., 1991). The blockage of glandular ducts leads to malnutrition syndrome, biliary liver cirrhosis, intestinal obstruction, and gastroesophageal reflux. In newborn infants, the presence of ileum-meconium can mark the first manifestation of CF (Feingold et al., 1999). Exocrine insufficiency of the pancreas occurs in 95% of cases (Mousia-Arvanitakis, 1999) and results in a decrease or the absence of lipolytic, proteolytic and amylolytic enzymes in the pancreatic juice, leading to chronic diarrhoea with bulky, greasy and fetid faeces. As a result, malnutrition becomes evident, owing to the loss of calories and proteins through poor digestion (Reis e Damasceno, 1998). The blockage can also affect the biliary ducts – the thick bile leads to difficulties of drainage, and there may be a full blockage of the ducts which may evolve into cirrhosis (Kopel, 1992).

Advances in genetic engineering and the development of transgenic animals during the last decade of the twentieth century, in conjunction with the prospect of early diagnosis, has contributed to the provision of proper and effective treatments that can increase the quality of life of patients with CF.

### 1.1 Incidence

The incidence of CF varies according to ethnicity, ranging from 1 CF individual per 2,000 to 5,000 Caucasian live births in Europe, the United States and Canada, 1 CF individual per 15,000 African Americans, and from 1 CF individual per 40,000 live births in Finland (Brunechy, 1972). In Brazil, the estimated incidence for the southern region is closer to that of Central Europe's Caucasian population, whereas for other regions it reduces to 1 per 10,000 live births. This is despite the fact that there are variations in the frequency of mutations in different geographic regions, which would probably reflect a different prevalence of the disease (Raskum et al., 1993). In the US and in European countries, early diagnosis – before the first year of life – allows affected children to be promptly treated and monitored with regard to the variables that directly influence the prognosis of the disease, such as the follow-up of the weight and height curve, and the presence of upper airway colonisation by pathogens (which is closely related to worse prognosis).

In Brazil, since 2001, and with the approval of the National Newborn Screening Program and its introduction by the laboratory of the Ecumenical Foundation for Exceptional Protection (FEPE-PR, Portuguese acronym), CF screening has been implemented in the State of Parana. Before the establishment of the National Newborn Screening Program for CF, the data showed that the average age receiving a diagnosis of the disease ranged at around 1.6 years (Santos et al., 2005).

### 1.2 Genetics

The isolation of the CFTR is a result of many years of study on the part of numerous research groups. Situated in long arm of the chromosome 7, at band q31.3 (Heng et al., 1993), with 250 Kb, the region codifier of the CFTR consists in 27 exons. Most of the exons are far from one another by between 50 and 250 base pairs, except for exon 13 which has 723 base

pairs of the genomic DNA. The CFTR protein has a molecular weight of 168,138 Da and it belongs to a transmembrane chloride ion channel superfamily protein, with 1,480 amino acids (Harris, 1992) present in apical membranes of those cells lining the surface of the gland tubes and the airways.

About 1,500 mutations have already been identified in the CFTR: the most frequent mutation is F508del, which is found in 30% of patients with CF (Zielenski, 2000). This mutation is caused by the deletion of three base pairs corresponding to the codon that translates a residue phenylalanine at position 508 of the CFTR polypeptide chain (Morral, 1994). Depending on ethnic groups in different geographic locations the relative frequency of this mutation vary among individuals affected by CF. In Northern Europe and North America, it reaches 70-90%; however it is less frequent in Southern Europe, where less of 50% of the CF chromosomes have this mutation (Morral et al., 1994).

The G542X mutation is considered to be the second most frequent mutation, and it accounts for 3.4% of alleles in CF (Tsui, 1992). At a molecular level, it leads to a replacement of nucleotides, which results in a stop codon at position 542 of the polypeptide chain, and thus the translation product is a non-functional peptide that will be degraded. According to the geographic location, the frequency of this mutation varies among the CF individuals. It may be found in the compound heterozygous with the  $\Delta$ F508 mutation. Raskin et al. (1993) have noted the frequency of the G542X mutation in 5% of the Brazilian population.

The G551D mutation affects 2.4% of the chromosomes of the population of individuals with CF - in general - and it leads to a replacement of guanine for adenine in nucleotide 1784 and, as with the G542X mutation, it also is located in exon 11. In Brazil, it presents a frequency of 1% (Raskin et al., 1993). Other, less frequent, mutations include: W1282X, N1303K, R553X, R1162X, and R334W (with their incidence varying according to population). Table 1 shows the molecular changes, and consequences of the more common mutations in the cystic fibrosis gene (Tsui, 1992).

### 1.3 Treatment

Approximately 50% of affected individuals are diagnosed in the age range from zero to six years but this percentage goes to 90% for those aged zero to eight years. Since reinforced nutrition is associated with a better prognosis, the screening of newborn infants is indicated. CF is one of most studied genetic diseases under the new therapy approaches, such as gene therapy which aims to restore the CFTR function. Clinical trials of gene therapy have been performed with viral vectors and cationic lipids. Despite advances in our knowledge of the disease, there is no specific treatment for CF yet. Due to its multi-systemic and chronic character, its treatment should be performed in reference centres and with a multidisciplinary team. Patients responding well to the treatment showed a median survival which has been increasing year to year, from over two years in 1950 up to between thirty and forty years today (Ribeiro et al., 2002). It is necessary to establish a strong and uninterrupted treatment program which is addressed to the prophylaxis of infections and complications. It should be started as soon as possible and it should be individualised, taking into account its severity and the organs affected. Early treatment decreases the evolution of the pulmonary lesions, improves prognosis, and increases the chances of survival.

NAME	MUTATION	CONSEQUENCE
ΔF508	Deletion of 3pb between 1652 and 1655 of the exon 10	Deletion of Phe in codon 508
G542X	G→T in nt 1756 of exon 11	Gly→ stop code in codon 542
G551D	G→A in nt 1784 in exon 11	Gly→ Asp in codon 551
W1282X	G→A in nt 3978 of exon 20	Trp→ stop code in codon 1282
3905insT	Insertion of T after the nt 3905 of exon 20	Change the reading chart.
N1303K	C→G in nt 4041 of exon 21	Asn→Lys in codon 1303
3849+10kbC → T	C→T in a Eco RI fragment at the attachment 5' of intron 19	ABERRANTE excision
R553X	C→T in nt 1789 of exon 11	Arg→ stop code in codon 553
621+1G→T	G→T in nt 1 of attachment 5' of intron 4	Excision mutation
1717-1G→A	G→A in nt 1 of attachment 3' of intron 10	Excision mutation
1078delT	Deletion of T in nt 1078 of exon 7	Change reading chart
2789+5G→A	G→A in nt 5 of end 5' of intron 14b	Excision mutation
3849+4A→G	A→G in nt 4 of end 5' of intron 19	Excision mutation
711+1G→T	G→T in nt 1 of attachment 5' of intron 5	Excision mutation
R1162X	C→T in nt 3616 of exon 19	Arg→ stop code in codon 1162
1898+1G→A	G→A in nt 1 of attachment 5' of intron 5	Excision mutation
R117H	G→A in nt 482 of exon 4	Arg→His in codon 117
3659delC	Deletion of C in nt 3659 of exon 19	Change the reading chart
G85E	G→A in nt 386 of exon 3	Gly→Glu in codon 85
2184delA	Deletion of A in nt 2184 of exon 13	Change the reading chart
Δ1507	Deletion of 3pb between nt 1648 and 1653 of exon 10	Deletion of Ile at codon 506 or excision mutation
R347P	G→C in nt 1772 of exon 7	Arg→Pro in codon 347
R560T	G→C in nt 1811 of exon 11	Art→Thr in codon 560 or excision mutation
A455E	C→A in nt 1496 of exon 9	Ala→Glu in codon 455
R334W	C→T in nt 1132 of exon 7	Arg→Trp in codon 334
S549R(T→G)	T→G in nt 1779 of exon 11	Ser→Arg in codon 549
Q493X	C→T in nt 1609 of exon 10	Gln→ stop code in codon 493
S549N	G→A in nt 1778 of exon 11	Ser→Asn in codon 549

Table 1. Table obtained from Tsui (1992).

*Pseudomonas aeruginosa* has been found in more than 80% of teenagers with CF (Dubouix et al., 2003). Once established in the airways, the *Pseudomonas aeruginosa* infection is not eradicated by antibiotics, which only reduce the number of the colonies of bacteria. In order to treat CF, antibiotics can be administered through oral, intravenous or inhalation routes – the choice of which route should be made according to demand, prophylaxis and maintenance.

Ribeiro et al. (2002) defined the main objective of the treatment of CF, namely: the continued education of patients and their relatives concerning the disease; prophylaxis of the infections with a full vaccination program; early detection and control of pulmonary infection; respiratory physiotherapy and the improvement of bronchial blockage; the correction of pancreatic insufficiency; nutritional support with guidelines for diet and vitamin supplementation; monitoring the progress of the disease and any complications; family genetic counselling; finally, the provision of any information to patients and relatives concerning any advances in knowledge of CF.

#### 1.4 Genetic counselling

Genetic counselling is essential in order to improve the understanding of the medical, psychological and familial implications of the disease. This process consists of the following steps: the interpretation of the familial medical history so as to assess the possibility of any occurrence or recurrence of the disease; the provision of education on heredity, genetic tests, the management of the disease, prevention, resources and research; the offer of counselling so as to properly inform the patient of the risks of having an affected child and its future life chances (Resta et al., 2006). Thus, counselling involves confirmation of the diagnosis, the estimated risk of recurrence, the provision of information about the disease, offers of support, and assistance with the acceptance of the diagnosis. It also suggests the provision of proper treatment and the offer of alternatives for prevention, such as pre-natal diagnosis and pre-implantation diagnosis. The first step for effective genetic counselling is the confirmation of the diagnosis of the affected individual (Harper, 2000). The definitive diagnosis of the disease is done with clinical features and the increased concentration of electrolytes (chloride and sodium) in sweat. The presence of the disease may be supposed from an altered neonatal screening, before the onset of symptoms. The CF hereditary pattern is autosomal recessive (i.e. in order to manifest the disease, the individual should present a mutation in two of the alleles of the CFTR). Therefore, both of the parents of the affected individual are carriers (heterozygous), and the risk of the recurrence of a CF child at birth is 25%. The individual with CF may show the same mutation in both of the alleles (homozygous for certain mutations) or else different mutations in each of the alleles (compound heterozygous). Healthy individuals carrying the mutation in only one of the alleles are called heterozygous (Saraiva-Pereira et al., 2011).

With the increase in the life expectancy of CF patients, many women carrying the disease have had pregnancies, whereas the affected men had shown infertility secondary to the obstructive azoospermia; nonetheless they may also have children because of assisted reproduction techniques. The risk of a CF individual having affected children depends upon her/his partner – if the partner is a carrier of the disease, that risk is 50%. The frequency of carriers for CF within the general population varies according to ethnic origin. In Caucasian populations, the frequency of heterozygous varies from 1/25 to 1/30

individuals (Saraiva-Pereira et al. 2011). The main difficulties for the genetic counselling of CF are those cases in which clinical confirmation is uncertain or else those cases in which is not possible to detect carriers within the family.

## 2. CF and infertility

### 2.1 Clinical features of infertility in CF

CF is a genetic disorder caused by mutations in the Cystic Fibrosis Transmembrane Regulator (CFTR) gene (Kerem et al., 1989; Riordan et al., 1989; Rommens et al., 1989). The CFTR is an anion channel regulated by cAMP-dependent phosphorylation, and it is expressed in the apical membrane of epithelial cells of a wide variety of tissues, including the reproductive tracts. The physiological role of CFTR in reproduction and its involvement in the pathogenesis of reproductive diseases remains largely unknown (Chan et al., 2009). The CF disorder is characterised by altered chloride and the bicarbonate transport of secretory epithelial cells (Chan et al., 2006; Li et al., 2010; Quinton, 1990, 1999).

Approximately 97% CF men are sterile due to the congenital bilateral absence of the vas deferens (CBAVD) and obstructive azoospermia (Wong, 1998). It has been suggested that the absence of vas deferens might be related to the requirement of the CFTR function at the embryonic stage (Li et al., 2010). It was demonstrated that the expression of the CFTR is developmentally regulated: cultured epithelial cells from the human foetal vas deferens have been shown to express the CFTR (Harris et al., 1991). Therefore, it is conceivable that fluid secretion by the Wolffian duct is required for the normal development of vas deferens. When secretion is impaired in CF individuals, normal development might be interrupted and so lead to vas agenesis (Wong, 1998).

Clinically, CF patients present a spectrum of genital phenotypes ranging from normal fertility to severely unpaired spermatogenesis and CBAVD (Xu et al., 2007). The diagnosis of CBAVD is based on the presence of azoospermia in subjects with normal or small size testes, non-palpable vas deferens, the characteristic ultrasonography view, and changes in the physical and biochemical properties of ejaculate (Jarzabek et al., 2004). Male CF patients' semen is characterised by azoospermia, low volume, low or normal viscosity, and increased turbidity. Testicular specimens show active spermatogenesis, although 50% of the spermatozoa have malformed heads. The pre-meiotic spermatogonia in CF patients appear to be morphologically normal, whereas the post-meiotic spermatogenic stages show malformation or the impairment of nuclear division (Denning et al., 1968). Testicular biopsies of post-pubertal men with CF have shown abnormal histological findings, such as pathological spermatogenesis and an increased number of dysmorphic spermatozoa (Mak et al., 2000).

The investigation of CFTR expression in male reproductive tissues showed that CFTR was present in the epididymis and vas deferens throughout post-natal life. High levels of CFTR expression were found in the head of the epididymis, but a variable expression was seen in distal epididymis. No CFTR was detected in the human testis. Accordingly, it was suggested that the anomalies in spermatozoa described in CF adult patients may result from epididymal dysfunction (Tizzano et al., 1994). Primary cultures of rat epididymal epithelial cells demonstrated the functional expression of CFTR and its involvement in the regulation of chloride secretion and fluid formation in the epididymis (Wong, 1998). Under basal

conditions, the epididymis generally reabsorbs fluid to concentrate sperm. However, the observation that neurohormonal factors stimulate CFTR-mediated chloride secretion by epididymal epithelia (Wong, 1998) suggests that epididymal fluid secretion may be stimulated so as to create the optimal fluid environment for sperm maturation, storage, and even transport during ejaculation (Chan et al., 2009). Approximately 18 % of non-clinical CF men with infertility due to a reduction of sperm quality, and 15 % men with azoospermia, have at least one mutation in the CFTR. The frequency of mutations in infertile males presents a significantly higher than expected 4 % of the CF carrier frequency within the population. This increased frequency of CFTR mutations in healthy men with reduced sperm quality, and in men with azoospermia without CBAVD, suggests that the CFTR protein might be involved in the process of spermatogenesis or sperm maturation, over and above playing a critical role in the development of the epididymal glands and the vas deferens (Van der Ven et al., 1996).

In order to fertilise eggs, mammalian sperm must acquire fertilising potential in the female reproductive tract through a process known as capacitation. Sperm capacitation is a prerequisite for the acrosome reaction, which is an exocytotic event releasing hydrolytic enzymes from the acrosome so as to enable spermatozoa to penetrate the egg investments and its plasma membrane (Jarzabek et al., 2004; Xu et al., 2007). Capacitation is associated with the elevation of intracellular pH and the hyperpolarisation of the sperm plasma membrane (Meizel & Deamer, 1978; Zeng et al., 1995). These events depend on extracellular bicarbonate, which activates adenylyl cyclase pathway producing cyclic adenosine monophosphate (cAMP) and various downstream cellular events (such as protein tyrosine phosphorylation) and results in sperm capacitation (Demarco et al., 2003; Xu et al., 2007).

As has been shown, CFTR secretes bicarbonate in the uterus and sperm, and its impairment leads to reduced sperm capacitation and the fertilising capacity of sperm (Wang et al., 2003; Xu et al., 2007). The interaction of the CFTR protein with its inhibitor or antibody significantly reduces sperm capacitation and associated bicarbonate-dependent events, including increases in intracellular pH, cAMP production, and membrane hyperpolarisation. The fertilising capacity of the sperm obtained from heterozygous CFTR mutant mice is also significantly lower compared with the wild-type. These findings suggest that sperm CFTR may be involved in the transport of bicarbonate important for sperm capacitation, and that CFTR mutations causing impaired CFTR function may lead to a reduced sperm fertilising capacity and male infertility other than CBAVD (Xu et al., 2007). A recent study showed that human sperm capacitation as facilitated by progesterone and acrosome reactions induced by recombinant human zona pellucida 3 peptides (rhuZP3) were both significantly inhibited by a CFTR inhibitor. In a group of fertile men, the percentage of spermatozoa expressing CFTR was significantly higher than that of the healthy and infertile men's group. In addition, the study showed that the presence of a CFTR inhibitor markedly depresses intracellular cAMP levels, sperm hyperactivation and the sperm penetration of zona-free hamster eggs (Li et al., 2010). Moreover, when spermatozoa from CF patients with CBAVD are used for intracytoplasmic sperm injection, fertilisation rates are not reduced, suggesting a specific defect in zona pellucid penetration or membrane fusion capacity in these spermatozoa (Silber et al., 1994; Li et al., 2010). CFTR appears to have a profound role in regulating sperm function (Chan et al., 2009).

In CF female patients, the cause of reduced fertility remains obscure. CFTR is expressed throughout the female reproductive tract in the cervix, oviduct, ovary, and uterus (Chan et al., 2002; Tizzano et al., 1994). CF has been associated with menstrual irregularities, including amenorrhea, irregular cycles, anovulation, smaller uteri, and delayed puberty (Johannesson et al., 1998; Stead et al., 1987). The absence of obvious anatomical abnormalities in the female reproductive tract – except for thick and tenacious cervical mucus with altered water and electrolyte content (Kopito et al., 1973) – has led to the general belief that abnormal mucus contributes to the reduced fertility of CF women by acting as a barrier to sperm passage (Chan et al., 2009). However, repeated and unsuccessful attempts with intrauterine insemination were also reported (Epelboin et al., 2001; Rodgers et al., 2000), suggesting that further abnormalities (such as inadequate fluid control throughout the rest of the reproductive tract) could also contribute to infertility in humans (Hodges et al., 2008).

As already noted, CFTR plays a crucial role in mediating uterine bicarbonate secretion and sperm capacitation, leading to the thought that CFTR bicarbonate secretion dysfunction might induce an impaired sperm fertilising capacity and reduced fertility in CF women. In a mouse sperm-endometrial epithelial cell co-culture system, it was demonstrated that endometrial epithelial cells possess a CFTR-mediated bicarbonate transport mechanism. A substantial decrease in apical fluid bicarbonate contents was observed after treatment with both blockers of CFTR and antisense oligonucleotides against CFTR when compared with the control. These results are consistent with the CFTR's mediating uterine bicarbonate secretion, and they indicate that defective CFTR might lead to impaired bicarbonate secretion in the uterus. *In vitro* fertilisation assays on zona-intact mouse eggs further demonstrated that the number of two-cell embryos obtained with sperm capacitated in a conditioned medium from CFTR antisense-treated endometrial cells was significantly reduced as compared with that obtained from sense-treated controls. Sperm capacitation and egg-fertilising ability depend – critically – on CFTR and bicarbonate content and defective CFTR-mediated bicarbonate secretion, and the lower fertilising capacity of sperm might also account for lower CF female fertility (Chan et al., 2009; Wang et al., 2003).

CFTR expression in the uterus is regulated by ovarian hormones with increasing expression in response to estrogen and decreasing expression in response to progesterone; this is a pattern that correlates with cyclic changes in uterine fluid (Zheng et al., 2004). Hormone changes have been observed in CF female adolescents, who displayed reduced estradiol and FSH levels (Reiter et al., 1981), and CF female adults, who displayed increased testosterone and reduced estradiol and progesterone levels, compared with age-matched controls (Johannesson et al., 1998). Interestingly, through observations in rodent uterus, CFTR was found to be co-expressed with the epithelial sodium channel (ENaC) in an out-of-phase fashion. With the maturation of ovarian follicles and the estrogen secretion phase, CFTR is highly expressed and ENaC is poorly expressed. Inversely, with corpus luteum activity and progesterone secretion, low CFTR expression and high ENaC expression are observed (Chan et al., 2002). This may explain maximal fluid secretion during the early phase of the oestrous cycle, when the level of oestrogen is at its highest. Similarly, at dioestrus, the attenuated fluid production with down-regulation of CFTR and increased reabsorption by up-regulation of ENaC may account for the disappearance of uterine fluid. These cyclic changes in CFTR and ENaC expression which result in uterine fluid volume variation have

physiological significance. Maximal CFTR expression and – therefore – high uterine fluid production may lubricate the cervical and vaginal lumen for sperm movement towards the oviduct as well as sperm capacitation. Equally, low CFTR expression and – consequently – reduced fluid volume may enhance close contact on the endometrial surface, facilitating embryo implantation. Dynamic changes in the fluid microenvironment, particularly the fluid volume, in the female reproductive tract are dictated by CFTR expression, which is normally regulated by ovarian hormones throughout the cycle and accommodating various reproductive events. The impairment of CFTR expression may lead to the disturbance of the fluid environment, resulting in various pathological conditions and infertility (Chan et al., 2007; Chan et al., 2009).

Interestingly, abnormalities in the reproductive endocrine axis have been viewed as an indirect consequence of CF, though they have been largely ignored as possible contributors to observed female infertility (Stead et al., 1987). CFTR expression was found in the areas of the rat hypothalamus (thalamus and amygdale) which are involved in the regulation of sexual maturation and reproduction. CFTR might increase the acidification of synaptic vesicles, and thus play an important role in the central regulation of sexual maturation and fertility (Johannesson et al., 1997b). Delayed pubertal increments of serum gonadotropin and sex hormone levels in CF patients suggest a late maturation of the reproductive system (Reiter et al., 1981). Anovulatory women showed significantly lower luteal oestradiol and progesterone, but higher total testosterone concentrations when compared to healthy controls and the ovulatory CF women (Johannesson et al., 1998). In a mouse model, increased FSH levels were found in CFTR mutant females as a result of a decreased number of ovulatory follicles, leading to less estradiol production and a lack of feedback inhibition of FSH secretion. CF female mice exposed to exogenous hormones showed a correction of organ size and ovulation. These findings suggests that the CF reproductive organs can respond to gonadotropins, but that an impaired hypothalamic-pituitary-gonadal (HPG) axis may be a direct cause of reduced fertility in women with CF (Hodges et al., 2008; Johannesson et al., 1997b).

In CF women, late puberty and amenorrhea are common clinical findings due to the deficit in their nutritional status. It has also been suggested that the lack of ovulation is a consequence of malnutrition and catabolism. Clinically, the anovulatory women presented more profound essential fatty acid deficiency (EFAD) and hypersecretion of insulin during an oral glucose tolerance test compared to the ovulatory women (Johannesson et al., 1998). However, it was shown that menarcheal age was also delayed in CF females in good clinical and nutritional condition. Homozygous patients for the most common mutation – F508del – and those with a pathological glucose tolerance test (OGTT) showed the most delay in menarcheal age (Johannesson et al., 1997a). This may be explained by the fact that ovarian cells express insulin receptors that mediate gonadal steroid production. Experimental data has shown that insulin has a gonadotropic effect through different mechanisms, such as a direct effect on steroidogenic enzymes, the modulation of FSH or LH receptor number, synergism with FSH and LH, and nonspecific enhancement of cell viability (Porestky & Kalin, 1987). Insulin appears to be necessary for the ovary to reach its full steroidogenic potential. The difference observed in the insulin pattern in the pathological OGTT group might alter ovarian function and thereby cause further delay in sexual maturation (Johannesson et al., 1998). Polycystic ovaries were also described in CF women (Stead et al., 1987).

CFTR mutations were previously associated with Congenital Absence of Uterus and Vagina (CAUV). CF mutations might affect the normal embryological development of the Müllerian ducts. During the seventh week of gestation, the cranial end of the Müllerian duct is immediately adjacent to the Wolffian duct, and both ducts share a common basement membrane. The Wolffian duct then guides the caudal growth of the Müllerian ducts. By the ninth week of gestation, the Müllerian duct reaches the caudal end of the adjacent Wolffian duct. At this time, these ductal systems separate from each other, form separate basement membranes, and continue to develop independently (Ludwig, 1998). The interdependency of these two systems suggests that the same genetic factors may control the early development of both systems. Failure of the development of the Müllerian duct causes CAUV in females. The incidence of most common CFTR mutations found in patients with CAUV (8%) is twice that which is found in the general population (4%), but much less than the incidence of CFTR mutations in men with CBAVD (80%). This suggests that it is unlikely for CFTR mutations to cause CAUV in females as they cause CBAVD in some males. As such, the effect of the abnormal CFTR protein product on the Wolffian duct must occur at a time when the development of the Müllerian duct is no longer dependent on the Wolffian duct (Timmreck et al., 2003).

CF female patients have such pregnancy complications as premature labour and delivery and increased maternal and prenatal mortality due to severe maternal pulmonary infection and maternal weight loss (Cohen et al., 1980; Kent & Farquharson, 1993). However, the risk of the deterioration of health during pregnancy for females with CF is considered to be small, if good medical care is provided and if women are in a stable and good clinical condition (FitzSimmons et al., 1996).

There remain many unanswered questions as to the cause of infertility in CF, and the exact role of CFTR in reproductive physiology and the contribution of CFTR dysfunction to infertility in both sexes is far from understood (Chan et al., 2009).

## **2.2 CFTR mutations closely related to CF infertility**

Infertility, or at least subfertility, in males with CF was first suspected in the 1960s (Denning et al., 1968; Radpour et al., 2008). Depending upon their molecular consequences, CFTR mutations may result in either a typical CF or else an atypical (often monosymptomatic) CF, such as congenital absence of the vas deferens (bi- or unilateral), bilateral ejaculatory duct obstruction, or bilateral obstructions within the epididymis (Jarzabek et al., 2004). Approximately 80% of CF male patients present CBAVD, a Wolffian duct anomaly (Radpour et al., 2008). Male infertility due to CBAVD has been shown to be commonly linked to CFTR mutations, and it is considered to be a genital form of CF or a CFTR-associated disease with incomplete CF expression (Dequeker et al., 2009; Kanavakis et al., 1998; Rave-Harel et al., 1997). Men with CBAVD are apparently healthy, with relatively normal lung and pancreatic functions. CBAVD appears to be a heterogeneous genetic condition, with many cases being mild forms of CF (DeBraekeleer and Férec, 1996).

Extensive studies have shown that patients with isolated CBAVD carry two CFTR mutations, usually in compound heterozygosity (Chillón et al., 1995; Claustres et al., 2000). Of isolated CBAVD patients, where the mutation is found on both CFTR, about 88% carry one severe mutation and one mild mutation, whilst the remaining 12% carry mild mutations

on both CFTR (Claustres et al., 2000). This is in contrast to classical clinically CF patients, where about 88% of the CF patients carry severe mutations on both CFTR, whilst about 11% carry a severe mutation on one CFTR and a mild mutation on their second one (Claustres et al., 2000; Radpour et al., 2008). The most frequent CFTR mutation conferring a mild phenotype found in CBAVD patients is the 5T polymorphism (Chillón et al., 1995), which is an allele found at the polymorphic Tn locus in intron 8 of the CFTR, and which can be found as a stretch of 5, 7, or 9 thymidine residues at this locus. Less efficient splicing will occur when a lower number of thymidines are found, resulting in CFTR transcripts that lack exon 9 sequences (Chu et al., 1993; Radpour et al., 2008). Men with the 5T variant in the non-coding region of the gene will produce an abnormally low level of CFTR protein in the epididymis. However, there may be sufficient proteins for the prevention of disease in other organs (such as the lung and the gastrointestinal glands) normally affected by CF, which might explain why the lung and pancreas are normal in CBAVD, but the epididymis is not (DeBraekeleer and Férec, 1996; Jarzabek et al., 2004; Wong, 1998). The analysis of the level of correctly spliced RNA transcribed from the 5T allele in different tissues (nasal and epididymal epithelium) and its correlation with CF disease expression, has shown that in infertile males with normal lung function the level of correctly spliced transcripts found in the nasal epithelium was higher than the level found in the epididymal epithelium. It indicates that there is variability in the efficiency of the splicing mechanism both between different individuals and between different organs of the same individual. In many human monogenic diseases, high variability in disease expression is found among patients carrying the same genetic defect (Levy et al., 2010; Rave-Harel et al., 1997). The molecular basis for this variability has been suggested to be allelic heterogeneity, additional genetic loci, and/or environmental factors. Accordingly, allelic variants of genes involved in the splicing regulation might contribute to the different efficiencies of alternative splicing found amongst different individuals (Rave-Harel et al., 1997).

CFTR mutations may represent one of the most common abnormalities associated with male infertility, especially with CBAVD but also with obstructive azoospermia (Kanavakis et al., 1998). A screening of the entirety of the CFTR in males with CUAVD (congenital unilateral absence of vas deferens), CBAVD and obstructive azoospermia of the vas deferens, has shown that almost 64% of patients carry two CFTR mutations. The most frequent mutations observed amongst those patients were F508del (44.7%), T5 allele (36.2%), and R117H (19.1%) (Jézéquel et al., 2000). In a large French cohort study, the most frequent allele mutations identified in CBAVD male patients were F508del (21.7%), the 5T allele (16.3%) and R117H (4.4%), followed by D1152H (1.19%) and D443Y (0.93%). Two CFTR mutations (including the 5T allele) were present in 47.7% and one mutation in 24.6% of CBAVD patients, while no mutation was reported in the remaining 27.7%. Approximately 43.5% of patients with CBAVD carried one F508del allele, and 31.7% had at least one 5T allele. Altogether, at least one CFTR mutation was identified in 72.25% individuals with CBAVD (Claustres et al., 2000). In an Italian multicentric study, a molecular screening of the most common CFTR mutations in infertile couples was performed. CFTR mutations were detected in 4.6% of subjects, a percentage that overlaps with the general population carrier frequency. However, it was found a mutation-frequency of over 37% amongst CBAVD individuals and of 6% in males with non-obstructive azoospermia (Stuppia et al., 2005). In another study, the carrier status of CBAVD patients for the F508del mutation was screened and 57% were found to be

carriers. Amongst these patients, 25% were later found to have compound heterozygotes for the F508del and R117H mutations (Williams et al., 1993). A study of the entire coding region of the CFTR of CBAVD patients found that 28.6% have mutations in both copies of the CFTR, 42.8% had one CFTR mutation, whilst in the remaining 28.6% no CFTR mutations were found (Kanavakis et al., 1998). These figures give an average of an eleven-fold increase of the carrier frequency compared to the population data on CFTR mutations in CBAVD patients. (Uzun et al., 2005). Amongst cases of obstructive azoospermia, 30% had one CFTR mutation whilst in the remaining 70% no mutations were found – this indicates an association between cases of obstructive azoospermia without CBAVD with CFTR mutations. The frequency of the IVS8(5T) allele was 14.3% for the CBAVD cases, which was three-fold higher than for normal chromosomes (Kanavakis et al., 1998). Similarly, another extensive analysis of the CFTR in CBAVD patients revealed that 42% of subjects were carriers of one CFTR allele and that 24% were compound heterozygous for CFTR alleles. The presence of only one CF allele in approximately 42% of CBAVD patients implies some role on the part of CF in CBAVD, although additional factors or genes are necessary for the development of CBAVD in those patients (Mercier et al., 1995; Van der Ven et al., 1996; Williams et al., 1993). The CFTR mutations commonly associated with male infertility are F508del, R117H, and the IVS8 (5T) polymorphism, each of which exhibit diverse frequencies among different cohorts (Van der Ven et al., 1996). Since the spectrum of CFTR mutations is markedly different amongst populations, the ethnic background of the patients should be taken into account so as to ensure that the most prevalent mutations appropriate to that particular population are included in the screening panel (DeBraekeleer & Férec, 1996). Altogether, the mutation-frequencies in infertile male patients are significantly higher than the expected carrier-frequencies in the general population (Van der Ven et al., 1996).

There are only a few studies on female CFTR mutation-frequency in the literature. It is generally assumed that fertility is reduced in CF women, although not as dramatically as in men. It was already proposed that CFTR mutations do not appear to be involved in female infertility (Morea et al., 2005) and CAUV condition (Radpour et al., 2008). The most common CFTR mutations – including the 5T allele – were tested in isolated CAUV female patients. These mutations were only found in 8% of the subjects, suggesting that it is unlikely that CFTR mutations cause CAUV in females (Timmreck et al., 2003). In a recent study, 24 women with altered fertility were screened for the F508del mutation. Amongst them, 37.5% showed reduced fertility without a known cause, 20.8% presented reduced fertility due to polycystic ovarian syndrome (although two of them demonstrated malformations of the reproductive tract), 37.5% had been pregnant previously although most of them had spontaneous abortions, and 8.3% presented early menopause. It was found that one patient who was a F508del mutation carrier and who had had an early menopause had also had a previous abortion. Unexpectedly – considering that Brazilian population is greatly mixed – the carrier frequency for the most common mutation in CF amongst infertile Brazilian women was similar to that of Caucasian populations. It was proposed that there are common clinical features between women with altered fertility and with CF women, and that CF mutations may be more frequent than expected amongst patients with fertility issues (Brunoro et al., 2010). Large cohort studies on CFTR mutation-frequency among infertile women are needed.

### 2.3 Considerations for CF mutation screening tests

According to the CF Mutation Database, around 20 mutations have individual worldwide frequencies greater than 0.1%, and can thus be considered to be common mutations (Lay-son et al., 2011). These common mutations vary by geographic and/or ethnic origin. Latin American countries have a high ethnic admixture and they show a wide distribution of 89 different mutations. Most of these mutations are frequent in Spain, Italy, and Portugal, and so is consistent with the origin of the European settlers. A few mutations found among Africans are also present in those countries which were part of the slave trade. This may be the result of the miscegenation of these populations. New mutations were also found which possibly originated in America (Pérez et al., 2007). As in most countries, F508del was the most common mutation detected, but in a lower proportion than the average frequency of 45–46% published for Latin-American countries (Keyeux et al., 2003; Pérez et al., 2007; Zielenski & Tsui, 1995), and the reported worldwide frequency of 66% (Lay-son et al., 2010; Zielenski & Tsui, 1995). The G542X mutation is the second most frequent mutation in Latin America, with a total frequency of 5.07%. N1303K, W1282X and R1162X are the next most frequent mutations, with variations from 0.59% to 3.95%. The frequency of the rest of the mutations varies from one country to another, but their overall frequencies are less than 1%, and could be considered to be rare in Latin America (Pérez et al., 2007). The carrier-rate and mutation-frequencies vary widely in different populations, and so screening tests with high detection-rates for CFTR mutations have to consider the population's ethnicity (Pieri et al., 2007).

There is increasing evidence that CFTR mutations may contribute etiologically to certain monosymptomatic disorders. Infertile men with isolate obstructive azoospermia may have mutations in the CFTR, many of which are rare in classical CF and not evaluated in most routine mutation screening. It was demonstrated that the routine mutation panel has failed to identify CFTR mutations and the IVS8-5T allele in 46% of CBAVD groups, 50% of CUAVD groups, and 79% of idiopathic epididymal obstruction groups. These results demonstrate that routine testing for CFTR mutations for infertile men may miss mild or rare gene alterations. The DNA sequence method detects more CFTR mutations than common mutation panels. This represents a significant problem because advances in assisted reproduction have allowed infertile male patients to conceive, raising the concern of transmitting - when present - pathogenic CFTR mutations onto progeny. The importance of accurate CFTR mutation detection in men with obstructive azoospermia and their partners has already been highlighted (Danzinger et al., 2004; Mak et al., 1999). Today, screening for a panel of CFTR mutations is offered to infertile men prior to in vitro fertilisation (IVF) or *intra cytoplasmic sperm injection* (ICSI), and includes only the most common mutations found amongst the CF patients of European and North American origin. The atypical CBAVD phenotype, however, is caused by milder mutations, most of them very rare or even not yet described, and thus not included in the panel of CF mutations usually screened. It was proposed that only an extensive CFTR screening can detect rare mutations that are not found by conventional screenings and commercial tests, and can thus improve the diagnosis and care of CF and CAVD as well as the prevention of new cases through the use of reproductive technologies (Pieri et al., 2007).

However, genetic testing should only be performed in the context of appropriate genetic counselling and laboratories should work in close association with clinical geneticists and reference laboratories so as to ensure that pertinent tests are performed and that proper information is provided to patients. There is no standard or preferred method, but laboratories should be aware of the limitations of their chosen method and they should know which mutations are not identified, whether the techniques are commercially available or else being developed within the laboratory. This means that individual laboratories should choose a method which is suited to their experience, workload, and scope of testing. In addition to the screen for frequent mutations, a complementary panel may be required to test population-specific mutations with a frequency above 1%. The knowledge of the ethnic or geographic origins of patients and their parents and grandparents is therefore important in order to determine the analysis to be performed (Dequeker et al., 2009). The knowledge of geographic or ethnic variations in the local population mutation-frequency is crucial so as to properly achieve effective genetic counselling and improve the cost-effectiveness of screening and diagnostic tests (Layson et al., 2011).

#### **2.4 Ethical implications of genetic testing**

According to the Patient Registry 2009 of the CF Foundation, USA there are growing numbers of CF adults 18 years of age and over. The percentage of CF patients aged 18 years or older has risen from 30% in 1990 to over 47% in 2009. It also have indicated that the median age of survival of patients with CF has risen from 27 years in 1985 to almost 36 years in 2009, leading to greater concern for the disease management of CF adults (Cystic Fibrosis Foundation Patient registry 2009: Annual data report, 2011). Coupled with an improved life expectancy, adult CF patients are more likely to seek independence from their families and pursue typical adult activities, such as attending college, entering serious relationships and pursuing careers (Modi et al., 2010). Issues related to sexual maturation, fertility, pregnancy and contraception have thus become important in the comprehensive care of CF patients (Tizzano et al., 1994). Fertility bears centrally to reproductive decision-making, determining whether natural conception is even an option or whether adoption or assisted reproductive technology must be considered (Hull & Kass, 2000). Along with the wish to conceive, CF parents and physicians confront major ethical issues regarding abortions, the premature termination of pregnancies, and possible arrangements in the event of morbidity or maternal mortality which should all be discussed prior to pregnancy (Barak et al., 2005).

There are still many paediatric CF clinics that continue to care for patients up to 18 years of age. Several studies have suggested that teenage patients and their parents have unmet information needs regarding the patient's sexual health. Usually, unplanned sex tends to be done without protection. An important priority for the CF team is to try to ensure that women with CF are aware of the risks of unplanned pregnancy. Collaboration between the family planning clinician and the teenager's CF physician is recommended (Roberts & Green, 2005). As such, reproductive counselling and reproductive health issues must be carefully addressed to CF adult patients (Hull & Kass, 2000; Sawyer, 1996). CF healthcare providers are an important source of information, and early discussion of sexual and reproductive health is indicated in paediatric settings for the adolescent patients, since a very high interest in future parenting is expressed by CF men. It has been suggested that

there should be greater emphasis on infertility, semen analysis, and the prevention of sexually transmitted infections, backed with a greater focus on reproductive options within adult healthcare services (Sawyer et al., 2005).

In the 1980s, it was thought to be too risky for a woman with CF to get pregnant and that it was impossible for a man with CF to father a child. Nowadays, improvements in the nutrition and lung function of these patients make it possible for CF women to have a healthy pregnancy and baby. In 2009, the Patient Registry reported that 226 women with CF were pregnant. Successful outcomes can be achieved for both the CF mother and the child with careful patient assessment, combined with the integration of a multidisciplinary team, composed of the CF physician, the fertility specialist and the obstetrician (CF Foundation Patient registry 2009: Annual data report, 2011). Close follow-up of the maternal and foetal condition, along with careful monitoring of ventilation, immunology, diabetes, glucose tolerance and nutrition is important since all these parameters may be adversely affected in a CF pregnancy (Barak et al., 2005; Rodgers et al., 2000).

For CF men, advances in fertility medicine have given them the option to father children (CF Foundation Patient registry 2009: Annual data report, 2011). The use of assisted reproductive techniques (such as testicular micro-aspiration and intracytoplasmic sperm injection (ICSI)) has enabled testicular spermatozoa to fertilise ova without the need to be capacitated, or to undergo acrosome reaction or else penetrate and fuse it with the egg (Wong, 1998). A report on CF men that have undergone ICSI coupled with IVF showed that 62% of the couples successfully achieved pregnancy (McCallum et al., 2000). A group of CF azoospermia males were submitted to ICSI and 63% of the couples had clinical pregnancy (Hubert et al., 2006). However, before such measures are taken, genetic screening and counselling for the men and their partners should be mandatory in safeguarding their offspring from the risk of clinical CF (DeBraekeleer and Férec, 1996). Moreover, CF men should be informed about their own health and any long-term issues (such as the likelihood of premature death) and this information should be clearly shared with their partner (Hubert et al., 2006). In the case of CBAVD patients - a genital form of CF - most carry a severe CF-causing CFTR mutation and, therefore, have a 0.5% chance of transmitting the CFTR mutation to the child. Assuming a risk of 1/25 of the partner being a CF carrier, and that a carrier has a chance of 0.5% of transmitting the mutant CFTR to the child, the combined risk of CBAVD couples of having a CF child is 1/100 when compared with a risk of 1/2500 amongst general population (Radpour et al., 2008). The detection of a CFTR mutation in CF male patients and their spouses is crucial, since the presence of a CFTR mutation would present a high-risk situation whereas its absence would present a low-risk situation (DeBraekeleer and Férec, 1996). In cases of oligozoospermia, it is also ideal to screen both partners. It was also recommended that - if resources are stretched - amongst couples with a CBAVD male only the female needs to be routinely CF screened because, if she is negative, then the couple's residual risk of having a CF or CBAVD child will be reduced to 1:960 (Lewis-Jones et al., 2000). The reproductive options for the majority of CF men who are infertile include not having children, adoption, in vitro fertilisation with donor sperm, and microscopic epididymal sperm aspiration (MESA) coupled with in vitro fertilisation. There is also a complementary option, namely pre-implantation genetic diagnosis (PGD). PGD refers to the genetic testing of embryos created through IVF for the purpose of selecting embryos that would lead to the birth of a child unaffected by a

detectable genetic defect. The notion of preventing a disease by preventing the birth of an individual with that disease is controversial. The CFF has no official position on this practice, and this type of decision is a personal choice to be made by the individual together with his/her physician (Davis et al., 2010).

### 3. Conclusion

The National Institutes of Health, USA recommends genetic counselling for any couple attempting assisted reproductive techniques where the man has CF or obstructive azoospermia and is positive for a CFTR mutation. It is important to analyse the clinical genetic conditions of the families by evaluating the full family history, by documenting the pregnancy and foetal, neonatal, and paediatric loss of life, as well as by cytogenetic studies of the couple and analysis for CFTR mutations. At this time, it is debatable whether it is better to perform the screening for mutations in the full gene or whether it is better to screening for typical local mutations of a population. It is also subject of debate if it is better to perform the screening for mutations in CF affected individuals only or also in their partner. All these choices have ethical and social implications and there may be better resolution with new population studies focused on the frequency of mutations in CF individuals with infertility. CF is now a disease of the adult population with many adult-specific issues. As such, adult CF patients must be treated by a well-trained interdisciplinary team of adult-care providers within the environment of the CF care network.

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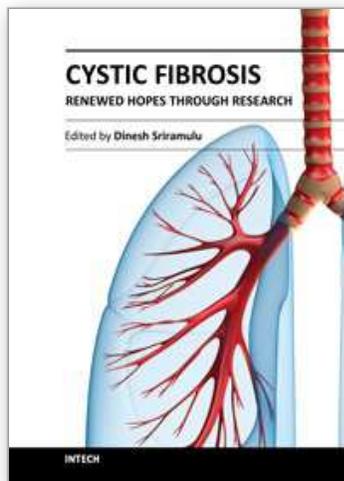
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## **Cystic Fibrosis - Renewed Hopes Through Research**

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Living healthy is all one wants, but the genetics behind creation of every human is different. As a curse or human agony, some are born with congenital defects in their menu of the genome. Just one has to live with that! The complexity of cystic fibrosis condition, which is rather a slow-killer, affects various organ systems of the human body complicating further with secondary infections. That's what makes the disease so puzzling for which scientists around the world are trying to understand better and to find a cure. Though they narrowed down to a single target gene, the tentacles of the disease reach many unknown corners of the human body. Decades of scientific research in the field of chronic illnesses like this one surely increased the level of life expectancy. This book is the compilation of interesting chapters contributed by eminent interdisciplinary scientists around the world trying to make the life of cystic fibrosis patients better.

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