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“CORRELATION OF CFTR GENE MUTATIONS AND PRIMARY MALE INFERTILITY”

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# TABLE OF CONTENTS

1. TITLE PAGE
2. TABLE OF CONTENTS
3. SUMMARY
4. ACKNOWLEGMENTS
5. CONFLICTS OF INTEREST
6. PERMISSION ISSUED BY THE EITHICAL COMMITTEE
7. ABBREVIATIONS
8. TERMS
9. INTRODUCTION
10. AIM AND OBJECTIVES
11. LITERATURE REVIEW
12. RESEARCH METHODOLOGY AND METHODS
13. DISCUSSION
14. CONCLUSION
15. REFERENCES
SUMMARY

RICHARD HILAL

“CORRELATION OF CFTR GENE MUTATIONS AND PRIMARY MALE INFERTILITY”

AIM: Our purpose in this study is to investigate the nature of the connection between CFTR and Primary male infertility. As well as creating a summary of the mechanism that connects CFTR and male infertility, using the most recent product of research.

OBJECTIVES:

1. To review literature on connections between CFTR gene mutations and primary male infertility.
2. To study literature for phenotype-genotype correlation of various CFTR gene mutations and the primary male infertility.
3. To study the mutation impact for the patients.

METHODOLOGY: Literature databases were searched for papers on the topics related to CFTR and male fertility and infertility with relevant keywords such as “CFTR”, “CBAVD”, “infertility”, “spermatogenesis”, “spermiogenesis”. The studies were derived from various databases such as PubMed, Medscape, ScienceDirect. The oldest article was published in 1997 and the latest article was published in March 2018. The date interval used was 10 years. The articles were checked for bias and proper quality.

RESULTS: 134 articles with relevant data were found. 32 articles were suitable to our topic. Articles were rejected on the fact that they were not related to the topic. CFTR has been proven involved in primary male infertility through a multitude of mechanisms, that in a direct and less direct way are capable of causing male infertility.

CONCLUSIONS: The analysis of the literature on the topic proved that different types of mutations are responsible for different phenotypes and that male infertility is one of the more benign pathology in the spectrum of the CFTR mutations. It is anyway noticeable that we cannot draw precise lines of demarcation because, the amount of possible variants that accounts for this gene, are responsible for some overlapping among phenotypes. The impact on the patient depends from the residual function of the protein and the particular form (obstructive or non-obstructive azoospermia) of infertility, because it directly affects the prospective of treatment and the methods with which the patient's partner can achieve a pregnancy.
ABBREVIATIONS LIST:

CF (Cystic Fibrosis)
CFTR (Cystic Fibrosis Transmembrane conductance Regulator)
CBAVD (Congenital Bilateral Absence of Vas Defferrens)
NBD (Nucleotide Binding Domain)
ICP (Idiopathic Chronic Pancreatitis)
OR (Odd Ratio)
PS (Pancreatic Sufficiency)
ICSI (Intra-Cytoplasmic Sperm Injection)
ABP (Androgen Binding Protein)
FSH (Follicle Stimulating Hormone)
BTB (Blood Testes Barrier)
VDAC1 (Voltage Dependent Anion Selective Channel 1)
ROS (Reactive Oxygen Species)
AC (Adenylyl Cyclase)
sAC (Soluble Adenylyl Cyclase)

KEY WORDS:
CFTR, CF, CBAVD, Male Infertility,
INRODUCTION

According to the World Health Organization, infertility is defined as the inability to achieve pregnancy after one year of unprotected intercourse. Mayo clinic states that up to 15 percent of couples are infertile. This means they aren't able to conceive a child even though they've had frequent, unprotected sexual intercourse for a year or longer. In up to half of these couples, male infertility plays a role.

![Chart representing the classification of male infertility](image)

Cystic fibrosis is an monogenic autosomal recessive inherited disease of the exocrine glands affecting primarily the GI and respiratory systems. It is caused by mutation of the CFTR protein. The cystic fibrosis transmembrane conductance regulator (CFTR) gene is located on chromosome 7q31.2 and contains 27 exons. Cystic fibrosis (CF) is the most common life-threatening genetic disease in the white population (Karnajit et al., 2015)
Congenital bilateral absence of the vas deferens (CBAVD) is found in more than 25% of men with obstructive azoospermia, involving a complete or partial defect of the Wolffian duct derivatives. The anatomical hallmarks of CBAVD include bilateral agenesis of vas deferens, atrophy or absence of seminal vesicles and a large portion of epididymis. A large number of clinical investigations indicate that CFTR mutations are responsible for the abnormalities.

In 80% of men with CBAVD, mutations are identified in the CFTR gene; moreover, more than 95% of patients with cystic fibrosis (CF) have CBAVD. Among the patients with two CFTR mutations, most of them have a combination of two different mutations. Almost all the presently known CFTR mutations have been observed in CBAVD, among which the frequency of the most common mutation in CF, F508del, is about 17%, while the frequency of a mild mutation, 5T allele, is about 25% (Yu et al., 2012).

Cystic fibrosis has interested scientists for many decades, pushing them to investigate the gene involved in it, and leading them to discover a spectrum of different phenotypes, associated with CFTR gene mutations and polymorphism. CBAVD affects more than 95% of patients with cystic fibrosis, as well as 80% of patients with CBAVD have CFTR mutations, leaving no doubt in the connection between CFTR mutations and CBAVD. Due to this anatomical defect, patients have difficulties reproducing. Thus, assisted reproduction techniques were theoretically capable of bypassing the problem. But, this proved to
be incorrect (Lu et al., 2014), possibly because of the CFTR mutations. Indeed, patients suffering of Non-Obstructive azoospermia also exhibited CFTR mutations (Jiang et al., 2017; Yu et al., 2014). It appears that CFTR may affect sperm quality/function directly. Taken together, these observations suggest that the mutations of CFTR may affect sperm production, maturation and fertilizing abilities as well.

AIM AND OBJECTIVES

Aim:
The purpose of this study is to investigate the various previous studies of CFTR and CBAVD. Thus, by performing searches in different databases such as PubMed, Medscape, ScienceDirect and collecting data on various investigations, reviews, experiments. As well as creating a summary of the mechanism that connects CFTR and male infertility, using the most recent product of research.

Objectives:

1. To review literature on connections between CFTR gene mutations and primary male infertility.
2. To study literature for phenotype-genotype correlation of various CFTR gene mutations and the primary male infertility.
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LITERATURE REVIEW

CFTR and CBAVD

Bilateral congenital aplasia of the vas deferens (CBAVD) is transmitted as an autosomal recessive trait and affects 1 / 1,000 males. It is responsible for 6-8% of cases of obstructive azoospermia. It is also
present in 95% of males affected by cystic fibrosis. An extended analysis of the 27 exons of the CFTR gene (responsible for cystic fibrosis) subclassified CBAVD patients into four groups:

1) patients with two mutations in the CFTR gene.
2) patients with a mutation in the CFTR gene and with the IVS8-5T allele in trans.
3) patients with a mutation in the CFTR gene or with the IVS8-5T allele.
4) patients without mutation in the CFTR gene and without the 5T allele.

This data have important implications on genetic counseling: the intracytoplasmic microinjection method of a single sperm has successfully solved the problem of male infertility. However, the identification of a CFTR mutation in patients with CBAVD implies that a possible CFTR mutation should always be sought in patient partners, and if identified, a pre-implantation diagnosis or prenatal diagnosis could be proposed.

CFTR mutations express their effects through a variety of molecular mechanisms that include either the failure of production of CFTR protein or the elaboration of a mutant protein with little or no functional CFTR activity on the apical membrane. CFTR alleles contribute to clinical variation in CF in various extents.

For example, the pancreatic function is determined primarily by the genotype at the CFTR locus. A severe mutation on a CFTR gene inherited from one parent confers a pancreatic insufficiency phenotype only if combined with another severe mutation on the gene inherited from the other parent, whereas a mild mutation confers a pancreatic sufficiency phenotype, even if the other mutation is severe.
As seen in the illustration above, CFTR defect type can be classified into various different classes. Mutations that belong to classes 1, 2, and 3 result in the complete functional loss of CFTR protein from the epithelial cell surface and fall in the category of ‘severe’ alleles. Mutations included in classes 4 and 5 are referred to as ‘mild’ and provide enough residual CFTR function to compensate for lack of function corresponding to a severe allele, so that compound heterozygotes of a severe and a mild mutation are, usually, pancreatic sufficient. Other symptoms, such as lung disease, are poorly correlated to CFTR genotype.

### CFTR mutations leading to CBAVD

There are more than 2000 different mutations of the CFTR gene. This gene variability translates in a wider range of clinical phenotypes. The majority of patients diagnosed with CF suffer of male infertility.
But, there are men who seek medical help due to infertility and happen to carry CFTR mutation without the classical manifestations of CF. The most common mutations of CFTR leading to CBAVD are: IVS8-T5 (polyT near the splice acceptor site at the end of the intron 8). This mutation causes an alternative splice that removes the exon 9 producing a non-functioning CFTR protein.

F508del. Most common mutation in CF


Gaikwad et al., 2017, wanted to detect the frequency of the CFTR gene variants poly-T, TG repeats and c.1408A>G p.Met470Val (M470V) in Indian men with congenital bilateral absence of the vas deferens (CBAVD). Men diagnosed with CBAVD (n = 76), their female partners (n = 76) and healthy men from general population (n = 50) were recruited.

- An increased frequency of heterozygous IVS9- c.1210-12T [5] (39.4%) was observed in CBAVD men as compared to controls (14%).
- The allelic distribution of c.1210-12T [5], c.1210-12T [7] and c.1210-12T [9] in CBAVD men was 21%, 64.4% and 13% and that in healthy controls was 7%, 73% and 20% respectively.
- Longest TG repeat c.1210-34TG [13] was found in association with c.1210-12T [5] with an allelic frequency of 5.9% in CBAVD men.

A total of 60 consecutive infertile males with a diagnosis of CAVD were subjected to CFTR gene analysis, by another Indian group (Sharma et al., 2014). These are the results

- 13 different CFTR gene mutations and 1 intronic variant that led to aberrant splicing.
- p.Phe508del (n = 16) and p.Arg117His (n = 4) were among the most common severe forms of CFTR mutations identified.
- The IVS8-T5 allele was found with an allelic frequency of 28.3%.
- Eight novel mutations were also identified in the CFTR gene.
- It is noteworthy that the spectrum of CFTR gene mutation is heterogeneous, with exon 4 and exon 11 as hot spot regions.

A similar study involved 106 Iranian patients with CBAVD. Radpour et al., 2006 the 5T allele and M470V exon 10 missense polymorphism.
• Five of the 106 patients with CBAVD had mutations in both copies of the CFTR gene, and none of them had the 5T allele.
• Eighty-five patients had a mutation in at least one copy of CFTR, and of these patients, 46 had one 5T allele (in 11 cases, two alleles and in 35 cases, just one allele of 5T was detected).
• In 21 patients, no CFTR and 5T mutations were found (19.81%).
• 5T/M470 genotype was found in 19 patients.
• 5T/V470 was found in 3
• 5T with heterozygote form of M470V was found in 24 CBAVD patients.
• 28 F508del carriers were identified.

Steiner et al., 2012 genotyped for common CFTR variants and tested for associations in two chronic pancreatitis (Idiopathic Chronic Pancreatitis-A: 126 patients, 319 controls; Idiopathic Chronic Pancreatitis-B: 666 patients, 1,181 controls) and a CBAVD population (305 patients, 319 controls).
• Haplotype H10 (TG11-T7-470V) conferred protection (ICP-A: OR 0.19, P<0.0001; ICP-B: OR 0.78, P = 0.06; CBAVD OR 0.08, P<0.001).
• Haplotype H3 (TG10-T7-470M) increased disease risk (ICP-A: OR 8.34, P = 0.003; ICP-B: OR 1.88, P = 0.007; CBAVD: OR 5.67, P = 0.01).
• The risk of heterozygous CFTR mutations carriers for ICP (OR 2.44, P<0.001) and CBAVD (OR 14.73, P<0.001) was fully abrogated by the H10/H10 genotype.

Samples obtained from 109 Chinese infertile males with CBAVD and 104 normal controls were analyzed for the presence of CFTR (TG)m(T)n, M470V and F508del by PCR amplification followed by direct sequencing. In this study, Ni et al 2012, showed that:
• F508del mutation was not found
• The 5T mutation was present with high frequency in Chinese CBAVD patients
• IVS8-5T linked to either 12 or 13 TG repeats was highly prevalent among CBAVD patients (97.22% of 72 cases and 96.91% of 97 alleles with IVS8-5T)
• A statistically significant relationship between TG12-5T-V470 haplotype and CBAVD was detected.
All these works show a prevalence of the 5T mutations among the patients with CBAVD around the world. Interestingly, a significant association between long TG repeats [TG12/13], combined with the 5T mutation, and was also found to be associated with CBAVD. The polymorphism M470 also shows a strong influence in the pathology when coupled with other mutations, even though the haplotype TG12/5T/V470 haplotype demonstrated a statistically significant involvement with the disease. P508del, the most common mutation among CF patients, and thus one of the most severe one, was really rare and in certain cases undetected among patients with CBAVD, demonstrating this condition, as a mild manifestation of the wide spectrum of phenotypes caused by CFTR mutations.

**The 5T variant**

The extent of CFTR exon 9 skipping was directly connected with the length of a polymorphic polythymidine tract (Tn) in intron 8.

People with 7T or 9T alleles at this locus produced up to a quarter of transcripts without exon 9, while carriers of a 5T allele produced around two-thirds of incomplete mRNAs. And homozygous carriers produced up to 90% aberrant mRNAs. These concepts were derived from the study of the CFTR transcript in a great number of individuals, with different combinations of the alleles. Apparently, the length of the polypyrimidine tract in the 5T variant is too short to qualify as a splicing acceptor site for the exon 9, making the CFTR non-functional.

The percentage of functional CFTR transcripts produced is affected by the length of the polypyrimidine locus Tn in intron 8, which could lead to CFTR mutations that could be considered quite ‘mild’, with decreased levels of expression of normal CFTR.

These outcomes demonstrate that a normal clinical phenotype can be achieved, even with a very small amount (10-25%) of functional full length CFTR.

CBAVD patients showed a high frequency of R117H mutations.

Tn alleles co-segregating with R117H in groups of patients with Cystic Fibrosis and CBAVD were analyzed and showed an interesting trend which is the following: CF patients showed to have the 5T variant, while CBAVD patients expressed the 7T variant.
Furthermore, the R117H-5T allele when coupled in trans with another CF mutation (carried on the other gene), a classic CF-PS phenotype would manifest due to the R117H-5T allele, while CBAVD would appear because of R117H-7T, and no disease would manifest from R117H-9T.

The presence of R117H and 7T might cause male infertility when paired with a severe mutation in trans, but leaves sufficient lung function. On the other hand, the same mutations in concomitance with 5T will produce a phenotype with respiratory problems.

Disease severity could theoretically be modulated with the specific IVS-Tn background where CFTR mutation is located.

Multiple works suggest that individuals with CBAVD happen to have a much more elevated frequency of the 5T allele compared to the normal population.

We can thus suppose that 5T allele combined with CFTR mutation seems to be one of the most important causes of CBAVD.

But, being a carrier of both CF and 5T doesn’t automatically make the patient infertile. Indeed, incidence of CBAVD would be much higher if this this variant would always cause CBAVD.

The fertility of 131 brothers of 105 men with CBAVD who tried to conceive was studied by Shin et al., (1997). They realized that only a very small number of brothers (7) had also CBAVD. This supposes that this prevalence is five times lower than the 25% expected for an autosomal recessive inheritance pattern.

Moreover, the genetic modifiers TGF-β1 and EDNRA, have been associated with the penetrance of CBAVD (Havasi et al., 2010; Sharma et al.,2014)

Tissues are differentially sensitive to exon 9 mis-splicing.

New perspectives into the relationship between levels of normal CFTR and phenotype variation was offered by the discovery of differential splicing efficiency between tissues that express CFTR (Mak et al., 1997),

1. the amount of CFTR required by each organ involved in CF or related diseases to maintain a normal phenotype may be variable. In some organs a small reduction in the level of normal transcripts might lead to dysfunction while others would not be affected.

2. The 5T allele may then become a disease causing mutation for the vas deferens due to the overall decrease in full-length CFTR mRNA in this tissue.

3. CBAVD patients have no pulmonary disease probably because the amounts of functional mRNA may exceed the necessary ‘threshold’ transcript levels for a non-CF phenotype in the lung.

4. The high frequency of mild CFTR mutations in patients with CBAVD suggested that the vas deferens was one of the tissues most susceptible to the effects of changes in CFTR activity.
What affects the 5T penetrance? The fact that it is accompanied, always in the intron 8, immediately upstream of the Tn site, of the CFTR gene, by another polymorphism, called TG, as constituted by the sequence of two bases, Thymidine and Guanine, which can be repeated a variable number of times and therefore have "length "Variable (from 9 to 13 repetitions, exceptionally up to 15). If the TG segment is repeated 12 or 13 times (TG12 or TG13), the 5T is more likely to cause pathological effects; if instead the TG stretch has length 9, 10, 11 (TG9, TG10, TG11), it is less probable that 5T will have consequences.

- These consequences are certain to occur only if the 5T-TG12 or 13 combinations is accompanied by a true "classical" mutation of the CFTR gene.
- And if this true mutation is "in trans", that is to say on the other chromosome 7, which contains "the other" copy of the CFTR gene. We all always have two copies of the CFTR gene, having all two chromosomes 7, one that comes from the father, one from the mother and the interaction between the two copies of the gene is the one that determines the total amount of the CFTR protein produced and therefore ultimately the presence or absence of disease manifestations.(G.Borgo 2015)

The longer the (TG)m tract the higher the proportion of transcripts without exon 9, but only when activated by the 5T allele

https://www.researchgate.net/figure/The-effect-of-particular-alleles-on-the-amount-of-functional-CFTR-For-different_fig1_228343163
The M470 locus is polymorphic at the amino acid level for the presence of methionine or valine.

http://www.pnas.org/content/101/10/3504

The M470 locus is polymorphic at the amino acid level for the presence of methionine or valine.
They have described potential functional differences for the two CFTR variants M and V470 (A or G at nucleotide position 1540 respectively), with the V470 variant resulting in decreased functional CFTR and the M470 maturing more slowly.

Numerous studies (Gaikwad et al., 2018; Du et al., 2014; Ni et al., 2012; Jiang et al., 2017) have demonstrated an association between CBAVD and the haplotype TG12-5T-V470.

It is still not well comprehended the mechanisms of how or which CFTR mutations help to cause the pathogenesis of CBAVD.

We can speculate that CBAVD is caused by either a morphogenic defect or because of obstruction of the tract from abnormal secretions.

By the 18th week of gestation, CFTR mRNA is well expressed in the epithelium of the epididymis; this can lead us to guess that CFTR could have a major job in the early development of the reproductive tissues.

Findings indicate that CFTR mutations are associated with 4/5 of cases of CBAVD (anomaly in the Wolffian duct), but do not show any association with any Müllerian duct abnormality, this hows further evidence on the delicate timing of CFTR damage in CBAVD.

Indeed, because the Müllerian and Wolffian ducts separate at the 9th week of development we can speculate that CFTR exercises its effect must occur after then.

**CFTR and Non-CBAVD infertility**

An investigation in the effects of CBAVD in assisted reproduction was performed, involving 945 patients with CBAVD. Results show, that, possibly as a result of CFTR mutation, patients with CBAVD have a significantly increased risk of miscarriage and stillbirth, and a reduce rate of live birth compared with non-CBAVD. This points to signs that anatomical defects might not be the only cause of infertility in CBAVD patients (Lu at al., 2014).

CFTR mutations have also been found to be associated with other types of male infertility, apart from CBAVD.

Sharma et al. screened 60 patients with obstructive azoospermia non-CBAVD and 150 patients with spermatogenic failure. Results showed an increased rate of heretozygote F508del mutation in both groups, with a slightly higher percentage in obstructive azoospermia (11.6%) in comparison to spermatogenic failure patients (7.3%). Furthermore, both groups showed significantly greater frequency of homozygous
IVS(8)-5T allele in comparison to healthy individuals. These results suggest that CFTR gene might be involved in various forms of male infertility (Sharma et al., 2014). The increased CFTR mutation frequency in non-obstructive azoospermia and oligospermia was further confirmed by a number of subsequent studies. The correlation between T[5] allele and spermatogenic failure emerged from them. Yu et al., analyzed 126 patients with non-obstructive azoospermia (NOA) and 213 control. Their group found that the frequencies of the T[5] allele and the T[5]+GT[12] combination in patients with non-obstructive azoospermia were both significantly higher than those in the fertile controls (13.1% versus 2.8%, P<0.01; 97.0 versus 41.7%, P<0.01, respectively), thus indicating a high risk susceptibility to non-obstructive azoospermia for males with T[5] allele or T[5]+GT[12]. This result was consistent with their meta-analysis (Yu et al., 2011).

Another group interested in the correlation between NOA and CFTR mutation, conducted a case-control study and reviewed other 12 case-control studies, finding again that the T5 allele was present at a significantly higher rate in NOA patients than in the control group (5.00% versus 0.00%, p < 0.01) with increased risk having NOA [Odds ratios (OR) 2.05, 95% confidence intervals (CI) 1.85–2.27]. The T5 variant was always accompanied by TG12 (10/10) and V470 allele participated in most TG12T5 haplotypes (8/10). TG12T5-V470 haplotype also enhanced risk of having NOA [OR 2.04, 95% CI 1.84–2.26] (Jiang et al., 2017).

**CFTR and spermatogenesis**

Spermatogenesis is the process by which haploid spermatozoa develop from germ cells in the seminiferous tubules of the testis. This process starts with the mitotic division of the stem cells located close to the basement membrane of the tubules. These cells are called spermatogonial stem cells. The mitotic division of these produces two types of cells. Type A cells replenish the stem cells, and type B cells differentiate into spermatocytes. The primary spermatocyte divides meiotically (Meiosis I) into two secondary spermatocytes; each secondary spermatocyte divides into four equal haploid spermatids by Meiosis II. The spermatids are transformed into spermatozoa (sperm) by the process called Spermiogenesis. The spermatogenic cells are in close contact with Sertoli cells which are thought to provide structural and metabolic support to the developing sperm cells. Sertoli cells serve a number of functions during spermatogenesis, they support the developing gametes in the following ways:
- Maintain the environment necessary for development and maturation, via the blood-testis barrier
- Secrete substances initiating meiosis
• Secrete supporting testicular fluid
• Secrete androgen-binding protein (ABP), which concentrates testosterone in close proximity to the developing gametes
• Testosterone is needed in very high quantities for maintenance of the reproductive tract, and ABP allows a much higher level of fertility
• Secrete hormones affecting pituitary gland control of spermatogenesis, particularly the polypeptide hormone, inhibin
• Phagocytose residual cytoplasm left over from spermiogenesis
• Sertoli cells have receptors for FSH and testosterone, the main hormonal regulators of spermatogenesis known to influence the germ cell fate
• As epithelial cells, Sertoli cells also maintain the blood–testis barrier (BTB)
• Secretion of anti-Müllerian hormone causes deterioration of the Müllerian duct
• Protect spermatids from the immune system of the male, via the blood-testis barrier
• Contribute to the spermatogonial stem cell niche

Defects in spermatogenesis may cause male infertility including azoospermia, oligospermia and teratospermia.

Sertoli cells express CFTR. Sertoli cells nurture the germ cell by secreting various proteins and growth factors. The function of Sertoli cells is tightly controlled by hormones, particularly FSH, an important regulator of spermatogenesis. The activation of the FSH receptor generate a signal cascade through the membrane-bound adenylyl cyclase (mAC) in Sertoli cell membranes, that triggers cAMP/PKA and thus, the expression of the downstream transcription factors, such as CREB, which promotes the genes expression and the proteins synthesis necessary for spermatogenesis.

In 2000, Chen et al., (2000) cloned a new type of adenylyl cyclase in rat, called soluble adenylyl cyclase (sAC).

Unlikely mAC, it is sensitive to HCO3− and calcium, and thus represents an alternative way in activating cAMP/PKA in a number of cell types.

CFTR is involved in HCO3− entry into Sertoli cell, which activates sAC and hence the cAMP/PKA/CREB pathway (Xu et al., 2011).

In addition, they observed that the FSH-induced cAMP production and CREB phosphorylation can be potentiated by the activation of CFTR and sAC, suggesting that CFTR may modulate the FSH signaling.
in Sertoli cells during spermatogenesis. Backing this hypothesis CFTR knock-out mice show down-regulated CREB expression, smaller testis size and reduced sperm production (Xu et al., 2011). Therefore defects in CFTR may result in insufficient activation of the FSH-induced signal transduction and gene expression, thus leading to impaired spermatogenesis.

In fact, lower CFTR expression, accompanied by reduced phosphorylated CREB and total CREB were found in the Sertoli cells in testicular samples collected from non-obstructive azoospermia patients (Xu et al., 2011).

These results strongly support the idea of a role of CFTR in regulating the FSH/cAMP/CREB pathway during spermatogenesis which might provide an explanation to the observed association of CFTR mutations with non-obstructive azoospermia.

FSH and testosterone signaling in Sertoli cells William Walker
**CFTR and blood-testis barrier**

The blood-testis barrier is formed by basal tight junctions between adjacent Sertoli cells; these serve to compartmentalize the seminiferous tubule into basal and adluminal compartments. Compartmentalization provides an environment in which developing germ cells are protected from external insults and the immune system.

Sertoli cells produce a number of junctional complex, structural, and extracellular matrix proteins. These proteins are important in maintaining the structural integrity and support for developing germ cells, forming the blood-testis barrier, mediating cell-to-cell interactions, and maintaining polarized secretion of products by Sertoli cells.

Disruption of these junctions leads to failure of spermatogenesis.

Cryptorchidism is a condition in which one or both testicles are retained in abdomen. It is associated with low fertility.

It is known that the temperature in the scrotum is lower than the body temperature, meaning that in the above mentioned condition the testes are located in an abnormally elevated temperature.

Interestingly it has been noticed that the product of the CFTR gene is susceptible to the heat. In particular the post-translational processing of CFTR (folding and insertion in the membrane) is performed at its best in lower temperature, while, at higher temperature there are occurrences of intracellular degradation.

To mimic the condition of cryptorchidism, mice models of testes underwent through hyperthermia (43 °C-heatshocked) and surgery. These models showed decreased level of CFTR, over activation of NF-kB, up-regulation of COX2, down-regulation of tight junction proteins (ZO1 and occludin). Moreover vacuoles inside the Sertoli cells and reduced number of germ cells were noted, indicating defective spermatogenesis.

To further corroborate these findings, rat’s Sertoli cells, cultivated at high temperature, showed decreased CFTR and increased COX-2.

Sertoli cells cultures were performed even with inhibition or knockdown of CFTR giving back similar results: increased PGE2, decreased ZO-1 and occluding. It is worth noting that these findings were reversed by the addition and NFkB and COX2 inhibition.

To summarize, cryptorchidism places the testes in a warmer environment in which CFTR gene is down-regulated. This leads to the activation of the inflammatory NFkB/COX2/PGE2 pathway, involved in the destruction of the tight junctions (ZO1 and occluding) between the Sertoli cells and ultimately the destruction of the blood testes barrier. The end product is a defective spermatogenesis.
Working model for the role of CFTR in spermatogenesis.

CFTR is expressed in both Sertoli cells and germ cells. In Sertoli cells, CFTR, on the one hand, allows the influx of HCO3− ion, directly or indirectly, and elevates cAMP level through activation of sAC. Together with the signals from FSH-mediated mAC activation, the increased cAMP level activates CREB which is required to support spermatogenesis. On the other hand, it modulates the expression of tight junction proteins, ZO-1 and occludin, through regulating the activation of NFkB, expression of COX-2 and production of PGE2. CFTR may also regulate the cell-junction dynamics through interaction with junctional complex proteins. In germ cells, CFTR-dependent HCO3− entry leads to the activation of CREM through the sAC/cAMP pathway. CREM, in turn, up-regulates the expression protamine-2 that is required for nuclear condensation during spermiogenesis. CFTR may also regulate cell size through Cl− flux.

Chun Ruan et al (2014) study showed that CFTR colocalizes and interacts with ZO-1 at the tight junctions of trachea and epididymis, and is expressed before ZO-1 in Wolffian ducts.

CFTR regulates tight junction assembly and controls tubulogenesis has shown in cultured epididymal epithelial cells treated with the CFTR inhibitor CFTRinh172. Interestingly, inhibition of CFTR with CFTRinh172 (10 and 50 µM) induced a marked reduction of ZO-1 expression. Knockdown of CFTR also reduced ZO-1 expression but the mRNA level of ZO-1 was not significantly changed, so the downregulation of ZO-1 occurred at the protein level. Indeed the downregulation of ZO-1 induced by CFTR knockdown was prevented in cells treated with the proteasome inhibitor MG-132 (10 µM) or the lysosome inhibitor leupeptin (10 µM) for 6 hours.
DC2 cells (dendritic cells type 2) co-cultures were studied with transepithelial electrical resistance (TER), measurement techniques for in vitro barrier model systems. CFTRinh172 significantly reduced TER from day 2 to day 5 in a dose-dependent manner and a mostly fragmented and cytosolic ZO-1 immunofluorescent labeling pattern.

DC2 form elongated structures when cultured under control conditions, CFTR knockdown inhibited the formation of these tubules and induced a disorganized cell growth with formation of cell masses lacking a lumen.

CFTR downregulations represses the transcription of ErbB2, a tyrosine kinase co-receptor important for epithelial differentiation and morphogenesis.

They also proved that the expression of CFTR preceded the one of ZO1 in Wolffian duct of mice and the lack of this expression leads to defective development.

These are convincing proves that CFTR is a direct regulator of the tight junction assembly, tubulogenesis and epithelial cell differentiation through modulation of the ZO-1–ZONAB pathway.

Proposed model for CFTR/ZO1 interaction in epithelial cells. (a) Through its interaction with ZO1, CFTR participates in the retention of ZONAB in TJs, which leads to a decrease in proliferation and activation of differentiation. (b) Retention of ΔF508-CFTR in the endoplasmic reticulum (ER) induces the redistribution of ZONAB from TJs to the nucleus. This increases proliferation and decreases differentiation.

Sylvie Breton, Regulation of epithelial function, differentiation and remodeling in the epididymis (2015).
Temperature has been shown to play a major role in the correct folding of CFTR (Chen et al., 2012). Heat shock proteins are the major contributors responsible for normal CFTR folding to the cell surface. Recent study showed that Grp78 is an ER stress signal that is related to mitochondrion function, possibly through mediation of VDAC1. Mitochondrial function defects may be important for the underlying mechanism of CF mutation. Therefore, it is possible that the heat shock protein GRP78(Bip) mediated VDAC1 function is altered. Changes in Grp78 and VDAC1 expression were detected in CF mutant mice. CFTR and VDAC1 can both bind to Grp78. Thus, the CFTR mutation could lead to an altered heat shock response and VDAC1 expression. VDAC1 plays critical roles in ATP production, which could induce oxidative stress. ATP concentration in mutant mice was significantly higher than in wild type controls. Higher level of ROS were also registered, indicating that the CFTR mutation leads to oxidative stress during spermatogenesis. It is well known that the CFTR mutation affects cytokine secretion in lung and other tissues. It was also found that IL-1α expression is significantly up-regulated in CF testis, indicating that the up-regulated expression of heat shock protein and over-activated VDAC1 mediated pathway could be responsible for the pro-inflammatory state in the CF testis. CFTR mutations could lead to an over-activated heat shock response, and reduced Grp78 and VDAC1 interactions to initiate the cascade leading to altered energy metabolism and ROS production. Furthermore, stimulated NF-κB activates the pro-inflammatory response and related interleukin increase. In conclusion the hypothesis is that CFTR plays a critical role in spermatogenesis through regulation of heat shock proteins and related pathways.

As discussed above, Sertoli cells function is crucial in order to achieve a normal spermatogenesis. Zhang et al., 2016 treated Sertoli cells of mice with CFTR(inh)-172. The expressions of F-actin and Ac-tub were decreased. The CFTR chloride channel plays an important role in maintaining the normal cytoskeleton of Sertoli cells. The reduced function and expression of the CFTR chloride channel may affect the function of Sertoli cells and consequently spermatogenesis of the testis. Also studying Sertoli cells, Sharma et al., 2018, found that CFTR immunofluorescence was most strongly observed within the Sertoli cells nuclei. Based on this peculiar localization it is possible to formulate a hypothesis about a CFTR independent role in gene regulation.
CFTR and germ cells/spermiogenesis

CFTR has been found in germ cells of the seminiferous epithelium. In particular, it is expressed by all the cells of seminiferous epithelium in the cytoplasm and in the membrane. It is also been noticed that the expression of CFTR was stronger after the diploid stage, probably due to the cell volume reduction during spermiogenesis (Teixera et al., 2012). CFTR is abundantly expressed in post-meiotic spermatocytes, including round spermatids and elongated spermatids. The stage-specific expression pattern of CFTR in seminiferous tubules suggests a possible involvement of CFTR in spermiogenesis, during which spermatids differentiate into spermatozoa by undergoing extensive remodeling, including chromatin condensation, acrosome formation, elongation, cell volume reduction and flagellar formation.

CFTR has been proved to influence another interesting protein MYS2. CFTR deletion in mice testes decreased the expression of MYS2, an RNA-DNA binding protein, that seems to be essential for spermiogenesis, indeed, its reduced expression leads to defective acrosome formation, reduced AKAP4, and decreased TBRBP. Moreover the alteration of its human homologue causes azoospermia (Yan et al., 2016).

The role of CFTR is spermiogenesis might not end here. An important collaboration has been proven with aquaporins. Aquaporins are water-selective channels that enable high permeability fluxes of water across plasma membranes. In the male reproductive tract, water movements and ion concentrations are determinants for the male reproductive function. Therefore, aquaporins expression and function play a key role in male fertility (Ching-Hei Yeung 2010).

CFTR activates aquaporins in a cAMP dependent pathway. (Jourdain et al., 2014). The water transportation, inside the cells, is inhibited by CFTR inh-172; is absent in cells lacking of CFTR, is profoundly altered in the presence of the F508del mutation restored after manipulation reversed the mutation. These findings suggests role of CFTR in cell volume regulation. Furthermore, Sertoli cells cultured from rats, analyzed with co-immunoprecipitation, showed a direct interaction between CFTR and Aquaporin 4 (Jesus et al. 2014), supporting the idea of a CFTR control of the water influx

CFTR might be involved in cell volume regulation also by mediating Cl− efflux (Gong et al., 2001). We already discussed the role of CFTR in HCO3− entry into the cells and its activation of the sAC, the latter also expressed in the germ cells(abundantly from spermatids to spermatozoa). The activation of sAC
generates an elevation of cAMP in spermatozoa, that has been proven, along with its downstream transcription factor CREM, to regulate a series of gene important to spermiogenesis. Round spermatids in CREM knockout mice fail to differentiate into spermatozoa, highlighting the essential role of the cAMP–CREM pathway in spermiogenesis.

It was observed a decreased in CREM and its downstream target protamine-2, the chromatin protein in spermatozoa, in homozygous CFTR knockout mice compared with wild-type, indicating that CFTR might be important in regulating the cAMP–CREM pathway in germ cells (Chen et al., 2012). This finding suggests a possible role of germ cell CFTR in signal transduction in addition to cell volume regulation during spermiogenesis. Nevertheless, the evidence gathered strongly indicates an important role of CFTR in spermatogenesis by regulating both Sertoli cell and germ cell functions at various stages of spermatogenesis, defects of which can lead to abnormal sperm production and function, as reported in patients with high frequency of CFTR mutations. (Chen et al., 2012).

**CFTR and sperm capacitation**

After the ejaculation the sperm cells go through several essential physiological changes during their time in the female genital tract before they, at the end, are able to penetrate the oocyte membrane. The first change in this cascade is capacitation. The sperm cells accomplish this during the ascension through the female genital tract (in contact with its secretions). It has to do with a physiological maturation process of the sperm cell membranes, which is seen as the precondition for the next step to follow, namely the acrosome reaction.

Capacitation is a functional maturation of the spermatozoon. The changes take place via the sperm cell membrane in which it may be that receptors are made available through the removal of a glycoprotein layer. The area of the acrosomal cap is also so altered thereby that the acrosome reaction becomes possible. Through the membrane alterations, the motile properties of the spermatozoon also change. (hyperactivity). The ejaculated sperm doesn't have any fertilizing capacity until it is exposed to the female reproductive tract. This suggests the possible presence of a capacitation-inducing molecule in the female reproductive tract. This molecule could be HCO3. In fact it is very abundant in the female reproductive tract and there is strong evidence of its important role in the capacitation. In co-culture of sperm and CFTR-suppressed endometrial epithelial cells, or defective pancreatic cell line (Cheng et al., 2010) the fertilization ability of
the spermatozoa was significantly lower and the capacitation was altered. To verify the role of HCO3 and CFTR, the ion was added and a wild type of CFTR was introduced restoring the spermatozoa fertility. A number of processes take place during capacitation: membrane hyperpolarization, increasing intracellular pH, modification of the plasmatic membrane, calcium influx, activation of the cAMP/PKA pathway, activation of the protein tyrosine phosphorylation. In most of these events HCO3 seems to have a role, with the mediation of sAC. HCO3 stimulates hyperactivity through the generation of a higher glycolitic flux and ATP production in cAMP/PKA fashion (Hereng et al., 2016). The same pathway is involved in the activation of various kinases, phosphatases and proteases during sperm capacitation (Signorelli et al., 2012). In conclusion there is important evidence of a HCO3/sAC/cAMP role, in intracellular signal transduction, mediating the capacitation of the ejaculated sperm (Wertheimer et al., 2013; Harayama et al., 2013), and the influx of the ion is made possible by CFTR.

CFTR is involved in bicarbonate flux in various organs, in a direct (Huang et al., 2018; O’Malley et al., 2018) or indirect way. (Garnett et al., 2013; Sellers et al., 2017). CFTR might also have a regulatory function establishing the rate of HCO3- flux (Shah et al., 2016). CFTR is known to conduct both Cl- and HCO3-. The channel is expressed in human and mice mature sperm (Figuerras-Fierro et al., 2013). We can propose the hypothesis of an involvement of CFTR in the entry of HCO3- inside the sperm cells.

As discussed earlier HCO3- induces capacitation related events. These events are inhibited by a CFTR inhibitor or antibodies against CFTR or CFTR deficient mice sperm. The latter showed reduced motility and fertilization capacity.

However HCO3- CFTR conduction is lower than the Cl- one. On the other hand CFTR works as a Cl-channel parallel with Cl-/ HCO3- exchangers. So, CFTR provides a Cl- recycling source to support these exchangers. Capacitation can be inhibited by removal of Cl-, by CFTR inhibitor or SLC26A3 inhibitor or antibodies against them(Chen et al., 2009). We discovered that both Cl– and HCO3– are required for sperm capacitation in guinea pig, suggesting the involvement of Cl––HCO3– exchangers (Chen et al., 2009).

In conclusion, CFTR is more likely to be involved in the HCO3- transportation in an indirect fashion, providing Cl- recycling pathway to drive the HCO3- entry through the Cl-/HCO3- exchangers.
**Alternative mechanisms**

One of the events included in the capacitation is the membrane hyperpolarization. Cl- is fundamental in the regulation of the plasmatic membrane voltage. Genistein, a CFTR agonist, is able to induce hyperpolarization and acrosome reaction, in non-capacitated mice sperm. Moreover Hernandez and Gonzales (2007) showed that without Cl- there is no hyperpolarization. They also demonstrated an increased concentration for Cl- during capacitation and a genistein induced hyperpolarization. Altogether, these results indicate a CFTR mediated Cl- influx, that contributes to the membrane voltage during capacitation.

Recently, increasing evidence suggests that CFTR may also participate in capacitation by interacting with other ion channels and transporters, especially the SLC26 family.

It was reported that CFTR inhibits ENaC activity in sperm, thereby promoting hyperpolarization by inhibiting Na+ influx through ENaC (Hernandez-Gonzalez et al., 2007).

CFTR interacts with SLC26A3, SLC26A6 and SLC9A3R1 (Chavez et al., 2011), as well as SLC26A8 (Rode et al., 2011), which are HCO3− transporters expressed in mouse sperm, suggesting alternative mechanisms of the CFTR-dependent HCO3− transport in capacitation.

Missense mutation in SLC26A8, encoding a sperm-specific activator of CFTR, are associated with asthenozoospermia.

To summarize, CFTR is an extraordinary factor, capable of a multitude of functions, not only via its role as a chloride channel, but also through the interactions with other cell constituents.

**RESULTS AND THEIR DISCUSSION**

**Mutations causing CBAVD**

CBAVD is the manifestation of a classic (severe, loss-of-function) CFTR pathogenic variant with a mild (retaining some function) CFTR pathogenic variant, in a heterozygous individual.

The most common mutations associated with obstructive azoospermia are:

- IVS8-T5 (polyT near the splice acceptor site at the end of the intron 8).
- F508del
• TG repeat c.1210-34TG [12]/c.1210-34TG [13]
• c.1408A>G p.Met470Val (M470V)

Despite, those mutations being the most prominent, they are not the only ones. There are many other variants capable of causing CBAVD.

**Sertoli cells**

Sertoli cells have a fundamental support role for the germ cells. 
Zhang et al., 2016 treated Sertoli cells of mice with CFTR(inh)-172, inducing abnormalities in the cytoskeleton and consequently the spermatogenesis.

Sertoli cells function is regulate, among the others, by FSH. This hormone is capable of activating an adenylyl cyclase that produces cAMP and, in turn, activates PKA. The latter is involved in the activation of CREB, which promotes spermatogenesis.

Chen et al., cloned a particular form of AC, sensitive to HCO3-, and later, Xu et al., proved that CFTR permits the influx of this ion inside the Sertoli cells and this activates the AC and thus the cAMP/PKA/CREB pathway. Finding in favor of a role of CFTR in spermatogenesis, via the Sertoli cells, were also obtained in both, human and mouse, defective of this gene, that showed lower levels of CREB and vice versa, higher levels of CREB were found when CFTR was potentiated.

So CFTR seems to be able to modulate the FSH/cAMP/CREB pathway during spermatogenesis and this provides an explanation to the observed association of CFTR mutations with non-obstructive azoospermia. 

![Sertoli cell and spermatogenesis](image-url)
Furthermore Sharma et al., 2018, found that CFTR immunofluorescence was most strongly observed within the Sertoli cells nuclei, conveying a CFTR independent role in gene regulation.

**Tight junction**

The blood testis barrier is a structure, form by junctional complexes between the Sertoli cells, assembled to create a protective environment for the germ cells. ZO-1 and occludin, are two of the protein forming this barrier. Experiments conducted by Chen et al.,demonstrated how a defective CFTR cause the destruction of the above cited proteins, via the inflammatory pathway NfkB/COX2/PGE2. Since the line of defense of the BTB is broken through, the spermatogenesis fails to be efficient. Moreover Chun Ruan et al., conducted a research in which it was seen that CFTR is expressed even in the epididymis, and regulates the expression of ZO1. Through the modulation of the ZO-1–ZONAB pathway it is able to affect the assembly of the tight junction, the differentiation of the epithelial cells (via erbB2), the tubulogenesis.
CFTR deficiency impairs tight junction assembly and reassembly, and prevents tubulogenesis in DC2 cells. (A) TER in DC2 cultures treated with CFTRinh172 (0.1–50 µM) or DMSO (Ctrl) at 2, 3, 4 and 5 days after seeding (n = 9). (B) DC2 confluent cultures were incubated in low-Ca²⁺ medium, and TER was measured every hour after switching the medium back to normal Ca²⁺ in the presence of CFTRinh172 (10 µM) or DMSO (Ctrl) (n = 9). (C) ZO-1 labeling (red) in DC2 cultures 24 hours after the Ca²⁺ switch experiment in the presence of DMSO (Ctrl; left) or 10 µM CFTRinh172 (right). (D) Immunoblotting for CFTR in DC2 cells transfected for 3 days with CFTR-specific siRNAs (siRNAcftr) and cells transfected with non-silencing siRNAs as a negative control (siRNAnc) (upper panel). The same membrane was re-blotted for actin (lower panel). (E) TER in siRNAcftr- or siRNAnc-treated DC2 cells 1, 2 and 3 days after transfection and seeding (n = 9). (F) TER values 1, 2 and 3 hours after the Ca²⁺ switch experiment in cells treated with siRNAcftr compared with those treated with siRNAnc (n = 9). (G) ZO-1 labeling 3 hours after the Ca²⁺ switch experiment in DC2 cultures treated with siRNAcftr (right) or in control cells (siRNAnc; left). (H) Brightfield microscope images of DC2 cells cultured on a Matrigel layer under control conditions (Ctrl) or in the presence of CFTRinh172 (1, 10 and 50 µM). (I) Laser scanning confocal imaging for ZO-1 in control (siRNAnc-treated) DC2 cells (left) and siRNAcftr-treated cells (right). Nuclei are labeled in blue with Topro-3. Data show the mean±s.e.m.; *P<0.05; **P<0.01; ***P<0.001 (by two-way ANOVA followed by Bonferroni's post hoc test). Scale bars: 20 µm (C,G,I); 50 µm (H). (Chun Ruan et al., 2014)
CFTR regulates ZO-1 expression. (A) Left, immunoblotting for ZO-1 in DC2 cells treated for 3 days with CFTRinh172 (10 and 50 µM) and in control cells (DMSO). The same membrane was re-blotted for actin (lower panel). Right, quantification of ZO-1 expression normalized for actin in the presence of CFTRinh172 (10 and 50 µM) and in controls (DMSO). (B) Left, immunoblotting for ZO-1 in triplicates of control DC2 cells (siRNAnc) and after CFTR knockdown (siRNAcftr). The same membrane was re-blotted for CFTR (middle panel) and for actin (lower panel). Right, quantification of ZO-1 expression normalized to actin. (C) qPCR analysis of ZO-1 mRNA (normalized to GAPDH) in controls (siRNAnc) and after CFTR knockdown (siRNAcftr). (D) Immunoblotting for ZO-1 in duplicates of control cells (siRNAnc) or siRNAcftr-treated cells that were treated with either vehicle (DMSO), the proteasome inhibitor MG-132 or the lysosome inhibitor leupeptin for 6 hours (left, upper panel). The same membranes were re-blotted for actin (left, lower panel). Quantification of ZO-1 normalized to actin (right). (E) Immunoblotting for CFTR and ZO-1 in DC2 cells after co-transfection with either siRNAnc or siRNAcftr and plasmid encoding human ZO-1 (pZO-1) or an empty vector (pVector). (F) TER measured after the Ca2+ switch experiment in DC2 cells under control conditions (siRNAnc) and after CFTR knockdown (siRNAcftr), in the absence (pVector) and presence of human ZO-1 overexpression (pZO1). Quantitative data show the mean±s.e.m.; *P<0.05; **P<0.01; ***P<0.001; ns, non-significant (two-way ANOVA). (Chun Ruan et al., 2014)

Additionally, there are data that suggest that CFTR expression precedes that of ZO-1 during embryonic development, so a lack of CFTR might cause deficient tight junction and misdevelopment of the structure that are going to originate from the Wolffian duct. This could be a possible mechanism of CBAVD.
CFTR and ZO-1 colocalization in the Wolffian duct. (A) CFTR immunofluorescence labeling (green) in mouse Wolffian duct and gonad at E13.5. Nuclei are labeled in blue with Topro-3. (B) Double-labeling for CFTR (green) and ZO-1 (red) in the proximal, middle and distal regions of the Wolffian duct delineated by the boxes in A. Scale bars: 100 µm (A); 10 µm (B). (Chun Ruan et al., 2014)

**Pro-inflammatory state in the testis**

CFTR down-regulation seems to be able to induce a pro-inflammatory state. In fact it appears to regulate two important protein: GRP78 (a heat shock protein) and VDAC1. Yan et al., demonstrated that CFTR malfunctioning is able to cause an altered heat shock response and VDAC1 expression, that culminates in abnormally high levels of ATP, and, in turn, the generation of ROS inside the testis. Furthermore it has been proven that CFTR mutations are responsible for increased IL-1α levels. In conclusion CFTR might cause a pro-inflammatory state and oxidative stress condition inside the testis, another, of the already illustrated mechanism by which this gene is able to affect spermatogenesis.
ER and Mitochondrial protein dysfunction may contribute to spermatogenesis defects in CF mice. 

(A) The expression of heat shock protein, ER and mitochondrial genes, including Grp78 and VDAC1 were increased in CFTR mutant mice. Right panel is the statistic result. (B) Upper panel: Co-IP result confirm that Grp78 could interact with immature band of CFTR in mice testis. Down panel: VDAC1 and Grp78 could interact with each other in mice testis. (Chen et al., 2016)

(A) ATP measurement shows that Cfr(-/-) mice has significantly increased ATP production compared with WT mice testis. (B) Immunohistochemistry staining of nitrotyrosine, an oxidative stress marker in testis shows that mutant mice has significant increased signal in seminiferous tubule. (C) cytokine profile further confirm that IL-1 level is significantly increased, while TNF-alpha expression shows no significant change in Cfr(-/-) mice testis. (Chen et al., 2016)
Proposed model to explain the specific function of CFTR in germ cell development.

In the current model, CFTR plays a critical role in spermatogenesis through regulation of heat shock proteins and related pathways. We propose that CFTR mutations lead to an over-activated heat shock response, and reduced Grp78 and VDAC1 interactions to initiate the cascade leading to altered energy metabolism and ROS production. Furthermore, over-activated NF-κB activates the pro-inflammatory response and related interleukin increase. Finally, the key RNA binding protein MSY2 expression was significantly reduced, indicating that CFTR affect spermiogenesis through regulation of MSY2 expression. (Chen et al., 2016)

**Spermiogenesis**

CFTR has been found in germ cells of the seminiferous epithelium. In particular, it is expressed by all the cells of seminiferous epithelium in the cytoplasm and in the membrane, in a stage-specific pattern. First of all, Yan et al., studying MYS2 (the mouse homologue of YBX2), demonstrated that the down-regulation of CFTR causes lower levels of MYS2 and this causes defective acrosome reaction and azoospermia.

In addition CFTR is able to activate Aquaporins in a cAMP dependent pathway. (Jourdain et al., 2014) and Sertoli cells show a direct interaction between CFTR and Aquaporin 4 (Jesus et al. 2014).

CFTR Regulation of Aquaporin-Mediated Water Transport. (Alves et al., 2015)
We already discussed the role of CFTR in activating the sAC through HCO3-. The cAMP is also able to activate another transcription factor called CREM in germ cells (Chen et al., 2012). CREM has been proven to be crucial for spermiogenesis in multiple ways. The CFTR gene keeps on showing how it interacts with multiple proteins and it is involved in multiple pathways that are able to compromise the germ cells development.

**Sperm Capacitation**

A number of processes take place during capacitation. In most of these events HCO3 seems to have a role, with the mediation of sAC. HCO3 stimulates hyperactivity through the generation of a higher glycolitic flux and ATP production, but also activates various kinases, phosphatases and proteases involved in sperm capacitation (Signorelli et al., 2012), in cAMP/PKA fashion (Hereng et al., 2016).

To summarize CFTR is the media by which cAMP can induce the capacitation of the ejaculated sperm.

**The indirect role of CFTR**

CFTR could be responsible of male infertility by interacting with other ion channels and transporters, especially: the SLC26 family (Chavez et al., 2011) (HCO3– transporters expressed in sperm) and ENaC (Hernandez-Gonzalez et al., 2007).

**CONCLUSION**

1. We analyzed the connection between CFTR and primary male infertility. To do this we scrutinized 134 articles in databases such as PubMed, Medscape, ScienceDirect etc. The latest article was published in March 2018. The date interval used was 10 years. 32 articles were suitable for our topic. Although a connection between CFTR and certain specific forms such as CBAVD was long time established, we analyzed also new pathways in which this gene involvement causes non-obstructive azoospermia.

2. CFTR is an extremely complex gene. Although it was discovered many decades ago, its implications of the determinism of bodily function are not yet fully known. We have seen how in some cases it goes directly to regulating organ function, for example in the pancreas. In other districts it produces an extremely variable phenotype, a consequence of a variety of factors, many still elusive. Its role in male infertility has been widely demonstrated. We have seen that CBAVD is nothing but the result
of a game between allelic variants, differently combined, within the CFTR gene. It has been understood how these mutations go to produce a qualitatively abnormal channel. However, the mechanisms by which it leads to the failure to form components of the male reproductive system remain unknown. Without these concepts the possibility of fetal therapy that prevents such defects is impossible. In reality a prenatal diagnosis would be possible? Given the amount of variables that can produce the same phenotype, as well as similar genotypes can generate such different clinics, is screening for the unborn child possible? Is it possible to distinguish pancreatic insufficiency from infertility (among which can be overcome by in vitro fertilization techniques)? The diagnostic difficulty and the ethical responsibility of the geneticist, faced with an error of evaluation becomes considerable. In recent years there have also been demonstrations of involvement of CFTR in the NOA. Our Cl and HCO3 transporter appears to influence spermatogenesis. Its pathogenic role is more intuitive in this case. Therefore, in vitro fertilization will still be necessary? Or, since the anatomical pathways are normally shaped, can one attempt to make up for the deficiency of certain ionic fluxes in precise districts by means of other channels or signal transduction pathways? Again, if we could control the function of CFTR, could we create a male contraceptive? Would we really be able to tame such a complex structure? In short, the possibilities that this gene hides are currently innumerable. Perhaps the real question is whether we will be able to interpret every aspect of it before natural selection makes this autosomal dominant character disappear.

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