



## Early View

Review

# **Sex Disparities in Cystic Fibrosis: Review on the Effect of Female Sex Hormones on Lung Pathophysiology and Outcomes**

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# **Sex Disparities in Cystic Fibrosis: Review on the Effect of Female Sex Hormones on Lung Pathophysiology and Outcomes**

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## **Review Summary for Social Media**

A disparity in survival has been noted in men and women with cystic fibrosis where female sex hormones may facilitate lung disease progression. There is strong evidence that implicate estrogen in numerous aspects of CF airway pathophysiology

## **Abstract**

Sex differences in morbidity and mortality have been reported in the cystic fibrosis (CF) population worldwide. However, it is unclear why CF women have worse clinical outcomes than men. In this review, we focus on the influence of female sex hormones on CF pulmonary outcomes and summarize data from *in vitro* and *in vivo* experiments on how estrogen and progesterone might modify mucociliary clearance, immunity and infection in the CF airways. The potential for novel sex hormone related therapeutic interventions is also discussed.

## **Introduction**

Cystic fibrosis (CF) is a multi-system genetic disease due to defects in the CF transmembrane conductance regulator (CFTR) protein, most commonly affecting the lungs. This manifests as chronic airway infection and inflammation, and recurrent pulmonary exacerbations, which account for the majority of morbidity and mortality seen in CF. Other risk factors associated with mortality includes certain CFTR genotypes, poor nutritional status, development of cystic fibrosis related diabetes (CFRD), low socioeconomic status, minority race or ethnicity and female sex<sup>[1,2]</sup>. Indeed, researchers have identified a persistent disadvantage in clinical outcomes and survival for female CF patients compared to their male counterparts. This disparity in outcomes between sexes is not limited to CF as a number of other respiratory diseases, such as asthma, also have worse reported outcomes in women<sup>[3]</sup>. While there are some anatomic differences between males and females, such as smaller airway diameter and lung volumes in women, that might contribute to differences in respiratory disease outcomes<sup>[4]</sup>, many have theorized that sex hormones may be contributing to differences in CFTR expression/function, mucous and airway fluid characteristics, as well as airway infection and inflammation. Here, we

provide a focused review of the literature on the influence of estrogen and progesterone on CF airway pathophysiology and pulmonary outcomes.

### **The “Gender Gap” in CF survival and pulmonary outcomes**

The gap in clinical outcomes between men and women with CF has been well documented in epidemiological studies<sup>[5]</sup> and confirmed over time with registry data worldwide. The 2017 CF Canada registry reported a median survival age of 56 years for males and just 49 years for females<sup>[6]</sup>. Similarly, this discrepancy is seen in registry data from the US, UK, Scandinavia and Australia where women also experience a higher rate of morbidity and mortality<sup>[5,7-10]</sup>.

Women demonstrate earlier or more frequent acquisition of organisms associated with worse outcomes including *Pseudomonas aeruginosa* (PsA), *Stenotrophomonas maltophilia*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Burkholderia* species, fungal and non-tuberculous mycobacterial species<sup>[8,9,11]</sup>. They also need more intense and complex pulmonary exacerbation treatment regimens, and require longer lengths of hospitalization<sup>[8,9]</sup>. As well, CFRD is a more common complication in women and they experience a larger drop in lung function and a greater mortality rate after developing CFRD than men<sup>[12,13]</sup>, though the cause for this remains unclear.

The cause of this “gender gap” is likely multifactorial, related to intrinsic physiologic differences as well as biochemical, nutritional, and behavioral differences between the sexes. Women, on average, have a smaller airway diameter with smaller lung volume, even adjusted for height, which has been speculated to represent an anatomic impediment to mucous clearance particularly from airways with sharp angled bifurcations or trifurcations<sup>[3]</sup>. In addition, women are also predisposed to a higher degree of airway hyperresponsiveness and

bronchoconstriction<sup>[3,4]</sup>. This increased propensity for bronchoconstriction in physiologically smaller airways may result in more frequent and exaggerated narrowing of inflamed airways during periods of exacerbations, leading to more mucous production and impaction, thus perpetuating the vicious cycle of inflammation and recurrent bacterial infections. As well, body mass index (BMI) has been well documented to be lower in CF women compared to CF men and is significantly lower than age matched non-CF women starting at a younger age<sup>[14]</sup>. Given the reported desirability of having a lower weight, CF women are likely more resistant to nutritional interventions that help to increase their weight<sup>[15]</sup>. Vertical growth, another indicator of nutritional status, is also stunted in CF girls compared to age matched non-CF girls, while the same disparity is not seen in CF boys<sup>[16]</sup>. Poor nutritional status and reduced BMI are associated with lower lung function and increased mortality in CF<sup>[9,14,17]</sup>, and therefore undoubtedly contribute to the sex disparity in CF survival. Furthermore, CF women have been found to have poorer medication and nutritional adherence, which may also contribute to reduced lung function<sup>[18,19]</sup> and worse health outcomes<sup>[20]</sup>.

Despite accounting for differences in BMI, lung function, CFRD status, and chronic bacterial infections between the sexes, female sex remains an independent risk factor for death<sup>[9]</sup>. It has been noted that sex differences in outcomes seem to only manifest after puberty as rates of pulmonary exacerbations and mortality are similar between boys and girls before puberty but are more common in women than men in adulthood<sup>[21,22]</sup>. Therefore, disparity in morbidity and mortality between sexes may also be related to post-pubertal female hormonal changes.

## **Physiology of Female Sex Hormones**

### The Biology of Female Sex Hormones

The predominant female sex hormones are estrogen and progesterone. Estrogen is mainly produced from cholesterol by the ovaries, the corpus luteum and the placenta<sup>[23]</sup> but can also be produced in extra-gonadal sites including peripheral adipose tissue and the lungs via the enzyme aromatase. Physiologic estrogens exist in three biochemical forms - estrone, estradiol (or 17- $\beta$ -estradiol) and estriol. Estradiol is the most potent and predominant form of estrogen found in premenopausal women, while estrone is the most active form of estrogen in menopausal women and estriol is the main active form of estrogen during pregnancy. All three forms of estrogen are transported in the bloodstream either bound to sex hormone binding globulin (SHBG) or in the unbound state<sup>[24]</sup> before binding to either estrogen receptors (ER) $\alpha$  or  $\beta$  expressed on the cell surface of the target tissue<sup>[25]</sup>. Notably, both ERs are expressed in lung epithelia, airway smooth muscle and the pulmonary vasculature<sup>[26,27]</sup>. The activated estrogen/ER complex has been implicated in fetal alveologenesis and surfactant production, pulmonary arterial hypertension, airway hyperresponsiveness and pulmonary inflammation<sup>[26]</sup>.

Progesterone is likewise produced from cholesterol<sup>[28]</sup>, and readily diffuses across cellular membranes and binds intracellular progesterone receptors (PR) -A and -B. PR is expressed in a number of target tissues, including lung airway epithelium and T lymphocytes<sup>[28-30]</sup>. Within the lungs, progesterone has been noted to mediate bronchodilation by reducing contractility and increasing smooth muscle relaxation, confer protection from viral injury, and reduce ciliary beat frequency *in vitro*<sup>[29-31]</sup>.

### Menstrual Physiology

In order to understand changes in CF clinical outcomes in the context of female sex hormone fluctuations, the physiological pattern of estrogen and progesterone change should first be

described. While the levels of these hormones are endogenously produced at a higher level in women than in men, estrogen and progesterone levels fluctuate with monthly menstrual cycles in females of childbearing age. At the start of the cycle, or menstruation, both estrogen and progesterone levels are low (Figure 1). Estrogen levels subsequently rise and peak while progesterone levels remain low during the follicular phase (the period of time pre-ovulation) and ovulation. In contrast, during the luteal phase (the period of time after ovulation), both progesterone and estrogen levels rise before falling again in concert with the start of the next menstrual cycle.

### **Effects of Female Sex Hormones on CF Pathophysiology**

As lung disease is a major determinant of survival in CF, sex differences in airway mechanics may contribute to the “gender gap”. The clinical observation of worsening airway infection and inflammation that correspond to increases in the physiologic levels of estrogen and progesterone in women suggest a plausible mechanistic link between sex hormones and CF airway pathophysiology<sup>[32]</sup>.

### **Impact of sex hormones on mucociliary clearance**

Mucociliary clearance is influenced by a number of factors in the CF patient. The CFTR protein is expressed on the mucosal surface of epithelial cells throughout the body, regulating the secretion and absorption of ions and fluids. Ion transport helps to regulate the pH and viscosity of airway surface liquid (ASL), a thin fluid layer that sits above the epithelium. Adequate ASL volume is essential for effective mucociliary clearance. In addition, mucous production is a physiologic response to the presence of airway debris and microorganisms. CF patients produce

more viscous mucous, in part due to CFTR dysfunction, which hampers mucociliary clearance. Suboptimal mucociliary clearance results in retained secretions, mediating an increased vulnerability to recurrent airway infection and inflammation. To understand the link between sex hormones and mucociliary clearance, a number of research groups have examined the effect of estrogen and progesterone on CFTR expression, CFTR-dependent and -independent ion transport, ASL, and mucous production.

*In vitro* studies have demonstrated that CFTR expression is either unchanged<sup>[33]</sup> or even increased in response to estrogen and/or progesterone exposure<sup>[33-35]</sup>. In a bronchial epithelial cell line expressing F508del mutated CFTR protein, Casavola and colleagues demonstrated that the defect in CFTR localization and CFTR mediated chloride secretion could be reversed upon estrogen stimulation, mediated through the up-regulation of the sodium hydrogen exchanger regulatory factor (NHERF1)<sup>[36]</sup>. Similar improvements in CFTR mediated chloride secretion can be seen in other cell types, such as rabbit pancreatic acinar cells<sup>[33]</sup>. Conversely, estrogen appears to have a negative effect on CFTR-independent chloride channel activity. Tarran and colleagues have demonstrated that 17- $\beta$ -estradiol can cause increased viscosity of ASL in a dose-dependent fashion by impairing Ca<sup>2+</sup>-mediated chloride secretion<sup>[37]</sup>. Similarly, KCNE3 (MinK-related peptide 2) is a protein expressed in tracheal epithelia that regulates KCNQ1 (potassium voltage-gated channel subfamily Q member 1) and has been implicated in the pathophysiology of CF as it indirectly mediates chloride secretion independent of CFTR<sup>[38]</sup>. Estrogen has been shown to disrupt the interaction between KCNE3 and KCNQ1, leading indirectly to reduced chloride ion secretion and therefore thicker mucous production<sup>[39]</sup>. The binding of estradiol to the estrogen receptor (ER)- $\alpha$  in airway epithelial cells has also been shown to increase mucin (MUC5B) production from the submucosal glands, potentially promoting mucous plugging in

the CF airways<sup>[40]</sup>. Finally, estrogen has also been shown to reduce the intensity of ciliary beat of the CF bronchial epithelium, further hampering the CF airways' ability to clear thickened secretions<sup>[41]</sup>. Conversely, in non-CF airway epithelium, estrogen has been shown to increase the intensity of cilia beat frequency and counteracts the negative effects of progesterone on ciliary function<sup>[29]</sup>. Thus, more work will be needed to understand why estrogen appears to play opposing roles in CF and non-CF airways.

Collectively, these findings suggest that while estrogen may directly improve CFTR expression and function, there are also a number of mechanisms whereby estrogen can indirectly reduce chloride secretion and thicken mucous/ASL to overall reduce mucociliary clearance.

#### Impact of sex hormones on immunity and infection

Female sex hormones, and estrogen in particular, have been studied in the context of a number of inflammatory, infectious and autoimmune diseases. Depending on the specific arm of the immune response and the disease model, estrogen has been implicated in both enhancing and suppressing immunity. In general, women tend to have more robust T cell immunity, which is particularly beneficial in anti-viral defense<sup>[42,43]</sup>, but exuberant T helper (T<sub>h</sub>)-2 response, eosinophilic inflammation and IL-33 production have been implicated in worsening asthma and autoimmune conditions<sup>[44]</sup>.<sup>[45]</sup> Conversely, estrogen appears to hamper the innate immune response to bacterial infections<sup>[46]</sup>. Sex hormones have also been shown to have pleotropic effects on CF inflammation and vulnerability to bacterial infections. As CF pulmonary exacerbations are frequently mediated by chronic bacterial infections, a sex disparity in bacterial infection response may therefore increase the risk of infection and inflammation in CF women.

The production of lactoferrin, an antimicrobial peptide that is part of the innate defense against invading pathogens, was found to be significantly reduced when estrogen stimulated mice were infected with PsA compared to mice without estrogen stimulation<sup>[47]</sup>. McElvaney and colleagues demonstrated that 17- $\beta$ -estradiol mediates an increase in secretory leucoprotease inhibitor (SLPI) production in CF bronchial epithelial cells, resulting in the down-regulation of IL-8 production via the Toll-like receptor (TLR) - NF $\kappa$ B signaling pathway<sup>[48]</sup>. As IL-8 is a chemokine responsible for neutrophil chemotaxis, inhibition of IL-8 production could therefore result in the down-regulation of the innate immune response to infections in CF patients. In addition to reduced neutrophilic chemotaxis, estrogen can also reduce neutrophil-mediated bacterial clearance as neutrophils isolated from estrogen-treated mice demonstrate increased oxidative burst but reduced PsA killing when compared to progesterone-treated controls<sup>[49]</sup>. Estrogen also appears to promote the T<sub>h</sub>-17 response during PsA infection as CF mice stimulated with estrogen demonstrated higher levels of IL-23 and IL-17 in lung tissue and bronchoalveolar fluid when compared to mice without estrogen stimulation, but despite this, the recovered bacterial load from lung homogenate was paradoxically higher<sup>[47]</sup>. This suggests that while estrogen may promote neutrophilic oxidative burst and T<sub>h</sub>-17 response resulting in increased host inflammation and presumably more lung tissue injury, neither response are effective in bacterial clearance. Consistent with this, estrogen treated ovariectomized female CF mice were found to have worse survival upon inoculation with PsA compared to progesterone treated mice<sup>[49]</sup>.

In addition to modulating the immune response, estrogen might also directly alter bacterial behavior. *In vitro* stimulation with estradiol was found to enhance the growth of PsA, promoting the development of the mucoid phenotype via the up-regulation of the bacterial *mucA* gene<sup>[50]</sup>. Sputum analyses performed during the CF patients' menstrual cycle demonstrated a

predominance of the mucoid phenotype in the follicular phase (high estrogen, low progesterone state) while the non-mucoid phenotype predominated during the luteal phase (low estrogen, high progesterone state)<sup>[50]</sup>. Mucoid PsA is associated with heightened production of alginate, an integral component of biofilm formation. More recently, estrogen has also been shown to promote virulence factor expression in PsA isolates, leading to increased PsA motility as well as increased biofilm formation<sup>[41]</sup>. Thus, estrogen may select for a form of PsA that is more resistant to host or antimicrobial killing.

Little is known about progesterone's effect on infection and inflammation in CF. During pregnancy in non-CF patients, progesterone has been demonstrated to promote T<sub>h</sub>2 cytokine production while reducing the T<sub>h</sub>1 cytokine response, playing a critical role the prevention of fetal rejection by the maternal immune system<sup>[51]</sup>. In addition, progesterone has been shown to act upon murine pulmonary epithelium to reduce cellular damage and improve lung function post influenza infection by increasing transforming growth factor (TGF)- $\beta$ , IL-6 and IL-22, as well as inducing the proliferation of CD39<sup>+</sup>T<sub>h</sub>17 cells<sup>[52]</sup>. Progesterone may also be involved in promoting serologic responses and T-cell memory as mice treated with progesterone demonstrate increased antibody titers and lower viral load upon influenza re-infection<sup>[53]</sup>. Thus, progesterone appears to play an important role in the host immune response to viral infection in murine models but its role in humans and more specifically CF remains to be seen.

While traditionally considered male sex hormones, androgens are also present in females but at a relatively lower level. "Androgen" is a broad term that encompasses a number of sex hormones including testosterone, dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEA-S)<sup>[27]</sup>. In non-CF men, testosterone treatment has been shown to be anti-inflammatory, reducing the T<sub>h</sub>2 response as well as markers of systemic inflammation such as C-reactive protein (CRP)

<sup>[54]</sup>. DHEA-S levels have been demonstrated to inversely correlate with levels of CRP<sup>[55]</sup>. Several groups have compared androgen levels between CF and non-CF patients. In one small study, serum testosterone levels in five ovulatory CF women were found to be comparable to fifteen ovulatory non-CF women<sup>[56]</sup>. More recently, Shaffer and colleagues reported lower DHEA-S levels in CF women compared to CF men and non-CF women despite comparable levels between non-CF women and men<sup>[57]</sup>. It is not clear currently what the clinical implications of the reduced DHEA-S level might be but this may be a contributing factor to increased inflammation seen in CF women.

### **Effects of Female Sex Hormones on Pulmonary Outcomes**

As estrogen has been linked to reduced mucociliary clearance and worsening bacterial infection, one could infer that any period of time when estrogen exposure is heightened there might be increased pulmonary complications and, on the contrary, attenuating the estrogen surge might mitigate this effect. However, studies examining the relationship between endogenous or exogenous sex hormones and pulmonary outcomes in CF have been conflicting and therefore the relationship is likely much more complicated (Table 1).

#### Endogenous changes

At clinical baseline, women with CF have comparable levels of serum estradiol as non-CF women<sup>[58]</sup>. Interestingly, Chotirmall and colleagues found that during periods of CF exacerbations, serum estradiol levels are significantly higher<sup>[50]</sup>. They also demonstrated a correlation between serum levels of estradiol and the frequency of infectious pulmonary exacerbations where the majority of events occurred during the follicular phase (high estrogen,

low progesterone)<sup>[50]</sup>. Furthermore, registry data collected during pregnancy, a period of time where estrogen and progesterone are both heightened (Table 2), suggest that annual rates of pulmonary exacerbations<sup>[59]</sup> and hemoptysis<sup>[60]</sup> are higher compared to non-pregnant female controls. However, it should be noted that the increased rates of exacerbations found during pregnancy might simply be a reflection of increased frequency and intensity of monitoring or other non-hormone related physiologic changes in pregnancy (such as increased acid reflux) that heighten the risk of pulmonary complications. Nevertheless, these observations support the hypothesis that elevations in estrogen may have a detrimental effect on pulmonary infections and presumably lung function. However, when Johannesson and colleagues monitored 12 CF women during their menstrual cycles, they found a 3-5% reduction in both forced expiratory volume in 1 second (FEV<sub>1</sub>) and forced vital capacity (FVC) during menstruation (low estrogen and progesterone) and ovulation (high estrogen, low progesterone), which recovered during the luteal phase despite representing a period of high estrogen and progesterone<sup>[61]</sup>. Thus, the physiologic changes in female sex hormones may have a more complex relationship with fluctuations in lung function and mucociliary clearance.

### Exogenous Changes

The most commonly used hormone-based combination oral contraceptive pills (OCP) works by inhibiting ovulation by providing 21 days of low dose estradiol and progesterone each month to abrogate the natural rise and fall of estrogen and progesterone during the menstrual cycle (Figure 1). Based on a large retrospective study involving 700 women with CF followed over 5 years, no statistically significant difference with regards to the annual rates of change in FEV<sub>1</sub> or in the numbers of pulmonary exacerbations requiring intravenous antibiotics were

observed between women using OCP compared to those who did not<sup>[62]</sup>. Paired analysis of the same women pre- and post-OCP over 3 years also failed to show any difference in clinical outcomes. A separate retrospective analysis involving the Irish CF registry also did not find a statistically significant difference in outcomes in women using OCP<sup>[50]</sup>. However, a trend towards reduction in the number of pulmonary exacerbations requiring treatment with either oral or intravenous antibiotics was seen with longer term OCP use<sup>[50]</sup>. While such retrospective registry studies can be helpful in identifying possible trends, it should be pointed out that there are a number of OCP products on the market that have different combined concentrations of estrogen and progesterone or are estrogen/progesterone monotherapies. As well, OCPs are categorized into monophasic (same dose for 21 days of the month) and multiphasic (escalation in dose throughout the month) products. Consequently, without capturing the details of the type of OCP used, potential effects of estrogen and progesterone on pulmonary outcomes can be challenging to study in observational studies.

A recent small prospective study presented in abstract form by Jain and colleagues demonstrated that FEV<sub>1</sub> increased by 2.5% in women who were started on OCP when compared to their pre-OCP ovulation phase FEV<sub>1</sub><sup>[32]</sup>. Clinically, assessment of patient reported symptom score using the CF questionnaire – revised (CFQ-R) as well as other patient reported quality of life scores demonstrated clinically significant improvements once OCP was initiated<sup>[63]</sup>. Furthermore, prior to OCP initiation, these scores were found to be lower in women during menstruation and ovulation when compared to men. Biochemically, sputum inflammatory markers (including tumor necrosis factor (TNF)- $\alpha$ , IL-8 and free neutrophil elastase) and PsA bacterial load were higher during ovulation than in the menstrual or luteal phases<sup>[32]</sup>. Importantly, a significant reduction in these sputum bacterial and biomarker findings were seen

once OCP was initiated. Given that OCP typically provides a consistently lower serum concentration of estrogen and higher concentration of progesterone than the physiologic rise and fall of either sex hormones (Figure 1), these clinical observations may suggest that reduction in inflammatory activity and bacterial load may result from either a higher level of progesterone, a combination of lower steady state of estrogen and higher steady state of progesterone, or the abrogation of physiologic hormonal fluctuations.

There has been a rise in the number of individuals identifying as transgender in the US over the past decade. Hormone therapies are commonly employed at the initial stage of gender transitioning from male to female to target a serum estradiol level in the range of 100-200ng/ml, similar to that seen during ovulation (Table 2). While there is currently no published data on how hormone therapies might influence CF relevant pulmonary outcomes, there is an increased lifetime risk of developing asthma in transgender females (male-to-female) relative to transgender males (female-to-male) or non-transgender individuals<sup>[64]</sup>. In our center, we currently have a patient who is receiving hormone therapy as part of sex reassignment. Anecdotally, she experienced persistent drops in FEV<sub>1</sub> with increased frequency of pulmonary exacerbations, coinciding with estrogen dose escalation<sup>[65]</sup>. Similarly, for women experiencing uncomfortable symptoms of menopause, estrogen and/or progesterone is often prescribed as hormone replacement therapy (HRT) for symptomatic relief. While there is no literature to date on the effects of HRT in the CF population, a prospective analysis involving non-CF individuals examining lung disease outcomes found an increased rate of adult onset asthma with estrogen only or combination estrogen/progesterone HRT use<sup>[66]</sup>.

### **Therapeutic targeting of Female Sex Hormones**

*In vitro* and *in vivo* studies have demonstrated that ER antagonism may be effective at reversing the estrogen mediated pro-inflammatory effects seen in CF airways. Tamoxifen, a selective estrogen receptor modulator that has both anti-estrogenic and pro-estrogenic effects depending on the target tissue, and other non-clinically available estrogen antagonists have been shown to counteract estrogen mediated increases in PsA virulence<sup>[41]</sup>. Tamoxifen has also been shown to improve chloride transport in CF epithelial cells in a CFTR-independent manner by increasing calcium-activated chloride channel currents<sup>[67]</sup>. While modulating estrogen levels or ER antagonism has the potential to reduce inflammation and improve pulmonary outcomes in CF females, estrogen has a wide range of physiologic effects including the regulation of menstrual cycles and fertility, bone health and the development of secondary sex characteristics. Therefore, a more targeted approach to altering the down-stream effect of estrogen-mediated inflammation is likely to be a more prudent strategy. In addition, it has been observed that the disparity between men and women with regards to pulmonary exacerbation rates can be improved or even abolished in women treated with CFTR modulators such as ivacaftor<sup>[68]</sup> or lumacaftor-ivacaftor<sup>[69]</sup>, suggesting that improvements in CFTR expression, localization or function might be sufficient to counteract the negative effects of estrogen on CFTR independent pathways, infection, inflammation and mucociliary clearance.

Unlike estrogen, much less attention has been paid towards the potential therapeutic benefits of progesterone treatment. Studies of female physiology during pregnancy demonstrate that progesterone has a bronchodilatory effect on the airways through relaxation of smooth muscle tone<sup>[70]</sup>. Thus, therapeutic dosing of progesterone to mimic levels achieved in pregnancy (Table 2) might be a viable intervention. As progesterone only OCP is much less popular than combined OCP due to the concern about accelerated bone loss with both short and long term

use<sup>[71,72]</sup>, there is little observational data on the effect of progesterone alone. Given that the therapeutic concentration of progesterone provided in the combined OCP is higher than the physiologic level of progesterone during ovulation (Table 2), the reduction in sputum inflammation seen with OCP use<sup>[32]</sup> might be attributable to higher progesterone levels alone. Alternatively, given that OCP provides a steady state concentration of progesterone, eliminating the typical rise and fall of sex hormones throughout the menstrual cycle, the improvement in sputum inflammation could also be due to the abrogation of large swings in sex hormone levels. Consequently, further understanding of the effect of progesterone on human lung inflammation will be important before considering the therapeutic use of progesterone in this context.

### **Future Research**

The study of sex hormones and CF holds much potential not only for furthering our understanding of inflammation and infection in CF host-pathogen interactions but also for the design of novel therapeutics to help reduce pulmonary-related morbidity and mortality. Several key areas warrant further investigation to achieve these goals. First, further research is needed to understand the mechanistic role that progesterone and the complex interaction between estrogen and progesterone stimulation might have on mucociliary clearance and bacterial response. While *in vitro* work demonstrates that estrogen alone is pro-inflammatory and promotes bacterial survival, clinical observations of improved pulmonary outcomes during the luteal phase (high estrogen and progesterone) when compared to the follicular phase or ovulation (both high estrogen and low progesterone states) suggest that the mere presence of high levels of estrogen does not always result in worse pulmonary outcomes. Given that estrogen and progesterone levels are similar in men and menstruating women, the fluctuations or surges in sex hormone

levels during the female menstrual cycle might be more important than the absolute level of the hormones themselves. Thus, the effect of progesterone and the cyclical changes in estrogen and progesterone exposure to CF airways merits further investigation. Second, as sex hormones have a myriad of endocrinologic effects, estrogen and progesterone based therapy may not be a viable intervention to reduce pulmonary exacerbations. Therefore, further research should be focused on finding down-stream components of the pro-inflammatory pathway mediated by ER activation that might be a more specific target for therapeutic intervention, such as the SLPI. Further mechanistic studies should be directed to better understand the ER pathway in bronchial epithelial cells to look for other specific localized targets without causing other unintended endocrinological effects.

## **Conclusion**

While it currently remains unclear why a sex disparity exists in CF survival, studies performed over the last several decades have provided evidence that sex hormones might contribute to poor pulmonary outcomes in females by impairing mucociliary clearance and the immune response to infection, increasing bacterial virulence, and increasing lung inflammation. Additional research is needed to clarify the role of progesterone on CF airways and to identify specific treatments that counteract the negative effects of female sex hormones on CF airway pathophysiology to improve outcomes in women with CF without causing broader endocrinological complications.

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Table 1. Summary of animal and human studies in CF examining the effects of female sex hormones or CFTR modulators

Intervention	Study Design	Study Model/No. of Subjects	Outcomes	Ref
<b>Animal Studies</b>				
Estradiol	<i>In vivo</i>	CFTR deficient Mouse model (CFTR <sup>tm1UNC</sup> )	- Decreased survival when infected with PsA due to reduced bacterial killing compared to progesterone treatment	[49]
	<i>Ex vivo</i>	CFTR deficient Mouse model (CFTR <sup>tm1UNC</sup> )	- Increased IL-23 and IL-17 expression in BAL and lung tissue post PsA infection - Increased PsA load - Decreased antimicrobial peptide, lactoferrin	[47]
<b>Human Studies</b>				
<i>Pre-clinical Studies</i>				
Estradiol	<i>In vitro</i>	Human CF bronchial epithelial cell line (CFBE41o-)	- Up-regulated SLPI and inhibited IL-8 production	[48]
	<i>In vitro</i>	Human CF Bronchial epithelial cell line (CuFi-1)	- Reduced intensity of ciliary beat frequency - Upregulated PsA virulence factors to promote motility and biofilm formation	[41]
	<i>In vitro</i>	Human CF bronchial epithelial cell lines (CFBE41o- and IB3-1)	- Reduced chloride transport in a CFTR-independent manner	[67]
	<i>In vitro</i>	Primary human CF bronchial epithelium (HBECs)	- Inhibited Ca <sup>2+</sup> influx - Reduced Cl <sup>-</sup> secretion and ASL volume	[37]
Tamoxifen	<i>In vitro</i>	Human CF bronchial epithelial cell lines (CFBE41o- and IB3-1)	- Improved chloride transport in a CFTR-independent manner	[67]
Tamoxifen	<i>In vitro</i>	Human CF	- Counteracted estrogen-mediated	[41]

and ICI182780		Bronchial epithelial cell line (CuFi-1)	increase in PsA virulence	
	<i>In vitro</i>	Primary human CF bronchial epithelium (HBECs)	- Increased Cl <sup>-</sup> secretion and ASL volume	[37]
<i>Clinical Studies</i>				
Ivacaftor	Retrospective case control	144 (77 males; 67 females)	- Decreased PEx rates in females to a greater extent than males and to a rate similar to that of males post-IVA - Greater improvement in sweat chloride in females compared to males 3 months post IVA - No significant sex-related differences in changes in FEV <sub>1</sub> or BMI post-IVA	[68]
OCP	Retrospective	77 (36 on OCP)	- Reduced antibiotics requirement	[50]
	Retrospective	114 (57 on OCP)	- Unchanged FEV <sub>1</sub> , BMI or number of PEx	[62]
	Prospective cohort	23 (13 on OCP)	- Improved CFQ-R scores	[63]
	Prospective cohort	23 (13 on OCP)	- Reduced sputum inflammatory biomarkers and PsA load - Increased FEV <sub>1</sub>	[32]

Definition of abbreviations: ASL = airway surface liquid, BAL = bronchoalveolar lavage, BMI = body mass index, CFQ-R = cystic fibrosis questionnaire revised, CFTR = cystic fibrosis transmembrane conductance regulator, FEV<sub>1</sub> = forced expiratory volume in one second, IVA = ivacaftor, OCP = oral contraceptive pill, PEx = pulmonary exacerbation, PsA = *Pseudomonas aeruginosa*, SLPI = secretory leucoprotease inhibitor

**Table 2. Serum concentrations of estradiol and progesterone during the menstrual cycle, pregnancy, and with oral contraceptive pill (OCP) use.**

	<b>Serum Estradiol (pg/ml)</b>	<b>Serum Progesterone (ng/ml)</b>
<b>Menstruation</b>	30-50	0.1 – 0.7
<b>Follicular Phase</b>	30-400	0.1 – 0.7
<b>Ovulation</b>	100-700	0.1 – 0.7
<b>Luteal Phase</b>	60-150	2 - 25
<b>Pregnancy (singleton; peak level)*</b>	1300 – 7200	65 - 290
<b>OCP (monophasic at steady state)**</b>	47 – 100	3.3 – 90
<b>Men</b>	14 – 54	0.1 – 1.0

\*During pregnancy, estrogen and progesterone levels rise steadily throughout the trimesters. Ranges provided in table represent peak levels documented in the third trimester.

\*\*Ranges of serum estradiol and progesterone reflect steady state serum concentrations achieved after multiple months of use with monophasic OCP from products with the lowest to the highest estradiol/progesterone concentration.

### **Figure Legend**

Figure 1. Changes in estrogen and progesterone levels in context of clinical, infectious and inflammatory findings during the menstrual cycle. During the menstrual phase, both estrogen and progesterone levels are low. In the follicular phase, estrogen begins to rise and peak just prior to ovulation. Progesterone begins to rise shortly before ovulation and peak during the luteal phase. Estrogen levels also increase during the luteal phase. Levels of both progesterone and estrogen decline until the start of the next menstrual cycle. FEV<sub>1</sub> and FVC are both noted to be reduced during menstruation and ovulation but recovers to baseline by the luteal phase. Rates of pulmonary exacerbations (PE<sub>x</sub>) are highest during the follicular phase, while markers of inflammation (TNF- $\alpha$ , IL-8 and free neutrophil elastase (NE)) and *Pseudomonas aeruginosa* (PsA) sputum bacterial load are highest during ovulation. PsA takes on the more virulent mucoid form during follicular phase while reverting to the less virulent non-mucoid form during the luteal phase.

## References

1. McColley, SA, Schechter, MS, Morgan, WJ, Pasta, DJ, Craib, ML, Konstan, MW . Risk factors for mortality before age 18 years in cystic fibrosis. *Pediatr Pulmonol* 2017; **52**: 909-915 [PMID: DOI:10.1002/ppul.23715 [doi]].
2. Kerem, E, Reisman, J, Corey, M, Canny, GJ, Levison, H . Prediction of mortality in patients with cystic fibrosis. *N Engl J Med* 1992; **326**: 1187-1191 [PMID: DOI:10.1056/NEJM199204303261804 [doi]].
3. Raghavan, D, Jain, R . Increasing awareness of sex differences in airway diseases. *Respirology* 2016; **21**: 449-459 [PMID: DOI:10.1111/resp.12702 [doi]].
4. LoMauro, A, Aliverti, A . Sex differences in respiratory function. *Breathe (Sheff)* 2018; **14**: 131-140 [PMID: DOI:10.1183/20734735.000318 [doi]].
5. Rosenfeld, M, Davis, R, FitzSimmons, S, Pepe, M, Ramsey, B . Gender gap in cystic fibrosis mortality. *Am J Epidemiol* 1997; **145**: 794-803 [PMID: DOI:10.1093/oxfordjournals.aje.a009172 [doi]].
6. CF Canada . The Canadian CF Registry 2017 Annual Data Report 2017 [PMID: .
7. Reid, DW, Blizzard, CL, Shugg, DM, Flowers, C, Cash, C, Greville, HM . Changes in cystic fibrosis mortality in Australia, 1979-2005. *Med J Aust* 2011; **195**: 392-395 [PMID: DOI:rei11229\_fm [pii]].
8. Olesen, HV, Pressler, T, Hjelte, L, Mared, L, Lindblad, A, Knudsen, PK, Laerum, BN, Johannesson, M, Scandinavian Cystic Fibrosis Study Consortium . Gender differences in the Scandinavian cystic fibrosis population. *Pediatr Pulmonol* 2010; **45**: 959-965 [PMID: DOI:10.1002/ppul.21265 [doi]].
9. Harness-Brumley, CL, Elliott, AC, Rosenbluth, DB, Raghavan, D, Jain, R . Gender differences in outcomes of patients with cystic fibrosis. *J Womens Health (Larchmt)* 2014; **23**: 1012-1020 [PMID: DOI:10.1089/jwh.2014.4985 [doi]].
10. . UK CF Registry Annual Data Report 2017 . *CF Trust* [PMID: .
11. de Jong, C C M, Slabbers, L, Engel, TGP, Yntema, JB, van Westreenen, M, Croughs, PD, Roeleveld, N, Brimicombe, R, Verweij, PE, Meis, JF, Merkus, PJ . Clinical relevance of *Scedosporium* spp. and *Exophiala dermatitidis* in patients with cystic fibrosis: A nationwide study. *Med Mycol* 2020 [PMID: DOI:myaa003 [pii]].
12. Miller, RJ, Tildesley, HD, Wilcox, PG, Zhang, H, Kreisman, SH . Sex disparities in effects of cystic fibrosis-related diabetes on clinical outcomes: a matched study. *Can Respir J* 2008; **15**: 291-294 [PMID: DOI:10.1155/2008/697186 [doi]].

13. Stephenson, AL, Tom, M, Berthiaume, Y, Singer, LG, Aaron, SD, Whitmore, GA, Stanojevic, S . A contemporary survival analysis of individuals with cystic fibrosis: a cohort study. *Eur Respir J* 2015; **45**: 670-679 [PMID: DOI:10.1183/09031936.00119714 [doi]].
14. Boelle, PY, Viviani, L, Busson, PF, Olesen, HV, Ravilly, S, Stern, M, Assael, BM, Barreto, C, Drevinek, P, Thomas, M, Krivec, U, Mei-Zahav, M, Vibert, JF, Clement, A, Mehta, A, Corvol, H, French CF Modifier Gene Study Investigators, European CF Registry Working Group . Reference percentiles for FEV(1) and BMI in European children and adults with cystic fibrosis. *Orphanet J Rare Dis* 2012; **7**: 64-64 [PMID: DOI:10.1186/1750-1172-7-64 [doi]].
15. Simon, SL, Duncan, CL, Horky, SC, Nick, TG, Castro, MM, Riekert, KA . Body satisfaction, nutritional adherence, and quality of life in youth with cystic fibrosis. *Pediatr Pulmonol* 2011; **46**: 1085-1092 [PMID: DOI:10.1002/ppul.21477 [doi]].
16. Zemel, BS, Jawad, AF, FitzSimmons, S, Stallings, VA . Longitudinal relationship among growth, nutritional status, and pulmonary function in children with cystic fibrosis: analysis of the Cystic Fibrosis Foundation National CF Patient Registry. *J Pediatr* 2000; **137**: 374-380 [PMID: DOI:S0022-3476(00)14536-6 [pii]].
17. Zemel, BS, Jawad, AF, FitzSimmons, S, Stallings, VA . Longitudinal relationship among growth, nutritional status, and pulmonary function in children with cystic fibrosis: analysis of the Cystic Fibrosis Foundation National CF Patient Registry. *J Pediatr* 2000; **137**: 374-380 [PMID: DOI:S0022-3476(00)14536-6 [pii]].
18. Patterson, JM, Wall, M, Berge, J, Milla, C . Associations of psychosocial factors with health outcomes among youth with cystic fibrosis. *Pediatr Pulmonol* 2009; **44**: 46-53 [PMID: DOI:10.1002/ppul.20925 [doi]].
19. Simon, M I S D S, Paulo, GCF, Marostica, JC . Body Mass Index and Albumin Levels are Associated with Pulmonary Function Parameters in Pediatric Subjects with Cystic Fibrosis. *Rev Paul Pediatr* 2019; **37**: 414-418 [PMID: DOI:S0103-05822019000400414 [pii]].
20. Patterson, JM, Wall, M, Berge, J, Milla, C . Gender differences in treatment adherence among youth with cystic fibrosis: development of a new questionnaire. *J Cyst Fibros* 2008; **7**: 154-164 [PMID: DOI:S1569-1993(07)00101-4 [pii]].
21. Viviani, L, Bossi, A, Assael, BM, Italian Registry for Cystic Fibrosis Collaborative Group . Absence of a gender gap in survival. An analysis of the Italian registry for cystic fibrosis in the paediatric age. *J Cyst Fibros* 2011; **10**: 313-317 [PMID: DOI:10.1016/j.jcf.2011.03.007 [doi]].
22. Sutton, S, Rosenbluth, D, Raghavan, D, Zheng, J, Jain, R . Effects of puberty on cystic fibrosis related pulmonary exacerbations in women versus men. *Pediatr Pulmonol* 2014; **49**: 28-35 [PMID: DOI:10.1002/ppul.22767 [doi]].
23. Cui, J, Shen, Y, Li, R . Estrogen synthesis and signaling pathways during aging: from periphery to brain. *Trends Mol Med* 2013; **19**: 197-209 [PMID: DOI:10.1016/j.molmed.2012.12.007 [doi]].
24. Laurent, MR, Hammond, GL, Blokland, M, Jardi, F, Antonio, L, Dubois, V, Khalil, R, Sterk, SS, Gielen, E, Decallonne, B, Carmeliet, G, Kaufman, JM, Fiers, T, Huhtaniemi, IT, Vanderschueren, D,

- Claessens, F . Sex hormone-binding globulin regulation of androgen bioactivity in vivo: validation of the free hormone hypothesis. *Sci Rep* 2016; **6**: 35539 [PMID: DOI:10.1038/srep35539 [doi]].
25. Hamilton, KJ, Hewitt, SC, Arao, Y, Korach, KS . Estrogen Hormone Biology. *Curr Top Dev Biol* 2017; **125**: 109-146 [PMID: DOI:S0070-2153(16)30204-6 [pii]].
  26. Assaggaf, H, Felty, Q . Gender, Estrogen, and Obliterative Lesions in the Lung. *Int J Endocrinol* 2017; **2017**: 8475701 [PMID: DOI:10.1155/2017/8475701 [doi]].
  27. Sathish, V, Martin, YN, Prakash, YS . Sex steroid signaling: implications for lung diseases. *Pharmacol Ther* 2015; **150**: 94-108 [PMID: DOI:10.1016/j.pharmthera.2015.01.007 [doi]].
  28. Taraborrelli, S . Physiology, production and action of progesterone. *Acta Obstet Gynecol Scand* 2015; **94 Suppl 161**: 8-16 [PMID: DOI:10.1111/aogs.12771 [doi]].
  29. Jain, R, Ray, JM, Pan, JH, Brody, SL . Sex hormone-dependent regulation of cilia beat frequency in airway epithelium. *Am J Respir Cell Mol Biol* 2012; **46**: 446-453 [PMID: DOI:10.1165/rcmb.2011-0107OC [doi]].
  30. Perusquia, M, Hernandez, R, Montano, LM, Villalon, CM, Campos, MG . Inhibitory effect of sex steroids on guinea-pig airway smooth muscle contractions. *Comp Biochem Physiol C Pharmacol Toxicol Endocrinol* 1997; **118**: 5-10 [PMID: DOI:10.1016/s0742-8413(97)00029-7 [doi]].
  31. Klein, SL, Flanagan, KL . Sex differences in immune responses. *Nat Rev Immunol* 2016; **16**: 626-638 [PMID: DOI:10.1038/nri.2016.90 [doi]].
  32. Jain, R, Sabanova, V, Keller, A, Schumacher, L, Holtrop, M, Fernandez, L, Cavazos, O, Gales, P, Abid, SN, Bose, M, Heltshe, S . EFFECTS OF SEX HORMONES ON MARKERS OF HEALTH IN WOMEN WITH CYSTIC FIBROSIS. *Pediatric Pulmonology* 2019; **54**: 221 [PMID: .
  33. Swezey, NB, Gauthier, C, Gagnon, S, Ferretti, E, Kopelman, H . Progesterone and estradiol inhibit CFTR-mediated ion transport by pancreatic epithelial cells. *Am J Physiol* 1996; **271**: 747 [PMID: DOI:10.1152/ajpgi.1996.271.5.G747 [doi]].
  34. Rochwerger, L, Buchwald, M . Stimulation of the cystic fibrosis transmembrane regulator expression by estrogen in vivo. *Endocrinology* 1993; **133**: 921-930 [PMID: DOI:10.1210/endo.133.2.7688293 [doi]].
  35. Ajonuma, LC, Tsang, LL, Zhang, GH, Wong, CH, Lau, MC, Ho, LS, Rowlands, DK, Zhou, CX, Ng, CP, Chen, J, Xu, PH, Zhu, JX, Chung, YW, Chan, HC . Estrogen-induced abnormally high cystic fibrosis transmembrane conductance regulator expression results in ovarian hyperstimulation syndrome. *Mol Endocrinol* 2005; **19**: 3038-3044 [PMID: DOI:me.2005-0114 [pii]].
  36. Fanelli, T, Cardone, RA, Favia, M, Guerra, L, Zaccolo, M, Monterisi, S, De Santis, T, Riccardi, SM, Reshkin, SJ, Casavola, V . Beta-oestradiol rescues DeltaF508CFTR functional expression in human cystic fibrosis airway CFBE41o- cells through the up-regulation of NHERF1. *Biol Cell* 2008; **100**: 399-412 [PMID: DOI:10.1042/BC20070095 [doi]].

37. Coakley, RD, Sun, H, Clunes, LA, Rasmussen, JE, Stackhouse, JR, Okada, SF, Fricks, I, Young, SL, Tarran, R . 17beta-Estradiol inhibits Ca<sup>2+</sup>-dependent homeostasis of airway surface liquid volume in human cystic fibrosis airway epithelia. *J Clin Invest* 2008; **118**: 4025-4035 [PMID: DOI:10.1172/JCI33893 [doi]].
38. Preston, P, Wartosch, L, Gunzel, D, Fromm, M, Kongsuphol, P, Ousingsawat, J, Kunzelmann, K, Barhanin, J, Warth, R, Jentsch, TJ . Disruption of the K<sup>+</sup> channel beta-subunit KCNE3 reveals an important role in intestinal and tracheal Cl<sup>-</sup> transport. *J Biol Chem* 2010; **285**: 7165-7175 [PMID: DOI:10.1074/jbc.M109.047829 [doi]].
39. Kroncke, BM, Van Horn, WD, Smith, J, Kang, C, Welch, RC, Song, Y, Nannemann, DP, Taylor, KC, Sisco, NJ, George, AL, Meiler, J, Vanoye, CG, Sanders, CR . Structural basis for KCNE3 modulation of potassium recycling in epithelia. *Sci Adv* 2016; **2**: e1501228 [PMID: DOI:10.1126/sciadv.1501228 [doi]].
40. Choi, HJ, Chung, YS, Kim, HJ, Moon, UY, Choi, YH, Van Seuningen, I, Baek, SJ, Yoon, HG, Yoon, JH . Signal pathway of 17beta-estradiol-induced MUC5B expression in human airway epithelial cells. *Am J Respir Cell Mol Biol* 2009; **40**: 168-178 [PMID: DOI:10.1165/rcmb.2007-0377OC [doi]].
41. Tyrrell, J, Harvey, BJ . Sexual dimorphism in the microbiology of the CF 'Gender Gap': Estrogen modulation of *Pseudomonas aeruginosa* virulence. *Steroids* 2019; **156**: 108575 [PMID: DOI:S0039-128X(19)30265-X [pii]].
42. Robinson, DP, Hall, OJ, Nilles, TL, Bream, JH, Klein, SL . 17beta-estradiol protects females against influenza by recruiting neutrophils and increasing virus-specific CD8 T cell responses in the lungs. *J Virol* 2014; **88**: 4711-4720 [PMID: DOI:10.1128/JVI.02081-13 [doi]].
43. Takahashi, T, Ellingson, MK, Wong, P, Israelow, B, Lucas, C, Klein, J, Silva, J, Mao, T, Oh, JE, Tokuyama, M, Lu, P, Venkataraman, A, Park, A, Liu, F, Meir, A, Sun, J, Wang, EY, Casanovas-Massana, A, Wyllie, AL, Vogels, CBF, Earnest, R, Lapidus, S, Ott, IM, Moore, AJ, Yale IMPACT Research Team, Shaw, A, Fournier, JB, Odio, CD, Farhadian, S, Dela Cruz, C, Grubaugh, ND, Schulz, WL, Ring, AM, Ko, AI, Omer, SB, Iwasaki, A . Sex differences in immune responses that underlie COVID-19 disease outcomes. *Nature* 2020 [PMID: DOI:10.1038/s41586-020-2700-3 [doi]].
44. Desai, MK, Brinton, RD . Autoimmune Disease in Women: Endocrine Transition and Risk Across the Lifespan. *Front Endocrinol (Lausanne)* 2019; **10**: 265 [PMID: DOI:10.3389/fendo.2019.00265 [doi]].
45. Watanabe, Y, Tajiki-Nishino, R, Tajima, H, Fukuyama, T . Role of estrogen receptors alpha and beta in the development of allergic airway inflammation in mice: A possible involvement of interleukin 33 and eosinophils. *Toxicology* 2019; **411**: 93-100 [PMID: DOI:S0300-483X(18)30331-7 [pii]].
46. Speyer, CL, Rancilio, NJ, McClintock, SD, Crawford, JD, Gao, H, Sarma, JV, Ward, PA . Regulatory effects of estrogen on acute lung inflammation in mice. *Am J Physiol Cell Physiol* 2005; **288**: 881 [PMID: DOI:288/4/C881 [pii]].

47. Wang, Y, Cela, E, Gagnon, S, Sweezey, NB . Estrogen aggravates inflammation in *Pseudomonas aeruginosa* pneumonia in cystic fibrosis mice. *Respir Res* 2010; **11**: 166-166 [PMID: DOI:10.1186/1465-9921-11-166 [doi]].
48. Chotirmall, SH, Greene, CM, Oglesby, IK, Thomas, W, O'Neill, SJ, Harvey, BJ, McElvaney, NG . 17Beta-estradiol inhibits IL-8 in cystic fibrosis by up-regulating secretory leucoprotease inhibitor. *Am J Respir Crit Care Med* 2010; **182**: 62-72 [PMID: DOI:10.1164/rccm.201001-0053OC [doi]].
49. Abid, S, Xie, S, Bose, M, Shaul, PW, Terada, LS, Brody, SL, Thomas, PJ, Katzenellenbogen, JA, Kim, SH, Greenberg, DE, Jain, R . 17beta-Estradiol Dysregulates Innate Immune Responses to *Pseudomonas aeruginosa* Respiratory Infection and Is Modulated by Estrogen Receptor Antagonism. *Infect Immun* 2017; **85**: 10.1128/IAI.00422-17. Print 2017 Oct [PMID: DOI:e00422-17 [pii]].
50. Chotirmall, SH, Smith, SG, Gunaratnam, C, Cosgrove, S, Dimitrov, BD, O'Neill, SJ, Harvey, BJ, Greene, CM, McElvaney, NG . Effect of estrogen on *pseudomonas* mucoidy and exacerbations in cystic fibrosis. *N Engl J Med* 2012; **366**: 1978-1986 [PMID: DOI:10.1056/NEJMoa1106126 [doi]].
51. Kumar, P, Magon, N . Hormones in pregnancy. *Niger Med J* 2012; **53**: 179-183 [PMID: DOI:10.4103/0300-1652.107549 [doi]].
52. Hall, OJ, Limjunyawong, N, Vermillion, MS, Robinson, DP, Wohlgemuth, N, Pekosz, A, Mitzner, W, Klein, SL . Progesterone-Based Therapy Protects Against Influenza by Promoting Lung Repair and Recovery in Females. *PLoS Pathog* 2016; **12**: e1005840 [PMID: DOI:10.1371/journal.ppat.1005840 [doi]].
53. Hall, OJ, Nachbagauer, R, Vermillion, MS, Fink, AL, Phuong, V, Krammer, F, Klein, SL . Progesterone-Based Contraceptives Reduce Adaptive Immune Responses and Protection against Sequential Influenza A Virus Infections. *J Virol* 2017; **91**: 10.1128/JVI.02160-16. Print 2017 Apr 15 [PMID: DOI:e02160-16 [pii]].
54. Bianchi, VE . The Anti-Inflammatory Effects of Testosterone. *J Endocr Soc* 2018; **3**: 91-107 [PMID: DOI:10.1210/js.2018-00186 [doi]].
55. Haring, R, Baumeister, SE, Volzke, H, Dorr, M, Kocher, T, Nauck, M, Wallaschofski, H . Prospective inverse associations of sex hormone concentrations in men with biomarkers of inflammation and oxidative stress. *J Androl* 2012; **33**: 944-950 [PMID: DOI:jandrol.111.015065 [pii]].
56. Johannesson, M, Landgren, BM, Csemiczky, G, Hjelte, L, Gottlieb, C . Female patients with cystic fibrosis suffer from reproductive endocrinological disorders despite good clinical status. *Hum Reprod* 1998; **13**: 2092-2097 [PMID: DOI:10.1093/humrep/13.8.2092 [doi]].
57. Shaffer, L, Abid, S, Jain, R . Evaluation of Dehydroepiandrosterone Sulfate (DHEA-S) Levels in Cystic Fibrosis . *American Thoracic Society International Conference Abstracts* 2019 [PMID: .
58. Schram, CA, Stephenson, AL, Hannam, TG, Tullis, E . Cystic fibrosis (cf) and ovarian reserve: A cross-sectional study examining serum anti-mullerian hormone (amh) in young women. *J Cyst Fibros* 2015; **14**: 398-402 [PMID: DOI:10.1016/j.jcf.2014.09.008 [doi]].

59. McMullen, AH, Pasta, DJ, Frederick, PD, Konstan, MW, Morgan, WJ, Schechter, MS, Wagener, JS . Impact of pregnancy on women with cystic fibrosis. *Chest* 2006; **129**: 706-711 [PMID: DOI:S0012-3692(15)52274-5 [pii]].
60. Renton, M, Priestley, L, Bennett, L, Mackillop, L, Chapman, SJ . Pregnancy outcomes in cystic fibrosis: a 10-year experience from a UK centre. *Obstet Med* 2015; **8**: 99-101 [PMID: DOI:10.1177/1753495X15575628 [doi]].
61. Johannesson, M, Ludviksdottir, D, Janson, C . Lung function changes in relation to menstrual cycle in females with cystic fibrosis. *Respir Med* 2000; **94**: 1043-1046 [PMID: DOI:S0954-6111(00)90891-6 [pii]].
62. Kernan, NG, Alton, EW, Cullinan, P, Griesenbach, U, Bilton, D . Oral contraceptives do not appear to affect cystic fibrosis disease severity. *Eur Respir J* 2013; **41**: 67-73 [PMID: DOI:10.1183/09031936.00018712 [doi]].
63. Jain, R, Sabanova, V, Holtrop, M, Keller, A, Schumacher, L, Fernandez, L, Cavazos, O, Gales, P, Abid, SN, Bose, M, Heltshe, S . SYMPTOM DIFFERENCES AND HEALTH PERCEPTIONS BETWEEN MEN AND WOMEN WITH CYSTIC FIBROSIS. *Pediatric Pulmonology* 2019; **54**: 424 [PMID: .
64. Morales-Estrella, JL, Boyle, M, Zein, JG . Transgender Status is Associated with Higher Risk of Lifetime Asthma. *American Journal of Respiratory and Critical Care Medicine* 2018; **197**: A34 [PMID: .
65. Lam, GY, Goodwin, J, Wilcox, P, Quon, BS . Worsening pulmonary outcomes during sex reassignment therapy in a transgender female with cystic fibrosis (CF) and asthma/allergic bronchopulmonary aspergillosis: a case report. *BMC Pulm Med* 2020; **20**: 234-x [PMID: DOI:10.1186/s12890-020-01272-x [doi]].
66. Barr, RG, Wentowski, CC, Grodstein, F, Somers, SC, Stampfer, MJ, Schwartz, J, Speizer, FE, Camargo, CA . Prospective study of postmenopausal hormone use and newly diagnosed asthma and chronic obstructive pulmonary disease. *Arch Intern Med* 2004; **164**: 379-386 [PMID: DOI:10.1001/archinte.164.4.379 [doi]].
67. Imberti, R, Garavaglia, ML, Verduci, I, Cannavale, G, Balduzzi, G, Papetti, S, Mazzanti, M . Antiestrogen- and tamoxifen-induced effects on calcium-activated chloride currents in epithelial cells carrying the F508-CFTR point mutation. *Respir Res* 2018; **19**: 198-1 [PMID: DOI:10.1186/s12931-018-0901-1 [doi]].
68. Secunda, KE, Guimbellot, JS, Jovanovic, B, Heltshe, SL, Sagel, SD, Rowe, SM, Jain, M . Females with Cystic Fibrosis Demonstrate a Differential Response Profile to Ivacaftor Compared to Males. *Am J Respir Crit Care Med* 2019 [PMID: DOI:10.1164/rccm.201909-1845LE [doi]].
69. Aalbers, BL, Hofland, RW, Bronsveld, I, de Winter-de Groot, K M, Arets, HGM, de Kiviet, AC, van Oirschot-van de Ven, M M M, Kruijswijk, MA, Schotman, S, Michel, S, van der Ent, C K, Heijerman, HGM . Females with cystic fibrosis have a larger decrease in sweat chloride in response to lumacaftor/ivacaftor compared to males. *J Cyst Fibros* 2020 [PMID: DOI:S1569-1993(20)30135-1 [pii]].

70. LoMauro, A, Aliverti, A . Respiratory physiology of pregnancy: Physiology masterclass. *Breathe (Sheff)* 2015; **11**: 297-301 [PMID: DOI:10.1183/20734735.008615 [doi]].
71. Cundy, T, Cornish, J, Roberts, H, Elder, H, Reid, IR . Spinal bone density in women using depot medroxyprogesterone contraception. *Obstet Gynecol* 1998; **92**: 569-573 [PMID: DOI:S0029784498002701 [pii]].
72. Petitti, DB, Piaggio, G, Mehta, S, Cravioto, MC, Meirik, O . Steroid hormone contraception and bone mineral density: a cross-sectional study in an international population. The WHO Study of Hormonal Contraception and Bone Health. *Obstet Gynecol* 2000; **95**: 736-744 [PMID: DOI:S0029784400007821 [pii]].

# Figure 1

