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FAILURE TO CONCEIVE IN WOMEN WITH CF IS ASSOCIATED WITH PANCREATIC INSUFFICIENCY AND ADVANCING AGE

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Running title: Failure to conceive in women with CF

Abstract

Objective: The causes of subfertility in women with CF though multifactorial are not well described. Our aim in this study was to determine the prevalence and factors associated with female subfertility among women with CF.

Methods: A retrospective multinational study from 11 CF centers in 5 countries (Israel, France, Spain, Italy, UK) including women with CF was undertaken. Sub/infertility was defined as not achieving a spontaneous pregnancy after one year of unprotected sexual intercourse. Data including genetics, pancreatic insufficiency (PI), prevalence of diabetes (CFRD) , lung function, nutritional status measured by body mass index (BMI), sputum bacterial colonization, and rate of pulmonary exacerbations were collected from patients' files.

Results: Out of 605 women, 241 attempted pregnancy. Of these, 84 (35%) had subfertility, and 67 of them eventually became pregnant. Females attempting conception were older but had better pulmonary function and nutrition compared to those who did not. In a multivariate analysis, PI (OR 1.9 [1.03-3.5], p=0.04) and older age (OR 3.9 [2.1-7.3] p<0.0001) were associated with subfertility. Lung function, BMI, CFRD, Presence of two class I-III mutations and number of exacerbations in the year prior to fertility attempts were not associated with subfertility.

Conclusions: The prevalence of subfertility among women with CF (35%) is higher than the expected 5-15% subfertility in the general population. Older age and pancreatic insufficiency are associated with subfertility in women with CF.

1. Introduction[1]

The majority of people with CF are adults[1,2]. They lead active lives similar to healthy adults of similar age including education, employment, sexual relationship and reproduction[3]. In men with CF, infertility due to complete bilateral absence of vas deferens (CBAVD) is almost universal. While normal fertility is expected in women with CF, a high rate of subfertility[4] is reported, although the exact prevalence is not known.

Subfertility in women with CF was first described in the 1970s, with findings of expression of cystic fibrosis transmembrane regulator (CFTR) in the cervical epithelium, and thick cervical mucus in CF patients[5–7]. It was hypothesized that the deficient CFTR in the cervical epithelium led to abnormal cervical mucus production resulting in reduced sperm permeability[7]. Studies in mice demonstrated the presence of CFTR in various organs of the genital tract, such as the oviduct and uterus[8,9], as well as the hypothalamus and pituitary gland [10] suggesting a role for CFTR function in the reproductive system. Girls with CF have delayed menstruation associated with delays in achieving pubertal levels of insulin-like growth factor-I (IGF-I), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and sex steroid hormones[11]. In a cohort of ten women with CF, it was found that five (50%) had anovulatory menstrual cycles, which were associated with lower essential fatty acids and a higher insulin response to an oral glucose challenge, compared with ovulatory CF women and healthy controls[12]. These findings suggested a role for pre- diabetes in CF related subfertility. Low levels of anti-Mullerian hormone (AMH), considered to be a marker of ovarian reserve[13], were found in women with CF compared to healthy controls [14]. In a recent survey of CF women and their sexual practices, the age of menarche was

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[1] Abbreviations: AMH- anti-Mullerian hormone; ART- Assisted reproduction therapy; BMI- body mass index; CFTR- cystic fibrosis transmembrane regulator; CFRD- cystic fibrosis related diabetes; FSH- follicle stimulating hormone; -1- insulin-like growth factor-1; LH- luteinizing hormone; OGTT- oral glucose tolerance test

significantly delayed in girls with CF compared to healthy girls (13 *vs.* 12 years of age in average)[3]. Whether the described endocrine abnormalities in CF are secondary to deficient CFTR function or are a result of impaired nutritional status is not known.

The increased prevalence of female subfertility has led to an increased use of assisted reproductive technology (ART). In recent surveys, high rates of subfertility and the use of ART were found in Israeli and French women with CF (34 and 40%)[15,16]. However, due to small numbers of patients, assessment of factors associated with subfertility could not be determined.

Our aim was to establish the prevalence of subfertility and to assess whether any specific factors are associated with female subfertility in CF.

2. Methods

We conducted a retrospective multinational-multicenter study, including women with CF from eleven CF centers in five countries, Israel (Six CF centers), France (Lyon), UK (Belfast), Italy (Milan), and Spain (Barcelona). The study protocol was approved by local Helsinki committees in all centers. We included female patients aged 18 and above with an established diagnosis of CF[17] and no prior lung transplantation. We assigned a reference date for every woman- the date of conception (calculated as 40 weeks before date of birth) of the oldest child. For a nulliparous woman, this was the date on which data was retrieved from the clinic registry. Fertility status was determined from interviewing CF center staff, ascertaining fertility investigations, including laboratory assessments of reproductive endocrine function, and reproductive treatments with data from fertility services in different centers. Women were assigned a fertility category according to the accepted definition of "fertility" by the American Society for Reproductive Sciences[18]: 1. Normal fertility - having achieved a spontaneous pregnancy; 2. Subfertility- having required assisted reproduction after 1 year of infertility or achieving spontaneous pregnancy after over 1 year of unprotected intercourse[18]; 3. Infertility- remained infertile despite

treatments; 4. Not attempted childbearing. Women who experienced termination of pregnancies, miscarriages etc. were categorized according to the mode of conception.

Data were collected from patients' hospital files or electronic medical records relevant from the reference date. These included: height, weight and BMI, lung function as forced expiratory volume in one second (FEV1) % predicted (best value during the year before the reference date). CF- related diabetes mellitus (CFRD) at the reference date, glycosylated hemoglobin for all of the women, and results of oral glucose tolerance test (OGTT) at 2 hours for women without CFRD taken as the last measurement prior to the reference date. Pancreatic insufficiency was determined by fecal elastase less than 200 mg/gr; lack of pancreatic enzyme replacement with no evidence of gastrointestinal (GI) complications was taken as a proof of pancreatic sufficiency. CFTR mutation class was categorized as: Class I-III: Both alleles carrying mutations classified as class I, II, or III; Class IV/V mutation: at least one allele carrying a mutation classified as class IV/V, the other allele with any identified mutation; Unknown mutation: at least one of the alleles with an unidentified mutation (despite standard or advanced genetic testing)[19–21]. Colonization with bacteria was defined by Leeds criteria: airway samples which are pseudomonas positive in ≥50% of the explored months [22].(Pulmonary exacerbations- the total number of pulmonary exacerbations (defined as a physician decision to treat with oral or intravenous (IV) antibiotics [23]) in the year ending at the reference date (ie, 92 weeks-40 weeks before birth for women who had concieved).

Statistical analysis was performed by using IBM statistics (SPSS) ,v24. The continuous variables were presented by mean, median & standard deviation. The categorical variables were presented in percentages. Demographical and clinical characteristics were compared between the normal fertility group and subfertile group by Chi Square test for the categorical variables and Independent-t-test, or

Mann-Whitney, as appropriate for the continuous variables. A logistic regression model was used to test factors associated with infertility. P<0.05 was considered statistically significant.

3. Results

3.1 Women included in the study:

622 women with CF were screened, eleven were excluded for failing to meet a confirmed diagnosis of CF, and fertility status could not be determined in 6 women. Out of the remaining 605 women, 241 attempted pregnancy. Of these, 157 had normal fertility, and 84 women were subfertile or infertile, representing a prevalence of 35% in women who attempted conception. Of the 84 sub/infertile women, 67 became pregnant with ART or after more than one year of attempts to conceive,, and 17 remained infertile. Table 1 summarizes the demographic characteristics of women in the study.

	Attempted conception N=241	Did not attempt conception N=364	p-value
Normal fertility: N (% of attempted conception)	157 (65%)	NA	
Subfertility: N (% of attempted conception)	67 (28%)	NA	
Infertility: N (% of attempted conception)	17 (7%)	NA	
Total sub/infertility: N (% of attempted conception)	84 (34.9%)	NA	
Age (mean ± SD)*	30±9	29±8	**0.032**
CFTR mutation type N (%)			
2 class I-III mutations	104 (44%)	244 (67%)	**<0.0001 †**
At least 1 class IV/V mutations**	94 (39%)	80 (22%)	
At least 1 unknown mutation	41 (17%)	39 (11%)	
Pancreatic insufficiency- N (%)	118 (49%)	270 (74%)	**<0.0001**
BMI (mean ± SD)*	N=155 (missing- 86) 22 ± 4	N=297 (missing- 67) 21±3	**<0.0001**
Presence of CFRD *(%)	(N= 228 missing- 13) 41 (18%)	(N=360 missing- 4) 96 (27%)	**0.021**
HbA1C (%)	(N= 140 missing- 101) 5.6 ± 0.71	(N= 299 missing- 65) 6.0 ± 1.3	**0.001**
FEV_1 (%predicted)* (mean± SD):	N=187 (missing- 54) 72 ± 22	N=356 (missing- 8) 67±24	**0.046**

Exacerbations (median (min, max))*§:	N=181 (missing- 60) 1 (0-5)	N=353 (missing- 11) 1 (0-11)	0.150
Bacterial colonization- N (%)*			
N with data (N missing)	215 (missing 26)	308 (missing 45)	
None	60 (28)	58 (16)	**0.003** †
Other	40 (19)	69 (19)	
P. aeruginosa¶	115 (54)	231 (65)	

Table 1. Demographic characteristics of women with CF

Abbreviations: Body mass index (BMI); CF-related diabetes (CFRD); Forced expiratory volume in one second (FEV1); HbA1C- % Glycosylated Hemoglobin. N- number of women.

§No. of pulmonary exacerbations.

*Data acquisition from the year preceding conception attempt. FEV_1 is the best value during that year. ** The second mutation was either class I-III or class IV/V, but not "unknown". † Comparison of 3 parameters. ¶ Women co-colonized with *Pseudomonas* and other bacteria were considered as colonized with *Pseudomonas.*

3.2   Comparison between women who attempted pregnancy and those who did not:

Women who did not attempt pregnancy were younger than women who did attempt pregnancy (age 28.7±8 years vs. 30.4±9 years, p=0.032) and had a higher frequency of 2 class I-III mutations compared to at least one class IV-V or unknown mutations, (p<0.0001), were more likely to be PI (74% vs. 49%, p<0.0001), have CFRD (26.6% vs. 18.3, p=0.021), have a higher HbA1C (6.0±1.3% vs. 5.6±0.7%, p=0.001) and be chronically colonized with bacteria (p=0.003). Women who did not attempt pregnancy had a lower BMI (20.6 ± 3.1 vs. 22.3 ± 3.6, p<0.0001) and FEV1 (67 ±24 vs. 72±22, p=0.046) (Table 1).

3.3 Factors associated with subfertility or infertility

Comparing women with normal fertility to women with subfertility or infertility (grouped together due to small numbers in the infertility group), pancreatic insufficient women had a higher prevalence of subfertility compared to pancreatic sufficient women (42% vs. 29%, p=0.033). Older age at first attempt of conception was also significantly associated with subfertility (age of fertile women 28.4 ± 8 years, age of subfertile women 34.1± 10 years, p<0.0001), as was colonization with *Pseudomonas aeruginosa* (p=0.041). Presence of two class I-III mutations (compared to at least one class IV/V, and to unknown mutations), and presence of CFRD were not significantly more common among women with subfertility than those with normal fertility, (p=0.261 and p=0.2, respectively). Weight , BMI, and HbA1C were similar between fertile and subfertile women (weight, 57±11 and 57±8 kg, p=0.747; BMI, 22.3±4 and 22.2 ±3 kg/m^2, p=0.899, respectively). Other factors not associated with sub/infertility were: lung function (mean FEV1: fertile: 72.6±22, sub/infertile: 71±22 % predicted), and number of exacerbations in the year prior to fertility attempts (fertile: median 0 (0,5); sub/infertile: 1(0,4), p=0.334), and mean 2 hour blood glucose OGTT (for women without CFRD) were available for only a minority of women, and not associated with the risk of subfertility (Table 2). In a logistic regression model assessing risks of

subfertility, age above 31 years and pancreatic insufficiency were independently associated with

subfertility (OR for pancreatic insufficiency 1.9(1.03-3.5), p=0. 04; Figure 1 and Table 3).

	Normal Fertility (N=157)	Sub/Infertility (N=84)	p value
Age at 1st attempt (years, mean± SD)	28±8	34±10	**<0.0001**
CFTR mutation type N (%)	N= 155 (missing- 2)	N=84 (missing= 0)	0.261
2 class I-III mutations	62 (40%)	42 (50%)	
At least 1 class IV/V mutations**	63 (41%)	31 (37%)	
At least 1 unknown mutation	30 (19%)	11 (13%)	
Pancreatic insufficiency- N (%)	N=157 69 (45%)	N=84 49 (58%)	**0.033**
Weight (Kg, mean± SD))	N=102 (missing= 55) 57±11	N=72 (missing= 12) 57±8	0.747
BMI Kg/m^2(mean ± SD)*	N=102 (missing= 55) 22.3	N=72 (missing= 12) 22.2	0.899
Presence of CFRD *(%)	N=145 (missing- 12) 23 (16%)	N=79 (missing- 5) 18 (23%)	0.2
HbA1C%	N=85 (missing- 72)	N=55 (missing, 29)	0.28

	5.6±0.7	5.7±0.7	
2 hr OGTT (mg/dL)	N= 25 (missing 122) 115±47	N=23 (missing 61) 122±51	0.984
FEV$_1$ (%predicted)* (mean± SD):	N= 112 (missing, 45) 73±22	N= 75 (missing, 14) 71±22	0.631
Exacerbations (median (min, max))*§:	N=111 (missing, 46) 0 (1,5)	N= 70 (missing, 14) 1 (0,4)	0.334
Bacterial colonization- N (%)*	N=138 (missing, 19)	N=77 (missing, 14)	**0.041**
None	46 (33%)	14 (18%)	
Other	26 (19%)	14 (18%)	
P. aeruginosa¶	66 (48%)	49 (64%)	

Table 2: Comparison between fertile and sub/infertile women.

Abbreviations: Body mass index (BMI); CF-related diabetes (CFRD); Forced expiratory volume in one second (FEV1); HbA1C- % Glycosylated Hemoglobin; OGTT- Oral glucose tolerance test (2 hour glucose).

N- number of women.

§No. of pulmonary exacerbations.

*Data acquisition from the year preceding conception attempt. FEV_1 is the best value during that year. **

The second mutation was either class I-III or class IV/V, but not "unknown". † Comparison of 3

parameters.

¶ Women co-colonized with *Pseudomonas* and other bacteria were considered as colonized with

Pseudomonas.

	Multivariate OR (95% CI)	P value
Age		
<=31	Reference	
>31	3.9 (2.1-7.3)	**<0.0001**
Bacteria		
no	Reference	
other	1.6 (0.64-4.2)	0.303
PA	1.9 (0.86-4.3)	0.113
Pancreatic status		
PS	Reference	
PI	1.9(1.03-3.5)	**0.040**
Class mutations		
unknown	Reference	
Class IV-V	1.2 (0.48-3.1)	0.676
Class I-III	1.4 (0.45-4.0)	0.549

Table 3. Multivariate analysis of factors associated with female subfertility

Abbreviations: *Pseudomonas aeruginosa* (PA); Pancreatic sufficiency (PS); Pancreatic insufficiency (PI)

Logistic regression model for factors influencing fertility in women with CF who attempted conception.

Twenty-seven women were excluded from this model due to lack of complete data.

Information on mode of conception was available for 29 women: in 20 women, pregnancy was achieved by *in vitro* fertilization (IVF), 5 with IUI, 3 with hormonal therapy, 1 woman experienced a spontaneous pregnancy after prolonged delay (over one year and thus was considered as subfertility).

4. Discussion

This is the first multicenter-multinational study to investigate female fertility, in a large cohort of over 600 women with CF. In this cohort 241 women (40%) attempted pregnancy which is in keeping with a recent survey of attitudes towards fertility and pregnancy among women with CF[24]. Compared to women who attempted pregnancy, women who did not attempt pregnancy were younger, but with greater CF severity reflected in a higher frequency of severe CFTR mutations, PI and lower BMI and lung function. The group of women who did not attempt pregnancy is likely comprised of two populations: young women who are yet to plan families and older women who have never attempted conception. It is likely that the differences between women who did not attempt conception to those who did are largely driven by the latter subgroup of older women with severe CF. These differences may reflect the greater disease burden among the severe patients, occupying them with the illness and treatment, and interfering with finding partners and planning a family. These findings may also reflect the practice of CF physicians discouraging women with low lung function from becoming pregnant, due to concerns of complications[25–27].

The rate of subfertility in our cohort was 35%. In comparison, established rates of subfertility in the general population are much lower- 5-15% [28,29]. The risk of subfertility significantly increases with age, a finding that is also well documented among non- CF women and attributed to diminishing ovarian reserve with ageing. The observed rate of subfertility in our cohort of women with CF with an average

age of 30.4 years is also higher compared to women of a similar age range in the general population-14% [28].

When assessing risk factors for subfertility, pancreatic insufficiency was significantly associated with subfertility (OR 1.9 [1.03-3.5], p=0.04). Pancreatic sufficiency is usually associated with residual CFTR function[30], but in our cohort, differences in mutation classes between fertile and sub/infertile women did not reach statistical significance. A possible explanation to the lack of significant association between mutation class and fertility might be misclassification of rare and poorly described CFTR mutations[19]. Colonization with PA was associated with an increased rate of subfertility, but in a regression model was not found to be an independent contributor, and may be influenced by the older age of the sub/infertile women. Due to incomplete data on lung function and BMI, it is not possible to determine whether these factors are associated with infertility. Due to the adverse obstetric outcomes of women with low lung function and poor nutritional status[26,27], women should be advised against pregnancy in these situations.

To the best of our knowledge, no previous study has tested associations between sub/infertility in women with CF and disease severity parameters. Multiple mechanisms have been suggested for the reduced fertility of women with CF- hormonal, as well as mechanical (altered composition of cervical mucus due to deficient CFTR in the cervical endothelium). In most of the women in this study, information regarding ovarian and hormonal reserve, as well as cervical mucus quality was not available. However, the strong association of subfertility to advancing age may suggest that the important contributor to female subfertility may be ovarian and hormonal reserve, rather than cervical mucus quality, which is not influenced by advancing age. While it is known that poor nutritional status may interfere with menstruation[31], in our cohort BMI was not associated with subfertility.

The cause of female subfertility was not investigated or not known for most of the women. Indeed, it is a common practice of many reproductive centers to minimize extensive reproductive assessments such

as analysis of cervical mucus, and proceed to ART. It may therefore be suggested that an attempt should be made to prioritize fertility treatment with ovulation induction and IUI, rather than IVF, which is associated with a higher risk and cost.

This study is limited by the lack of formal and uniform assessment of the reason for subfertility in most of the women. In addition, sub/infertile females were identified by CF center multidisciplinary teams, and it is likely that some attempts of conception will not have been recorded, possibly resulting in underestimation of sub/infertility rate. Some of the factors, for example BMI and measures of glycemic control, were available for only part of the women, and this may underestimate a possible effect. However, we do not expect that women with missing data were unevenly distributed between the fertility groups, and therefore we do not expect that there was a bias in the interpretation of results. Due to the nature of CF care, attempts to conceive are usually discussed by patients with the CF staff, and it is common for fertility and obstetrics services to request the assessment of CF physicians for each referral. These practices make the assignment of fertility status reasonably robust and allow us to assess prevalence and risk factors. We were able to obtain information on a large number of women with CF in a multinational cohort, minimizing the risk of bias by different practices across different centers.

In conclusion, we report an elevated prevalence of subfertility in women with CF that is increasing with advancing age and is associated with pancreatic insufficiency. Future studies exploring the mechanisms and the response to CFTR modulation on female subfertility may further improve our knowledge in this field.

References

1. Zolin A, Naehrlich L van RJ et al. Annual Reports | European Cystic Fibrosis Society (ECFS). 2017. https://www.ecfs.eu/projects/ecfs-patient-registry/annual-reports. Accessed March 8, 2018.

2. Flume PA. US Cystic Fibrosis Foundation and European Cystic Fibrosis Society consensus recommendations for the management of non-tuberculous mycobacteria in individuals with cystic fibrosis. *J Cyst Fibros*. 2016;15(2):139-140.

3. Kazmerski TM, Sawicki GS, Miller E, et al. Sexual and reproductive health behaviors and experiences reported by young women with cystic fibrosis. *J Cyst Fibros*. 2017;17(0):57-63.

4. Durieu I. Patients becoming parents: Reproduction and Pregnancy. In: Dominique Hubert NS, ed. *Living Longer with CF*. Vol European Cystic Fibrosis Society; 2015:119-126.

5. Kopito LE, Kosasky HJ, Shwachman H. Water and electrolytes in cervical mucus from patients with cystic fibrosis. *Fertil Steril*. 1973;24(7):512-516.

6. Oppenheimer EA, Case AL, Esterly JR, Rothberg RM. Cervical mucus in cystic fibrosis: a possible cause of infertility. *Am J Obstet Gynecol*. 1970;108(4):673-674.

7. Gervais R, Dumur V, Letombe B, et al. Hypofertility with thick cervical mucus: another mild form of cystic fibrosis? *JAMA*. 1996;276(20):1638.

8. Chan LN, Tsang LL, Rowlands DK, et al. Distribution and regulation of ENaC subunit and CFTR mRNA expression in murine female reproductive tract. *J Membr Biol*. 2002;185(2):165-176.

9. Tizzano EF, Silver MM, Chitayat D, Benichou JC, Buchwald M. Differential cellular expression of

cystic fibrosis transmembrane regulator in human reproductive tissues. Clues for the infertility in patients with cystic fibrosis. *Am J Pathol*. 1994;144(5):906-914.

10.   Johannesson M, Bogdanovic N, Nordqvist AC, Hjelte L, Schalling M. Cystic fibrosis mRNA expression in rat brain: cerebral cortex and medial preoptic area. *Neuroreport*. 1997;8(2):535-539.

11.   Arrigo T, Rulli I, Sferlazzas C, De Luca F. Pubertal development in cystic fibrosis: an overview. *J Pediatr Endocrinol Metab*. 2003;16 Suppl 2:267-270.

12.   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(8):2092-2097.

13.   Hampl R, Šnajderová M, Mardešić T. Antimüllerian hormone (amh) not only a marker for prediction of ovarian reserve. *Physiol Res*. 2011;60(2):217-223.

14.   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(3):398-402.

15.   Lemonnier L, Cazes M-H, Bellis G, Sponga M, Durieu I. WS18.1 Evolution of pregnancies in the French CF Registry: 1992–2011. *J Cyst Fibros*. 2013;12:S36.

16.   Ben Lulu A, Blumenfeld Z, Livnat G, Gur M, Bentur L, Mussaffi H, Blau H, Sarouk I, Efrati O, Cohen-Cymberknoh M, Kerem E, Aviram M, Pickard E, Shteinberg M, Ben Lulu A, Blumenfeld Z, Livnat G, Gur M, Bentur L, Mussaffi H, Blau H, Sarouk I, Efrati O, Cohe SM. Infertility among women with cystic fibrosis: prevalence and risk factors. In: *The 39th European Cystic Fibrosis Conference*. Vol Basel, Switzerland; 2016.

17.   De Boeck K, Wilschanski M, Castellani C, et al. Cystic fibrosis: terminology and diagnostic algorithms. *Thorax*. 2006;61(7):627-635.

18.   Practice Committee of American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss: a committee opinion. *Fertil Steril*. 2013;99(1):63.

19.   CFTR2@Johns Hopkins - Home Page . 2013(12/27/2013).

20.   Cystic Fibrosis Mutation Database. http://www.genet.sickkids.on.ca/home.svc. Accessed January 1, 2018.

21.   Fanen P, Wohlhuter-Haddad A, Hinzpeter A. Genetics of cystic fibrosis: CFTR mutation classifications toward genotype-based CF therapies. *Int J Biochem Cell Biol*. March 2014.

22.   Lee TWR, Brownlee KG, Conway SP, Denton M, Littlewood JM. Evaluation of a new definition for chronic Pseudomonas aeruginosa infection in cystic fibrosis patients. *J Cyst Fibros*. 2003;2(1):29-34.

23.   Bilton D, Canny G, Conway S, et al. Pulmonary exacerbation: towards a definition for use in clinical trials. Report from the EuroCareCF Working Group on outcome parameters in clinical trials. *J Cyst Fibros*. 2011;10 Suppl 2:S79-81.

24.   Kazmerski TM, Gmelin T, Slocum B, Borrero S, Miller E. Attitudes and Decision Making Related to Pregnancy Among Young Women with Cystic Fibrosis. *Matern Child Health J*. 2017;21(4):818-824.

25.   Edenborough FP, Mackenzie WE, Stableforth DE. The outcome of 72 pregnancies in 55 women with cystic fibrosis in the United Kingdom 1977-1996. *BJOG*. 2000;107(2):254-261.

26.   Edenborough FPP, Borgo G, Knoop C, et al. *Guidelines for the Management of Pregnancy in Women with Cystic Fibrosis*. Vol 7. 2008.

27. Thorpe-Beeston J, Madge S, Gyi K, Hodson M, Bilton D. The outcome of pregnancies in women with cystic fibrosis-single centre experience 1998-2011. *BJOG An Int J Obstet Gynaecol*. 2013;120(3):354-361.

28. Fertility: assessment and treatment for people with fertility problems. *NICE Clin Guidel*. 2013. https://www.nice.org.uk/guidance/cg156/evidence/full-guideline-pdf-188539453. Accessed February 28, 2018.

29. Münster E, Letzel S, Passet-Wittig J, et al. Who is the gate keeper for treatment in a fertility clinic in Germany? -baseline results of a prospective cohort study (PinK study)-. *BMC Pregnancy Childbirth*. 2018;18(1):62.

30. Kerem E, Kerem B. Genotype- phenotype correlations in cystic fibrosis. *Pediatr Pulmonol*. 1996;22(6):387-395.

31. ESHRE Capri Workshop Group. Nutrition and reproduction in women. *Hum Reprod Update*. 2006;12(3):193-207.