Female patients with cystic fibrosis suffer from reproductive endocrinological disorders despite good clinical status

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Ten women with cystic fibrosis (CF) were evaluated with regard to hormonal profiles during a natural and a clomiphene citrate (CC) stimulated cycle. Five of the women were found to be anovulatory during a natural cycle. All women except one did respond with ovulation to CC stimulation indicating adequate ovarian response. Neither did they show increased follicle-stimulating hormone (FSH) concentrations on day 10 after CC treatment confirming normal ovarian reserve. Clinically the anovulatory women differed from the ovulating in two aspects: more profound essential fatty acid deficiency (EFAD) and higher peak/basal insulin response during an oral glucose tolerance test. The anovulatory women had significantly lower luteal oestradiol and progesterone but higher total testosterone concentrations when compared to healthy controls and the ovulatory CF women. The pathological insulin response and high testosterone concentrations resemble those seen in women with polycystic ovarian (PCO) syndrome. However, the CF patients in our study had normal ovaries, as deduced from ultrasound examination and normal luteinizing hormone (LH)/FSH ratio. It is suggested that EFAD as well as hypersecretion of insulin may be of importance for the observed ovarian dysfunction. Further studies are needed to evaluate the relation between ovulatory mechanisms and EFAD in CF women as well as studies to compare anovulatory CF women with women with PCO syndrome.

Key words: cystic fibrosis/female fertility/hormones/polycystic ovarian syndrome/reproduction

Introduction

Cystic fibrosis (CF) is the most common autosomal recessive hereditary lethal disease in the Caucasian population (Welsh et al., 1995). It is caused by mutations of the gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR), located on chromosome seven (Rommens et al., 1989; Riordan et al., 1989; Kerem et al., 1989).

CF is clinically characterized by elevated concentrations of sweat chlorides and abnormal thick mucus with symptoms mainly from the respiratory and gastrointestinal tracts (Welsh et al., 1995). The prognosis for women with CF is more positive than before due to intensified symptomatic treatment and emphasis on nutritional aspects (Davis et al., 1996) as well as to new methods such as organ transplantation (Egan et al., 1995), and hope for gene therapy (Wagner and Gardner, 1997).

Delayed puberty and amenorrhoea is common in CF females (Stead et al., 1987). Malnutrition has been suggested to be the main cause (Shwachman et al., 1965; Mitchell-Heggs et al., 1976; Neinstein et al., 1983; Stead et al., 1987). The hypothalamic pituitary axis has been studied in pubertal CF boys and girls. In one study it was found that pubertal increments of serum gonadotrophin and sex steroids were delayed suggesting late maturation of the reproductive endocrine system as a result of chronic inanition (Reiter et al., 1981). However, in a recent study we have shown that menarcheal age was also delayed in CF females in good clinical and nutritional condition. The patients who were homozygous for the most common mutation, deltaF508, and those with a pathological glucose tolerance test (OGTT) showed the most delay in menarcheal age. The majority of the patients had essential fatty acid deficiency (EFAD) which may cause pubertal delay (Johannesson et al., 1997).

Most men (98%) with CF have congenital bilateral absence or atrophy of the vas deferens (CBAVD) (Denning et al., 1968; Kaplan et al., 1968). It appears that CBAVD, as an isolated entity, may be a very mild phenotype of CF. It has been shown that up to 70% of patients with CBAVD have mutations in the CFTR gene (Anguiano et al., 1992; Chillon et al., 1995). However, a recent study has also shown that CFTR protein might be important for spermatogenesis or sperm maturation (van der Ven et al., 1996). Both normal and decreased spermatogenesis have been shown in histological studies from testicular biopsies from male CF patients (Kaplan et al., 1968; Gottlieb et al., 1991). Men with CF can today become fathers with the help of microsurgical epididymal sperm aspiration (MESA) or percutaneous epididymal sperm aspiration (PESA) combined with intracytoplasmic sperm injection (ICSI) (Oates et al., 1992, Fogdestam et al., 1994, Hamberger et al., 1995).

The low fertility in CF females has been thought to be caused mainly by tenacious impermeable cervical mucus due to defective CFTR protein expressed in the cervix (Kopito et al., 1973; Tizzano et al., 1994). Multiple ovarian cysts have previously been described in women with CF (Shawker et al., 1983; Stead et al., 1987). Hormonal profiles were not reported in these studies.

Pregnancy in CF women is a subject of medical debate.
Maternal mortality associated with pregnancy and increased incidence of spontaneous abortions, prematurity and still-births were previously of great concern (Grand et al., 1966; Larsen, 1972; Cohen et al., 1980). Today the risk for deterioration of health during pregnancy for females with CF is considered small if good medical care is provided and the women are in a stable good clinical condition (FitzSimmons et al., 1996). However, premature labour and delivery remain a risk for pregnant women with CF (Kent and Farquharson, 1993). Pre-pregnancy lung function and body weight appear to be useful indicators of outcome for both mother and child (Kotloff et al., 1992). So far, the experience concerning lung transplantation and pregnancy is limited. There are no known side-effects of the immunosuppressive medicines on the fetus, and the transplants appear to be unaffected by pregnancies (Zirbes and Wielinski, 1995).

Artificial reproductive techniques can today be offered to CF women who want to become pregnant (Kredentser et al., 1986).

Females with CF are known to be less fertile compared to normal healthy women. We aimed to study the possibility of endocrine/ovarian dysfunction in women with CF. Such an abnormality, if confirmed, would help in future management of their subfertility. We therefore studied hormone profile and follicle growth during one complete spontaneous menstrual cycle and during a subsequent cycle of clomiphene citrate (CC) treatment to assess their ovarian reserve and response (Navot et al., 1987; Scott and Hofmann, 1995).

### Materials and methods

#### Patient selection

Ten female patients attending the Stockholm CF Center fulfilled the inclusion criteria for the study and gave their consent to participate. The criteria were: (i) diagnosis of CF in childhood with positive sweat test (Gibson and Cooke, 1959), (ii) known age at menarche, (iii) 20–35 years of age, (iv) regular menstruation (25–35 days), (v) stable good clinical condition without signs of acute exacerbation of the illness, (vi) no liver or thyroid dysfunction, (vii) no organ transplants, (viii) no glucocorticoid treatment and (ix) no hormonal contraceptives for at least 3 months prior to the study. Fifteen healthy women with regular menstruation and proven fertility not using hormonal contraceptives for at least 3 months prior to the study, 20–35 years of age, were used as controls. For clinical data see Table I.

### Evaluation of patient characteristics

Body mass index (BMI = weight/height²) was used as a marker for nutritional status. The CF patients were further characterized by their genotype, pancreatic function, duration of chronic bacterial colonization by pseudomonads, and their Shwachman score. This clinical score includes results from pulmonary auscultation, pulmonary radiological examination, nutritional status and physical fitness. Maximal scores are 100 and scores above 85 are considered excellent (Shwachman and Kulczycki, 1958). Lung function tests, oral glucose tolerance test (OGTT) (eight patients) and analysis of serum phospholipid fatty acid pattern were performed as part of their yearly medical check-up at the Stockholm CF Center. The CF patients’ seventh chromosome pair was analysed for five of the most common mutations in Swedish: deltaF508, R553X, G542X, G551D and 394 delTT (Dahl et al., 1993). Pulmonary function was evaluated by forced expiratory manoeuvres (Sensor Medics Corporation, Anaheim, CA, USA) giving forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV₁).

Data are expressed as percentage of the reference data of the laboratory (Solymar et al., 1980; Quanjer, 1983). OGTT was performed in the morning after an overnight fast. Plasma glucose and serum insulin were measured before and after (15, 30, 45, 60, 90, 120 and 180 min) an oral glucose load of 1.75 g/kg body weight (maximum 100 g) was given. Plasma glucose was measured by a hexokinase method (glucose, BM/Hitachi® 747 system; Boergering Mannheim, Germany), and insulin by radioimmunoassay (Insulin RIA, Pharmacia, Sweden). An insulin peak to basal ratio higher than eight was considered a sign of insulin resistance (Travis, 1980).

Serum was drawn in the morning after an overnight fast and stored at −20°C until analysis of the fatty acid pattern in serum phospholipids by gas chromatography after Folch extraction (Ellin et al., 1991).

#### Study plan and cycle monitoring

All women participating in the study were studied during one natural cycle and nine of the CF women continued with a second cycle in which a CC challenge test was performed by administration of 100 mg CC (Pergotime®; Serono Nordic AB, Stockholm, Sweden) on which a CC challenge test was performed by administration of 100 mg CC (Pergotime®; Serono Nordic AB, Stockholm, Sweden) on cycle days 5–9 (Navot et al., 1987). For determination of the luteinizing hormone (LH) peak, morning urine samples were collected from cycle day 10–15, using a self-test (OvuQvick®; Monoclonal Antibodies Inc., Sunnyvale, CA, USA). All the controls and seven of the CF women gave their permission for vaginal ultrasound examinations 3–4 days post menstruation to confirm ovaries of normal appearance. An additional ultrasound examination was performed mid cyclic during the natural cycle to assess follicular growth pattern prior to expected ovulation. The ultrasound equipment used was a Siemens Sonoline® Si/200 real-time scanner with mechanical sector rotating 5–7.5 MHz transducer.

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### Table I. Clinical parameters of 15 fertile healthy females and 10 cystic fibrosis (CF) female patients (median and range)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls</th>
<th>Patients</th>
<th>Statistical analyses²</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Menarcheal age (year)</td>
<td>12 (11–14)</td>
<td>14.5 (13–17)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Age (year)</td>
<td>32 (24–35)</td>
<td>23.5 (22–31)</td>
<td>P = 0.001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165 (155–176)</td>
<td>165 (152–178)</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64 (57–78)</td>
<td>59 (42–73)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 (21–27)</td>
<td>21.5 (16–28)</td>
<td>P = 0.01</td>
</tr>
<tr>
<td>Cycle length (days)</td>
<td>28 (24–31)</td>
<td>29 (23–38)</td>
<td>NS</td>
</tr>
<tr>
<td>No. spontaneous pregnancies</td>
<td>15</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

²Mann–Whitney two-tailed. NS = not significant.

BMI = Body mass index.
Peripheral blood samples were collected by venepuncture in the morning between 07.30 h and 08.30 h during the follicular phase days 3–5, estimated time of LH peak days 10–15, and during the luteal phase days 20–22.

**Hormonal assessment**

Serum total testosterone was measured by a Radio Immuno Assay (Diagnostic Products Corporation, Los Angeles, CA, USA). Serum oestradiol, progesterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and sex hormone binding globulin (SHBG) were measured in a solid-phase chemiluminescent enzyme assay using the Immunolite System® (Diagnostic Products Corporation). Ovulation was defined according to Landgren et al. (1980) by plasma progesterone concentrations higher than 16 nmol/l during the luteal phase.

Local ethic committee approval was obtained prior to the study.

**Statistical analysis**

Results are presented as median and range. Statistical evaluations were performed using Mann–Whitney two-tailed test and Kruskal–Wallis one-way analysis of variance by ranks with correction for multiple comparisons. Differences were considered significant when P < 0.05.

**Results**

The CF women were older at the time of menarche (P < 0.001) and had a lower BMI (P = 0.01) when compared to the controls (Table I). The healthy controls were older (P = 0.01) than the CF women (Table I) at the time of the study.

All controls did ovulate normally. Five of the CF women had ovulatory cycles. Their serum oestradiol and progesterone concentrations did not differ significantly compared to the healthy controls (Table II). Five of the CF women did not ovulate (Landgren et al., 1980). Their luteal phase serum oestradiol and progesterone were significantly lower compared with the controls and the ovulating CF women (P = 0.01) respectively (P = 0.003). The anovulatory patients had statistically higher total serum testosterone concentrations (P = 0.004) compared with the controls and the ovulating CF women (Table II). However, the difference in SHBG concentration was not statistically significant. Neither did the LH/FSH ratio differ between the groups (Table II).

The five anovulatory CF women only differed in two parameters compared to the ovulating CF women. They had lower concentrations of linoleic acid (P = 0.016) and higher insulin peak to basal ratio during OGTT (P = 0.043) compared to the ovulating group (Table III).

None of the CF patients had polycystic appearance of their ovaries on ultrasound performed post-menstrually or mid-cyclic. Only one of the anovulatory CF women had no follicular growth at all as depicted by ultrasound.

Of the five anovulatory women, two had low oestradiol concentrations without variations whereas one had a small increase in oestradiol concentrations during the follicular phase. Two of the anovulatory women exhibited follicular activity followed by luteinization as deduced by slightly elevated progesterone concentrations in the luteal phase.

A total of nine CF women were treated with CC. Eight of them ovulated with increased serum progesterone concentrations in the luteal phase in the CC stimulated cycle compared to the natural cycle with a median and range of 79 nmol/l (18–143) and 20 nmol/l (4–42) respectively (P = 0.004). They had low FSH concentrations on day 3 (basal) and day 10 (after CC stimulation) with median and range of 2.5 mIU/ml (2.1–4.3) and 2.7 mIU/ml (1.6–4.8) respectively.

The only patient who did not ovulate presented with high FSH concentrations on basal cycle day 3 (6.0 mIU/ml) and day 10 after CC stimulation (7.5 mIU/ml), indicating ovarian

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Controls ovulating</th>
<th>Patients ovulating</th>
<th>Patients not ovulating</th>
<th>Statistical analyses$^a$</th>
</tr>
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<tbody>
<tr>
<td>$n$</td>
<td>15</td>
<td>5</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td>Oestradiol follicular phase</td>
<td>168</td>
<td>(77–742)</td>
<td>(90–128)</td>
<td>(117–193)</td>
</tr>
<tr>
<td>Oestradiol luteal phase</td>
<td>512</td>
<td>(225–940)</td>
<td>(257–944)</td>
<td>(184–292)</td>
</tr>
<tr>
<td>Progesterone luteal phase</td>
<td>36</td>
<td>(21–72)</td>
<td>(23–42)</td>
<td>(2–17)</td>
</tr>
<tr>
<td>Total testosterone</td>
<td>0.83</td>
<td>(0.7–1.8)</td>
<td>(0.6–1.0)</td>
<td>(1.0–3.8)</td>
</tr>
<tr>
<td>SHBG</td>
<td>57</td>
<td>(48–105)</td>
<td>(25–70)</td>
<td>(35–130)</td>
</tr>
<tr>
<td>LH/FSH</td>
<td>1.1</td>
<td>(0.7–1.6)</td>
<td>(0.3–1.6)</td>
<td>(0.4–2.1)</td>
</tr>
</tbody>
</table>

$^a$Kruskal–Wallis test.

$^b$Patients not ovulating significantly lower serum oestradiol luteal phase compared with controls and patients ovulating.

$^c$Patients not ovulating significantly lower serum progesterone luteal phase compared with controls and patients ovulating.

$^d$Patients not ovulating significantly higher total testosterone compared with controls and patients ovulating.

NS = not significant.

SHBG = sex hormone binding globulin.

LH/FSH = luteinizing hormone/follicle-stimulating hormone.
insufficiency. She was the most severely affected by CF. Her Shwachman score was 70; her FVC was 61% and FEV1 36% of predicted. She had a BMI of 20, low EFA concentrations (linoleic acid 12.4 mol% and arachidonic acid 4.2 mol%) and a pathological OGTT (peak glucose 16.9 mmol/l and high insulin response with a peak to basal ratio of 14).

**Discussion**

The lowered fertility observed in women with CF has been thought to be due mainly to impermeable cervical mucus which does not undergo the typical changes during menstrual cycle (Kopito et al., 1973). Anovulation due to malnutrition and catabolism has been suggested as a secondary cause for the infertility (Stead et al., 1987; Scott et al., 1994). Anovulation due to malnutrition with a pathological OGTT (peak glucose 16.9 mmol/l and high insulin response with a peak to basal ratio of 14).

The anovulatory CF women differed from those who ovulated in that they had more profound EFAD and high pathological insulin response during OGTT. The elevated serum testosterone concentrations in our anovulatory patients may be related to hyperinsulinaemia. Most CF patients with pathological OGTT have low concentrations of insulin due to the islets of Langerhans being replaced with fibrous and fatty tissue (Iannuci et al., 1984; Lanng, 1996). However, studies on insulin sensitivity in CF patients have given divergent results (Lanng et al., 1994; Moran et al., 1994; Austin et al., 1994). It is possible that a transient period of hyperinsulinaemia is succeeded by a diabetic state in a subgroup of CF patients. At least in these anovulatory CF patients, it appears as if they have a state of hyper-insulin secretion during OGTT similar to the one seen in patients with borderline abnormal glucose tolerance or chemical diabetes (Reaven et al., 1976). This in turn may be related to the more marked EFAD in the anovulatory group. EFAD and their prostaglandin metabolites have been reported to exhibit an inhibitory effect on insulin secretion (Robertson, 1986; Turk et al., 1988). Studies on rats with EFAD have shown hypersecretion of

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ovulating</th>
<th>Not ovulating</th>
<th>Total</th>
<th>Statistical analysesa</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Genotypeb</td>
<td>3+/+, 1=+/–, 1–/–</td>
<td>3+/+, 2–/–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21 (16–23)</td>
<td>23 (19–28)</td>
<td>21.5 (16–28)</td>
<td>NS</td>
</tr>
<tr>
<td>Pancreas functionc</td>
<td>5 PI</td>
<td>4 PI/1 PS</td>
<td>9 PI/1 PS</td>
<td>NS</td>
</tr>
<tr>
<td>Shwachman score</td>
<td>90 (75–90)</td>
<td>90 (70–90)</td>
<td>90 (70–90)</td>
<td>NS</td>
</tr>
<tr>
<td>FVC (% of predicted)</td>
<td>80 (67–122)</td>
<td>94 (61–105)</td>
<td>87 (61–122)</td>
<td>NS</td>
</tr>
<tr>
<td>FEV₁,₉ (% of predicted)</td>
<td>72 (36–99)</td>
<td>53 (48–113)</td>
<td>66.5 (36–113)</td>
<td>NS</td>
</tr>
<tr>
<td>Years of pseudomonad colonization</td>
<td>17 (7–20)</td>
<td>11 (0–12)</td>
<td>11.5 (0–20)</td>
<td>NS</td>
</tr>
<tr>
<td>Linoleic acid 18:2 (mol %)</td>
<td>19.2 (14.8–20.3)</td>
<td>13.8 (12.4–18.1)</td>
<td>16.5 (12.4–20.3)</td>
<td>P = 0.016</td>
</tr>
<tr>
<td>Arachidonic acid 20:4 (mol %)</td>
<td>6.4 (4.2–9.5)</td>
<td>6.2 (3.8–9.5)</td>
<td>6.5 (3.8–9.5)</td>
<td>NS</td>
</tr>
<tr>
<td>OGTT-glucose peak concentration (mmol/l)</td>
<td>10.0 (6.1–12.2)</td>
<td>11.6 (10.7–16.9)</td>
<td>11 (6.1–16.9)</td>
<td>NS</td>
</tr>
<tr>
<td>OGTT-insulin peak/basal concentrationd,e</td>
<td>6.5 (4–11)</td>
<td>13 (9–18)</td>
<td>10 (4–18)</td>
<td>P = 0.043</td>
</tr>
<tr>
<td>Patients with IDDM</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>NS</td>
</tr>
</tbody>
</table>

*a Mann–Whitney two-tailed.

b Homozygous for deltaF508, heterozygous for deltaF508, –/– unknown mutations.

c PI = pancreas-insufficient, PS = pancreas-sufficient.

d Reference value: >20.3.

e Reference value: >5.9.

Patients with anovulation, PS pancreas-sufficient, PI pancreas-insufficient, PS pancreas-sufficient.
insulin during glucose challenge (Apkan et al., 1981; Hjelte et al., 1990).

Recent studies have indicated that CF patients might have inflammation in their tissues prior to infection (Khan et al., 1995; DiMango et al., 1997) with an imbalance of pro-inflammatory cytokines versus the anti-inflammatory ones (Bonfield et al., 1995; Moss et al., 1996). Involvement of cytokines or a disturbed hypothalamic–pituitary–ovarian axis cannot be excluded as factors behind reduced fertility in CF women. CFTR mRNA expression has recently been found in areas of rat hypothalamus which are involved in the regulation of sexual maturation and reproduction (Johannesson et al., 1997). These areas contain high concentrations of gonadotrophin-releasing hormone (GnRH) and oestrogen-rich neurons (Shivers et al., 1986; Simerly and Swanson, 1988). CFTR might augment acidification of synaptic vesicles (Barasch and Al-Awqati, 1993) and thereby be of importance for central regulation of sexual maturation and fertility. In addition, physiological and/or psychological stress in these chronically ill women may influence ovulation (Speroff et al., 1994b).

In conclusion, our findings suggest that the cause of reduced fertility among these CF women is much more complex than previously thought. The data indicate that an ovarian dysfunction is involved. Further studies are needed to evaluate the relation between ovulatory mechanisms and EFAD in CF women as well as studies to compare anovulatory CF women with women with PCO syndrome.

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References


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