Fertility of Tall Girls Treated with High-Dose Estrogen, a Dose-Response Relationship

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Context: High-dose estrogen treatment to reduce final height of tall girls increases their risk for infertility in later life.

Objective: The aim was to study the effect of estrogen dose on fertility outcome of these women.

Design/Setting: We conducted a retrospective cohort study of university hospital patients.

Patients: We studied 125 tall women aged 20–42 yr, of whom 52 women had been treated with 100 μg and 43 with 200 μg of ethinyl estradiol (EE) in adolescence.

Main Outcomes: Time to first pregnancy, treatment for infertility, and live birth rate were measured.

Results: The time to first pregnancy was increased in treated women. Of untreated women, 80% conceived within 1 yr vs 69% of women treated with 100 μg EE and 59% of women treated with 200 μg EE. This trend of increased time to pregnancy with increasing estrogen dose was significant (log rank trend test, \( P = 0.01 \)). Compared with untreated women, fecundability was reduced in women treated with both 100 μg EE [hazard ratio = 0.42; 95% confidence interval (CI), 0.19–0.95] and 200 μg EE (hazard ratio = 0.30; 95% CI, 0.13–0.72). We also observed a significant trend in the incidence of treatment for infertility with increased estrogen dose (\( P = 0.04 \)). Fecundity was affected in women treated with 200 μg EE who had reduced odds of achieving at least one live birth (odds ratio = 0.13; 95% CI, 0.02–0.81), but not in women treated with 100 μg EE.

Conclusions: We report a dose-response relationship between fertility in later life and estrogen dose used for the treatment of tall stature in adolescent girls; a higher estrogen dose is associated with increased infertility. (J Clin Endocrinol Metab 97: 3107–3114, 2012)
At the Department of Pediatric Endocrinology of the University Medical Center Groningen, two dosages of EE have been used for the treatment of tall stature: 100 and 200 μg/d. In the late 1980s, girls here were treated with 200 μg/d until a large study in the beginning of the 1990s showed similar effectiveness of 100 μg/d, after which in the early 1990s girls were also treated with the latter dose (7). We therefore initiated the current study to evaluate whether there is a dose-response relationship between estrogen treatment of tall girls and fertility later in life.

Subjects and Methods

Subjects

We identified women who during childhood had sought medical attention for their tall stature at the Department of Pediatric Endocrinology of the University Medical Center Groningen between 1979 and 1999. All evaluations at initial presentation had been performed by pediatric endocrinologists who assessed skeletal age according to Greulich-Pyle using hand and wrist radiography to predict final heights (8). Women with a predicted height above the 97th percentile according to Dutch standards were eligible to participate in the current study if an underlying disease as cause of their tall stature was excluded (9). They included girls who received estrogen treatment of either 100 μg EE (treated 100) or 200 μg EE (treated 200) daily (plus cyclic 10 mg progestin) in adolescence, and girls who did not (untreated group). In general, parents decided along with their daughters whether treatment was initiated or not. Common reasons for choosing not to have treatment were satisfaction with the predicted adult height or uncertainty about possible side effects. Excluded were women with endocrine or metabolic disorders, chromosomal defects, and primary or secondary growth disorders.

Data collection

Eligible women were traced using municipal registries and invited by mail to participate. Participants received a questionnaire assessing their personal and family history as well as relevant demographics. They were invited to visit the outpatient clinic of the University Medical Center Groningen. Clinical examination included a standardized interview to evaluate reproductive history and fertility problems. Two approaches were used to assess fertility outcome. First, we asked closed (yes/no) questions regarding fertility. Second, we asked women to estimate the number of months of unprotected intercourse before their first pregnancy was established, either spontaneous or planned. Height was measured using a SECA 225 stadiometer (SECA, Hamburg, Germany). Target height was calculated from midparental height corrected for secular trend according to Dutch standards (9, 10). The study received ethical approval by the institutional medical ethics review boards of the University Medical Center Groningen and the Erasmus Medical Center, and all participants provided written informed consent.

Statistical analysis

To study the differences between the three treatment categories, the linear by linear association χ² statistic was used to test for linear relationships of ordered categorical variables. For normally distributed continuous variables, one-way ANOVA with Bonferroni correction was used. For ordered categorical variables, the Kruskal-Wallis one-way ANOVA was used. Correlation of two continuous variables was studied using Pearson’s correlation coefficient. Multiple binomial logistic regression was used to adjust for the confounders age, body mass index (BMI), and smoking. Exact logistic regression was used when expected counts in cells of the two-by-three tables were less than five. In all analyses, a two-tailed P value of less than 0.05 was regarded to be statistically significant.

Analysis of time to first pregnancy (TTP) was done using the Kaplan-Meier survival method to compare periods of not achieving a pregnancy between the three treatment categories. Cumulative probabilities of conception were calculated by Kaplan-Meier life-table analysis. The log rank trend test was used to test the null hypothesis of no difference between the treatment categories in the probability of conception at any time point. Censoring was used for the following conditions: 1) TTP less than 24 months; and 2) still having not conceived a pregnancy at the time of interview. For spontaneous pregnancies with less than 1 month of unprotected intercourse, TTP was recorded as 1 month. Cox proportional hazard model with TTP as time variable was used to account for possible confounders. Variables were entered into the model and retained in a backward stepwise manner based on the likelihood ratio if their presence significantly improved the fit or if their presence in the model substantially modified the estimate of the treatment effect. Log-minus-log plots were made per treatment category to inspect possible deviations from the proportional hazard assumption. The resulting hazard ratio (HR) represents the fecundability of treated subjects compared with untreated subjects. Exact logistic regression was performed using SAS version 9.1 (SAS Institute Inc., Cary, NC). All other calculations were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL).

Results

Participants

From the patient records of the Department of Pediatric Endocrinology at the University Medical Center Groningen, we identified 222 eligible tall women. We were able to trace 219 (99%) women who were invited to participate in our study; 137 of these women had been treated with high-dose estrogen (76 with 100 μg EE, and 61 with 200 μg EE). In total, 125 women (57%) agreed to participate; this included 52 women (68% of the original cohort) treated with 100 μg EE and 43 women (70%) treated with 200 μg EE. Nonparticipating women were slightly younger than participating women (29.3 vs. 30.1 yr; P = 0.03) but were not different with regard to predicted final height (187.0 vs. 187.7 cm; P = 0.1).

Table 1 shows the general characteristics of the participating women by treatment categories. Treated and untreated women were mostly similar with regard to
However, women treated with 200 μg EE were older than the other women. Table 2 shows the clinical parameters of the women when they first presented as girls at our department of pediatric endocrinology. Age, bone age, and target height did not differ between treated and untreated women. However, treated women were taller at presentation and had a higher predicted height than untreated women. Tanner breast stage and pubic hair stage at presentation did not differ between treated and untreated women (Kruskal-Wallis, \( P < 0.01 \) and \( P = 0.52 \)), nor did the number of girls who had experienced menarche (\( P = 0.68 \)). Table 2 also shows the treatment specifics. Treatment details were similar for both treatment categories, except that the mean period of follow-up after cessation of treatment was lon-

<table>
<thead>
<tr>
<th>TABLE 1. Characteristics of the participating women</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>Untreated</td>
</tr>
<tr>
<td>n</td>
<td>30</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>28.8 (2.0)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>183.5 (4.1)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.1 (12.3)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 (3.5)</td>
</tr>
<tr>
<td>Marital status(^a)</td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>8 (27%)</td>
</tr>
<tr>
<td>Married or living together</td>
<td>21 (70%)</td>
</tr>
<tr>
<td>Divorced or widowed</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Highest education level</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>Medium</td>
<td>9 (30%)</td>
</tr>
<tr>
<td>High</td>
<td>19 (63%)</td>
</tr>
</tbody>
</table>

Values are expressed as mean (SD) or number (percentage).

\(^a \) \( P < 0.05 \) compared with both untreated and treated (100) women.

\(^b \) Missing: one treated (100) woman.

<table>
<thead>
<tr>
<th>TABLE 2. Clinical parameters at first presentation and treatment specifics</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>30</td>
</tr>
<tr>
<td>At first presentation*</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>12.6 (1.5)</td>
</tr>
<tr>
<td>Bone age (yr)</td>
<td>12.7 (1.2)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.9 (6.5)</td>
</tr>
<tr>
<td>Predicted height (cm)</td>
<td>184.5 (3.2)</td>
</tr>
<tr>
<td>Target height (cm)</td>
<td>182.0 (5.9)</td>
</tr>
<tr>
<td>Tanner breast stage**</td>
<td></td>
</tr>
<tr>
<td>Stages 1 and 2</td>
<td>8 (30%)</td>
</tr>
<tr>
<td>Stage 3</td>
<td>3 (11%)</td>
</tr>
<tr>
<td>Stages 4 and 5</td>
<td>16 (59%)</td>
</tr>
<tr>
<td>Tanner pubic hair stage***</td>
<td></td>
</tr>
<tr>
<td>Stages 1 and 2</td>
<td>6 (22%)</td>
</tr>
<tr>
<td>Stage 3</td>
<td>8 (30%)</td>
</tr>
<tr>
<td>Stages 4 and 5</td>
<td>13 (48%)</td>
</tr>
<tr>
<td>Menarche*</td>
<td>10 (33%)</td>
</tr>
<tr>
<td>Treatment specifics</td>
<td></td>
</tr>
<tr>
<td>Age at start (yr)</td>
<td>12.9 (1.2)</td>
</tr>
<tr>
<td>Duration (months)</td>
<td>23.6 (7.8)</td>
</tr>
<tr>
<td>Follow-up (yr)</td>
<td>15.9 (1.9)</td>
</tr>
</tbody>
</table>

Values are expressed as mean (SD) or number (percentage).

\(^a \) \( P < 0.01 \) compared with untreated women.

\(^b \) \( P < 0.05 \) compared with treated (100) women.

* Missing: four treated (100) women and seven treated (200) women.

** Missing: three untreated women, five treated (100) women, and eight treated (200) women.

*** Missing: three untreated women, nine treated (100) women, and nine treated (200) women.
ger in women treated with 200 µg EE, reflecting that treatment with 100 µg EE is a more recent therapy. All treated girls were followed until regular menstrual cycles after cessation of the treatment. Anosmia in participants or their family members was not reported. In our study population, treatment had on average been initiated in the year 1989 (range, 1983–1999) in women treated with 200 µg EE and in the year 1993 (range, 1986–1996) in women treated with 100 µg EE.

Time to pregnancy

Of the 125 participating women, 71 women had attempted to conceive a pregnancy. Fourteen of these women were untreated (47% of untreated women), 25 were treated with 100 µg EE (48%), and 32 were treated with 200 µg EE (74%). Results of the TTP analysis in women who had attempted to conceive are shown in Fig. 1. Time to pregnancy was significantly increased in treated women compared with untreated women. Although 80% (n = 11) of the untreated women conceived in the first year, only 69% (n = 17) of the women treated with 100 µg EE and 59% (n = 19) of the women treated with 200 µg EE had conceived their first pregnancy within 1 yr. The observed trend of increased time to pregnancy with increasing estrogen dose was statistically significant (log rank trend test P = 0.01).

While accounting for several possible confounders in a discrete Cox model, we were able to calculate fecundability for each dose. The model was adjusted for age, BMI, and smoking. The curves for the three treatment categories in the log-minus-log plots ran parallel, indicating that the proportional hazards assumption was met. Compared with untreated women, fecundability was reduced in both women treated with 100 µg EE (HR = 0.42; 95% confidence interval (CI), 0.19–0.95; P = 0.04) and women treated with 200 µg EE (HR = 0.30; 95% CI, 0.13–0.72; P = 0.007).

Neither age at initiation of treatment nor duration of treatment was correlated with TTP in treated women.

Fertility outcome

Figure 2 shows the fertility outcome of the women who had attempted to conceive. Treated women were more likely to report fertility problems than untreated women were. Although both groups in general had been equally able to conceive a pregnancy, treated women were more likely to have needed 12 months or more to achieve that pregnancy. As a consequence, treated women had more often visited a doctor because of fertility problems and more often received infertility treatments. We observed a significant trend of increasing fertility problems with increasing estrogen dose; i.e., women treated with 200 µg EE experienced more fertility problems than women treated with 100 µg EE, who in turn experienced more fertility problems than untreated women.

Because women treated with 200 µg EE were older and had longer follow-up time than women treated with 100 µg EE, we corrected for age in an exact logistic regression model. The effect sizes were not significantly influenced by adding age or BMI or smoking to the model. In addition, we performed a sensitivity analysis to see whether differential follow-up could explain the observed difference in fertility outcome between women treated with 100 µg EE and women treated with 200 µg EE. We assumed that participating women, treated with both 100 and 200 µg EE, who had not attempted to conceive had an overall fertility outcome comparable to women treated with 200 µg EE. When this hypothetical group of treated women who had not attempted to conceive was added to the analysis, the significant trends of increasing fertility problems with increasing estrogen dose remained (trend P < 0.05).

Women treated with 200 µg EE had significantly reduced odds of achieving at least one live birth compared
with untreated women (odds ratio = 0.13; 95% CI, 0.02–0.81; \( P = 0.03 \)). Although the percentage of women treated with 100 \( \mu g \) EE who had achieved a live birth was similar to the percentage of women treated with 200 \( \mu g \) EE, the difference with untreated women was not statistically significant. The median duration of involuntary childhood in women treated with 200 \( \mu g \) was 25 months. There were no differences between the treatment categories and the risk of miscarriages. In treated women who had visited a doctor because of fertility problems, 56% of these visits had resulted in a diagnosis. Identified causes of reduced fertility included fallopian tube abnormalities, endometriosis, or ovulatory problems such as polycystic ovary syndrome. Prevalence of these causes was not significantly different between both treatment groups. As for the partners of these women, a low prevalence of a contributing male factor was reported (5%), and mean height of the partner was similar in all groups (untreated 187.9 \( \text{vs.} \) treated (100) 189.4 \( \text{vs.} \) treated (200) 187.1 cm; \( P = 0.49 \)).

There were no differences in mean age at initiation of treatment or mean duration of treatment between treated women requiring infertility treatments or not, or between treated women achieving a live birth or not.

**Discussion**

It has been shown that high-dose estrogen treatment to reduce final height of tall girls increases their risk for infertility in later life (3, 4). Here, we studied the effect of estrogen dose on fertility outcome of these women. We compared women who received no treatment to women who received either 100 \( \mu g \) EE or 200 \( \mu g \) EE. Our study confirms that tall women treated with high-dose estrogen have an increased time to pregnancy and experience more fertility problems compared with untreated women. We demonstrate for the first time that the association between estrogen treatment and the obstetric outcomes in women is dose-dependent.

As previously shown, we also observed fecundity to be reduced in women treated with 200 \( \mu g \) EE because they had significantly reduced chances of achieving at least one live birth. At the time of the study, one third of these women were suffering from involuntary childhood for a median of 25 months. Based on our results, it seems probable that women treated with 100 \( \mu g \) EE are also at risk of reduced fecundity. However, currently we did not observe a significantly reduced live birth rate, possibly due to a relatively small sample size.

Although only a few studies have reported on fertility outcome after high-dose estrogen treatment for tall stature, multiple studies have been performed on the long-term effects of low-dose estrogens used for contraceptive treatment. In the 1960s, several reports suggested that oral contraceptive pill (OCP) use may cause secondary amenorrhea; however, this was not established by later studies (5, 11, 12). Interestingly in these early papers estrogen doses equal to 50–100 \( \mu g \) of EE were given; however, due to a lack of proper study design, no conclusions could be drawn based on these observations. In the late 1970s and early 1980s, several studies reported on fertility after discontinuation of OCP. These studies have demonstrated some delay in the time to pregnancy in previous OCP users (13, 14). However, this impairment was typically seen in the early months after discontinuation of OCP use, whereas 12-month pregnancy rates were within the normal range (5). However, a few early studies on estrogen doses equal to 50 \( \mu g \) EE reported pregnancy rates of 75% in the first year, which is lower than the generally accepted pregnancy rates of 85% (15). Only one study has reported on the effect of estrogen dose on fertility after cessation of OCP (16). They observed consistently longer conception delays in women discontinuing OCP containing at least 50 \( \mu g \) of estrogen compared with women who had used less than 50 \( \mu g \) of estrogen (16). In addition, one other study has reported that OCP users in the lower weight percentiles have longer conception delays, suggesting a possible dose effect (17). These studies are in line with our results of a dose-dependent increased time to pregnancy in women treated with high-dose estrogen.

Although human studies on the effects of treatment with estrogens have mostly focused on OCP users, animal studies have focused on environmental exposure to EE as an endocrine-disruptor and on the effects of diethylstilbestrol (DES). In rodents, both in utero and postnatal exposure to EE or DES produces permanent adverse effects on the developing female reproductive system (18–20). Animal studies on in utero exposure to DES have shown disruption at the follicle level. In DES-exposed mice, reduced numbers of primordial follicles and of oocytes after ovulation induction have been found (19, 21, 22). Neonatal exposure to DES in lambs reduces the primordial follicle pool by stimulating their initial recruitment, resulting in increased numbers of atretic follicles (23). Finally, DES induces transient changes in gene expression during gestation; these changes could be involved in follicle development, rate of atresia, or patterns of secretion or metabolism of steroid hormones (24). These animal studies suggest that pharmacological doses of estrogens may influence fertility in many ways and at various time points. This knowledge, although difficult to extrapolate, may help in better understanding the mechanism behind
the observed infertility in tall women treated with high-dose estrogen.

Apart from the dosage used, high-dose estrogen treatment to reduce final height also differs from low-dose contraceptive treatment with regard to regimen (continuous vs. cyclic), timing (prepubertal vs. peri/postpubertal), and duration (1–2 yr vs. several months to many years). A recent study on the return to fertility after continuous use of OCP for up to 1 yr established a normal 12-month pregnancy rate of 81% and concluded that there was no delay in fertility (25). In addition, several studies have shown that there is no evidence that increased duration of OCP use delays subsequent fertility (5, 26).

At first presentation, treated women were taller and had higher predicted heights than untreated women. Therefore, the possibility of an association between tall stature and reduced fertility needs to be considered. We found no difference between treated and untreated women with respect to clinical parameters representing gonadal function at first presentation such as Tanner stage and age at menarche. In the available literature, height is negatively correlated with reproductive success in Western society; however, this is attributed to sexual selection and the fertility of these women, which has not been studied in detail (27). Finally, the height difference between untreated and treated women equals 1 percentile (98th vs. 99th percentile) according to Dutch standards (9). From a population perspective, it seems unlikely that this could explain a 15–25% reduction in achieving a first pregnancy in the first year.

The possibility of partial hypogonadotropic hypogonadism (HH) in these women needs to be considered because it is associated with both tall stature and reduced fertility. However, HH is a secondary growth disorder, and its growth pattern is distinctively different from constitutional tall stature. In HH, growth during childhood is unremarkable, and tall stature does not become evident until the teenage years when growth continues because of the lack of epiphyseal closure (28). Constitutional tall stature is characterized by accelerated growth velocity in early childhood, and tall stature becomes apparent at the age of 3 to 4 yr (6). Although we excluded women with secondary growth disorders, we cannot fully exclude the possibility of partial HH in some women because serum gonadotropin and sex steroid concentrations were not measured at initial presentation.

Previously, it has been shown that a considerable number of tall women treated with high-dose estrogen in adolescence suffer from primary ovarian insufficiency with concomitant early follicle pool depletion diagnosed by increased serum FSH levels, decreased serum anti-Müllerian hormone levels, and low antral follicle counts (3). Although the mechanism behind this accelerated follicle loss observed in these women remains unknown, based on our results we conclude that estrogen may play a key dose-dependent role. This is supported by a study on in utero exposure of women to DES, who reported an earlier age at menopause with cumulating doses of DES (29). We hypothesize that the effects of high-dose estrogen could be directly at the follicle level or indirectly through other intraovarian regulatory hormones such as IGF-I or anti-Müllerian hormone. Several studies have reported on these effects of estrogen; however, none have studied the effects of supraphysiological levels of estrogen. Future research is needed to test these hypotheses. Studies on physiological levels of estrogen have shown that human granulosa cells are a site of estrogen reception, whereas it is still uncertain whether the human oocyte is also estrogen responsive (30). Among the local intrafollicular actions of estrogen is its responsibility for facilitating the differentiation of granulosa cells, including the induction of receptor systems for FSH and LH, and it can influence postreceptor mechanisms (31). Studies on estrogen-depleted ovaries have shown that folliculogenesis halts in the antral stage, causing infertility due to the inability to ovulate (31). Animal studies have shown that IGF-I is required for reproduction. It has been suggested that IGF-I promotes fertility by limiting the recruitment of primordial follicles in the growing pool, thus conserving the resting pool (32). Interestingly, it has been shown that serum IGF-I levels are greatly reduced during high-dose estrogen treatment (33). One study, in fact, has shown that serum IGF-I levels are lower in girls receiving 200 µg EE compared with 100 µg EE after 12 months of high-dose estrogen treatment (34). In addition, in boys treated with high-dose androgen, no effect on IGF-I levels is seen and there are no effects on later fatherhood (33, 35).

Women treated with 200 µg EE had started treatment in earlier years and, as a result, had a longer duration of follow-up and were older at the time of study. We cannot therefore fully exclude the possibility of bias due to differential follow-up. Although this is corrected for by the use of the time to event analysis in the calculation of TTP, it is possible that with similar follow-up time, women treated with 100 µg EE would have had overall fertility outcome similar to women treated with 200 µg EE. However, this seems unlikely because correcting for age and the use of a sensitivity analysis in the calculation of fertility outcome did not significantly influence our results.

In conclusion, we report a dose-response relationship between fertility in later life and estrogen dose used for the treatment of tall stature in adolescent girls. Treated women had an increased time to pregnancy and more of-
ten sought medical attention for infertility. Of these, women treated with 200 μg of EE experienced fertility problems significantly more often than women treated with 100 μg. Our results suggest an important role of estrogen dose on fertility outcome and a possible lead toward explaining the mechanism behind the loss of fertility in tall women treated with high-dose estrogen in adolescence.

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