EFFECT OF F-6103 ON IMPLANTATION AND EARLY GESTATION IN WOMEN

By

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ABSTRACT

The influence of large doses of F-6103 (bis-/p-acetoxyphenyl/-2-methylcyclohexylidene methane) on implantation and early gestation was studied in clinical experiments.

In the first study, 600 mg of F-6103 were administered daily for 2 to 6 days beginning from 43 to 72 days after the first day of the last menstrual period. Spontaneous abortion, or foetal death occurred in 6 out of 26 women, as compared to 3 out of 24 untreated cases. The difference is statistically not significant.

In the second study 10 women exhibiting a positive pregnancy diagnosis test were treated with 600 mg of F-6103 daily for 7 days beginning from 32 to 48 days after the first day of the last menstrual period. Repeated immunological pregnancy tests were positive in all of them until curettage was performed 18 to 38 days following discontinuation of the treatment. Histopathological examination revealed in all subjects, but one, the presence of normally developed chorionic tissue exhibiting no signs of degenerations. In three cases well preserved foetal tissues were also found.

In the third study 16 women with proven fertility were treated from the 14th day of the cycle daily for 7 days with 600 mg of F-6103. Pregnancy occurred in 4 women after 1 to 6 courses of treatment.

The data indicate that in the dose schedule employed F-6103 has neither abortifacient nor postovulatory contraceptive effect in women.

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The antifertility effect of the weak non steroidal oestrogen, bis-(p-acetoxyphenyl)-2-methylcyclohexylidene methane (F-6103) in experimental animals is well documented (Einer-Jensen 1968). This compound was an effective abortifacient when a few mg/kg were given to rats and mice during various 2-day periods from days 1 to 12 of pregnancy. Ethinyl oestradiol, diethyl stilboestrol and dienoestrol were more effective than F-6103, although they were relatively less active when given on days 11–12, than on days 3–4 of gestation. Furthermore, when given to rats on days 17 and 18, F-6103 was as active as ethinyl oestradiol. These data suggested that the abortifacient effect of F-6103 in the later period of pregnancy might be due in part to a property not shared by other oestrogens. This assumption seemed to gain additional support from the specific uptake of F-6103 by corpora lutea as well as from its inhibitory effect on the conversion of pregnenolone to progesterone by luteal tissue (Larsson & Stensson 1967; Appelgren 1967).

The clinical experiments reported in this paper were performed in order to investigate the effect of F-6103 on implantation and early gestation in women.

**EXPERIMENTAL**

**Abbreviations and trivial names**

BBT: basal body temperature.

Three types of clinical studies were carried out as follows.

**First study**

In an exploratory study 26 women were treated with F-6103 in a daily dose of 600 mg for 2-6 consecutive days. The drug was administered orally in gelatine capsules according to a 3 X 200 mg schedule. The treatment commenced 43-72 days after the first day of the last menstrual period. The duration of treatment was 2 days in 2 subjects, 3 days in 20, 4 days in 1 and 6 days in 3 subjects.

The influence of F-6103 on the course of pregnancy was evaluated on the basis of the histological appearance of the foetal and placental tissues obtained at curettage, the occurrence of vaginal bleeding during or following treatment and hormone assays, (HCG and pregnanediol). Urinary HCG was estimated according to the method of Wide (1962) and pregnanediol according to the technique described by Klopper et al. (1955).

**Second study**

In the second study 10 women exhibiting early amenorrhoea (7 to 20 days past the expected period) were treated with a daily dose of 600 mg (3 X 200 mg) of F-6103 for 7 consecutive days. An immunological pregnancy diagnosis test was carried out on the
first day of the treatment and was repeated twice with an interval of 8-16 and 7-21 days, respectively. A curettage was performed 53 to 79 days following the first day of the last menstrual period and the tissues obtained were subjected to a histopathological evaluation.

Third study

Sixteen women with proven fertility and with a cycle length of 26 to 32 days were instructed to take from the 14th day of the cycle 600 mg F-6103 daily for 7 consecutive days. Basal body temperatures were recorded continuously during at least 8 cycles. On the first day of each cycle, the subjects were given a BBT-diagram, on which the 7 days of medication were marked. The subjects were requested to record their BBT and to indicate the date of each intercourse on the diagram. The experimental subjects were examined on the first day of each subsequent cycle, when a new 7-days’ dose of F-6103 was given to them. An endometrial biopsy was carried out at the end of the third treatment cycle in all subjects, who did not become pregnant.

A pregnancy diagnosis test was carried out whenever a menstrual bleeding did not occur within four weeks after the administration of the last dose of F-6103. In such cases a curettage was performed and the tissues obtained were examined histologically.

In each of the subjects the haematological status, liver function and kidney function were controlled before, during and immediately after medication.

RESULTS

First study

Curettage was carried out within 4–36 days after cessation of F-6103 treatment. All the 26 women were pregnant at the commencement of treatment as indicated by repeated pregnancy diagnosis tests. Hormone assays were performed in 18 of the 26 cases. In 16 of them no hormonal changes indicating a disturbed pregnancy were detected. Vaginal bleeding occurred in three cases. In two of these there was a greatly diminished excretion of HCG, reaching undetectable levels and the pregnanediol excretion decreased below 1.0 mg/24 h. No hormone assays were performed in the third case. Slight bleeding was reported by two additional subjects. Spontaneous abortion occurred in three cases. Furthermore, missed abortion was diagnosed on clinical and histological criteria in one subject, and foetal death in two cases. In these two cases the findings included an amniotic sac with necrotic contents and no recognizable foetal tissues. Thus foetal death was recorded in a total of 6 cases.

The above findings were compared with observations made in a similar group of 24 women in whom a legal abortion had to be carried out for medical social indications. The pregnancies were interrupted during weeks 8–12th. The results obtained are indicated in Table 1.

The data of Table 1 indicate that 6 foetal deaths occurred in 26 women treated with F-6103, whereas 3 foetal deaths occurred in the control group of 24 women. The difference is statistically not significant.

Among the unspecified cases indicated in Table 1, some histopathological
Table 1.
Relative frequency of foetal death in a group of pregnant women treated with F-6103 as compared to that observed in a control group.

<table>
<thead>
<tr>
<th></th>
<th>F-6103</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Foetal death</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>Clear-cut histological changes</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Not specified</td>
<td>16</td>
<td>62</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>26</td>
<td></td>
</tr>
</tbody>
</table>

alterations were observed in a further 5 cases of the treatment group and in 4 cases among the controls.

Second study
The 10 women in this group all had a positive pregnancy test at the commencement of the treatment. The pregnancy tests remained positive in all the subjects during and following the treatment until a curettage was performed. Curettage was carried out within 18 to 38 days after discontinuation of the treatment. The results of this study are indicated in Table 2.

Vaginal bleeding occurred in four cases. In two subjects (Cases No. 2 and 6) the bleeding was transient and lasted only one day. A third subject (Case No. 9) reported scanty bleeding (spotting) for 12 days, beginning on the 5th day of medication. The fourth subject (Case No. 1) had a prolonged bleeding from the 5th day of treatment which continued until curettage was performed 16 days later. At this time a partial abortion was diagnosed.

Histological examination of the material obtained at curettage indicated in all cases typical decidual changes of the endometrium and the presence of placental tissue (e.g. Figs. 1 and 2). With the exception of Case No. 4, no degenerative changes were observed. The development of the chorionic villi corresponded to the stage of gestation. Occasionally some fibrin deposition was found in the intervillous space, as well as between the inner and outer epithelial layers of the villi. The blood vessels of the chorionic villi were well preserved (Fig. 3).

In at least three cases well preserved foetal tissues were found. Figs. 4 and 5 indicate the appearance of the foetal tissues obtained from Cases No. 3 and 6.
The picture shown in Fig. 4 indicates that the epithelial cells are extremely well preserved. The nuclei as well as the cytoplasm reveal distinct borders. Furthermore, the cartilaginous cells are intact and do not show any signs of degeneration.

The histological finding presented in Fig. 5 (Case No. 6) reveals a similar picture of intact foetal tissue.

Side effects were reported by 5 of the 10 subjects; slight abdominal pain (Case No. 3), nausea and vomiting (Cases No. 5, 6 and 8) and nausea, meteorism and tinnitus (Case No. 9). Whether these effects are attributable to the treatment or to the pregnant condition as such, remains to be determined.

Third study

In an attempt to assess the effect of F-6103 on fertilization, tubal transport and implantation, a third study was carried out, in which administration of the drug commenced on the 14th day of the cycle. Five of the 16 women in this group discontinued treatment during or soon after the first treatment cycle. Of the remaining 11 subjects 4 became pregnant after 1–6 cycles of treatment. A survey of some of the data obtained in this group is presented in Table 3.

The data of Table 3 do not reveal any important differences between the 4 pregnant and 7 non-pregnant cases with regard to the indices of fertility, or frequency of intercourse. The side effects were slight and non-specific in both groups.

Clinical laboratory tests were carried out in 14 of the 16 women. The data indicated no adverse effects on the blood picture, or on the renal and hepatic function.

In the non-pregnant group the duration of treatment varied between 3 and 5 cycles (e. g. Table 3). There were no deviations from the normal cycle length. Endometrial biopsies taken on days 22–26 revealed a secretory transformation of the endometrium in 6 cases and a proliferative endometrium in one case.

Short case histories of the 4 subjects in the pregnant group are presented below.

Case No. 12. – One previous normal pregnancy and 8 induced abortions, the last one in August 1968. She received the first of her 6 courses of F-6103 November 23 to 29th, 1968, and the last course from March 31st to April 6th, 1969. Cycle lengths during the 5 courses were 23 to 28 days, her normal range: 24-28. Last menstrual period prior to the last treatment course: March 18th to 22nd. Pregnancy diagnosis tests: Positive on April 25th and May 5th (cycle days 39 and 49). A spontaneous abortion occurred on May 7th, 1969. Histological examination of the expelled conceptus indicated marked infiltration of inflammatory cells into the endometrial stroma. Some degenerative changes were also found in the placental tissue.


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**Table 2.**
Evaluation of F-6103 as an abortifacient in the human species. Treatment: 200 mg F-6103 orally 3 times daily for 7 days, from the day of the first positive pregnancy diagnosis test.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Children</th>
<th>Previous cycles (days)</th>
<th>Interval between last menstrual period and pregnancy diagnosis test (days)</th>
<th>Interval between last menstrual period and curettage (days)</th>
<th>Histopathological findings</th>
<th>First menstrual period following curettage (cycle day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>1</td>
<td>24</td>
<td>35 (+)</td>
<td>—</td>
<td>—</td>
<td>53</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>1</td>
<td>30</td>
<td>37 (−) 47 (+) 59 (+)</td>
<td>71</td>
<td>Decidual tissue and chorionic villi. No degenerative changes.</td>
<td>34</td>
</tr>
<tr>
<td>3</td>
<td>36</td>
<td>2</td>
<td>28</td>
<td>48 (+) 59 (+) 76 (+)</td>
<td>78</td>
<td>Decidual and foetal tissue without degeneration. Fibrin deposition in some chorionic villi.</td>
<td>31</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>0</td>
<td>28</td>
<td>38 (+) 46 (+) 66 (+)</td>
<td>68</td>
<td>Decidual tissue only. Marked degeneration with necrosis.</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>2</td>
<td>24</td>
<td>40 (+) 56 (+) 63 (+)</td>
<td>65</td>
<td>Decidual and placental tissue. A small placental area revealing intervillous fibrin deposition.</td>
<td>27</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>1</td>
<td>28</td>
<td>47 (+) 60 (+) 76 (+)</td>
<td>78</td>
<td>Decidual tissue, chorionic villi and foetal tissue. No degenerative changes.</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>0</td>
<td>26</td>
<td>32 (+)</td>
<td>41 (+)</td>
<td>67 (+)</td>
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<td>28</td>
<td>41 (+)</td>
<td>48 (+)</td>
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<td>8</td>
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<td>30</td>
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<td>75 (+)</td>
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<tr>
<td>9</td>
<td>36</td>
<td>1</td>
<td>28-30</td>
<td>41 (+)</td>
<td>—</td>
<td>78 (+)</td>
<td>79</td>
</tr>
</tbody>
</table>

Decidual tissue and chorionic villi. No degenerative changes.

Chorionic villi only. No degeneration. Minimal deposition of fibrin in some villi.

Decidual tissue and chorionic villi. No degenerative changes.

Well preserved foetal and placental tissue. Some oedematous chorionic villi with fibrin deposition.
Fig. 1.
Decidual tissue with well preserved epithelium (Case No. 2). No signs of degenerative changes.

Fig. 2.
Chorionic villi showing a normal development typical for the 9-10th weeks of gestation (Case No. 3). The villi are large and covered with a double layer of epithelium. The stroma at this time is made up of loosely knit fibroblasts. Note the absence of any degenerative changes.
Fig. 3.
Chorionic villi from early gestation (Case No. 5) showing a few blood vessels lined with large, intact, immature endothelial cells.

Fig. 4.
Embryonic tissue showing well preserved cartilaginous cells and an intact epithelial lining without any signs of degeneration (Case No. 3).
Embryonic tissue from Case No. 6 showing an intact neural cavity lined with columnar epithelial cells. Note the absence of degenerative changes.

(cycle day 39). Curettage was carried out on February 1st (cycle day 52). Histological examination revealed the presence of well preserved placental and foetal tissues.

*Case No. 19.* – Two previous normal pregnancies. The duration of her previous normal cycles was 30 days. She received her first course of F-6103 January 7-13th, and the second February 5-11th, 1969. Last menstrual period prior to the second course of F-6103: January 23-27. Elevated body temperature (39.6°C) on cycle day 23, and bleeding on day 30. Positive pregnancy diagnosis test: February 21st (cycle day 30). Curettage: March 15th (cycle day 52). Histological examination: well preserved placental tissue.

*Case No. 25.* – One previous pregnancy with delivery after 7½ months. Duration of 4 preceding cycles prior to medication: 30 days. Last menstrual period: January 30th. Treated with F-6103: February 5-11th, 1969. Positive pregnancy tests: March 6 and 13th (cycle days 43 and 50). Curettage: March 17th (cycle day: 54). Histological examination: decidual transformation of the endometrium and placental tissue without any signs of degeneration.

**DISCUSSION**

There is a plethora of data indicating that in rodents oestrogens and so-called anti-oestrogens are capable of interfering with tubal passage of the fertilized ovum (*e.g.* Greenwald 1961; Deanesly 1963) and/or with its implantation.
<table>
<thead>
<tr>
<th>Outcome</th>
<th>Case No.</th>
<th>Age</th>
<th>Previous pregnancies</th>
<th>F-6103 cycles</th>
<th>Intercourse per treated cycle (average)</th>
<th>Intercourse during cycle days 14–20 (average)</th>
<th>Histopathological findings</th>
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<tbody>
<tr>
<td>Pregnancy</td>
<td>12</td>
<td>38</td>
<td>9</td>
<td>6</td>
<td>8.8</td>
<td>2.5</td>
<td>Decidual tissue revealing severe inflammatory changes. Some chorionic villi without degeneration. Foetal tissue, chorionic villi and decidual tissue. No degeneration. Decidual tissue and chorionic villi. No degenerative changes. Decidual tissue and chorionic villi corresponding to the placental age. No degeneration.</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>25</td>
<td>1</td>
<td>1</td>
<td>12.0</td>
<td>3.0</td>
<td></td>
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<tr>
<td></td>
<td>19</td>
<td>31</td>
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<td>2</td>
<td>16.0</td>
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<td>25</td>
<td>22</td>
<td>1</td>
<td>1</td>
<td>10.0</td>
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<td></td>
</tr>
<tr>
<td>Mean</td>
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<td>2.5</td>
<td>11.7</td>
<td>3.8</td>
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<td></td>
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<tr>
<td>No pregnancy</td>
<td>14</td>
<td>25</td>
<td>3</td>
<td>3</td>
<td>9.7</td>
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<td>1</td>
<td>3</td>
<td>10.7</td>
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<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.3</td>
<td>3.7</td>
<td>11.6</td>
<td>3.4</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
(Emmens et al. 1962; Pincus 1965; McLean Morris et al. 1967; Kar 1969). Data are also available indicating a similar effect of oestrogens in monkeys and women (McLean Morris & vanWagenen 1966). On the basis of these findings it has been recommended that large doses of oestrogens can be used for the purposes of postcoital contraception in women (McLean Morris & vanWagenen 1967; Szontagh & Kovacs 1969; Haspels 1969).

Since F-6103 possesses a marked antifertility effect in rodents (Einer-Jensen 1968), it appeared of considerable interest to find out, whether or not this compound is also effective in the human species. The experiments reported in the present communication indicate that in the dose schedule employed F-6103 possesses no abortifacient effect in women. In this respect our findings are similar to those reported recently on the failure of large doses of ethinyl oestradiol to interfere with early embryonic development in the human species (Bačič et al. 1970). Furthermore, our present data also indicate that F-6103 is not capable of interfering with fertilization and tubal transport of the fertilized human ovum. No such data are available as yet with regard to ethinyl oestradiol. It is possible, that the very low solubility and relatively poor absorption of F-6103 were also instrumental in the failure of this drug in the human species. Therefore, its pharmacokinetics seems to deserve additional studies.

The data of our present study together with those reported previously (Bačič et al. 1970) seem to justify the conclusion, that there is a significant difference between the human and other species with regard to the abortifacient effect of synthetic oestrogens. The balance of evidence available at present suggests that the use of large doses of oestrogens is not a practical approach to interfere with early gestation in the human species. Other approaches, for instance the administration of prostaglandin F2α (Karim & Filshie 1970; Roth-Brandel et al. 1970) appear to be much more promising in this respect.

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