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THE RELATION BETWEEN PLASMA PROGESTERONE AND TOTAL URINARY OESTROGENS FOLLOWING INDUCTION OF OVULATION IN WOMEN

By

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ABSTRACT

Plasma progesterone levels have been measured before and after a single injection of 9000 IU of human chorionic gonadotrophin (HCG)* at different levels of total oestrogen excretion (TE) in 67 treatments with human pituitary gonadotrophin (HPG) in amenorrhoeic women. With a TE excretion of less than 70 µg/24 h the rise in plasma progesterone was similar to that found in a normal menstrual cycle indicating the formation of only one corpus luteum. With more than 70 µg/24 h of TE several corpus lutea was formed as judged by the rise in plasma progesterone levels. No further increase was seen above 300 µg/24 h. The luteal phase was shorter than normal in the group with 75–300 µg/24 h of TE.

Since the report of Gemzell et al. (1958), the use of human pituitary gonadotrophin (HPG) in infertile women lacking gonadotrophins has been generally accepted. However, one of the drawbacks of the treatment has been super-ovulation leading to multiple births. The mechanisms that control the release

* The following abbreviations are used:
HCG: human chorionic gonadotrophin.
HPG: human pituitary gonadotrophin.
FSH: follicle stimulating hormone.
LH: luteinizing hormone.
s: standard deviation.
TE: total urinary oestrogens.
of only one ovum per cycle are incompletely understood and research in this field has been hampered by the lack of sensitive methods for the assay of the steroid hormones involved. The development of a rapid method for total urinary oestrogens (Brown et al. 1968) and a sensitive method for plasma progesterone (Johansson 1969) have made it possible to follow the ovarian response in more detail.

This study was started with the purpose of investigating the rise in plasma progesterone concentration after one injection of human chorionic gonadotrophin (HCG) at different levels of total urinary oestrogen (TE) excretion.

**MATERIALS AND METHODS**

During a five month period in 1968, 51 women with primary or secondary amenorrhoea and oligomenorrhoea were treated 89 times with HPG and HCG in order to induce pregnancy. Following treatment 19 women conceived. Nine treated cycles were not included due to failure of ovulation in two and spontaneous ovulation in seven cases. In 13 treated cycles the data were incomplete and the information obtained from these cycles was only used for estimation of the duration of the luteal phase.

HPG was prepared by the method of Roos & Gemzell (1965). One mg contained 28 IU of FSH activity, when the 2nd IRP-HMG was used as a reference standard and about 30 IU of LH activity, when an international standard of HCG was used. Consequently, the ratio between FSH and LH was close to 1.0. All the women included in this study were treated with a preparation of HPG obtained from the same batch of human pituitary glands prepared by Dr. Roos. The daily doses of HPG varied between 100 and 300 IU.

HCG (Pregnyl®, Pharmacia, Sweden) in a single dose of 9000 IU was given intramuscularly at various levels of TE excretion. No woman was treated with HPG for less than six days.

The women were seen and examined daily and the changes in cervical mucus and size of the ovaries were noted. The women kept a basal body temperature chart and continuous 24 h urine collections were made. These were delivered to the laboratory each day by 8 a.m., where the content of TE was measured using the rapid method of Brown et al. (1968). The results were available at noon on the same day, when the women were examined and treated.

A venous blood sample (3–5 ml) was taken just before the HCG injection (P₀) and 24 h later (P₁). The plasma was assayed for progesterone using the method of Johansson (1969). From the P₀ samples 0.50 ml plasma was extracted and from the P₁ samples 0.25 ml. In order to rule out any interference from other steroids, parallel determinations were made using the thin layer chromatographic step used by Neill et al. (1967). No difference was found between the samples assayed by the rapid method and the samples further purified by thin layer chromatography, except in two patients with the polycystic ovary syndrome, where the thin layer chromatography gave a somewhat lower yield due to the presence of another steroid with the same Rₚ value as testosterone, and displacement properties similar to testosterone in a competitive protein binding system for testosterone (Johansson, unpublished).
RESULTS

The progesterone levels in the peripheral plasma of 67 treated cycles just before the HCG injection and 24 h later are seen in Fig. 1. The progesterone values are grouped according to the TE excretion on the day of the HCG injection.

Before the level of urinary excretion of total oestrogens had reached 70 µg/24 h the rise in plasma progesterone following the HCG injection during a 24 h period was only moderate. Above this TE level the plasma progesterone showed a marked increase. In Table 1 the treated cycles are arranged in groups according to the level of urinary excretion of total oestrogens on the day of the HCG injection. At an average TE level of 54 µg the plasma level of progesterone rose from 0.51 to 3.08 ng/ml during the 24 h period. It also follows from Table 1 that above an average TE level of about 300 µg there is no further increase in plasma progesterone. When the results are plotted on a logarithmic scale (Fig. 2) an increase in the plasma progesterone before the HCG injection is more clearly seen; the data fit a line with a regression coefficient of 0.5652. The line that satisfied the $P_1$ values had a regression coefficient of 0.5456.

The average length of the luteal phase in the 70 treated cycles that did not
The mean plasma progesterone concentration before \( (P_0) \) and 24 h after \( (P_1) \) the injection of 9000 IU of HCG at different levels of total oestrogen excretion on the day of HCG administration.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean TE ± s on the day of HCG injection</th>
<th>Mean progesterone ( P_0 ) ng/ml ± s</th>
<th>Mean progesterone ( P_1 ) ng/ml ± s</th>
<th>Number of treated cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>TE &lt; 75 µg</td>
<td>54.0 ± 17.5</td>
<td>0.5 ± 0.3</td>
<td>3.1 ± 1.8</td>
<td>13</td>
</tr>
<tr>
<td>TE 75–150 µg</td>
<td>105.5 ± 18.8</td>
<td>0.8 ± 0.6</td>
<td>5.2 ± 3.9</td>
<td>22</td>
</tr>
<tr>
<td>TE 150–300 µg</td>
<td>218.0 ± 14.1</td>
<td>1.7 ± 1.7</td>
<td>9.5 ± 6.6</td>
<td>23</td>
</tr>
<tr>
<td>TE &gt; 300 µg</td>
<td>475.0 ± 269.0</td>
<td>1.8 ± 1.7</td>
<td>9.3 ± 4.6</td>
<td>9</td>
</tr>
</tbody>
</table>

*Fig. 2. The same values as in Fig. 1 plotted on a logarithmic scale. Progesterone levels just before (black spots) and 24 h after the injection of HCG (open circles).*

Result in pregnancy was 12 days. The luteal phase was considered to last from the day after the HCG injection to the day before the first day of bleeding. The distribution of the duration of the luteal phases is shown in Fig. 3, and for comparison the length of 20 luteal phases following spontaneous ovulation is included (Johansson 1969). Out of 70 treated cycles 33 were below the lower range of the normal cycles.

In 14 treated cycles the plasma progesterone levels were measured throughout the luteal phase. Only women with primary amenorrhoea (n = 10) or...
Fig. 3.
The distribution of the length of the luteal phase in 70 FSH treated cycles compared to 20 normal cycles from Johansson (1969).

Secondary amenorrhoea (n = 4) of long duration were followed up. The results are shown in Fig. 4. In the first group (n = 4) the mean TE excretion on the day of HCG injection was 62 µg/24 h. Five days after the HCG injection the plasma level of progesterone reached a plateau of about 20 ng/ml. The luteal

Fig. 4.
Plasma progesterone levels during the luteal phase. The TE excretion on the day of HCG (Pregnyl®) injection was in group 1, mean 62 µg (n = 4); group 2, mean 122 µg (n = 3); group 3, mean 207 µg (n = 5) and group 4, mean 344 µg/24 h (n = 2).
M: onset of bleeding.
phase lasted for 14 days. The second group \((n = 3)\) with a mean TE excretion of 122 \(\mu g/24\) h showed a more rapid initial rise but this did not eventually attain a value above the plateau level of the first group. The luteal phase lasted for only 10 days. In group 3 \((n = 5)\) the mean TE excretion was 207 \(\mu g/24\) h. The increase in plasma progesterone was more rapid with a maximum value twice as high as in groups one and two. The luteal phase lasted for 10 days. The fourth group \((n = 2)\) had a mean TE excretion of 344 \(\mu g/24\) h. The increase in plasma progesterone following the HCG injection was very rapid, reaching a level 4 times as high as the levels of groups one and two. The plasma progesterone levels was above 70 ng/ml plasma for at least 6 days. The luteal phase lasted for 12 days.

An effort was made to follow more closely the changes in plasma progesterone levels after the HCG injection (Fig. 5). Three patients were selected who at the time of the HCG injection had an average TE excretion of 257 \(\pm 53\) (s) \(\mu g/24\) h. Eight h after the injection, the plasma progesterone levels were already significantly higher than the starting values. Between 20 to 24 h after the HCG injection, a slight break in the upward trend was observed. In one patient the progesterone level at 24 h after the HCG injection was actually lower than the level at 20 h while in the two other patients the levels were about the same.

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**Fig. 5.**

The rise in the plasma progesterone concentration in three patients with a mean TE excretion of 257 \(\pm 53\) (s) \(\mu g/24\) h on the day of the HCG injection.
DISCUSSION

There is a clear relationship between the TE excretion obtained after HPG stimulation and the rise in plasma progesterone following the administration of a single injection of HCG. However, the relation is not linear. There seems to be a threshold effect at about 70 µg/24 h of TE. At higher levels of TE excretion there is a marked increase in plasma progesterone, but no further increase seems to occur above 300 µg of TE.

When the same method for the determination of TE as used in this study was applied to 26 normal menstrual cycles, the mid-cycle TE peak preceding ovulation had a mean of 38 ± 28 (s) µg/24 h urine. This TE peak also coincided in time with the LH peak (Gemzell et al., to be published). Using the mid-cycle peak of TE as reference Johansson (1969) found that the levels of plasma progesterone 24 and 48 h later were 2.1 and 3.8 ng/ml respectively. The rise in plasma progesterone in the group with less than 75 µg/24 h urine found in this study was therefore similar to that in the normal cycle. Furthermore the maximum levels of plasma progesterone and the length of the luteal phase were not different from a normal cycle. Thus, it appears that the threshold effect observed following HCG, when the TE is above 70 µg/24 h, represents the formation of more than one corpus luteum.

It should be pointed out that the treated women belong to a heterogenous group with highly individual likelihood of ovulation. The TE excretion may originate from one or several developing follicles, which makes the timing of the HCG injection crucial and can in fact result in highly variable progesterone levels if the TE excretion is the same. To avoid this fluctuating effect it may be important to have the ovaries in a state of rest at the beginning of the treatment.

There are ample indications that at a certain time before rupture, the ripening follicle can produce progesterone (Zander et al. 1958) and that some of this progesterone will reach the circulating blood (Mikhail 1967; Johansson & Wide 1969). However, the rise in the progesterone concentration in the normal cycle does not occur before the rise in LH levels (Johansson & Wide 1969).

Many of the high P₀ values (Figs. 1 and 2) were found in women with secondary amenorrhoea or oligoamenorrhoea. Following the rise in plasma progesterone, some LH may be released from the pituitary gland and stimulate the production of progesterone in the ripening follicle. On the other hand, the elevated progesterone levels before the HCG injection in patients with a high TE excretion may very well be explained by a baseline production of progesterone from several stimulated follicles in which the LH content of the HPG preparation can play a role.

The short luteal phase in almost half of the non-pregnant HPG cycles is of particular interest as it might explain why these patients did not conceive in
spite of a well-timed ovulation. The majority of the short luteal phases occur in women with a TE excretion of between 75 and 300 µg/24 h at the time of the HCG injection. As seen in Fig. 4 these women showed a rapid rise in plasma progesterone levels following the HCG injection as compared to the women with a lower TE excretion. The plasma plateau of progesterone concentration was therefore reached at an earlier stage and as the plateau seemed to have a fairly constant duration the decrease would occur earlier and result in a short luteal phase. Consequently, the likelihood of a fertilized egg becoming implanted properly would decrease.

In a previous study (Gemzell unpublished) ovulation was induced by HPG and 3000 IU of HCG daily for 3 days in 31 women when the TE level ranged between 75 and 300 µg/24 h urine. The length of the luteal phase of these women was 12.8 ± 1.1 (s) days. As the amount of HCG administered in the present and in previous studies was the same, it might be suggested that the period of the HCG administration was responsible for the prolongation of the luteal phase. The drawback of 3 injections of HCG coupled with a high TE response was the high number of multiple births, and it was hoped that a single injection would reduce this number. To prolong the luteal phase a second HCG injection might be administered 4–5 days after the first one. In this case a prolonged HCG effect might be achieved without any risk of additional eggs being fertilized. At that stage of treatment the cervical mucus would block further penetration of sperms.

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