Fluctuation of Urinary Level of Follicle Stimulating Substance and of Estrogen during Pregnancy

Seiji Nagai and Yukichi Haryu

Department of Obstetrics and Gynecology (Prof. K. Kushima), Tohoku University School of Medicine, Sendai

Urinary estrogen level in various stages of human pregnancy increased after pregnant mare serum (PMS) injection. This increase in the estriol fraction was statistically significant, whereas the level of urinary FS-substance in the first and second trimesters of pregnancy rose remarkably following temporary fall after injection of estradiol benzoate. This suggests a feed-back mechanism between FSH and estrogen secretion during pregnancy.

The level of urinary FS-substance reached a peak in the third month of gestation and thereafter it fell to a relatively constant level. Later it again increased and reached the second peak in the 8th month and decreased toward the 9th month. In the 10th month the level was higher than that in the 3rd and 8th months. FS-substance contained in the placenta began to increase from the second trimester and it reached a peak in the 8th month, and then decreased in the last 2 months.

These results indicated that the FS-substance was predominantly derived from the anterior pituitary during the early months of gestation, whereas considerable amounts of it were delivered from the placenta at least near the end of gestation.

It was previously thought that the human anterior pituitary ceases its normal function of releasing gonadotrophins after the formation of the placenta; the feedback mechanism between gonadotrophins and ovarian steroid hormones in normal non-pregnant women disappears in pregnancy.\textsuperscript{1-3} Therefore, the placental function consists not only in gas exchange and metabolite transit between mother and fetus, but also in endocrine activity. In fact, a number of reports\textsuperscript{4-6} have indicated that it is possible to detect steroid hormones and proteohormones in the human placenta.

There are, however, very few reports as to whether placental gonadotrophin has a function of regulating steroidogenesis in the same way as pituitary gonadotrophin has in non-pregnant women. There has been also little information about the mechanism of regulation of neogenesis and secretion of estrogen, although a large amount of estrogen is excreted in the urine of pregnant women, and estrogen metabolism is regarded as an index of the function of fetoplacental unit.

The following study was undertaken to investigate the relation between FSH and estrogen metabolism in pregnant women and to investigate the level of

Received for publication, December 20, 1968.
follicle stimulating substance (FS-substance) in urine and placenta at the various stages of gestation.

**Materials and Methods**

Estrogen was extracted from the urine by Suzuki and Takahashi's method, partitioned by column chromatography and estimated by fluorometry. Urinary FS-substance was extracted by Albert's method and measured by Steelman-Pohley's method.

Placental FS-substance was prepared in the following way: the placenta was washed in running water and perfused with 600 ml of water via the umbilical vein. After the fetal membranes and fetal cords were removed, the placenta was cut into pieces, washed again in running water, and then minced in acetone (300 ml/100 g of placenta) by a blender for one minute at 0°C. The last procedure was repeated and acetone powder of the placental tissue was obtained. The remnants of membrane, large vessels and infarct tissues were sieved out with a mesh of 2 × 2 mm.

Fine acetone powder prepared in this way was homogenized by a glass homogenizer for one minute at 0°C and extracted by strong stirring in 500 ml of saline (500 ml/10 g of acetone powder) at pH 6.5 for 30 minutes at 0°C. This extraction procedure was repeated, and 1,000 ml of extract per 10 g of acetone powder were made. FS-substance was prepared from the extract by Albert's method and measured by the Steelman-Pohley's method.

**Results**

1) **Effect of PMS on the level of urinary estrogen in pregnant women**

Urine specimens were collected from total daily excretions of 15 pregnant women. 2,000 i.u. of PMS (Serotropin, Teikoku Zoki) were injected on the morning of the 3rd day of the experiment.

The results were as follows. (1) The level of total estrogen increased after the day of the injection. (2) The comparison of the estrogen level at the peak after the injection of PMS with the average level in the control urine gave the following results; the levels in pregnancy increased 1.8-5.0 times (0.05 < p < 0.1) in the first trimester group (4 women), 1.3–11.1 times (0.1 < p < 0.2) in the second trimester group (7 women), and 1.7–1.9 times (0.05 < p < 0.1) in the third trimester group (4 women). The increased total estrogen after the injection of PMS dropped on the 7th day of the experiment and approached the level of the control (Figs. 1–3).

The increase of the level in the estriol (Et) fraction after the injection of PMS was remarkable and the comparison described above showed that the levels increased 3.0–6.7 times (0.02 < p < 0.05) in the first trimester group and this increase was statistically significant at 5% level. In the second trimester group the increase was 1.3–10.7 times (0.1 < p < 0.2); however, the levels in the third trimester group increased 1.8–2.4 times (0.02 < p < 0.05) and therefore the increase in this group was also statistically significant. The levels of the estrone (Eo) fraction and the estradiol (Ed) fraction tended to increase after the injection of PMS in all trimesters, but the increase was not statistically significant at 5% level.

2) **Effect of PMS on the urinary estrogen levels of newborn infants**

Five healthy mature male infants delivered spontaneously were injected intramuscularly with 300 i.u. of PMS immediately after the umbilical cords were cut
Fig. 1. Fluctuation of the level of daily urinary estrogen in the first trimester group before and after injection of 2,000 i.u. of PMS.

Fig. 2. Fluctuation of the level of daily urinary estrogen in the second trimester group before and after injection of 2,000 i.u. of PMS.

(Experimental group). Another six healthy mature male infants who did not receive the injection were used as a control group. All the infants were fed artificially on the same powdered milk (Neomilk P-F, Meiji).

The daily urine output was collected carefully from these infants by use of a pediatric urine collector (Sterilon Co.) successively for 4 days, and the estrogen was extracted, partitioned and estimated.

The results were that there were no statistically significant differences in the urinary levels of total estrogen and the fractions of Eo, Ed, and Et between the experimental and the control groups (Fig. 4).

3) Effect of estradiol-17β-benzoate (Ed-benz; Ovahormon, Teikoku Zoki) on the level of urinary FS-substance during pregnancy

Ed-benz in 2.0 mg was injected intramuscularly into pregnant women in the first and second trimesters of gestation and daily urine was collected successively
before and after the injection, and then the urinary FS-substance was extracted and measured. Experimental results showed that the level of urinary FS-substance was changed markedly by the injection of Ed-benz (Fig. 5).

4) Urinary FS-substance during pregnancy

Daily urine was collected from healthy pregnant women at various stages of pregnancy and urinary FS-substance was measured. The level of urinary FS-substance showed a peak in the 3rd month of gestation, and then it tended to a decrease and remained relatively constant during the second trimester. In the 8th month, the level rose to reach a second peak, then decreased steadily until the 9th month, but increased again until the 10th month. Urinary FS-substance level in the postdate pregnant women was almost equal to that in the normal full term (Fig. 6).

5) Placental content and concentration of FS-substance during pregnancy

FS-substance was determined in 134 placentas at various stages of pregnancy (Table 1). FS-substance contained in a placenta and its concentration per unit placental weight (per 10 g of acetone powder) were calculated.
The total content of the substance in one placenta began to increase from the 5th month and reached a peak in the 8th month, thereafter decreasing toward the expected date of delivery. However, there were several postdate placentas which had a relatively high content. The concentration of FS-substance was high in the 2nd month, but thereafter it decreased and remained relatively constant during the second trimester. In the 8th month it reached the second peak and then decreased toward the expected date. However, there were several postdate placentas which contained the substance in a relatively high concentration (Fig. 7).

**Discussion**

Though it is well known that urinary estrogen increases markedly during pregnancy, there are very few reports on the hormonal regulation of neogenesis and secretion of estrogen. This scarcity of reports may be due to the following reasons. In research of the metabolism of hormones during pregnancy we have to consider not only the influence of maternal and fetal endocrine organs, but also that of the placenta. Careful analysis of the result is necessary. However, fetus and
placenta are enclosed in the uterine cavity, so to speak in an isolated cell, and accordingly it is very difficult to approach them directly from outside the uterus.

In the present investigation, urinary estrogen, especially of Et fraction which increased markedly by the injection of PMS, tended to increase. Dassler\textsuperscript{10} reported that urinary estrogen during pregnancy increased after the injection of ACTH into the mother, and Nakayama\textsuperscript{11} reported that the urinary estrogen level rose when HCG was injected directly into the fetus. However, the injection of HCG into the mother or injection of ACTH into the fetus did not cause an increase in the urinary estrogen. For an explanation of these findings, these two authors supposed a stimulatory action of injected hormones on the adrenal cortex. However, Akasu et al.\textsuperscript{12} reported that both HCG and ACTH-Z had no effect on estrogen neogenesis in the adrenal cortex of castrated women and PMS accelerated estrogen neogenesis in the adrenal cortex after priming with ACTH.

In the investigation of estrogen during pregnancy, we have to consider the influence not only of the placenta or the fetus but also of other possible factors. Timonen et al.\textsuperscript{13} reported that urinary estrogen level might be influenced by a urine volume increase of more than 20%. In the present investigation, the

Fig. 5. Fluctuation of the level of daily urinary FS-substance in the pregnant women at various weeks of gestation before and after injection of 2.0 mg of Ed-benz.
Fig. 6. The level of daily urinary FS-substance during pregnancy.

urine volume on the day when the estrogen level showed a peak after injection of PMS was not always larger than that on the control days, or rather there might be no relationship between urine volume and estrogen level in the same urine, as far as the urine after the injection was concerned.

To determine the fetal factor, PMS was injected into new-born infants, and the urinary estrogen level was measured, but there was no difference in the level between the experimental and control groups. Diczfalusy et al.14 also reported that urinary estrogen level of new-born infants was not influenced by injection of 750 i.u. of HCG.

There has been no report indicating the possibility that FSH injected into the mother is transferred through the placenta to the fetus at least near the expected date. Even if FSH transit takes place through the placenta, it may not have much influence on estrogen metabolism in the fetus.

It could be probably supposed from the present investigation that estrogen neogenesis or secretion may be regulated by FSH even in pregnancy. It was also suggested that FSH secretion in pregnancy was influenced by estrogen injection. The present investigation showed that urinary FS-substance decreased on the first day of injection of Ed-benz, which has a strong biological activity, then increased steadily from the second day. It may be assumed from these results that there is a feed-back mechanism between FSH and estrogen in pregnant women just as in cycling women.
To study FSH fluctuation during pregnancy, the level of urinary FS-substance was measured. The level was relatively high during the first trimester, but fell to some degree and remained constant during the second trimester, and then rose.

<table>
<thead>
<tr>
<th>Months of gestation</th>
<th>Number of placentas used for one extraction procedure</th>
<th>FS-substance per 10g of acetone powder</th>
<th>FS-substance per one placenta</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>51</td>
<td>54</td>
<td>1.3</td>
<td>Artif. miscar.</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>12</td>
<td>0.27</td>
<td>Artif. miscar.</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>Artif. miscar.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>Artif. miscar. &amp; spont. abort.</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>Artif. miscar.</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>40</td>
<td>34</td>
<td>Spont. abort.</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>Spont. abort.</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>Spont. abort.</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>8</td>
<td>16</td>
<td>Spont. abort.</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>14</td>
<td>32</td>
<td>Cesar. sect.</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>89</td>
<td>116</td>
<td>Spont. prem. labor</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>44</td>
<td>80</td>
<td>Cesar. sect.</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.4</td>
<td>4</td>
<td>Cesar. sect.</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>Spont. prem. labor</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.3</td>
<td>13</td>
<td>Spont. prem. labor</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>55</td>
<td>72</td>
<td>Spont. prem. labor</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>24</td>
<td>44</td>
<td>Spont. prem. labor</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.3</td>
<td>5</td>
<td>Cesar. sect.</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>Spont. deliv.</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>Spont. deliv.</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>16</td>
<td>32</td>
<td>Spont. deliv.</td>
</tr>
<tr>
<td>Over dated</td>
<td>1</td>
<td>64</td>
<td>64</td>
<td>Spont. deliv.</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.12</td>
<td>4</td>
<td>Spont. deliv.</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>34</td>
<td>76</td>
<td>Cesar. sect.</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>21</td>
<td>91</td>
<td>Vacuum extr.</td>
</tr>
</tbody>
</table>
toward the expected date. The urinary FS-substance level of postdate pregnant women was almost equal to that of pregnant women in the 10th month.

Butt et al.\textsuperscript{15} reported by measuring GA that there was FSH activity in the urine of pregnant women, and Imoto\textsuperscript{16} also reported by measuring the same substance that FSH activity could be detected in the urine throughout the whole duration of pregnancy and it was considerably high in the 3rd month of gestation.

Although GA does not always show FSH specificity, it may be assumed that there is FSH secretion during pregnancy. Crooke any Butt\textsuperscript{17} reported that FSH activity was detectable, although its level was low, in six pregnant women during the whole duration of pregnancy. Anyway, it seems that increase of FSH secretion might cause a marked rise in estrogen secretion in the first trimester and serve to maintain a high level of estrogen secretion in the third trimester.

Butt et al.\textsuperscript{15} presumed that the FSH secreting organ during pregnancy was the pituitary. Goss and Lewis\textsuperscript{18} were also of the same opinion based on their finding that FSH activity could not be detected in the urine of hypophysectomized pregnant
women and in placental tissues of normal pregnant women.

From animal experiments, Parlow et al.\textsuperscript{19} reported that FSH concentration in the swine pituitary on the 25th day of gestation was equivalent to that on the 25th day of castration. Greenwald et al.\textsuperscript{20} measured pituitary FSH concentration in the pregnant hamster and reported that it dropped steadily during the first half of pregnancy to reach its lowest value on the 8th day. This is followed by a plateau on the 8th to 14th days of pregnancy, and then by a steady increase to peak values on the 14th day of lactation. During the second half of pregnancy there were twice as many vesicular follicles in the ovary as during the first 8 days of pregnancy. This morphologic change is correlated with the pituitary concentration of FSH during pregnancy. These results indicate that FSH is contained in the pituitary of pregnant animals and secreted from this organ during pregnancy.

As for organs other than the pituitary, Lyon et al.\textsuperscript{21} measured FSH activity in three human placentas on the 61st (one) and the 68th days (two) of gestation and detected slight FSH activity in the placenta of the 61st day. However, he reported at the same time it was possible that the FS-substance was derived from circulating hormone of pituitary origin.

In the present investigation, FS-substance was measured in the placenta at various stages of gestation and was detected in more than half the number of placentas studied. The concentration of placental FS-substance per unit weight of placenta was relatively high during early stages of gestation. However, the total content of the substance in the placenta was small, because the weight of the placenta during these stages was smaller. After the second trimester of gestation, the concentration showed a peak in the 8th month. Since the placenta grew as pregnancy progressed, there were many placentas which contained a considerable amount of the substance.

The rise and fall of the content of placental FS-substance do not always show directly the degree of secretion of this substance from the placenta. However, when the level of FS-substance in the placenta is in parallel with the urinary level of the substance, it may be concluded that FS-substance is secreted predominantly from the anterior pituitary during the first trimester of gestation, and after the second trimester a considerable quantity of this substance is secreted from the placenta.

Little et al.\textsuperscript{22} measured successively urinary estrogen of a pregnant woman who had been hypophysectomized in the 26th week of gestation, and reported that the level of urinary estrogen fell after the operation until the 32nd week of gestation, and then rose in the last weeks and reached the normal level at the date of delivery.

Whether follicle stimulating activity in the urine of pregnant women is an intrinsic capacity of HCG or whether there is really FSH secretion during pregnancy has already been discussed. Albert and Derner\textsuperscript{23} reported follicle stimulating activity of HCG preparations.

Butt et al.\textsuperscript{15} obtained two fractions from the urine: one possessed principally HCG activity and the other principally FSH activity. Tojo et al.\textsuperscript{24} reported that the
fraction with follicle stimulating activity contained in his HCG preparations during the early stages of gestation was higher in concentration than that during the last stages of gestation. Isersky et al. demonstrated that FS-substance contained in his preparations was immunologically similar to that found in human menopausal urine.

In the present experiments, the level of urinary FS-substance reached a peak in the first trimester of gestation. This finding is in agreement with the conventional pattern of urinary HCG level during the first trimester, whereas after the second trimester the pattern of urinary FS-substance differs from that of urinary HCG and also differs from the finding of Tojo et al. that the fraction with follicle stimulating activity decreased in the later stages of gestation.

If FSH secretion independent of HCG secretion exists during pregnancy, the possibility cannot be excluded that FSH is present concomitantly in HCG preparations.

References


