URINARY FSH AND LH EXCRETION FOLLOWING COMBINED TREATMENT WITH NORETHISTERONE ACETATE AND ETHINYL OESTRADIOL* AND NORETHISTERONE** ACETATE ONLY

By

William G. McCormick***, Lars Carlborg and Carl Gemzell

ABSTRACT

The urinary excretions of follicle stimulating hormone (FSH), luteinizing hormone (LH) and pregnanediol were studied for five consecutive months in six young healthy females following combined treatment with norethisterone acetate and ethinyl oestradiol and norethisterone acetate alone. The following effects were observed:

1. The "rebound effect" of gestagen-oestrogen therapy was confirmed and shown to be due to an increase in FSH and not LH secretion.
2. The "rebound effect" was not demonstrated with gestagen therapy alone.
3. Depression of FSH and LH excretion was shown during the cycles in which either a gestagen-oestrogen combination or gestagen alone was given.
4. Loss of midcycle peaks of FSH and LH was also shown during treatment with the gestagen-oestrogen combination and gestagen alone.

* As Anovlar® (Norethisterone Acetate 4 mg and Ethinyl Oestradiol 0.05 mg) Schering A.G., Berlin.
** Norethindrone in the U.S.A. (17a-ethinyl-19-nortestosterone).
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5. A loss of midcycle peaks of LH was also found in the untreated cycles following treatment.
6. A close association of FSH midcycle peak and basal body temperature (BBT) shift was observed.
7. The possibility of random spontaneous ovulation during treatment was inferred from the pregnanediol data.
8. The interaction of FSH and LH is discussed in relation to super-ovulation.
9. The single determination of gonadotrophin has been shown to be of little value clinically.

In the last few years there have been conflicting reports on the effect of »the contraceptive pill« on fertility. Rice-Wray et al. (1963, 1965) and Goldzieher et al. (1962) have shown an increase in fertility after administration of the pill was discontinued. Rice-Wray et al. (1965) found a pregnancy rate of 54% in the first month after cessation of treatment as compared with 34% after cessation of other methods of contraception. Goldzieher et al. (1962) report that 62% of patients become pregnant in the first month after discontinuation of the pill as compared with 34% after cessation of various mechanical means of contraception. Watts et al. (1964) report an increase in the rate of twinning from the usually accepted rate of 1:90 for Caucasians to a rate of 1:22 after cessation of treatment. On the other hand Whitelaw et al. (1966) reported 24 cases of infertility following the use of this type of treatment and further estimates a much higher incidence of infertility in the general population after the pill has been discontinued.

Although combination therapy with gestagen and oestrogen for fertility control has been in use for a number of years, the exact mechanism of their actions is still a matter of debate. Several studies (Loraine et al. 1965; Brown et al. 1958; Taymor 1964) have been reported on the urinary excretion of total gonadotrophins after the use of various gestagen-oestrogen combinations. Stevens et al. (1965) reported on a study where FSH and LH were studied separately.

This paper deals with the follicle stimulating hormone (FSH), luteinizing hormone (LH) and pregnanediol excretion in a group of young, healthy females before, during and after the administration of norethisterone acetate-ethinyl oestradiol combination and norethisterone acetate alone over a continuous period of time.

During the same time period the sialic acid concentration of the cervical mucus, sperm penetration rate and basal body temperature were followed in the same subjects. These results will be reported in a separate paper (Carlborg et al. 1967, to be published).

The results reported here show some effects not yet reported after discontinuation of gestagen-oestrogen combination therapy. These findings will
unfortunately raise more questions than they will answer, but they seem of interest in gaining some insight into the mechanisms of gonadotrophin action.

MATERIAL AND METHODS

The urinary excretions of FSH, LH and pregnanediol were studied for five consecutive months in six young, healthy females between 20 and 22 years of age except for I. L. who was 38 and had an infertility problem. L. J. had been on combination therapy 13 months before this study.

The general plan of the experiment was as follows:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1st month</th>
<th>2nd month</th>
<th>3rd month</th>
<th>4th month</th>
<th>5th month</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment / Anovlar® / No treatment / acetate alone / No treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Treatment started on day 5 of the menstrual cycle and lasted to day 25. Five subjects followed this plan while the sixth subject was treated in the same way for the first three months of the study and was then placed on a regime for forty consecutive days of Anovlar® followed by a control period.

For the entire period of the study each subject collected all her urine in 48 hour pools. Each 48 hour pool from each subject was then individually analyzed for FSH, LH and pregnanediol.

The FSH content of the urine was determined by the Steelman-Pohley ovarian weight HCG augmentation test (Steelman & Pohley 1953) with the modifications described elsewhere (Persson & McCormick, to be published). The gonadotrophins were extracted by a modification of the kaolin-acetone method (Albert 1955) also described in the same article.

A three point assay with five animals (Sprague-Dawley immature female rats 21–23 days old weighing 50–60 g) per point was carried out on each 48 hour specimen from each subject. A total of 418 extractions were performed. One specimen was lost during the experimental period thus resulting in 417 assays. All the extractions were done within two weeks of the time of urine collection and all the assays within three weeks of extraction. The urine was stored in a cold room (+3° C) while waiting for processing.

A human pituitary FSH preparation (FSH-20) was used as a provisional standard (Roos & Gemzell 1964). One microgram FSH-20 is equivalent to 0.022 IU of the 2nd-IRP-HMG standard. (From National Institute for Medical Research, Mill Hill London N. W. 7). FSH (75 μg and 150 μg), HCG (40 IU)* and saline controls were also done for each week's assays.

LH was determined by the immuno-assay method of Wide & Gemzell (1962) on each of the 418 specimens.

Pregnanediol determinations were done on all 48 hour specimens by the method of Goldzieher & Nakamura (1962). Methylene chloride was used instead of chloroform for the extraction (Carstensen 1962).

* as Pregnyl from Pharmacia, Uppsala, Sweden.
A total of 80 ml was removed from each 48 hour specimen for the LH and pregnanediol determinations. These were stored at -20° C until analysis.

RESULTS

The results of this investigation are shown in detail in Figs. 1–6. These Figs. represent the raw data. No statistical analysis of the data was attempted.

Fig. 1.
Urinary excretion of FSH, LH and pregnanediol in Subject P. C. (22 years) during 3 control cycles, one cycle with Anovlar® and one cycle with gestagen (Progestin).

Fig. 2.
Urinary excretion of FSH, LH and pregnanediol in Subject S. S. (21 years) during 3 control cycles, one cycle with Anovlar® and one cycle with gestagen (Progestin).
Fig. 3.
Urinary excretion of FSH, LH and pregnanediol in Subject B.S. (21 years) during 3 control cycles, one cycle with Anovlar® and one cycle with gestagen (Progestin).

Fig. 4.
Urinary excretion of FSH, LH and pregnanediol in Subject L.J. (20 years) during 3 control cycles, one cycle with Anovlar® and one cycle with gestagen (Progestin).

because of the relatively small number of subjects involved and the variability of the data from day to day.

**Follicle Stimulating Hormone (FSH)**

In order to demonstrate the effect of gestagen-oestrogen and gestagen therapy alone, straight line curves of the FSH data were made. The area under these curves was then determined by the use of a planimeter (mechanical
Urinary excretion of FSH, LH and pregnanediol in Subject I. L. (38 years old with an infertility problem) during 3 control cycles, one cycle with Anovlar® and one cycle with gestagen (Progestin).

Urinary excretion of FSH, LH and pregnanediol in Subject M. S. (20 years) during 3 control cycles, one cycle with Anovlar® and a 40 days period of Anovlar® administration.

integrator). Table 1 shows the results of this analysis. The numbers reported are the planimeter readings and have no units. This represents the quantity of FSH excreted over the time periods shown. Day 1–6 means the first 6 days of the cycle with day one taken as the first day of menstruation. Day 1–1 represents an entire cycle from the first day of menstruation to, but not including the next first day of menstruation. The numbers show only relative changes in urine content of FSH and should not be used for any other purpose.

By comparing untreated cycle 1 and 2 before and after Anovlar® therapy,
Table 1.
Planimeter readings of FSH excretion during the first 6 days of the cycle (day 1–6) and the whole cycle (day 1–1) following the administration of Anovlar® and Norethisterone acetate.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Cycle</th>
<th>Untreated (1)</th>
<th>Anovlar®</th>
<th>Untreated (2)</th>
<th>1 mg Norethisterone Ac.</th>
<th>Untreated (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1–6</td>
<td>Day 1–1</td>
<td>Day 1–6</td>
<td>Day 1–1</td>
<td>Day 1–6</td>
<td>Day 1–1</td>
</tr>
<tr>
<td>B. S.</td>
<td>.92</td>
<td>4.13</td>
<td>1.07</td>
<td>6.00</td>
<td>1.15</td>
<td>5.37</td>
</tr>
<tr>
<td>P. C.</td>
<td>1.03</td>
<td>6.40</td>
<td>.64</td>
<td>5.36</td>
<td>2.00</td>
<td>7.33</td>
</tr>
<tr>
<td>S. S.</td>
<td>.70</td>
<td>4.37</td>
<td>1.54</td>
<td>4.49</td>
<td>2.74</td>
<td>8.24</td>
</tr>
<tr>
<td>L. J.</td>
<td>1.64</td>
<td>6.99</td>
<td>1.65</td>
<td>9.10</td>
<td>1.66</td>
<td>6.45</td>
</tr>
<tr>
<td>I. L.</td>
<td>2.27</td>
<td>5.58</td>
<td>.81</td>
<td>3.90</td>
<td>1.06</td>
<td>4.67</td>
</tr>
<tr>
<td>M. S.</td>
<td>1.14</td>
<td>7.05</td>
<td>1.21</td>
<td>4.34</td>
<td>1.34</td>
<td>7.54</td>
</tr>
<tr>
<td>Mean</td>
<td>1.28</td>
<td>5.75</td>
<td>1.15</td>
<td>5.33</td>
<td>1.65</td>
<td>6.60</td>
</tr>
</tbody>
</table>
an increase is found in FSH excretion in all subjects except one (IL) during day 1-6 in the second untreated cycle. An increase in FSH excretion is found during the entire second untreated cycle (day 1-1) in all but two subjects (L.J, IL). These two subjects will be discussed later in more detail, thus in general the «rebound effect» of gestagen-oestrogen therapy has been confirmed for FSH.

Norethisterone acetate alone does not seem to produce this «rebound phenomenon». There are no larger peaks in FSH and the cycle length is shorter following norethisterone acetate alone (28 vs. 25 days average of 5 subjects).

During the cycle on Anovlar® the amount of FSH excreted was decreased in three (PC, IL, MS) and increased in the other three subjects. In each case the curve of excretion was «smoother», indicating a more stable excretion of FSH with less day-to-day variation in secretion. This is also the case during norethisterone acetate administration alone. The midcycle peaks (Table 2) of FSH are not present in any subject during gestagen-oestrogen therapy and only occurred in one subject during gestagen therapy alone (SS). Before therapy all subjects except (IL) had this FSH peak between day 13 and 15. One of the effects of the gestagen in combination therapy would thus seem to be a loss of the midcycle peak of FSH.

**Luteinizing Hormone (LH)**

The changes in LH excretion were more uniform than those in FSH excretion. In the first control cycles four subjects showed a midcycle peak of LH. The two subjects (L.J, IL) who did not show this rise will be discussed later. During gestagen-oestrogen administration one of the subjects (MS) had a LH peak. This was not seen in any of her subsequent cycles. In the following untreated (second) cycle, only one subject (PC) showed a midcycle peak, and even in this subject the rise was much less than that in her control cycle.

During gestagen therapy alone none of the subjects had a midcycle peak of LH. During the last untreated cycle only (PC) and (SS) had a midcycle rise of LH. Two subjects had a rise at the end of this cycle (PC, L.J) two days before the onset of menstruation.

This loss of LH midcycle peak in the untreated post-medication cycles is probably the most interesting finding in this study. Its significance will be considered in more detail later. Again, because this loss of midcycle peak is demonstrated with both combination therapy and norethisterone acetate alone, this effect is attributed to the presence of the gestagen.

**Pregnanediol**

Table 2 shows the day on which the pregnanediol peak was assumed to occur as determined from the data presented in Figs. 1-6. The close correlation between the rise in basal body temperature (BBT) and pregnanediol reported
### Table 2.

Day of Midcycle FSH Peak, Midcycle LH Peak, Pregnanediol Rise and Basal Body Temperature Rise and Cycle Length in days.

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Untreated (1)</th>
<th>Anovlar®</th>
<th>Untreated (2)</th>
<th>1 mg Norethisterone Ac.</th>
<th>Untreated (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FSH² LH² P₂ T C</td>
<td>FSH² LH² P₂ T C</td>
<td>FSH² LH² P₂ T C</td>
<td>FSH² LH² P₂ T C</td>
<td>FSH² LH² P₂ T C</td>
</tr>
<tr>
<td>Subject</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. S.</td>
<td>14 14 20 13 26</td>
<td>– – – – 28</td>
<td>16 – 18 14 29</td>
<td>– – – – 24</td>
<td>– – 15 11 23</td>
</tr>
<tr>
<td>S. S.</td>
<td>15 15 17 17 28</td>
<td>– – 13 26</td>
<td>17 – 23 16 30</td>
<td>12 – 18 30</td>
<td>13 15 13 15 21</td>
</tr>
<tr>
<td>L. J.</td>
<td>15 – 18 – 30</td>
<td>– – – – 29</td>
<td>16 – 17 17 32</td>
<td>– – – – 23</td>
<td>– 23 18 16 25</td>
</tr>
<tr>
<td>I. L.</td>
<td>– – 16 11 27</td>
<td>– – – – 28</td>
<td>17 – 21 17 31</td>
<td>– – 15 26</td>
<td>12 – 16 14 28</td>
</tr>
<tr>
<td>M. S.</td>
<td>15 13 17 x 27</td>
<td>– 13 7 x 28</td>
<td>17 – 24 x 31</td>
<td>– – 39 x 48</td>
<td>17 – 19 x 30</td>
</tr>
</tbody>
</table>

- Not present
- Not done

FSH² – Midcycle FSH Peak
LH² – Midcycle LH Peak
P₂ – Pregnanediol Rise
T – Basal Body Temperature Rise
C – Cycle Length Days

(40 days of Anovlar®)
by Brown et al. (1958) was not found but in each case the temperature rise coincided with or preceded the pregnanediol rise. There was a much closer association between the BBT shift and the second FSH peak in each cycle.

It is interesting to note that two subjects (PC, MS) had a pregnanediol rise with a stable temperature during the Anovlar® cycle. (SS) had a rise in temperature but her pregnanediol remained stable.

During the administration of 1 mg of norethisterone acetate, two subjects (PC, SS) both showed peaks of pregnanediol. Subject (MS) during the period in which she was receiving Anovlar® for 40 days demonstrated a pregnanediol rise on her 39th day (33rd day of drug).

This finding probably represents spontaneous random ovulation. This phenomenon has been reported previously (Rudel et al. 1965; Holmes & Mandl 1962; Rice-Wray et al. 1963).

DISCUSSION AND CONCLUSION

From the data of Table 2 a sequence of events in the majority of patients is noted in the control cycle (untreated cycle No. 1). A primary FSH rise with menses and a secondary midcycle rise in FSH either preceding the midcycle LH rise or coincident with it, and finally 2–6 days after the LH rise, a rise in pregnanediol.

It may be postulated that the primary rise in FSH causes follicle formation. The secondary FSH rise followed by LH then results in follicle rupture and an increase in pregnanediol excretion some days later.

A complex interaction between hypothalamus, pituitary gland and ovaries is necessary to achieve ovulation. Anything that can change this interaction or «desynchronize» it will alter fertility. The reversibility of the physiological interplay is one of the most important considerations to be taken into account when choosing any of these types of drugs. It has not been shown by this study how long it will take any one subject to return to a «normal» pattern of secretion of pituitary and ovarian hormones. The findings in subject (MS), treated with long-term (40 days) therapy, suggest that perhaps long-term therapy is no better or worse than a short-term 28-day cycle as far as pituitary activity is concerned.

The subject (IL) who had a decreased FSH and LH in her control cycle was the oldest subject in this group and also had an infertility problem. Because of the pattern of FSH and LH secretion and the FSH response to the gestagen-oestrogen administration it was postulated that the simple administration of human chorionic gonadotrophin at midcycle should result in pregnancy. This was done and at present the patient is approximately five months pregnant.

The depression of both FSH and LH during therapy with norethisterone
acetate-ethinyl oestradiol has been confirmed. A depression of FSH and LH when 1 mg of norethisterone acetate alone is given has also been demonstrated. The previously reported total gonadotrophin rebound effect after administration of gestagen-oestrogen combination therapy has been confirmed. Further it has been shown that this «rebound phenomenon» is due to an increase in FSH excretion alone and not LH. The depression of gonadotrophin excretion is seen during combined gestagen-oestrogen therapy and gestagen alone. These data tend to confirm that the pituitary depressant effect of «the pill» is due to the presence of the gestagen norethisterone acetate and not to the oestrogenic component of this type of medication. The effect of oestrogen administration on this same parameter must also be done to test this hypothesis fully.

The most intriguing and significant finding is the demonstration of a loss of the LH peak in the cycle following cessation of both norethisterone acetate-ethinyl oestradiol combination and norethisterone acetate therapy alone. This finding was consistent in all the study subjects except (PC) where the height of the peak was markedly decreased. One subject (LJ) had been on combination therapy for 13 months before the study period. One month before beginning the study, the treatment was stopped. Her first control period represents the second month during which she was without treatment. It should be noted that there is still no significant rise in LH in this control cycle.

This finding of suppression of LH after cessation of the «contraceptive pills» raises a question about the role of LH in the ovulation cycle, particularly in the light of the increased fertility and twin rates reported by others after stopping the pill.

It is interesting to speculate on the significance of this finding when discussing the use of human chorionic gonadotrophin in the induction of ovulation (Gemsell 1964; Crooke et al. 1963). Is LH needed? Does the absence of LH contribute to superovulation? Does one find superovulation when HCG is too low, rather than too high, so that the ratio of FSH to LH is not correct for controlled single ovulation?

It is interesting to speculate further about the significance of the absence of a LH peak in the light of the increased fertility and twinning noted by other investigators. Does this mean that there is no need for a midcycle boost of LH but only a high level of total gonadotrophins no matter what they are? Is there a need for a precise ratio of FSH to LH for a single ovulation to occur? All these are questions which will have to be answered before there is a complete understanding of the role of the gonadotrophins in human fertility.

That this loss of the LH peak is the cause of sterility in patients after discontinuation of contraceptive treatment is still another possibility. One might postulate that the patients in this study have «borderline» pituitary function
and that the depression of LH is more long-lasting in these subjects than in the patient with normal pituitary function.

It should be remembered that this finding of depressed LH was a consistent finding in five of six study subjects following both the 4 mg dose and in three of five subjects following the 1 mg gestagen alone. This would lead one to infer that this effect is the result of the gestagen rather than the oestrogen component. One can object the conclusion that 1 mg of gestagen causes inhibition of LH activity on the grounds that the LH effect of the 4 mg dose of norethisterone acetate and ethinyl-oestradiol combination has been shown to last through at least two cycles (subject L.J.). The FSH level on the other hand has been shown to »rebound« dramatically after »the pill« treatment has been terminated, and this too, was inhibited by the 1 mg dose. It will be of some importance to continue this type of long-term, continuous study at still lower doses of gestagen and to follow the patients over longer post treatment control periods.

Some insight into the action of the lower dose will be gained from the sialic acid studies of the cervical mucus reported on these same patients (studied at the same time as these data were collected) and on other patients to be reported at a later date.

An interesting sidelight of this study is that the normal data and those on subject (IL) tend to support the concept that a single determination of FSH on any individual patient is of little or no value in the period between puberty and menopause. This concept will be discussed in more detail in a subsequent paper (McCormick & Persson, to be published).

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