CONTINUOUS MONITORING OF PLASMA
FSH, LH, HCG AND PLACENTA LACTOGEN
DURING HMG-INDUCED OVULATORY CYCLES
AND SUBSEQUENT PREGNANCIES

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ABSTRACT
This study gives a description of the pattern of plasma FSH, LH/HCG, and HPL in four patients with sterility problems suffering from ovarian insufficiency. Plasma values were assayed daily during HMG- and HCG-treatment, in the luteal phase and in the first few weeks of subsequent pregnancy by the dioxane and propanol precipitation radioimmunoassays. From the data obtained the following conclusions may be drawn:
1) HMG-treatment induces no or only a slight increase in plasma LH as compared with normal follicular phases. A cumulative effect does not become evident, probably due to the short half-life of 21 minutes and 4 hours respectively.
2) Injection of 10,000 to 15,000 IU HCG causes a marked increase in plasma level above 200 mU/ml and a subsequent depression in 4 to 5 days, corresponding to the long half-life of 5 and 23 hours respectively.
3) During the five days before the rise in endogenous HCG/LH, values are in the same range or somewhat higher than during HMG-treatment and than in normal luteal phase.
4) Between the 10th and 11th day after HCG injection a very sharp rise in endogenous HCG is observed, reaching 10 IU/ml within 15 to 20 days.
5) FSH levels in the treatment period are augmented above the levels observed in normal follicular phases. Though there is no peak around the time of ovulation, a relative high FSH content can be noted.
6) During the luteal phase FSH-values remain elevated and are higher than in normal cycles. Parallel to the rise in HCG, the FSH falls and is found to be very low during early pregnancy. In one patient with severe ovarian hyperstimulation the FSH concentration was different, with high levels in the first few weeks of pregnancy.

7) Measurable HPL production was first detectable between day 28 and 32 after the ovulatory HCG peak. By the 10th week of gestation this hormone showed a constant increase.

Possible explanations for the differences between the induced and physiological cycles are discussed.

Contrary to numerous data which have been published on plasma FSH and LH in normal menstrual cycles, only few papers deal with gonadotrophin levels in cycles spontaneously followed by pregnancies (Jaffe et al. 1969; Parlow et al. 1970). Furthermore only little is known about the urinary gonadotrophin excretion in pregnancies following induced ovulations by exogenous gonadotrophin therapy (Sele & Starup 1970; Schmidt-Elmendorff 1970). In one patient with irradiation of the pituitary gland Vande Wiele et al. (1970) presented plasma gonadotrophin patterns during HMG treatment and after conception. Following previous observations on FSH and LH/HCG levels in patients with ovarian insufficiency (Czygan et al. 1971) an attempt was made in this study to clarify, whether there are differences between physiological or induced ovarian cycles and pregnancies. This observation therefore may elucidate problems connected with gonadotrophin stimulation.

MATERIAL AND METHODS

Four patients with sterility problems because of ovarian insufficiency were studied. In all cases gonadotrophin therapy was first started after various treatment schedules with other ovulation inducing substances (clomiphene, Sexovid®) had failed.

Patient A. R., 29 years old, had a secondary post partum amenorrhoea for 2 years. The urinary gonadotrophin excretion (mouse uterus test, expressed in terms of IU IRP HMG) was found to be in the normal range. Neither seven treatment cycles with clomiphene and one with Sexovid® nor the first HMG-treatment induced an ovulation. In the second treatment presented here a total dose of 1200 IU HMG (FSH and LH) and 10 000 IU HCG was administered according to the treatment schedule given in Fig. 1.

Patient No. 2, H. M., 31 years old, suffered from anovulatory cycles for 2 years after 2 miscarriages. Clomiphene therapy was followed by one ovulation but no pregnancy resulted. Mobilization of the Fallopian tubes was achieved by removal of peritoneal adhesions. Subsequently the patient was treated for the first time with HMG in a total dose of 1450 IU FSH and LH and 15 000 IU HCG (Fig. 2).

The first menstrual bleeding in the 29 years old patient No. 3 T. W. occurred at the age of 18. She was 151 cm in height, the secondary sex organs developed after treatment with sexual steroids. The total urinary gonadotrophin excretion was 32 and 15 IU/24 hours. The patient did not respond to clomiphene or Sexovid®
Culdoscopy which showed a normal state, was followed by two HMG and HCG treatment cycles, both of which are presented here. In the first one a total dose of 2325 HMG was injected. The patient ovulated but did not become pregnant. In the second treatment cycle she received 1650 IU HMG and 10 000 IU HCG. In this ovulatory cycle, however, the patient conceived (Fig. 3).

Patient No. 4, E.-A. S., 30 years old, had a secondary amenorrhoea since the age of 16. The urinary gonadotrophin excretion was normal with 33 and 19 IU/24 hours. After culdoscopy which displayed a normal anatomy the patient was treated with 1800 IU FSH and LH and 10 000 IU HCG (Fig. 4). During the first 3 weeks after ovulation this patient developed a severe hyperstimulation syndrome.

Subsequently the pregnancies in all cases developed normally and the patients were delivered of healthy children (in patient T. W. twins).

Clinical controls and treatment schedule

In all cases the HMG preparations were injected intramuscularly either in the morning (1 amp./day) or twice, in the morning and in the afternoon, when more than one ampoule was given. The patients were examined daily by observation of the cervical mucus and the basal body temperature. The total urinary oestrogen excretion was measured daily by the method of Brown et al. (1968). The dose, timing, and duration of HMG administration were controlled by these parameters. Heparinized blood samples were drawn every morning. After centrifugation the serum was stored at -22°C until processed.

Radioimmunoassay of FSH and LH/HCG

All the gonadotrophin estimations were carried out according to the dioxane precipitation method of Thomas & Ferin (1968) and Thomas et al. (1969) with minor modifications.

Labelling of antigens

The antigens were labelled with ¹³¹I (IBS 31, The Radiochemical Center, Amersham) according to the chloramine-T-method of Greenwood et al. (1963) with modifications described by Saxena et al. (1969). Purification of the tracer was achieved by gel-filtration using a 10 × 300 mm column of Sephadex G-75. The specific activity of the fraction used ranged between 150 and 250μCi/μg.

Antigens

For the FSH assay a highly purified pituitary FSH (kindly supplied by Dr. Saxena) with a biological activity of 5000 IU/mg and for the LH/HCG-test a preparation of 14 000 IU/mg was used (Batch PTF 10 D supplied by Organon).

Antisera

Because of cross reaction to LH and HCG the FSH antiserum had to be absorbed before use with 12.5 IU HCG/ml of the 1:400 dilution. For testing the samples of early pregnancy with high HCG levels an absorption with 20 IU HCG/ml of 1:400 dilution was necessary. This procedure reduced the cross reaction to less than 4% with HCG at a dose of 10 IU. LH/HCG determinations were carried out with an antiserum (kindly supplied by Dr. Thomas), which had only negligible cross reaction with FSH (less than 5% at the 200 mU FSH level) after absorption with 5 IU FSH/ml of the 1:10 dilution.
Standards

The standards in all the assays were the previously mentioned pituitary FSH antigen and the pituitary Research Standard A (National Institute for Medical Research, Mill Hill, London). The standards are expressed in mU in reference to the II. IRP HMG. The sensitivity was 3 mU/ml for FSH and 2 mU/ml for LH.

Assay procedure

In order to increase the sensitivity and to reduce the blank we used incubation volumes of 300 to 400 μl instead of 200 μl as described originally. After checking various sera, a pooled bovine plasma proved most useful as a control plasma for the standards. As the binding rate in the dioxane assay is greatly dependent on the protein concentration, we paid particular attention to the identical protein content in each test tube. An increased sensitivity was achieved by pre-incubation for 24 hours before adding the tracer, which was followed by a second incubation period of 48 hours. The strictly terminated incubation time of all the tubes is of major importance for comparison with the standard curve. After adding the cold dioxane dilution and rigorous stirring, all the tubes were centrifuged at 2800 g for 30 min. The supernatant was sucked off and the precipitate was measured in an Autowell II (Packard Company) and related to 10 000 counts of the total activity, which facilitated the calculation. All the samples were assayed in duplicate or triplicate at least twice in separate assays. The variation between the values obtained in two such assays was less than 10 %.

Radioimmunoassay for HPL

HPL in the plasma was determined by a recently described method (Thomas, presented at »The International Symposion on Gonadotrophins« in New York, June 1971, in press) i.e. a procedure, which involves the use of iso-propanol as the precipitating agent. The assay is in good agreement with the dioxane method used for the assay of gonadotrophins, except that 66 % (v/v) propanol aqueous solution was used instead of dioxane. In our hands this test proved practicable, precise and reproducible with results comparable to the data described in the literature. The blanks showed less binding with propanol than with dioxane. A HPL preparation (supplied by ICN Nutritional Biochemicals, Cleveland, USA) was used for iodination \(^{131}\text{I}\) as well as for standard dilutions, which are expressed as ng of this substance because of the lack of a reference preparation. The antiserum (kindly supplied by Dr. Keller) did not show any major cross reaction with gonadotrophins. It was used in a final dilution of 1:2000 giving a 40–60 % binding of the labelled hormone. The incubation period of the test tubes without pre-incubation was 72 hours at 4°C. After adding the 66 % propanol dilution and rigorously shaking the samples were centrifuged at 2800 g for 30 min. The subsequent steps are identical with the dioxane assay. All the samples were assayed in duplicate and in two dilutions; the plasma of a non-pregnant subject was added as control plasma.

RESULTS

In Figs. 1–4 the gonadotrophin patterns and the treatment schedules of all patients are documented. In Fig. 1 (pat. A. R.) the LH/HCG level shows high normal values as compared to normal cycles during the treatment period.

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One day after HCG administration a rapid increase reaching a maximum of 260 mU/ml was noted. During the following four days the curve declined. Until day 10 surprisingly high LH plasma values ranging between 17 and 21 mU were found, which are significantly higher than those found before and during treatment. One day 12 after the ovulatory HCG peak a sharp rise of presumably endogenous HCG occurred reaching values up to 10 IU/ml during the next 16 days. The plasma FSH concentration during the treatment period demonstrates an increase to values higher than before the HMG injection. This elevated FSH content persists during the ovulatory and post-ovulatory period with small daily variations. Starting on day 14 a pronounced depression of FSH becomes evident. From day 18 to 21 the values remain low ranging from less than 2 to 15 mU/ml. HPL was first detectable on day 28 after ovulation. The rise in this placental hormone is much slower than that of HCG. It reached values of 1 µg on day 110.

The pattern of FSH and LH/HCG in the plasma of the second patient (H. M., Fig. 2) is essentially comparable to that of the first patient. The values of LH under treatment are relatively high and the typical HCG peak occurs after the injection of HCG. Subsequently a stepwise decrease can be observed.
Plasma FSH (——), LH/HCG (•••), HPL (−−−) pattern, BBT, and HMG therapy schedule in patient H. M. (aged 31 years, anovulatory cycles).

The plasma FSH is elevated to about 40 mU/ml during administration, but a continuous increase while under treatment can not be noted. Despite a moderate decrease in the luteal phase the values are still higher than in normal cycles. In this patient, too, FSH falls to very low values parallel to the rise in HCG on day 10. On day 29 the endogenous HCG is higher than 10 IU/ml. The first measurable HPL secretion starts on day 30 and increases slowly to 150 ng on day 50 which is slower than found in the first patient.

Fig. 3 combines two treatment cycles of patient T. W., the first which revealed ovulation but no pregnancy and the second which was successful in inducing a pregnancy. Although a high HMG dose was administered in the first cycle, there was no significant augmentation of plasma LH. This hormone remains in the range of normal follicular phase values. Unfortunately in this cycle no blood samples were taken in the first two days after HCG injection. A relative low HCG value on the third day after injection was found. Starting on day 8 after the ovulatory HCG peak, plasma LH/HCG was detected in the range of normal luteal phases. In contrast, FSH was elevated during HMG therapy to a level of about 20 to 25 mU/ml. The
Plasma FSH (---), LH/HCG (•••), HPL (- - -) pattern, BBT, and HMG therapy schedule during two treatment cycles in patient T.W. (aged 29 years, secondary amenorrhoea).

Luteal FSH values ranged between 7 and 15 mU/ml which is still higher than in normal cycles. The shape of the gonadotrophin curves in the second treatment cycle was quite comparable to the first one. A moderate increase of LH under treatment was followed by a sharp peak caused by the HCG injection. The luteal LH values, even higher than during the treatment, were followed by the first endogenous HCG increase on day 11. In this treatment, too, the FSH plasma concentration reached values around 20 mU/ml which essentially persisted during the ovulatory and post-ovulatory phase apart from a slow regression. Parallel to the rise in the HCG, the FSH concentration falls to very low and partially not detectable values which lasted at least until the 70th day of gestation. HPL production started on day 31 and showed a moderate increase comparable to that of the other patients.

The plasma LH profile in the 4th patient (E.-A.S., Fig. 4) reflects the patterns of the first 3 patients, although a slight increase during the treatment phase in the days before HCG injection can be noted. From a relatively high LH level between day 6 and 9 after the ovulatory peak endogenous HCG production starts on day 10, after which there is a rapid increase in the following days. The FSH pattern in this patient was markedly different. High values around 25 mU/ml could not only be observed in the treatment and post-
ovulatory phase but also during the first 14 days of pregnancy. Subsequently the FSH decreases, but the values remain still higher, fluctuating around 15 mU/ml in the next few weeks as compared with the levels of patient 1, 2 and 3. The elevation in FSH coincides with the ovarian hyperstimulation. HPL could first be detected on day 32 and the ensuing rise of this hormone in the next two months was slower than in the other patients examined.

**DISCUSSION**

A separate discussion of the gonadotrophin curves during the follicular, ovulatory, luteal and the phase of early pregnancy should be of help. All the four patients were suffering either from secondary amenorrhoea or anovulatory cycles because of normo-gonadotrophic ovarian insufficiency. We therefore were primarily interested in the changes in plasma FSH and LH during daily HMG injections. When compared with the LH values in normal follicular phases, which range between 5 and 15 mU/ml in our assay, the HMG therapy
obviously causes no or only minor changes in the plasma LH content. In two patients (T. W. and A. R.) no elevation could be observed, while in the two other subjects a small increase appeared in the days preceding ovulation. It is remarkable, that the elevation in LH according to the amount of HMG injected, does not seem to be dose-dependent. The weak response of plasma LH to therapy can be explained by the very short half life of this hormone. Yen et al. (1968) described a double exponential curve with a fast \( t^{1/2} \) of 21 min) and a slow component \( t^{1/2} \) of 235 min) for endogenous LH after hypophysectomy. Other investigators (Kohler et al. 1968; Schalch et al. 1968) reported a half life between 30 and 70 min for the exogenously administered hormone. Coble et al. (1969) confirmed these data in metabolic experiments with iodine-labelled FSH and LH preparations. From these results we made conclude, that 12 hours after a daily HMG administration of 225 IU LH or less, only small changes in the plasma are detectable. In contrast to LH, FSH in all the cases investigated showed a distinct increase during HMG treatment as compared with either the levels before therapy or with our values in normal follicular phases (10–16 mU/ml). The half life for exogenous (Kohler et al. 1968; Coble et al. 1969) and for endogenous FSH (Yen et al. 1970) was shown to be much longer than for LH. These investigators described a \( t^{1/2} \) of 3.9 hours and 70.4 hours respectively. The elevation of FSH during gonadotrophin treatment in our patients can well be related to this fact. Nevertheless it remains noteworthy, that FSH rises to a plateau thus indicating no cumulative effect. According to our experimental data we can neither prove nor exclude a possible participation of endogenous gonadotrophins which might account for the elevated plasma levels. This might prove to be an interesting aspect of central regulation. In any case among the cycles described here, the FSH plasma levels proved to be sufficient for stimulating a Graafian follicle. There was no evidence in patient T. W., that an additional elevation in FSH or LH could be regarded as a trigger for the twin pregnancy.

The shape of the gonadotrophin curves during the ovulatory period is characterized by a uniform high level of FSH without any surge like that found during the midcycle peak in normal subjects. But the intramuscular injection of 10,000–15,000 IU HCG causes a very high plasma LH/HCG content when determined 12–16 hours later. Even though an expression of HCG concentration in terms of the LH standard is unusual, it can be stated from the curves, that the induced peak is much higher with more than 200 mU/ml than found in the endogenous ovulatory LH peak. Another difference should be taken into account. Probably effected by the longer half life of HCG, which has been reported to be 11 and 23 hours (Midgley & Jaffe 1968) and 5.6 and 24 hours (Rizkallah et al. 1969) the duration of the high ovulatory HCG concentration is prolonged by 2 to 3 days as compared with the midcycle LH peak. Apart from the qualitative differences between LH and HCG, this
long lasting plasma surge following the formation of the corpus luteum may be responsible for the hyperstimulation syndromes. From the clinical examinations, the basal body temperature, and the plasma steroid patterns it is likely, that in all the cycles studied here, ovulation occurred 12 to 36 hours after the first HCG injection.

In the post-ovulatory period, too, marked differences in our patients can be noted. The data of many authors and our own investigations in normal cycles demonstrate that the mean luteal values of FSH and LH are lower than during the follicular phase. Surprisingly the post-ovulatory FSH level in all the cycles studied here remains elevated with values higher than before HMG treatment or than in normal luteal phases. Similar statements can be made from the plasma LH/HCG. The shape of the curve after HCG injection with the sharp peak and a stepwise decline in the following 6 days corresponds well with the results of Midgley & Jaffe (1968), who described a t \( \frac{1}{2} \) of 32 hours after a single im injection of 5000 IU HCG. As the plasma HCG in this study was less than 10 mU/ml after the sixth day, we assume that it is mainly HCG which is measured until day 6 in our patients. But our findings of increased LH/HCG concentration in the following days until endogenous HCG production starts, remains quite obscure. It is possible that this effect is dependent on a cumulation caused by HMG injections, though the fact that the FSH plateau shows no increase during follicular and a decline during the luteal phase does not support this hypothesis. It is also possible that the metabolic clearance rate in exogenously stimulated cycles is increased as proposed by Sele & Starup (1970). Their results of an elevated urinary gonadotrophin excretion 8–10 days after discontinuing HMG therapy are parallel to our finding of increased plasma FSH and LH in the luteal phase. Though a prolonged binding of gonadotrophins to exogenously stimulated ovaries is conceivable, there are no data up to the present on differences in the gonadotrophin half life of spontaneous and induced cyclees. Another hypothesis to explain the increase of the FSH and LH levels in the luteal phase involves the possible release of endogenous hormones in the pituitary. In this context it is of interest that Vande Wiele et al. (1970) in a patient with irradiation of the pituitary gland, had to substitute the luteal phase with HLH in order to prevent a short luteal phase. They postulate a definite LH level after the ovulatory LH peak for the stimulation of adequate steroid production by the corpus luteum. In contrast all our patients with normal urinary gonadotrophin excretion before treatment, showed a normal luteal function. The plasma LH was increased instead of depressed, thus indicating a possible role of the pituitary secretion. The mean luteal I.H values in the first cycle of patient T.W. followed by menstrual bleeding were found to be lower than in the second cycle, when she became pregnant.

The rise in plasma HCG can be regarded as a first signal for the inception
of pregnancy. On day 10 or 11 in all cases, a rapid increase of immunoreactive endogenous HCG can be observed. The curve is very steep and exceeds the 10 IU/ml level between day 25 and 30. This is in good agreement with the results of Jaffe et al. (1969) and Parlow et al. (1970) in spontaneous pregnancies. Another parallelism to these results is our finding of an FSH depression during the HCG rise in 3 of 4 patients. The observation that in these subjects the plasma FSH falls quickly to very low or not detectable values, can be explained by the negative feedback action of the increasing amounts of steroids produced in early pregnancy. Furthermore the divergent behaviours of FSH and HCG give support for the specificity of the antisera used.

The FSH plasma curve of one patient (E.-A. S.) shows a different behaviour. After the rise in endogenous HCG, the FSH values remain as high as in the follicular and luteal phase until day 25, followed by a slow decrease in the next few weeks to values which are still higher than those found in the three other patients. This is in accordance with Faiman et al. (1969) who described a relatively constant FSH plasma content of about 12 mU/ml until the end of pregnancy. As the unusual FSH elevation during the first 14 days of early pregnancy of our patient was accompanied by a severe hyperstimulation syndrome, a possible retention of this hormone by the ovaries must be taken into consideration.

The first measurable HPL secretion was found to start in all patients between day 28 and 32. When compared with the very rapid rise in the HCG level, the HPL seems to be augmented more slowly. The plasma concentration of one µg/ml is reached around day 100 of gestation. This is in agreement with the curve presented by Keller et al. (1970) thus demonstrating no quantitative difference between spontaneous and induced pregnancies. In patient T. W. with a twin pregnancy neither the increase of HCG nor that of HPL seemed to be more rapid in the first few weeks as compared with the other subjects.

The results of gonadotrophin monitoring in 4 patients treated with HMG and HCG indicate curves qualitatively similar to the physiological events of ovulation and the onset of pregnancy when compared with spontaneous cycles. But when they are analysed in detail, however, there are some differences which cannot fully be explained by the pharmacological treatment. Further investigations concerning the role of endogenous secretion by the pituitary, the HMG induced plasma steroid patterns, and the possible gonadotrophin binding activity of the stimulated ovaries may lead to a more physiological treatment schedule, thus preventing complications due to treatment. Such investigations may also answer questions regarding the regulation mechanisms involved in the normal ovulatory cycle.
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