OVARIAN STIMULATION TEST USING HUMAN MENOPAUSAL GONADOTROPHIN (HMG)

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(Received 21st January 1969)

Summary. A stimulation test involving the administration of HMG has been used to investigate ovarian function in patients with amenorrhoea. A total of twenty-seven subjects was studied and two different test designs were employed. It was concluded that good results were obtained even when the HMG injections were not followed by hCG. With the standard procedure which was eventually adopted, fifteen out of sixteen patients responded satisfactorily.

The ovarian response to HMG was greater in patients in whom pre-treatment gonadotrophin levels were within the normal range. For the same dosage of FSH, HMG o was more potent and had a longer duration of effect than HMG s. HMG o sometimes provoked luteal stimulation but this effect was not noted with HMG s. It is presumed that this difference between the two preparations results from their varying LH content.

INTRODUCTION

In recent years, gonadotrophins of pituitary and urinary origin have been shown to be of value for the treatment of human sterility caused by failure of follicular development and ovulation. So far, however, only a few attempts have been made to develop a standard diagnostic test of ovarian function using these hormones, since the majority of investigators have been especially concerned with the therapeutic use of gonadotrophins and with means of reducing to a minimum the hazards of hyperstimulation and multiple pregnancies. The authors used PMSG (Swyer, Little, Lawrence & Collins, 1968) or hMG (Cox, Cox & Blanck 1966). It should, however, be emphasized that experience with this type of therapy may be unreliable and therefore costly, because the ovaries do not appear to respond identically during successive courses of treatment. Our approach to the use of gonadotrophins in human infertility has been somewhat different from that of other workers and is concerned with the assessment of the anatomical-functional state of the ovary.

The functional status of the ovary is believed to be important, for the difficulty of treating some cases of amenorrhoea is well known. For instance, 'total urinary gonadotrophin' output can be normal in gonadal dysgenesis, and normal signs of puberty associated with several years of apparently normal menstruation is often observed in such cases. Moreover, laparoscopy and ovarian biopsy are not always performed in such individuals and when performed the results of these investigations are not easy to interpret.
A test which could demonstrate whether the ovary is full of quiescent follicles or not, and which would be simple, harmless, clear-cut and reliable, is still needed. We have tried to assess the possibility of developing such a test.

MATERIALS AND METHODS

Tests were performed on two groups of patients. The first group consisted of cases of amenorrhoea with a normal sella turcica, absence of urinary oestrogens and absence of any response to the standard dexamethasone and hCG test. The second group consisted of adolescent girls who required reassurance regarding their amenorrhoea, since they were severely disturbed by the fear of not being able to become pregnant when married. In these cases, the test was partly used therapeutically, by virtue of its beneficial effect on the psychology of the patients.

Altogether, twenty-seven patients have been studied. Fifteen of them had primary amenorrhoea: five had delayed puberty, four were cases of hypogonadotrophic primary amenorrhoea, four showed eugonadotrophic primary or secondary amenorrhoea (probably psychogenic in origin), there was one case of gonadal dysgenesis 46,XX/Xiso-X, and one of Marfan’s syndrome with gonadal dysgenesis and with a chromosomal karyotype of 46,XY. The remaining twelve patients had secondary amenorrhoea which had lasted for more than 2 years. Of these, eight were psychogenic in origin, three were suspected of a premature menopause and there was a case of the Chiari-Frommel syndrome.

In the patients with primary amenorrhoea, the output of ‘total gonadotrophic activity’ measured by the mouse uterus test was normal in six cases, low in seven, high in only one case and fluctuating in one case. In the group of patients with secondary amenorrhoea, the levels were normal in seven cases, high in two, normal or low in two and normal or high in one.

Of eleven patients in whom the chromosomal karyotype was investigated, nine were normal (46,XX); one showed a mosaic of 46,XX/Xiso-X and one was 46,XY. In four other cases, the Barr body was present.

Two kinds of human menopausal gonadotrophin (hMG) were used (hMG s and hMG o); both contained 75 2nd IRP units FSH per ampoule. In hMG s, the FSH/LH ratio is approximately 1/1; in hMG o, the FSH/LH ratio is approximately 1/5.

Two main designs were used in the investigation:

1. The first method was identical to the sequential type of therapy used in the treatment of infertility associated with amenorrhoea. The patient received a daily dose of hMG, sufficient to promote follicle stimulation. She was then injected with 5000 i.u. of hCG, this being repeated 2 to 4 days later; she also received 3 mg of dexamethasone daily during the administration of hCG. Patients were examined at 3-day intervals in order to determine the most suitable dosage of hormone to produce an ovarian response. Such a response was judged initially according to the clinical criteria such as the vulvo-vaginal appearance, abundance and nature of cervical mucus, size of the ovaries, basal body temperature and subsequent menstruation. Additional investigations involved examination of vaginal cytology, endometrial biopsy in the luteal
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phase of the cycle, and urinary steroid assays. The latter assays were performed using the method of Jayle, Scholler & del Pozo (1962) following HMG administration the day after the last injection of HCG. Assays were conducted for oestrone, oestradiol, oestriol, pregnanediol, pregnantriol, androsterone, etiocholanolone and 17-hydroxycorticosteroids.

This design was used in eight patients; six were given HMG s and two HMG o before HCG administration.

The procedure described has the advantage that it allows for the great variability in ovarian sensitivity to stimulation. On the other hand, the procedure has its drawbacks. These are that it is costly and time-consuming for both patient and physician, and also that there is a risk of hyperstimulation. This risk could be greatly diminished by omitting HCG injections and it was because of this danger that we attempted to devise a standardized test to which the majority of patients would respond. The following procedure is the one selected for this purpose.

(2) Five ampoules of HMG were injected daily on Days 1, 4 and 7. According to Crooke, Butt & Bertrand (1966), this total of 15 ampoules is roughly equivalent to 20 to 25 ampoules of human pituitary gonadotrophins (Hpg), being given as seven daily doses. On the other hand, it was found that 20 to 25 ampoules of HMG was the dose which, in patients treated for sterility, had an efficiency of 80% in inducing ovulation.

The response of the ovary to this regimen was assessed by examining cervical mucus, vaginal cytology and the size of the uterus and ovaries on Days 3, 6 and 9 following the start of treatment. Urinary assays of total oestrogens were performed on Day 9 and subsequently after the rise in basal body temperature denoting ovulation.

Three types of ovarian response to HMG were found.

(a) Negative: there was no evidence of oestrogenic effects.

(b) Positive: cervical mucus was ++++, vaginal cytology showed mainly superficial cells, and the excretion of ‘total oestrogens’ was approximately 20 \( \mu g/24 \) hr.

(c) Intermediate (weakly positive): cervical mucus was +, there was a change in vaginal cytology towards the superficial type of cell, and the excretion of urinary oestrogens ranged from 10 to 20 \( \mu g/24 \) hr.

RESULTS

First design

Individual dosages necessary to obtain a positive response by the ovary were found to vary between 9 and 51 ampoules of HMG and 5000 to 16,500 i.u. of HCG. In five patients, there was evidence of ovulation and corpus luteum formation, although two patients did not respond to 35 and 39 ampoules, respectively. However, one of the latter had a precocious menopause and the other was a case of Marfan’s syndrome. One patient received HMG in the absence of HCG and had a purely oestrogenic response.

Second design

Nineteen patients were tested, ten with HMG s and nine with HMG o. Of the
responses, eleven were positive (six with 10 to 15 ampoules HMG s, and five with 15 to 17 ampoules of HMG o). Four responses were of the weakly positive or intermediate type (two with 15 ampoules HMG o, one with 20 ampoules HMG o, and one with 25 ampoules of HMG o). Four of the responses were negative, and in one case, the test was interrupted after the second injection of HMG s due to a febrile reaction. Among the three others, who received 15 to 25 ampoules, two had high excretion values for ‘total gonadotrophic activity’.

In conclusion, it may be considered that fifteen patients out of nineteen responded positively to this treatment. It appears reasonable to eliminate the patient in whom the treatment was interrupted prematurely, and the two women with high endogenous gonadotrophin levels in whom an ovarian response was unlikely. In these fifteen patients, the test (using a dosage of 15 ampoules of HMG) demonstrated the existence of a stock of oocyte-containing follicles that were capable of being stimulated by the treatment administered.

DISCUSSION

With respect to the type of response in the patients studied, it must be pointed out that, among the five women in whom the first test design was used and in whom the test resulted in corpus luteum formation, two showed a slight hyperstimulation. With the second design, when a test was positive there was good agreement between clinical, cytological and biochemical indices of function. In these patients, oestrogen output varied between 20 and 425 µg/24 hr, although the duration of follicular stimulation appeared to be very short as judged by assays carried out on Days 10, 13 and 16. The level of oestrogens returned to normal within 5 days after the last injection, a peak of excretion occurring 1 to 2 days after this injection.

With regard to the relationship between endogenous gonadotrophin levels and ovarian response, the only cases, using the second design, in which no response was obtained, were two women in whom gonadotrophin levels before the treatment were in the menopausal range and one in whom readings were abnormally low. The four weakly positive tests were cases of primary amenorrhoea, two of which were 'hypogonadotrophic', the third showed mosaicism in her karyotype, and the fourth had fluctuating gonadotrophin levels. In contrast, there was a good and clear-cut response in every woman in whom pituitary gonadotrophin excretion before the test was normal. This is probably of importance in view of the fact that with the first design a response could be obtained in three 'hypogonadotrophic' women, only with very large doses of HMG (between 39 and 50 ampoules).

A comparison between HMG s and HMG o revealed significant differences between the effects of the same doses of the hormones on the different indices used to assess response. Although caution is demanded in the interpretation of these results on account of the small number of observations and expected individual differences between patients, it appears that there is a difference between the two preparations in terms of their activity and the duration of the response evoked.

It appears that HMG o induces a more intense stimulation (with a range of 45
to 425 \(\mu g/24\) hr of oestrogen output) than \(\text{HMG}\) s (with a range of 20 to 115 \(\mu g/24\) hr). Moreover, with \(\text{HMG}\) o, two patients had a thermal plateau, and an increase in urinary pregnanediol output followed by menstruation; two others menstruated after the test. With \(\text{HMG}\) s, on the other hand, one patient bled after the test without any sign that a corpus luteum had been formed previously. \(\text{HMG}\) o seemed to stimulate the ovaries for a longer period than \(\text{HMG}\) s, and with \(\text{HMG}\) o rather large quantities of oestrogens were excreted for some time. For example, in three cases high levels of oestrogens were still being excreted on Days 4 and 7 following the last injection. With \(\text{HMG}\) s, however, assays performed on Days 3, 5 and 9 showed a return to basal levels of excretion within 5 days. It is presumed that the longer duration of the effect of \(\text{HMG}\) o, as well as its ability to produce luteal development, is due to its higher content of \(\text{LH}\).

Finally, it should be emphasized that with the first design, hyperstimulation may result. On the other hand, the second design is apparently harmless. \(\text{HMG}\) s induced four febrile reactions, two at the second injection and two owing to faulty resorption of the injected fluid. Other than these, however, no side effects were observed as a result of the injections.

It is therefore concluded that a standardized diagnostic test of ovarian response to \(\text{HMG}\) has proved to be satisfactory. However, there is a great difference between patients in their sensitivity to \(\text{HMG}\). If the test happens to be negative (which occurred once in the study of sixteen patients) several procedures might be adopted. First, a test can be performed with daily injections of \(\text{HMG}\) without \(\text{HCG}\). The difficulty here is to know at what point the test should be stopped if it proves negative. Another approach is to carry out a laparoscopy, or better still a laparotomy, and to perform an ovarian biopsy in order to determine the number of oocytes present in the ovary. It should, however, be pointed out that microscopic observation of a biopsy specimen may not necessarily permit a solution to this problem because of the great heterogeneity of the ovary.

\textit{Note.} Tables of results will be sent by the authors on request.

ACKNOWLEDGMENTS

\(\text{HMG}\) o (Humegon) was kindly supplied by Laboratoire Organon. \(\text{HMG}\) s (Pergonal) was kindly supplied by Laboratoire Clin-Byla.

REFERENCES


