INDUCTION OF OVULATION BY HUMAN GONADOTROPHINS

By

N. Pasetto and G. Montanino

ABSTRACT

The results reported in the literature show that ovulation in cases of primary and secondary amenorrhoea can be induced by treatment with human gonadotrophins. From these reports, it can be concluded that human menopausal gonadotrophins (HMG), as well as preparations from post-mortem human pituitary (HPG), are potent ovarian stimulators. In order to avoid cyst formation, the authors of this paper used smaller doses of HMG and HCG. The human menopausal gonadotrophin used was Pergonal-25 E 35. In 6 cases of primary and secondary amenorrhoea, induced ovulation was followed by two pregnancies.

The capacity of various human gonadotrophins to stimulate human ovaries has been shown by many investigators. Gemzell et al. (1958) reported that treatment with human pituitary gonadotrophic extracts (HPG), followed by HCG, produced ovulation, in 4 out of 5 patients, accompanied by a remarkable increase in urinary oestrogen and pregnanediol excretion.

The first pregnancy obtained by this treatment was reported by Gemzell et al. (1960). Buxton & Herrmann (1960, 1961) also reported the induction of ovulation in 6 out of 7 patients treated with human pituitary FSH and HCG.

Similar studies were carried out by Gemzell (1962), Crooke (1962) and Bettendorf et al. (1962), while Lunenfeld et al. (1962 a, b) reported three pregnancies obtained with the combined treatment of HMG (human menopausal gonadotrophins) and HCG.

Staemmler (1961), Palmer & Dorangeon (1962), and Rosemberg et al. (1962) demonstrated the effectiveness of human urinary gonadotrophins in conjunction with HCG.
However, the scarcity of post-mortem human pituitary glands required for the preparation of HPG, eliminates the possibility of a wide-scale use of this treatment. Hence, attention was directed to the extraction of gonadotrophins from postmenopausal urine.

We have tested the effectiveness of treatment with HMG and HCG in anovulation, in 2 cases of primary amenorrhoea and 4 cases of secondary amenorrhoea. The effectiveness of this treatment was determined by the oestrogen and pregnanediol elimination pattern, by the basal temperature, and by the pregnancies that occurred in 2 cases.

The dosage schedule differed from that used by other investigators both in the amount of HMG and HCG used and in the form of administration. At first, we followed the schedule suggested by Lumenfeld et al. (1962 a) and obtained, in one pregnancy, the formation of a luteo-follicular cyst, with rupture, haemoperitoneum, and interruption of pregnancy. We then used successively smaller doses and obtained good results.

At present, our patients include 14 cases of amenorrhoea, from which we have drawn the 6 cases described in this report. In other cases, we obtained bleeding after 10-14 days, following withdrawal of treatment, but no sign of ovulation. It must be emphasized that 5 patients had primary amenorrhoea, and that a particular treatment schedule was used, based on a small amount of HMG and HCG, in order to avoid cyst formation.

MATERIALS AND METHODS

Pergonal – 25 E 35 was the human menopausal gonadotrophin used.

The biological activity of each ampoule was the following:
1) Total gonadotrophic activity (mouse uterus test): 83 mg-eq. of HMG-IRP.
2) FSH activity (augmentation test): 414 mg-eq. of HMG-IRP.
3) LH activity (ventral prostate weight in hypophysectomized rats): 134 mg-eq. of HMG-IRP.

The chorionic gonadotrophin was a common, commercially available preparation. Hormonal excretion were determined in each case, prior to and after treatment. Total urinary gonadotrophins were assayed according to Borth et al. (1961).

Oestrogens (oestrone, oestradiol-17β and oestriol) were estimated according to Brown (1955). Pregnanediol was assayed according to Klopper et al. (1955).

17-kctosteroids were assayed by means of the micro-method described by Vestergaard (1951).

RESULTS

1) C. B., 28 years old, married, secondary amenorrhoea and galactorrhoea for 5 years.

Urinary gonadotrophins (mouse uterus) were 6.4 mg-eq. of IRP. Total
oestrogens were 12 μg/24 h and pregnanediol 1.47 mg/24 h; 17-ketosteroids were 10 mg/24 h. Endometrial biopsy revealed an atrophic endometrium. A very intense ovarian response followed treatment with HMG-HCG, as is seen in Fig. 1. We can assume that the high level of urinary oestrogens and pregnanediol was due to cyst formation. In fact, 20 days following the beginning of therapy, a bilateral enlargement of the ovaries was observed. Pregnancy occurred in this patient and was ascertained by means of the Galli-Mainini pregnancy test and the haemagglutination inhibition pregnancy test.

On the 44th day after the introduction of treatment, rupture of a cyst with haemoperitoneum occurred; laparotomy performed immediately confirmed the diagnosis, and a partial bilateral ovariectomy was performed. 42 days after the beginning of treatment, protective therapy was started with progesterone by the intramuscular route; this was intensified up to 200 mg/d in the following days. In spite of this, abortion occurred on the 52nd day.

2) Q. L., 32 years old, married, secondary amenorrhoea for 9 years.

Urinary gonadotrophins (mouse uterus) were 3.6 mg-eq. of IRP. Total oestrogens were 8.1 μg/24 h, pregnanediol was 2 mg/24 h and 17-ketosteroids were 7 mg/24 h. Endometrial biopsy revealed an atrophic endometrium. The
Q. L. 32 y. SECONDARY AMENORRHOEA

B. TEMP. (°C)

PREGNANEDIOL
mg / 24 h

TOTAL OESTROGEN
µg / 24 h

DAYS

COITUS

BLEEDING

0

5

10

15

20

25

30

Fig. 2.

S. V. 18 y. PRIMARY AMENORRHOEA

B. TEMP. (°C)

PREGNANEDIOL
mg / 24 h

TOTAL OESTROGEN
µg / 24 h

DAYS

COITUS

BLEEDING

0

5

10

15

20

25

30

Fig. 3.
treatment induced a significant ovarian response with an increase in oestrogens (up to 200 µg/24 h) and in pregnanediol (7 mg/24 h). The basal temperature curve showed a biphasic slope.

On the 27th day of the treatment, bleeding occurred and lasted for 4 days (Fig. 2).

3) S. V., 18 years old, unmarried, primary amenorrhoea.

Urinary gonadotrophins (mouse uterus) were not detectable. Total oestrogens were 3 µg/24 h, pregnanediol was 1.1 mg/24h, and 17-ketosteroids were 4.8 mg/24 h. Determination of chromatine sex, performed on an oral smear, gave a positive Barr’s chromatine.

There was a good ovarian response to treatment, with an increase in oestrogens, pregnanediol and basal temperature. Bleeding lasting 4 days occurred 25 days after the introduction of treatment (Fig. 3).

4) M. M., 21 years old, unmarried, primary amenorrhoea.

Urinary gonadotrophins were 8 mg-eq. of IRP. Total oestrogens were 6 µg/24 h, pregnanediol 0.57 mg/24 h, and 17-ketosteroids 4 mg/24 h.

The determination of sex chromatin, performed on an oral smear, showed a positive Barr’s chromatin.

There was an intense ovarian response to treatment, as shown by the steady
increase in oestrogens, and subsequently in pregnanediol. On the 14th day of treatment, an increase in basal temperature occurred. Menstruation lasting for 5 days began 11 days after the cessation of treatment (Fig. 4).

5) P. F., 33 years old, married, secondary amenorrhoea for 3 years, diabetes. There were no detectable urinary gonadotrophins. Total oestrogens were 20 µg, pregnanediol was 1.84 mg/24 h. and 17-ketosteroids were 6 mg/24 h. Endometrial biopsy revealed an atrophic endometrium. A good ovarian response to the treatment resulted, with gradual increase in oestrogens and subsequently in pregnanediol. The basal temperature curve was biphasic. A biopsy, performed 8 days after the interruption of therapy, showed a scanty endometrium with signs of the secretory type. Bleeding did not occur, probably because of the scanty endometrial layer (Fig. 5).

6) P. C., 27 years old, married, secondary amenorrhoea for 1 year. Gonadotrophins were 7 mg-eq. of IRP. Total oestrogens were 7 µg/24 h, pregnanediol was 0.97 mg/24 h and 17-ketosteroids 7.5 mg/24 h. Biopsy revealed an atrophic endometrium. There was good ovarian response to treatment, with gradual and steady increase in oestrogens and pregnanediol. The basal temperature showed a biphasic curve. Since bleeding did not occur for 18 days after the interruption of treatment, an immunologic pregnancy test
was performed, which gave positive result. At the time when the final copy of this report was being prepared, the patient gave birth to a healthy baby girl weighing three and a half kilogram (Fig. 6).

**DISCUSSION**

Our results prove that ovulation can be induced, in cases of dysfunctional amenorrhoea (primary and secondary), by combined treatment with HMG and HCG.

In the 6 cases reported in this paper, the gonadotrophic pituitary function was normal in 3 cases (1–4–6) and below normal in 3 cases (2–3–5).

Urinary oestrogen excretions was below normal levels in 5 cases. Urinary pregnanediol excretion was limited to the basal values in all cases.

The cases of primary amenorrhoea were tested for chromatin sex with positive results. The basal temperature was monophasic in all cases.

Histological examination of the endometrium, performed on the married patients only (cases 1–2–5–6), revealed an atrophic endometrium.

A control of ovulation was performed by observation of the basal tempera-
ture, pregnanediol and oestrogen excretion. The histological control of the endometrium was performed only in case number 5. In the others (1–2–6) it was not performed for fear of interrupting a possible pregnancy. We wish to emphasize that, since we started using this reduced dosage schedule, we have been carrying out the dynamic ovarian function exploration before starting treatment.

With regard to the dosage and treatment schedule, our cases must be divided into two groups, i.e. group 1 (cases 1 and 2) and group 2 (cases 3, 4, 5 and 6).

The first group received:

a) HMG (Pergonal): a total of 10 764 FSH mg-eq. of HMG-IRP for 13 days.

b) HCG: in a total dose of 25 000 IU for 3 days.

The second group received:

a) HMG (Pergonal): in a total dose ranging from 4140 to 10 764 FSH mg-eq. of HMG-IRP, for 13–14 days.

b) HCG: in a total dose ranging from 4500 to 10 000 IU for 3 days.

Even though the minimum amount of HMG (414 FSH mg-eq. of HMG-IRP/d) is lower than the amount used by other investigators (Lunenfeld et al. 1962 a), the ovarian stimulation is quite clear. With this treatment side effects and the danger of cyst formation are eliminated.

In 6 cases in which ovulation occurred, two became pregnant, two were unmarried (patients 1 and 4) and in one the husband was absent (patient 5).

Our results thus confirm that HMG can produce ovarian stimulation and, with the addition of HCG, can induce ovulation in patients with primary or secondary amenorrhoea.

ACKNOWLEDGEMENT

We wish to thank Dr. P. Donini, Istituto Farmacologico Serono, Rome, for supplying the Pergonal – 25 E 35 which was used in these experiments.

REFERENCES


Received on February 10th, 1964.