TREATMENT OF AMENORRHOEA WITH MENOPAUSAL URINARY GONADOTROPHIN EVALUATED BY THIN-LAYER CHROMATOGRAPHIC DETERMINATION OF PREGNANEDIOL IN THE URINE

By

Helge G. Berthelsen and H. O. Bang

ABSTRACT

Three young amenorrhoeic hypogonadal women, aged 19–24 years, all nulliparous, with a monophasic basal-temperature curve for at least 2 months, a low hypotrophic vaginal cytology, and a constantly low pregnanediol excretion were treated with an intramuscular injection of human menopausal urinary gonadotrophin (HMG) for ten days, followed by daily injections of chorionic gonadotrophin for four days. All the patients had previously been treated unsuccessfully with chorionic gonadotrophin. The clinical effect was checked by daily thin-layer-chromatographic determination of the pregnanediol excretion in the morning urine, vaginal cytology, and daily recording of the basal temperature. Vaginal examination was done every day. One patient exhibited enlargement of the ovaries during the treatment, but apart from that there were no side effects, in particular no abnormal increase in basal temperature and no local reactions in the treated patients. All the patients developed vaginal bleeding following an increase of the pregnanediol excretion. One woman conceived.

The follicle-stimulating effect of pregnant mare’s serum gonadotrophin has proved insufficient in human subjects, mainly because of the antigenic property of the hormone (Østergaard 1942, 1964). Gemzell et al. (1958) have demonstrated the follicle-stimulating effect of gonadotrophin extracted from human hypophyses. This has been substantiated by a number of investigators (Bettedorf 1964; Crooke et al. 1963; Diczfalusy et al. 1964; Gemzell 1962).
Owing to the difficulty of obtaining sufficient material for producing the hormone from human hypophyses, Lunenfeld et al. (1961) have used Pergonal® prepared by Donini & Marchetti (1952), a follicle-stimulating hormone from the urine of post-menopausal women (HMG). The clinical use of this hormone has been demonstrated by several investigators (Crooke et al. 1963; Diczfalusy et al. 1964; Lunenfeld et al. 1961; Rosenberg & Engel 1961).

For assessing the effect of such stimulatory treatment on amenorrhoeic women, it is essential to detect ovulation. The methods of detecting ovulation have hitherto been unreliable, and in particular premenstrual endometrial biopsy cannot be carried out in patients under treatment for sterility. Determination of the urinary excretion of pregnanediol daily or every second day has been shown to be an adequate method for ascertaining whether ovulation has taken place (Berthelsen & Bang 1965).

MATERIAL AND METHOD

3 young women, previously treated with chorionic gonadotrophin without effect, were treated with HMG as a follicle-stimulating principle. The preparation was made by Leo Pharmaceutical Products, Copenhagen, and contains 50–60 international FSH units per ampoule. The ratio between the amount of the preparation that increases the ovarian weight in infantile female rats up to 50 milligrams, and the amount that increases the uterine weight in female rats up to 50 mg (Johnsen & Hamburger 1959), was 2.8–2.9. The dosage was 150–180 international FSH units daily for 10 days, followed by chorionic gonadotrophin (Physex®) 6000 IU daily for 4 days.

Before treatment the patients had x-ray examination of the sella turcica and the excretion of 17-ketogenic and fractionated 17-ketosteroids were determined. Smears of oral mucosa were examined for chromatin content. These findings were normal. The rectal temperature curves were found to be monophasic and the vaginal cytology was of a low hypotrophic type. The urinary excretion of pituitary gonadotrophin was abnormally low in all 3 cases.

During treatment, the patients were checked daily by vaginal palpation. The pregnanediol excretion in the urine was determined daily by means of thin-layer chromatography as described by Bang (1964). In 54 double determinations the coefficient of variance was found to be 7.7 per cent.

Owing to the practical difficulties in collecting the entire 24-hours' urine from out-patients, all the pregnanediol determinations were performed on morning urine, i.e. the total amount of urine voided in the morning after the patients had not voided for about 8 hours (Wide 1962), and the results were expressed as the absolute amount of pregnanediol in milligrams found in the morning urine.

In 11 normally menstruating women, aged 21–37 years, the pregnanediol excretion in the morning urine was determined every day of a cycle.

Postmenstrually, but before ovulation, the pregnanediol excretion was low, as a rule less than 0.5 mg in the morning urine, and after ovulation there was a marked increase, often more than 0.7 mg. Immediately before the next menstrual period the level fell to the preovulatory value. It was found that in all ovulatory cycles the curve ran a biphasic course, but the level of the individual curves varied somewhat.
In order to obtain a reliable evaluation of whether ovulation has taken place, it is necessary – because of the fairly marked individual variations of pregnanediol excretion – to perform pregnanediol analyses every day or at least every other day for 4 weeks (Berthelsen & Bang 1965).

CASE REPORTS

450331. J: A single woman, aged 19, with primary amenorrhoea. Secondary sex characters not developed. Height 159 cm. Weight 52.2 kg. Gynaecological examination showed the uterus to be the size of a hazel nut. The ovaries were unpalpable. X-ray examination of the hands showed an osseous development corresponding to 13 years. Urinary gonadotrophin excretion: <3 M.U.U./d. B.M.R. −10 per cent and −5 per cent. Protein-bound iodine: 6.4 μg/100 ml. Exploratory laparotomy: Uterus 1.5 cm × 2 cm, tubes slender, but normal. Both ovaries less than pea size and polycystic. No corpus luteum.

The patient was treated with HMG 180 internat. FSH units for 10 days, followed by chorionic gonadotrophin 6000 IU for 4 days. The treatment was well tolerated. During treatment there was slight growth of the breasts and uterus, and 23 days after the first injection she had vaginal bleeding for 5 days. Fig. 1 shows the pregnanediol excretion during the treatment.

391220. M: A single woman, aged 24, with secondary amenorrhoea of 2 years' duration. Height 169 cm, weight 57.5 kg. Secondary sex characters normal. Gynaecological examination: Uterus small, virginal, ovaries not enlarged. B.M.R. +10 percent and

![Fig. 1.](image)

Pregnanediol excretion in mg in morning urine before, during and after HMG + chorionic gonadotrophin therapy. M = vaginal bleeding. ↑ days of HMG administration. p = days of Physex administration. (Case 450331. J).
5 per cent. Protein-bound iodine 4.9 μg/100 ml. Urinary gonadotrophin excretion: < 4 M.U.U./d.

The dosage of HMG was 150 international FSH units. The treatment was well tolerated. 26 days after its institution, she had vaginal bleeding for 4 days. Fig. 2 shows the pregnanediol excretion and the temperature curve during the treatment.

400424. C: A married woman, aged 24, with secondary amenorrhea of 5 years' duration. Primary sterility for 5 years. Owing to hypothyroidism she had received thyroid hormone for 4 years. Height 148 cm, weight 38.3 kg. Secondary sex characters normal. Uterus infantile, ovaries not enlarged. X-ray examination of the hands: Osseous development corresponding to her age. Urinary gonadotrophin excretion: < 4 M.U.U./d. B.M.R. -15 per cent. Protein-bound iodine 5.1-7.0 μg/100 ml. Metopirone-corticotrophin tests normal.

The patient was treated with HMG 180 international FSH units daily for ten days. During this treatment the ovaries increased in size, and at the completion of this treatment, both ovaries were 1½ times the normal size. During treatment with chorionic gonadotrophin (6000 IU) their size doubled. Three days after the conclusion of treatment the patient complained of pelvic pain. Both ovaries were now the size of oranges and very tender. Otherwise her condition was unaffected, and there was no peritoneal reaction. There was no abnormal increase in temperature. Three weeks after the onset of the symptoms both adnexa were normal to palpation. The patient was able to have sexual intercourse during the chorionic gonadotrophin therapy and for 3 days thereafter. Fig. 3 gives the results of the vaginal cytological study, the recording of the basal temperature, and the pregnanediol excretion during the treatment. No vaginal bleeding occurred, and 5 weeks after the institution of the treatment, pregnancy was demonstrated.

![Fig. 2.](image)
Pregnanediol excretion in mg in morning urine (---) and rectal temperature (x-----x) before, during, and after HMG + chorionic gonadotrophin therapy. M = vaginal bleeding. † and p as in Fig. 1. (Case 391220. M).
Fig. 3.
Pregnanediol excretion in mg in morning urine (\(\cdot\)\(\cdot\)\(\cdot\), rectal temperature (x\(\cdot\)x), and vaginal cytology (acidophilic index) (0\(\cdot\)0) before, during, and after HMG + chorionic gonadotrophin therapy. ↑ and ↓ as in Fig. 1. (Case 400424. C).

**DISCUSSION**

In many gynaecological cases, a reliable method of detecting ovulation is essential. Measurement of rectal temperature and examination of vaginal cytology are the methods most commonly used. As the urinary excretion of pregnanediol follows the production of corpus luteum hormone to some extent, the quantitative determination of this excretion may be used as a method of detecting ovulation. Because of the rather large individual variation in pregnanediol excretion, frequent determinations of pregnanediol – preferable daily or every second day – during the menstrual cycle is necessary. The simple and rapid thin-layer chromatographic method of Bang (1964) makes this analysis valuable as a reliable method of detecting ovulation.

The present investigations have shown that administration of pituitary gonadotrophin from human postmenopausal urine was able to replace the deficient pituitary gonadotrophic activity in 3 young amenorrhoeic women. Immediately after the institution of the treatment, a previously low, hypotrophic vaginal cytology changed to high, acidophilic type, indicating follicular stimulation. Furthermore, the treatment made it possible to alter the sensitivity of the ovaries to chorionic gonadotrophin so that this hormone could induce a change in the basal temperature and the pregnanediol excretion as well as cause vaginal bleeding, presumably due to the formation of a corpus luteum.
At least in one case a corpus luteum was formed, since the patient became pregnant. Except for this one patient, in whom ovarian enlargement was demonstrated, presumably because of excessive stimulation, the treatment did not cause any side effects. The complication mentioned stresses the significance of daily vaginal palpation during the treatment. The two other patients deliberately avoided conception during and after the hormone administration.

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