THE OVARIAN RESPONSE IN WOMEN TO LARGE SINGLE INJECTIONS OF HUMAN MENOPAUSAL GONADOTROPHIN AND HUMAN CHORIONIC GONADOTROPHIN

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Summary. A large single dose (3000 i.u.) of human menopausal gonadotrophin (HMG) was injected subcutaneously into each of twelve women. Seven days later, 19,500 i.u. human chorionic gonadotrophin was injected intramuscularly and laparotomy was performed 48 to 96 hr later. The response of the ovaries was determined by assaying urinary oestrogen and pregnanediol, by inspecting the ovaries, and by examining histological sections from multiple ovarian biopsies.

Eight of nine women, who were currently having normal menstrual cycles, showed evidence of ovulation, the number of corpora lutea varying from one to seven. In three women, who received injections of HMG on the 14th day of a normal puerperium, there was no evidence of ovulation or of a significant increase in steroidogenesis.

INTRODUCTION

Numerous workers have treated infertility successfully by inducing ovulation with human follicle stimulating hormone (FSH) obtained from pituitary glands or post-menopausal urine, since the report by Gemzell, Diczfalussy & Tillinger (1958). Despite a variety of regimens for administration of the gonadotrophins, the inadvertent production of multiple pregnancies remains a problem. Attempts to control this problem by regulating the dosage of FSH have been based on the assessment of vaginal cytology, cervical mucus (Wiele & Turksoy, 1965), retrospective assay of urinary steroids (Gemzell *et al.*, 1958) and the predetermination of individual sensitivity (Crooke, Butt & Bertrand, 1966a). Such practices, even including the daily adjustment of dose by concurrent oestrogen assay, have been only partially successful in reducing the incidence of multiple pregnancies (Brown, Evans, Adey, Taft & Townsend, 1969). All such studies have had the disadvantage of having to employ indirect means in assessing the incidence and degree of multiple ovulation.

The intensity of supervision required in multiple injection regimens has limited the number of patients treated because of geographical considerations and the availability of suitable laboratory facilities.

This study was designed to investigate whether multiple ovulation occurs after single injections of human menopausal gonadotrophin (HMG) using more direct observation than is available during therapy and thereby to assess the suitability of single-injection therapy for use with less stringent supervision than is required for multiple-injection regimens.

MATERIALS AND METHODS

The twelve women in the study were volunteers, aged from 22 to 35 years, who were to undergo laparotomy for incidental reasons. Nine of the women were menstruating regularly and spontaneously. Three women were at the 14th day of a normal puerperium, following a normal term pregnancy and delivery. The three puerperal women were not lactating and had not received any steroid therapy during the 7 days before the first injection.

The HMG (Humegon, Organon Laboratories) contained FSH and luteinizing hormone (LH) in a ratio of 1 i.u. : 3 to 10 i.u. respectively. A dose of HMG equivalent to 3000 i.u. FSH was suspended in 8 ml high molecular weight dextran and injected subcutaneously either on the 2nd day of a menstrual period in those women who were having periods or on the 14th day of the puerperium in the three women who had been recently delivered. Seven days after each initial injection, 19,500 i.u. human chorionic gonadotrophin (HCG-Pregnyl, Organon) were injected intramuscularly and laparotomy was performed 48 to 96 hr later.

Urinary oestrone, oestradiol and oestriol were measured according to the method of Brown (1955) and urinary pregnanediol by the method of Klopper, Michie & Brown (1955) in 24-hr specimens collected during and after the treatment.

Pelvic and abdominal examinations were performed on all women before each injection to detect the presence of excess peritoneal fluid and to detect changes in the size or tenderness of the ovaries. At laparotomy, the ovaries were inspected and note taken of the number and nature of any cysts present. Those cysts showing a marked vascularity, haemorrhagic contents or recent rupture were classed as corpora lutea (CL). Multiple, small biopsies were taken of the cysts from each ovary and examined histologically. Those cysts showing luteinization of the granulosa cells were classed as CL.

RESULTS

In one woman just before the injection of HCG, the ovaries were noted to have enlarged to approximately three times their original size. These ovaries became further enlarged to 10×6×6 cm by 72 hr after the HCG injection. Ovarian enlargement before the HCG injection was not detected in the other women and no woman experienced pain or produced a detectable excess of peritoneal fluid.

The nine non-puerperal women all had enlarged ovaries containing multiple, small ovarian cysts (up to 15 mm in diameter). The size of the ovaries varied from approximately 50% greater than normal to a gross enlargement in the case of one woman (H.A.B.). The ovaries of another woman (M.P.) measured

TABLE 1
URINARY STEROIDS

Patient	Total oestrogens ($\mu\text{g}/24\text{ hr}$)			Pregnanediol ($\mu\text{g}/24\text{ hr}$)
	Day 0 to 1	Day 7 to 8	Day 13 to 14	Day 13 to 14
Regular menstrual cycles				
P.G.	15	101	71	4.1
M.L.	27	218	—	—
M.S.	18	125	45	5.1
M.P.	26	173	108	13.0
S.L.	8	267	62	3.3
M.B.	17	51	30	1.6
H.A.B.	17	392	417	19.5
J.W.	8	143	82	2.4
L.W.	16	72	41	3.1
Puerperal				
T.H.	23	42	22	0.4
B.D.	11	9	6	0.3
J.C.	24	27	13	0.5

An injection of HMG containing 3000 i.u. FSH given on Day 1 followed by 19,500 i.u. HCG on Day 8.

Total oestrogen excretion from patient H.A.B. = 720 $\mu\text{g}/\text{hr}$ on Days 9–10.

TEXT-FIG. 1. Urinary total oestrogen and pregnanediol in a cycle in which a single CL was produced after a single injection of HMG followed by HCG.

5 × 4 × 4 cm and contained four CL. Aspiration of the peritoneal cavity yielded 250 ml of pink-stained fluid from H.A.B. and 200 ml from M.P.

The urinary total oestrogen levels in the nine women with normal cycles rose to 51 to 392 µg/24 hr on the 7th day after the HMG injection (Table 1). In general, this was followed by a fall with a subsequent rise in all except one woman (M.B.), whose ovaries showed no definite CL formation. The 7th-day level of 218 µg from patient M.L. was followed by the production of two CL, whereas M.P. produced four CL after an oestrogen excretion of 173 µg. The five women with single ovulations had urinary pregnanediol levels from 2.4 to 5.1 mg/24 hr on the 6th day after the HCG (Text-fig. 1), whereas those cycles producing four and seven CL had pregnanediol levels of 13.0 and 19.5 mg/24 hr, respectively.

TABLE 2
ESTIMATED NUMBER OF CORPORA LUTEA

Patient	Estimated number of corpora lutea based on:		
	Urinary pregnanediol	Inspection	Histology
Regular menstrual cycles			
P.G.	1	1	1
M.L.	—	1	2
M.S.	1	1	1
M.P.	Multiple	4 to 5	4
S.L.	1	1	1
M.B.	0	0	0
H.A.B.	Multiple	3 to 4	7
J.W.	1	1	1
I.W.	1	1	1
Puerperal			
T.H.	0	0	0
B.D.	0	0	0
J.C.	0	0	0

Urinary pregnanediol of 2 to 7 mg/24 hr 6 days after the HCG injection was considered to indicate the presence of one CL.

Marked vascularity, haemorrhagic contents or signs of recent rupture noted on inspection of a cyst were regarded as evidence of recent CL formation.

Cysts showing luteinization of the granulosa cells were classed as CL.

Two of the three puerperal women showed no significant change in the urinary excretion of total oestrogen while patient T.H. had a rise from the pre-treatment level of 23 µg/24 hr to 42 µg/24 hr on the 7th day of treatment. There was no significant change in pregnanediol excretion throughout the cycles of treatment in the three puerperal women.

The ovaries of the three puerperal women contained no visible cysts greater than 3 mm in diameter, were normal in size, had a flattened contour and presented the general appearance of quiescence.

Theca luteinization was present in most of the biopsy specimens. Luteinization of the granulosa cells representing definite CL formation was apparent in eight women (Table 2), but in M.B., the granulosa cells had an atypical appearance, suggesting either an atypical CL or failure of ovulation.

DISCUSSION

The reason for multiple ovulation in women treated with gonadotrophins is still uncertain and the true incidence of multiple ovulation is difficult to determine. The pregnanediol excretion from one CL 6 days after ovulation may vary between 2 and 7 mg/24 hr (Klopper, 1957) and can, therefore, provide only an approximate indication of the number of ova released (Crooke, 1964). This is indicated by the two women producing four and seven eggs in this trial. Moreover, since not all the eggs released will necessarily be fertilized, the number of foetuses formed can provide only a rough indication of the number of ovulations.

It has been postulated that, in the normally ovulating woman, some primordial follicles change each month so that they become capable of responding to FSH from the anterior pituitary gland (Eckstein, 1962). These changed primordial follicles thus form Graafian follicles. During subsequent maturation over the next 7 to 10 days, usually all but one follicle become atretic leaving that one follicle to go on to maturity and to ovulate in response to the surge of LH. It has been considered probable that there is an inherent mechanism, governed by the steroid production from the developing follicles themselves, which selects the single follicle for maturation.

This trial with HMG was designed to test, by direct observation, the effect of a single large stimulus on this natural process of selection.

Crooke, Butt & Bertrand (1966b) reported that several women became pregnant during sensitivity tests using single injections of human pituitary gonadotrophin (HPG). Crooke *et al.* (1966b) found that a dose of FSH was about 50% more effective when given as a single injection than when divided into three equal injections. Daume & Kaiser (1965) induced ovulation in seven amenorrhoeic women with HMG (Humegon) using 858 to 2860 i.u. in divided doses, followed by HCG. Thus, a single dose of HMG containing 600 to 1800 i.u. FSH might be expected to be similarly effective. The dose of FSH (3000 i.u.) in the first injection in this trial was therefore equivalent to one and a half to five times the dose used by Daume & Kaiser and was greater than that employed in seven out of eight women treated by Crooke *et al.* (1966b) using single doses of HPG. This dose was deliberately excessive in order to determine whether the process of single-follicle-selection remained efficient despite a large initial stimulus.

The dose of HCG has been thought by some workers to be critical in determining the number of ovulations (Crooke, 1964; Brown *et al.*, 1969). The dose of 19,500 i.u. HCG in this trial, more than six times that used in previous therapy schemes in New Zealand (Liggins & Ibbertson, 1966), was exaggerated so that there would be a greater chance of producing multiple CL if selection of single follicles for maturity had not been well defined.

Five of the nine women with normal cycles produced convincing evidence of single ovulation. However, in one instance, evidence of ovulation was inconclusive suggesting that a single stimulus, even though large, may be inadequate in some individuals. Furthermore, the production of superovulation in two women suggests that a natural process of single-follicle-selection cannot be

relied upon, at least when large single doses of HMG and HCG are used. As with multiple-injection schemes, individual adjustment of dosage is necessary if multiple ovulation is to be avoided in single-dose schemes but dosage may be less critical.

Puerperal amenorrhoea is usually attributed to the low level of circulating pituitary gonadotrophin after pregnancy persisting for a variable time into the puerperium (Sharman, 1966). However, Forbes, Henneman, Griswold & Albright (1954), Keetal & Bradbury (1961), Johnsen & Fuchs (1961) and Keller (1968) have demonstrated relatively normal levels of gonadotrophin excretion in most lactating women who were amenorrhoeic. The failure to induce ovulation or significantly to increase steroidogenesis by the ovaries of puerperal women in response to massive doses of HMG and HCG also implies that puerperal amenorrhoea is due to the ovaries being refractory to FSH rather than resulting from FSH deficiency.

The cause of the refractoriness of the ovary to HMG in the puerperium is uncertain.

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