PAPERS AND ORIGINALS

Attempts to Programme Ovulation with Exogenous Oestrogens and LH-RH Analogue

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Summary

An attempt was made to programme ovulation in women on a predetermined day of the menstrual cycle by treatment used to induce ovulation in anovulatory sterility. At laparotomy for elective sterilization the ovaries were observed to assess the occurrence of ruptured follicles and ovulation. Histological analysis of ovaries and endometrium was performed, and ova were recovered from some women. Several regimens were tested but ovulation seldom occurred as planned. Clomiphene citrate, human chorionic gonadotrophin (HCG), synthetic luteinizing hormone releasing hormone (LH-RH), and ethinyloestradiol were ineffective. Human menopausal gonadotrophin followed by HCG was more effective, but

multiple ovulations occurred. When one single injection of a potent long-acting LH-RH analogue was given on day 13 of the cycle to 10 women pretreated with ethinyloestradiol signs of recent ovulation were observed on day 15 in seven and on day 16 in two.

Introduction

Assurance that ovulation will occur in a given cycle and its dating are two of the most desirable goals in the management of infertility. Accurate dating of ovulation might also be useful in research on human reproduction and help those who want to rely on the rhythm method for family planning. Dating of spontaneous ovulation in women is still difficult and inaccurate, however, and attempts to induce ovulation in patients with anovulatory cycles have been only partially successful.

An alternative solution to the problem of ovulation dating might be ovulation programming—that is, inducing ovulation on a given day of the menstrual cycle. The possibility of using human gonadotrophins has been extensively explored, and human menopausal gonadotrophin (HMG) associated with human chorionic gonadotrophin (HCG) has been found to induce ovulation. We studied this effect by direct observation of both ovaries at laparotomy for elective tubal sterilization and found multiple ovulations in five of the 11 women who responded to treatment.¹

We thought that the positive feedback effect of exogenous oestrogen on LH release²⁻⁴ might increase the effect of synthetic LH-RH; synthetic LH-RH has an effect similar to the natural hormone in releasing endogenous follicle-stimulating hormone (FSH) and LH.⁵⁻⁶ We report here our attempts to programme ovulation in normal women using current treatments for anovulatory sterility and oestrogen followed by a potent synthetic gonadotrophin-releasing peptide, D-leu-6-des-gly-10-LH-RH ethylamide (D-leu⁶ LH-RH ethylamide)—an analogue of LH-RH which has a more intense and prolonged effect on the release of FSH and LH in experimental animals than LH-RH.⁷⁻⁸ The gonadal response was then observed at laparotomy for elective tubal sterilization.

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Patients and Methods

Healthy fertile women with ovulatory menstrual cycles who had previously been accepted for sterilization by our department's committee on ethics because of high multiparity (mean 7 pregnancies, range 5-13), obstetrical risk, or medical reasons took part in the study. Husband and wife gave informed consent and agreed to have surgery on a programmed date. The average duration of menstrual cycles could not be accurately estimated, since intrauterine devices or progestogens were being used for contraception and were discontinued and replaced by vaginal or mechanical methods only one or two cycles before surgery. Ten untreated women who were surgically sterilized on day 15 of their menstrual cycles acted as controls.⁹

Routine laboratory tests were made before surgery and ovulation was studied by cervical mucus tests, endometrial biopsy, and urinary pregnanediol excretion on day 21-25 of the cycle before the experimental cycle. We chose to programme ovulation to occur on day 14 of the menstrual cycle, and laparotomy was performed on day 15 or early on day 16.

At laparotomy both ovaries were carefully examined and a wedge biopsy specimen was made for histological analysis of ovarian tissue in the gonads with evidence of a corpus luteum or maturing follicle. Endometrial tissue was obtained immediately after surgery by curettage. In several cases the oviducts were excised and the uterine cavity flushed in situ to obtain eggs from the uterus and uterine tubes. Eggs were fixed and prepared for electron microscopy (to be reported elsewhere). The ovarian and endometrial tissues were fixed and stained with hematoxylin and eosin. These tissues were dated independently by the pathologist (H.D.C.) and the clinician (J.Z.) according to Corner's criteria for corpus luteum¹⁰ and by Noyes *et al.*'s for endometrium.¹¹

The following regimens were used in an attempt to induce ovulation on day 14 of the cycle: (a) clomiphene citrate 100 mg/day from day 3 to day 7 was given to two women; (b) HCG 5000 IU was given intramuscularly on days 11, 12, and 13 to two women; (c) synthetic LH-RH 100 mg injected subcutaneously twice a day from day 11 to day 13 was given to two women; (d) ethinyloestradiol 0.050 mg was given by mouth every 12 hours from day 9 to day 11 to two women; (e) ethinyloestradiol 0.050 mg was given every 12 hours on days 9-11 and synthetic LH-RH 0.600 mg given by repeated nasal sprays on days 12 and 13 to four women; (f) ethinyloestradiol 0.050 mg was given every 12 hours on days 9-11 followed by one injection of synthetic LH-RH on days 12 and 13 to two women; (g) HMG 150 IU was

injected intramuscularly every other day in three to six doses followed by 10 000 IU of HCG intramuscularly 24-48 hours later in the same cycle to 10 women; (h) ethinyloestradiol 0.050 mg was given every 12 hours on days 10 and 11 or days 9-11 followed by one injection of D-leu⁶ LH-RH ethylamide on day 13 to 10 women. In this group, the lengths of two or three previous menstrual cycles were registered; they ranged from 21 to 43 days with an average of 28 days duration.

Results

Five of the untreated women had a ruptured follicle on day 15, and one ovum was recovered from the oviduct in three. Ovulation was observed in only one of the two women treated with HCG (table I). Ovulatory responses were observed in six women treated with H.M.G. and H.C.G., but multiple ovulations occurred in two (table II). Ethinyloestradiol followed by D-leu⁶ LH-RH ethylamide gave a more consistent ovulation rate (table III).

Discussion

The date of ovulation is related to the length of the proliferative phase of the menstrual cycle and depends on follicle maturation and endogenous oestradiol secretion that may, together with endogenous LH-RH, result in the release of large amounts of LH and FSH in a preovulatory peak, which in turn may release the oocyte and cumulus as the mature follicle ruptures. Unfortunately, the timing of this physiological process is unpredictable, so we tried to fix the rupture of the mature follicle at a predetermined date of the cycle. For convenience we followed the normal timing of physiological events and chose day 14 of the cycle as the target date for the programmed ovulation.

As expected, five of the 10 untreated women from the control group showed a fresh corpus luteum at laparotomy on day 15. Only one of the 14 women treated with clomiphene, HCG, or ethinyloestradiol and synthetic LH-RH alone or combined (treatments (a)-(f)) ovulated before or on day 15. Four of the eight women treated with HMG followed by HCG who were examined on days 13-16 had ovulated. In nine out of 10 women

TABLE I—Results of Programming Ovulation with Various Regimens (Treatments (a)-(f)) in Normal Women. Results in Untreated Controls are also Shown.

No. of Women	Treatment		Surgery		
	Compound	Day of Cycle	Day	Signs of Ovulation (No. of Women)	Findings on Endometrial Biopsy*
10	None		15	Corpus luteum (day 1-4) (5), maturing follicles (5)	P. (16)
2	Clomiphene 100 mg	3-7	15	Cystic follicles (2)	E.
2	HCG 5000 IU	11-13	15	Corpus luteum (day 2) (1)	E.
2	LH-RH 100 mg × 2	11-13	15	Maturing follicles (2)	P. (17)
2	Ethinyloestradiol 0.05 mg × 2	9-11	15	Maturing follicles (2)	E.
4	LH-RH nasal spray 0.6 mg	12, 13	15	Cystic follicles (4)	E.
2	Ethinyloestradiol 0.05 mg × 2	9-11	15	Cystic follicles (2)	E.
	LH-RH 0.5 mg I.M. × 2	12, 13			

P. = Secretory endometrium. Number in parentheses indicates day of normal 28-day cycle to which findings corresponded. E. = Proliferative second week of normal cycle

TABLE II—Results of Programming Ovulation in 10 Women with Exogenous Human Gonadotrophins (Treatment (g))

Case No.	Days of Treatment		Surgery			
	HMG 150 IU I.M.	HOG 10 000 IU	Day	Signs of Ovulation	Ova Recovered	Endometrial Biopsy†
1	3, 7, 10	11	13	Maturing follicle	—	E.
2	4, 8, 11	12	14	Maturing follicle	—	E.
3	5, 7, 11	13	15	2 Corpora lutea (day 3 and 4)*	+ ‡	P. (16)
4	6, 8, 10	13	16	Maturing follicle	—	E.
5	5, 9, 11	13	16	1 Corpus luteum (day 3)	—	P. (16)
6	4, 8, 10	11	13	Maturing follicle	—	E.
7	3, 6, 8, 10	11	13	2 Corpora lutea (day 1 and 3)*	+	P. (18)
8	3, 5, 7, 9, 11	12	14	1 Corpus luteum (day 2)	—	E.
9	25, 27, 29, 31, 33, 34	34	36	1 Corpus luteum (day 8)	—	P. (21)
10	26, 28, 30, 32, 33, 34	35	37	1 Corpus luteum (day 8)	—	P. (irregular)

*Multiple ovulations.

†One ovum recovered from uterine cavity.

‡See footnote to table I for key.

TABLE III—Results of Programming Ovulation in 10 Women with Oestrogens and D-leu⁶ LH-RH Ethylamide (Treatment (h))

Case No.	Ethinyloestradiol 0.050 mg × 2 (Days of Cycle)	D-leu ⁶ LH-RH Ethylamide		Surgery			Findings on Endometrial Biopsy†
		Dose (mg)	Day of Cycle	Day	Signs of Ovulation	Ova Recovered	
11	9-11	0.8	13	15	Corpus luteum (day 2)	—	P. (15)
12	9-11	0.5	13	15	Corpus luteum (day 1)	—	E.
13	9-11	0.5	13	15	Corpus luteum (day 3)	+	P. (16)
14	10, 11	0.6	13	15	Corpus luteum (day 1)	—	E.
15	10, 11	0.6	13	15	Corpus luteum (day 3)	—	P. (16)
16	10, 11	0.6	13	15	Cystic Follicle	—	E.
17	10, 11	0.6	13	16	Corpus luteum (day 3)	—	P. (16)
18	10, 11	0.6	13	15	Corpus luteum (day 4)	—	P. (17)
19	10, 11	0.5	13	16	Corpus luteum (day 3)	+	P. (15)
20	10, 11	0.5	13	15	2 Corpora lutea (day 4)*	—	P. (17)

*Multiple ovulation.

†See footnote to table I for key.

treated with ethinyloestradiol and D-leu⁶ LH-RH ethylamide, however, there was evidence of ovulation. These results are surprising but support the concept that exogenous oestrogens and the more potent LH-RH analogue, in the order we gave them, facilitated ovulation by strengthening each others effects.

We could not establish accurately whether ovulation had occurred spontaneously or as a result of treatment. Women in whom a corpus luteum was dated as day three or four may have had a precocious spontaneous ovulation unrelated to treatment, but we cannot rule out the possibility that pituitary overstimulation might have accelerated development of this structure. Neither ethinyloestradiol given alone on days 9-11, nor synthetic LH-RH alone, nor the more potent D-leu⁶ LH-RH ethylamide given alone on day 13 induced ovulation. Ethinyloestradiol and synthetic LH-RH given on day 13 subcutaneously or by nasal spray were also ineffective. Furthermore, no ovulation was observed in a woman receiving ethinyloestradiol 0.050 mg every 12 hours on days 9-11 plus 0.500 mg of the LH-RH analogue given by mistake on day 12 of the cycle. These findings suggest that exact timing and dosage of the exogenous oestrogen to obtain the proper subsequent positive feedback, and the exact timing of the LH-RH analogue to strengthen the effect, are crucial for obtaining an effective LH and FSH release that will rupture the mature follicle as planned—on day 14 of the cycle.

To conclude that treatment with ethinyloestradiol and LH-RH analogue is truly successful for programming ovulation more ovulating women should be treated and compared with a similar untreated group. Plasma gonadotrophin and steroid concentrations should also be measured to assess the temporal relations between treatment, pituitary ovulatory discharge, and ovarian ovulatory response, which should be observed directly and by recovery of the ova. Such studies should leave no doubt about the efficacy of the treatment.

A useful approach to regulating human fertility might be in sight. Ethinyloestradiol and D-leu⁶ LH-RH ethylamide treatment has been useful in obtaining pregnancy in ovulatory women who were artificially inseminated because of severe oligospermia or azoospermia in their husbands.¹² The possibility of using this simple regimen for fixing ovulation, and its potential value in making the rhythm method of contraception more effective, will be investigated more thoroughly.

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