SYMPTOMS AND HORMONAL CHANGES ACCOMPANYING OVULATION

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Summary To determine whether normal women could predict and identify symptomatically the occurrence of ovulation, twenty-two volunteers were instructed in a pattern of vaginal "mucus symptoms" which had been established previously. Plasma luteinising hormone and urinary œstrogens and pregnanediol were measured to provide a "hormonal estimate" of the day of ovulation. A characteristic "lubricative" mucus identified by all the women occurred on the day of ovulation in five, 1 day before in nine, and 2 days before in four. The onset of mucus symptoms occurred 6·2 days (mean) before ovulation. It is concluded that the time of ovulation can be identified clinically, without recourse to temperature measurement or more specialised tests.

Introduction

THE ability of a woman to predict and identify the time of ovulation, by means of her own symptoms, would have obvious applications in fertility control and in the management of infertility. "Natural" methods of family planning are usually based on a calendar record [1,2] of past menstrual cycles (the "rhythm method"), from which the fertile time of the cycle can be calculated, or on a basal body temperature (B.B.T.) record,[3,4] from which the occurrence of ovulation can be judged retrospectively, or a combination of the two. The rhythm method is restrictive in that it must take account of the woman's longest and shortest recorded cycle; the B.B.T. method lacks specificity, and does not identify ovulation prospectively.

We have found that women can be taught to recognise a pattern of vaginal mucous discharge which occurs at about the time of ovulation, that there is a close correlation between those symptoms and the day of ovulation as estimated from hormone-excretion patterns, and that impending ovulation can be predicted for a period long enough to be of practical use for fertility control. The clinical application of the combination of symptoms and changes in B.B.T. has been described previously [5] as the "ovulation method".

Methods

Symptoms of Ovulation

In the past fifteen years two of us (E. L. B. and J. J. B.) have interviewed several hundred women to define the mucous-discharge pattern occurring at the periovulatory period of the menstrual cycle. The major features of these symptoms are as follows:

(*a*) Menstrual bleeding is followed by a variable number of days on which no vaginal loss is present ("dry days").

(*b*) The onset of the "mucus symptoms" is characterised by the appearance of increasing quantities of "cloudy" or "sticky" secretion; the duration of this phase is variable (see below).

(*c*) This is followed by the occurrence of clear, slippery, lubricative mucus, having the physical characteristics of raw white of egg (*Spinnbarkheit*); this has been termed the "peak symptom". It characteristically lasts for 1–2 days, and the last day of its occurrence is referred to as the day of the peak symptom in the results presented.

(*d*) The "peak symptom" is followed by the presence of thick, "tacky", opaque mucus, the duration of which is variable.

Women Studied

Twenty-two women attending the Catholic Family Planning Centre, Melbourne, were chosen for the study solely because of their willingness to cooperate, and the series studied is a consecutive one. Several were unconvinced, after initial instruction, that they could readily identify mucus symptoms, but all were well motivated to assist in the investigation. All were married, their ages ranged from 25 to 42 years, and they were of proven fertility (1–7 children; modal number, 2). Menstrual cycle lengths varied from 22 to 35 days, mean 27·6. Each woman was asked to keep a daily record of her mucus symptoms, and to measure vaginal B.B.T. before rising in the morning. Serial daily blood-samples were collected on 9–10 consecutive days around the expected time of midcycle, and urine collections (24-hour) were made throughout the cycle in all except the first six women, who collected only at midcycle. The blood was collected by venepuncture, by a nurse visiting the home. The women were, therefore, normal volunteer housewives of proven fertility, studied in their own homes.

Hormone Assays

Luteinising hormone (L.H.) was measured by a solid-phase radioimmunoassay similar to that previously described [6] for human growth hormone, using radioiodinated human L.H. (donated by Dr. A. Stockell Hartree), and anti-L.H. antiserum. Results were expressed as m.I.U. per ml., using a laboratory standard of pituitary gonadotrophin which had been characterised biologically in terms of the second international reference preparation of human menopausal gonadotrophins (L.H. activity,[7] 144 I.U. per mg.). Total urinary œstrogens [8] and pregnanediol [9] were measured by previously published methods.

Hormonal Estimate of Day of Ovulation

The midcycle peak of plasma-L.H. occurs about 16 hours before ovulation, as judged by biopsy of the corpus luteum,[10] and the midcycle peak of urinary œstrogen excretion usually occurs on the day before the plasma-L.H. peak or on the same day.[11] Urinary pregnanediol excretion rises with the establishment of luteal function.

We defined the day of ovulation as the day after the midcycle plasma-L.H. peak; this usually corresponded to the day on which urinary pregnanediol excretion had reached

DR. CHATURVEDI AND OTHERS: REFERENCES—*continued*

3. Norris, R. M., Brandt, P. W. T., Caughey, D. E., Lee, A. J., Scott, P. J. *Lancet*, 1969, i, 274.
4. Lawrie, D. M., Greenwood, T. W., Goddard, M., Harvey, A. C., Donald, K. W., Julian, D. G., Oliver, M. F. *ibid.* 1967, ii, 109.
5. Mackenzie, G. J., Ogilvie, B. C., Turner, T. L., Foulton, M., Lutz, W. *ibid.* 1971, ii, 200.
6. Pentecost, B. L., Mayne, N. M. C. *Br. med. J.* 1968, i, 830.
7. Fortieth Annual Report of the Registrar General. H.M. Stationery Office, 1961.
8. Linn, B. S., Yurt, R. W. *Br. med. J.* 1970, ii, 25.
9. Adgey, A. A. J., Geddes, J. S., Webb, S. W., Allen, J. D., James, R. G. G., Zaidi, S. A., Pantridge, J. F. *Lancet*, 1971, ii, 501.
10. Pantridge, J. F., Geddes, J. S. *ibid.* 1967, ii, 271.
11. Kernohan, R. J., McGucken, R. B. *Br. med. J.* 1968, iii, 178.

a value more than twice the mean follicular-phase level. With one exception, the peak of urinary œstrogen excretion occurred 0·2 days before the estimated day of ovulation. In two women (nos. 3 and 10), it was clear that blood-samples had not been obtained at midcycle (cycle lengths were 35 and 33 days, respectively), and the day of ovulation was assumed to be the day after the total œstrogen peak, confirmed by a pregnanediol rise in subject no. 10.

B.B.T. Rise

For each woman, a line was drawn on the B.B.T. chart ("the marginal line"),[5] below which the temperatures in the late follicular phase were found, and above which the temperature had reached luteal-phase levels. The first day on which the temperature lay above the marginal line was termed the day of rise in B.B.T.

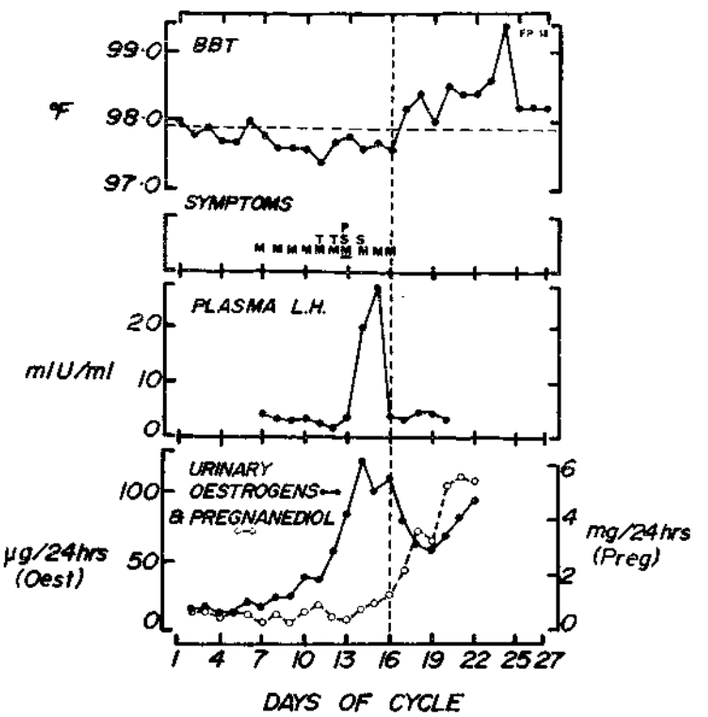
TABLE I—ANALYSIS OF HORMONAL AND SYMPTOMATIC INDICES OF OVULATION

Subject	Cycle length	Day of L.H. peak (plasma)	Day of total œstrogen peak (urine)	Day of significant rise in pregnanediol (urine)	Day of peak symptom	Hormonal estimate of ovulation (days)	Day of first recorded symptom	Day of rise in B.B.T.
1	30	16	15	..	16	17	10	18
2	28	14	14	..	14	15	11	16
3	35	..	24	..	23	25	16	24
4	25	14	13	15	16	15	11	4
5	27	12	11	13	11	13	6	13
6	25	13	13	16	12	14	9	14
7	25	13	13	16	12	14	7	14
8	28	13	12	18	13	14	6	15
9	27	15	15	18	13	16	8	?16*
10	33	..	19	20	17	20	11	23
11	22	10	9	12	11	11	8	12
12	29	14	14	16	14	15	11	16
13	26	14	12	12	11	12 (15)	9	14
14	27	11	10	12	12	12	9	14
15	26	11	10	12	12	12	7	13
16	25	12	12	13	12	13	7	16
17	27	13	13	13	13	14	5	15
18	28	15	14	16	14	16	7	17
19	31	17	17	17	18	18	10	19
20	25	11	11	13	12	12	6	14
21	27	11	10	13	14	12	9	13
22	33	19	20	20	19	20	10	22

.. Sample collected at inappropriate part of cycle.
* Unsatisfactory B.B.T. record.

TABLE II—CORRESPONDENCE BETWEEN DAY OF OVULATION AS ESTIMATED FROM SYMPTOMS AND HORMONAL MEASUREMENTS: INTERVAL FROM ONSET OF SYMPTOMS TO OVULATION

Subject	Interval: peak symptom to day of ovulation (days)	Interval: first recorded symptom to day of ovulation (days)
1	1	7
2	1	4
3	2	9
4	−1	4
5	2	7
6	1	4
7	2	7
8	1	8
9	3	8
10	3	9
11	0	3
12	1	4
13	1 (4)	3 (6)
14	0	3
15	0	5
16	1	6
17	1	9
18	2	9
19	0	8
20	0	6
21	−2	3
22	1	10
Mean ..	0·9	6·2



Subject 18: B.B.T., mucus symptoms, plasma-L.H., and urinary œstrogens and pregnanediol.

Symptoms are characterised as mucus (M), tacky mucus (TM), slippery or lubricative mucus (SM), and P (pain). M indicates maximum quantity of mucus; the day of the peak symptom was taken as the second day marked SM. The vertical dotted line indicates the day of ovulation estimated hormonally. The horizontal dotted line on the B.B.T. record is the "marginal line".

Results

The results are summarised in tables I and II, and a typical cycle is shown in the figure. In subject no. 9, peak excretion of pregnanediol in the luteal phase reached only 1·1 mg. per 24 hours, suggesting that a fully functioning corpus luteum was not established; there was, however, a clear peak in urinary œstrogen excretion and in plasma-L.H. (23·5 m.I.U. per ml.). In subject no. 13 there were inconsistencies in the hormonal measurements; thus, although there was a peak in plasma-L.H. (10·6 m.I.U. per ml.) on day 14, urinary pregnanediol had increased significantly on day 12; the day of ovulation in this subject was taken as day 12 or possibly day 15 (although on day 15 pregnanediol was already 5·6 mg. per 24 hours).

Table II shows that ovulation, as assessed hormonally, occurred 0·9 days (mean) after the occurrence of the peak symptom, with a range from 3 days after to 2 days before (in one subject). Furthermore, the mean interval from the onset of mucus symptom to ovulation was 6·2 days, with a range from 3 to 10 days. The rise in B.B.T. generally occurred 1–2 days after ovulation.

Discussion

We have found that twenty-two normal housewives, selected only because they were willing to participate in the study, could be taught to recognise a pattern of vaginal mucous secretion during the menstrual cycle, and to distinguish the occurrence of a particular

symptom—namely, lubricative clear mucus. This " peak symptom " was closely correlated with the day of ovulation, as estimated from measurements of plasma-L.H. and urinary total œstrogens and pregnanediol. Furthermore, the mucus symptoms began, on average, 6·2 days before the putative occurrence of ovulation, thus providing a useful indication of impending ovulation. The peak symptom was closely associated with the peak of urinary œstrogen excretion, which is consistent with the effects of œstrogen on the cervical mucus glands.

These observations provide a basis for a method of birth regulation which depends solely on observations which can be made by most normal women, and on the avoidance of sexual intercourse from the time of onset of these symptoms until after the occurrence of the peak symptom. Such a method (the " ovulation method ") is now being studied: women are instructed to abstain from intercourse at the onset of the mucus symptoms, and sexual activity may be resumed on the fourth day after the " peak symptom ". Furthermore, a couple who are having difficulty in conceiving can be instructed to concentrate their efforts on the days of the " peak symptom ".

The widespread application of such a method clearly requires confirmation of its reliability in practice; in addition, it demands that women be taught to recognise their symptoms, and thus its applicability to all classes in society must be demonstrated. Such studies are in progress.

Our findings have also proved useful in physiological studies of the hormonal events which surround ovulation. A World Health Organisation list [12] of research needs in fertility control by periodic abstinence included " a formula that may give a better estimate of the time of ovulation than the current formulæ " and " simple tests for the accurate prediction of ovulation ". Our results suggest that these needs may be met by simple clinical means.

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REFERENCES

1. Ogino, K. *Zbl. Gynäk.* 1930, **54**, 464.
2. Knaus, H. *ibid.* 1929, **53**, 2192.
3. Ferin, J. *Brux. méd.* 1947, **27**, 2786.
4. Marshall, J. *Lancet*, 1968, ii, 8.
5. Billings, J. J. The Ovulation Method. Melbourne, 1971.
6. Catt, K. J., Tregear, G. W., Burger, H. G., Skermer, C. *Clinica chim. Acta*, 1970, **27**, 267.
7. Parlow, A. F. *in* Human Pituitary Gonadotrophins: a Workshop Conference (edited by A. Albert); p. 301. Springfield, Illinois, 1971.
8. Brown, J. B., MacLeod, S. C., Macnaughton, C., Smith, M. A., Smyth, B. *J. Endocr.* 1968, **42**, 5.
9. Barrett, S. A., Brown, J. B. *ibid.* 1970, **47**, 471.
10. Yussman, M. A., Taymor, M. L. *J. clin. Endocr. Metab.* 1970, **30**, 396.
11. Burger, H. G., Catt, K. J., Brown, J. B. *ibid.* 1968, **28**, 1508.
12. *Wld Hlth Org. tech. Rep. Ser.* 1967, no. 360.

APLASTIC ANÆMIA TREATED BY MARROW TRANSPLANTATION

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Summary 4 patients with complete marrow failure, 3 due to unknown cause and 1 associated with hepatitis, showed no indication of spontaneous recovery after 7 to 52 weeks of conventional and supportive treatment. They were conditioned for engraftment by the administration of cyclophosphamide, 50 mg. per kg., on each of 4 days, followed in 36 hours by marrow infusion. The marrow donor in each instance was a sibling of opposite sex who matched the recipient with regard to the major human histocompatibility locus (HL-A) as shown by cytotoxicity testing of the family and confirmed by non-reactivity in the mixed-leucocyte test. Methotrexate was given for a limited period after grafting as an immunosuppressive agent to modify the graft-versus-host disease. All 4 patients showed prompt engraftment indicated by a return of marrow cellularity and a rise of peripheral blood-counts, confirmed by cytogenetic analysis. 1 patient died of graft-versus-host disease with a cellular marrow 45 days after grafting. Another patient rejected his graft and died 67 days after grafting. 2 patients have excellent-functioning marrow grafts without graft-versus-host disease and are apparently well 138 and 215 days after grafting.

Introduction

MARROW transplantation has for a long time offered the hope of permanent correction of marrow failure whether due to disease or to drug reaction or of unknown cause.[1] However, fulfilment of this hope has been limited to those few cases in which the patient had an identical twin to serve as a marrow donor.[2] We describe here 4 cases of marrow failure prepared for marrow grafting by administration of cyclophosphamide (cy.) followed by marrow from a sibling of opposite sex. In each instance the sibling donor was shown to have inherited the same 2 HL-A haplotypes as the patient, and the match was confirmed by non-reactivity in a mixed-leucocyte culture.

Methods

Techniques used in this laboratory have been given in detail in publications describing the technique of marrow-grafting,[3] the mixed-leucocyte test,[4] the general management of patients undergoing marrow engraftment,[5] and the cytogenetic methods.[6] Buffy-coat cells from 1 unit of the donor's blood were given to the recipient the day before cy. administration. The cy. (' Cytoxan ', Mead Johnson, Evansville, Indiana) was administered according to the regimen of Santos et al.[7] The day of marrow infusion was designated " day 0 " and subsequent days were numbered from that point. After marrow grafting all fresh-blood products were given 1500 rad in vitro before