PREDICTION OF OVULATION USING A DIPSTICK SENSITIVE TO URINARY LUTEINISING HORMONE

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Summary: Accurate prediction of ovulation is essential for patients receiving donor insemination (AID). In 9 patients, detection of the urinary luteinising hormone (LH) surge by monoclonal antibody immunospecific dipstick tests (Ovustick) for both home and laboratory use was assessed with simultaneous quantitative estimations of plasma and urinary LH. In all patients the Ovustick accurately detected within 24 hours the beginning of the plasma LH surge. This test may prove to be a useful method for ovulation prediction but further evaluation is required.

Success rates in patients having treatment by donor insemination are dependant mainly on the quality of the inseminate and the timing of the insemination. A number of methods have been used to predict ovulation including a urinary luteinising hormone (LH) haemagglutination assay which was not entirely problem free ([4]). It has been shown that there are advantages in using a rapid radioimmunoassay (RIA) to measure the LH surge in blood or urine which occurs about the time of ovulation ([1, 7]). The chances of predicting the optimum time for donor insemination are increased by daily monitoring of LH. However, patients living some distance from the laboratory have to be hospitalised and this adds to the cost of the investigation and is inconvenient for the patient. As an alternative to hospitalisation patients may attempt to predict the day of ovulation by keeping daily records of their basal body temperature and observing changes in cervical mucus. It is the opinion of some observers that these methods are not suitable for many patients ([8, 9]). The need for a simple method which would establish when ovulation was most likely to occur has long been recognised. Such a test would be particularly useful for women who at present have LH levels monitored as " in patients ", since distance may make it impossible for them to travel daily to hospital.

Recent development in monoclonal antibody technology have made available a number of new tests for the measurement of gonadotrophins in plasma and urine. These methods have been shown to be most useful for pregnancy diagnosis. More recently tests have been produced which give semi quantitative measurements in urine. These tests may have a useful application in the investigations of women with infertility and for women who wish to know when they may be about to ovulate. We made a preliminary investigation with two such tests, the Ovustick Urine LH kit for laboratory use and the Ovustick Self Help test for use by the patient. The Ovustick test was assessed against a rapid RIA for plasma LH ([3]) and the Ovustick Self Help test against an RIA for urinary LH.

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MATERIAL AND METHODS

Nine patients (aged 25-35 years) with normal ovulatory cycles ranging from 25 to 35 days were investigated. They each collected a first morning and a last evening specimen of urine from cycle day 9 until the second day after their plasma LH peak. 73 morning and 71 evening urines were tested with an enzyme immunoassay dipstick test (Ovustick) for urinary LH. One patient was investigated for two successive cycles. All women had a daily morning blood sample taken for plasma LH assay from the 9th day of the menstrual cycle until 2 days after the plasma LH surge was detected. Insemination was performed in the evening when the first significant rise in LH was detected. A significant rise was one in which the plasma LH levels were double the mean of all previous values. The patient using the Ovustick Self Help kit tested an early evening specimen of urine from the 10th to 18th day of her cycle. Urinary LH was measured in each sample by RIA and the concentration expressed as IU LH per g creatinine (IU LH/g^c). Ovustick and Ovustick Self Help tests are based upon the following principle; urinary LH is sandwiched between an alpha subunit specific antibody which has been immobilised on a plastic dipstick and beta hLH specific antibody which has been linked to the enzyme alkaline phosphatase. Unbound enzyme conjugate is removed by washing and the dipstick is incubated in a substrate solution, which reacst by depositing a blue product on the reactive end of the dipstick.

Using Ovustick, a semi quantitative estimation of urinary LH can be made by comparing the colour intensity produced by the urine under test, with that produced by standard solutions containing 0, 20, and 40 mIU LH/ml. A surge was defined as an Ovustick result that equalled or exceeded the colour intensity produced by the 40 mIU/ml standard. The Ovustick Self Help test reaction was read against a colour chart coded 1, no colour, to 5 when maximum blue colour reaction was obtained.

RESULTS

The length of the menstrual cycle in 9 women receiving donor insemination was between 25 and 35 days (means ± s.e.m. 28 ± 1 days). In 8 of these women the surge in plasma LH occurred between days 12 and 14 of the menstrual cycle. In the 9th patient with a cycle of 35 days the surge was detected on the 19th day of the cycle. When morning and evening urines from these 9 women were tested with Ovustick the surge occurred in the evening specimens of 8 of the women. The urinary LH surge, not less than 40 IU LH/1, occurred on or within ± 1 day of the observed plasma LH surge (table 1). The mean ± s.e.m. plasma LH values on pre surge days 1 and 2 were 10.6 ± 0.64 (N = 20), on the day of the surge 34.5 ± 6.6 (N = 10), at the peak 51.1 ± 9.1 (N = 10) and 18.9 ± 5.2 one day after the peak. The surge in urinary LH paralleled that of plasma LH and occurred within 1 day of it (table 1). The results obtained by one patient using the Self Help version of the Ovustick test at home are shown in Figure 1. Urinary concentration of LH, expressed in IU/g^c, showed steadily increasing preovulatory levels until a peak was reached on day 17. In spite of the semi quantitative nature of the test, the shape of the LH curve was similar to that obtained by RIA.

DISCUSSION

The inter-relationship between the LH surge and ovulation has resulted in the routine measurement of this gonadotrophin for women with infertility problems. The ability to predict the onset of ovulation is of prime importance particularly for those women receiving donor insemination. However, the time at which ovulation occurs, when related to LH measurements is extremely variable. In patients treated with gonadotrophins ovulation follows 29 - 44 hours later [5]. In untreated patients the time interval between the first significant rise in plasma LH and ovulation is 24-36 hours [1] and 20-44 hours after the onset of the urinary LH surge [2]. The diurnal rhythm in the beginning of the LH surge in women may be further complicated by an observed seasonal variation in this rhythm [4, 10]. Edwards [6] measured

Table 1. – *Showing menstrual cycle length, day of AID, surge and peak levels of plasma LH and semi-quantitative estimation of urinary LH.*

Cycle number	Menstrual cycle length days	Plasma LH IU/l and day of		Urinary LH surge and day	Day of AID
		Surge	Peak		
1	25	16.2 (12) ***	24.1 (13)	40 (12) ***	12
2	26	19.6 (12)	20.9 (13)	40 (13)	12
3 *	26	41.0 (12)	41.0 (12)	40 (12)	13
4	27	84.0 (12)	84.0 (12)	40 (13)	12
5	25	53.0 (13)	53.0 (13)	40 (12)	13
6 *	26	20.0 (13)	31.2 (15)	40 (14)	14
7	29	37.4 (14)	110.0 (15)	40 (13)	14
8 **	29	30.0 (14)	60.0 (17)	40 (15)	15
9	30	21.5 (14)	27.2 (15)	40 (15)	14
10	35	22.8 (19)	59.5 (21)	40 (20)	19

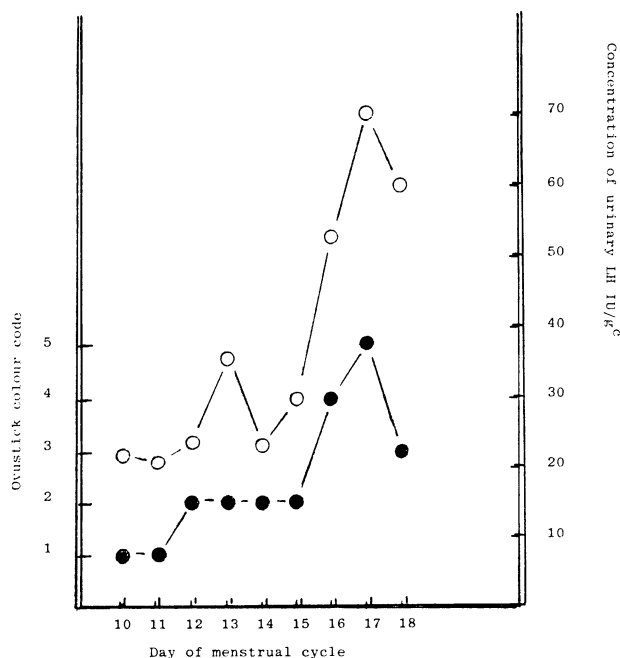* same patient;   ** patient pregnant after AID;   *** day of peak or surge.

Fig. 1. — Measurement of urinary LH by RIA (○) and Ovustick Self Help test (●) during a normal menstrual cycle.

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urinary LH excretion at approximately 3-hourly intervals and reported that a rise occurred in 70 percent of women in the early morning (00.30 - 00.90 hours). In the present investigation, in which 7 morning and 7 evening specimens from each patient were tested, the surge in urinary LH occurred in the evening specimen in 9 out of 10 cycles (table 1). The difference between our results and those of Edwards ([6]) may be due to differences in the number of women investigated and the frequency with which samples were collected and assayed. In this study all 9 women were inseminated within 12 - 24 hours of the commencement of the plasma LH surge, one patient becoming pregnant. If the urinary LH surge had been used instead donor insemination would have been performed at the same time, in relation to the plasma LH surge, in 3 cycles, 24 hours earlier in 2 cycles and 24 hours later in 5 cycles. Considering the wide variation between the time of the LH surge and subsequent ovulation the difference between using plasma or urinary LH measurements may be of little consequence in obtaining a conception.

A more comprehensive investigation is needed to confirm the potential usefulness of Ovustick for detecting the midcycle surge of LH and the " Self Help " test for out-patient use as an aid to conception.

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