Shimane J. Med. Sci., Vol.11, pp. 49-52, 1987/88

DETECTION OF URINARY LUTEINIZING HORMONE IN SUBJECTS WITH A NORMAL OVULATORY CYCLE USING A SIMPLE TEST KIT

(sol particle immunoassay/urinary LH/monoclonal antibody)

Kentaro TAKAHASHI, Kazuo YOSHINO, Arata NISHIGAKI, Takaaki SHIRAI, Akihiro UCHIDA, Masuo KUSAKARI, and Manabu KITAO

Department of Obstetrics and Gynecology, Shimane Medical University, Izumo 693, Japan

(Received March 1, 1988/Accepted May 15, 1988)

We have evaluated the usefulness of a simple test to detect urinary LH, ie, sol particle immunoassay (Discretest). The Discretest was positive 16-24 hours before the LH-surge in the blood and in eight cases, the test results of Discretest changed from positive to negative, before and after ovulation. Discretest seems to be a useful tool as ovulation can be monitored accurately and rapidly.

Measurement of human luteinizing hormone (LH) is the most accurate method for predicting ovulation when attempting to treat cases of infertility. Radioimmunoassay (RIA) is the most accurate method available to measure LH in the sera and urine but this approach is complicated and time consuming. Hence, daily tests present problems for the physician. Urinary LH can be measured using Hi-Gonavis (Mochida Pharmaceutical Co., Ltd.)(3), however, LH is not thus directly measured, rather this is an indirect approach by means of a cross-reaction between anti-hCG and LH. We have now evaluated the relationship between ovulation and changes of urinary LH level, measured using a simple test to detect urinary LH, i.e. sol particle immunoassay (SPIA). The clinical usefulness of the test (Discretest) was examined.

MATERIALS AND METHODS

Eight Japanese volunteers aged 20-26 years with a normal

ovulatory cycle were evaluated. Basal body temperature (BBT) was continuously recorded. The menstrual cycles averaged 26-33 days, in 3 menstrual cycles which occurred before the study. Measurements of urinary LH levels were made twice a day (9:00 A.M. and 5:00 P.M.) before and after the estimated ovulation date. LH levels in sera and urine were simultaneously measured, using RIA and Hi-Gonavis, respectively. Ovulation was confirmed by Ultrasonography. The principle of Discretest (Chefaro International, Holland) is based on SPIA. In the absence of sufficient quantities of LH in the urine, the non-agglutinated coated gold particles, producing a transparent reddish-purple colour, will remain as such. The unchanged reddish-purple colour constitutes a negative test result indicative of the absence of sufficient LH in the urine to be a determinant of an upcoming ovulation. In the presence of sufficient quantities of LH, the coated gold particles will aggregate resulting in a colour change. If sufficient quantities of LH are present, the aggregates will enlarge and from agglutinates resulting in discolouration. If the test result is positive then ovulation will soon occur (Fig. 1). The procedure is as follows:

1) Squeeze liquid from the dropper into the well.
2) Withdraw urine using the empty dropper.

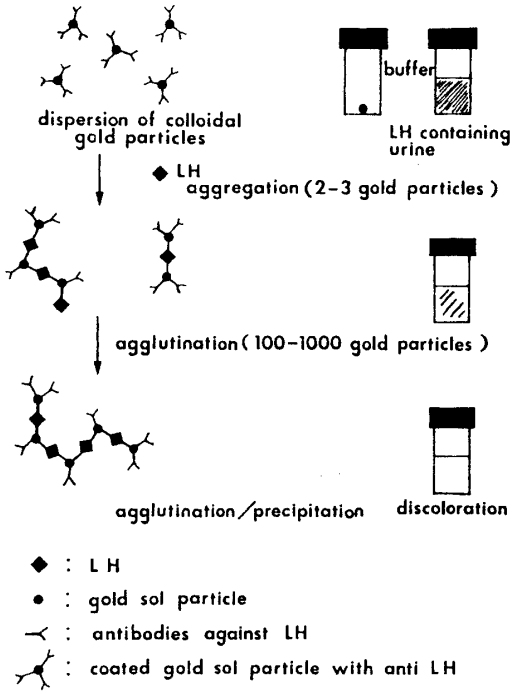

Fig.1 Discretest —— related chemical actions

3) Put four drops of urine into the well, by gently squeezing the dropper. Squeeze out the remaining urine.
4) Withdraw the entire mixture from the well using the dropper
5) Add the entire contents of the dropper to the test tube.
6) Press the stopper of the test tube back into place. Shake the tube until the contents are well mixed.
7) Put the tube into the stand.

The result can be attained 30 minutes after start of the test.

RESULTS

The LH-surge in sera and urine were present in all eight volunteers and ovulation then occurred. Hi-Gonavis revealed positive results (at least a 4-fold dilution) of a positive test with Discretest. As the LH level was thus 50 IU/L, the detection limit of Discretest was estimated to be 50 IU/L. The Discretest was positive 16-24 hours before the LH-surge in the blood and these results are in fairly good agreement with the onset of the LH-surge evident in sera and urine samples. The time to ovulation after the first positive result with Discretest ranged from 32-48 hours (average 40 hours). In all cases, the test results of Discretest changed from positive to negative, before and after ovulation. Changes of LH levels in sera and urine and the test results with Discretest for one week, in one volunteer are shown in Fig. 2.

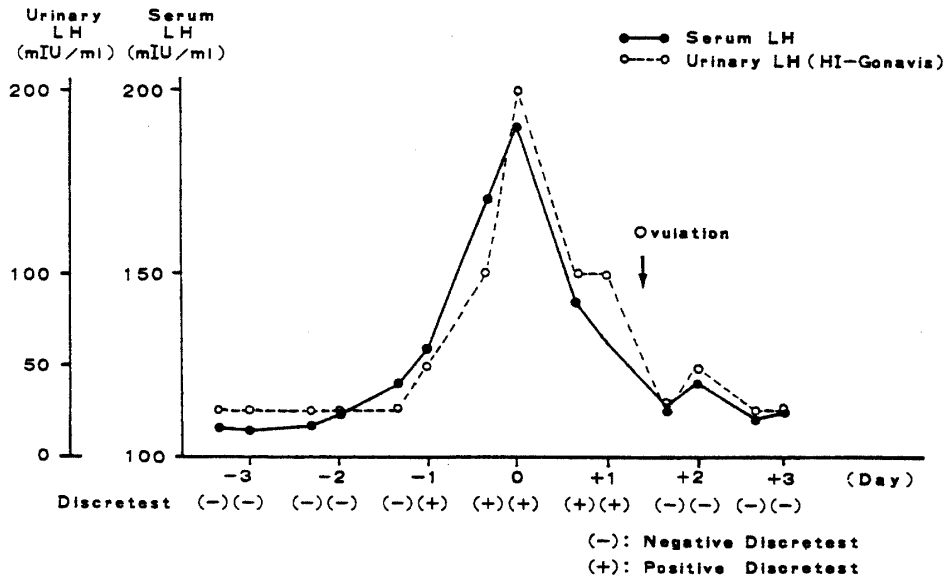

Fig.2 Changes in LH levels in sera and urine and results of Discretest. The day of the highest serum LH level was used as the reference point (day 0)

DISCUSSION

The purpose of this study was to confirm that the Discretest could detect the LH-surge to predict ovulation in Japanese volunteers. The results of Discretest correlated well with the clinical data in the prediction of ovulation and also showed a good correlation with Hi-Gonavis results of urinary LH levels and RIA results of serum LH levels. Recently, a simple, rapid, non-radioactive, and semiquantitative enzyme-linked immunoassay (Ovustik, Monoclonal, Antibodies, Inc., Mountain View, CA) has been developed and ovulation can be predicted using this test(2). Nakamuro et al.(1) reported that this test was useful for the detection of LH-surge in the urine of Japanese volunteers. This test involves two steps and the procedure is complicated and time consuming as a longer incubation period is required, (80 min.).

The test kit we evaluated warrants further use as the this test can be done in only 30 minutes and urinary LH can be measured directly. Ovulation can be accurately assessed as we noted changes from positive to negative before and after ovulation, in all cases. It is also evident that ovulation can be expected to occur 40 hours after the positive results with Discretest.

Discretest seems to be a useful tool for the clinical diagnosis of infertility, as ovulation can be monitored accurately and rapidly.

REFERENCES

1. Nakamuro, K., Kato, M., Saji, F. and Tanizawa, O. (1985) Simple, rapid and semiquantitive enzyme-linked immunoassay for detection of the preovulatory LH excretion in the urine, Acta Obst. Gynaec., 37: 2137.
2. Singh, M., Saxena, B.B. and Rathnam, P. (1984) Clinical validation of enzymeimmunoassay of human luteinizing hormone (hLH) in the detection of the preovulatory luteinizing hormone (LH) surge in urine, Fertil. Steril., 41: 210.
3. Varma, T.R., Patel, R.H. and Everard, D. (1982) Determination with Hi-Gonavis of luteinizing hormone levels in urine compared with those in plasma, Br. J. Obstet. Gynaec., 89: 87.