

Reproductive hormonal levels in Malay women with superovulatory cycles

Htay Htay Nwe, MBBS
Lecturer,¹

Hairunnishah Abdul Wadood, BSc
Scientific Officer,²

Ramli Bin Abdullah, PhD
Associate Professor and Head,³

Hamid Arshad, MBBS, FRCOG
Professor and Director General²

- 1 *Department of Physiology, Universiti Kebangsaan Malaysia (UKM), Kuala Lumpur*
- 2 *National Population and Family Development Board, Malaysia (NPFDB).*
- 3 *Department of Zoology, Faculty of Science, University of Malaya, Kuala Lumpur*

Summary

This paper presents a study of cycles with spontaneous luteinizing hormone (LH) surge in superovulatory Malay women during in vitro fertilization and gamete intrafallopian transfer programmes. Sixteen Malay women underwent ovarian stimulation for ovum pick up at the National Population and Family Development Board, Malaysia. Two cycles showed spontaneous LH surge, and comparisons were made between these two cycles and the cycles without surge. Further observations were made in their characteristics and differences in these two cycles as well as the relationship between progesterone and LH at the time and before the surge.

Key words: Spontaneous luteinizing hormone surge, superovulatory Malay women.

Introduction

In vitro fertilization and embryo transfer (IVF-ET) and gamete intrafallopian transfer programmes (GIFT) are enabling many infertile couples to conceive. The improvement in the success rate over Steptoe's original method that used the natural cycle is due largely to superovulation (ovarian stimulation).¹ The chances of starting a pregnancy increase dramatically with increased number of embryos replaced in the uterus.² Clomiphene citrate (CC), human menopausal gonadotrophin (hMG) and follicle stimulating hormone (FSH) are presently being used alone or in combination for the recruitment of multiple fertilizable oocytes.¹

It has been postulated that exogenously stimulated ovaries secrete a substance that may block the oestrogen-induced LH surge.³ However, an endogenous LH surge before human chorionic gonadotrophin (hCG) administration has been reported from various centres.^{1,4,5} Thus the occurrence of an endogenous LH surge necessitates prompt retrieval before spontaneous ovulation.¹

Appropriate timing of oocyte retrieval is essential to obtain matured oocytes. Since follicle rupture generally occurs about 36 hours after the detection of the onset of the surge,⁶ accurate timing for retrieval can be achieved by appropriate administration of hCG which is followed by a timely retrieval.

Once an LH surge has occurred, decisions must be made concerning the time of administration of hCG and when to perform the retrieval. Each case must be considered individually.¹ When an LH surge occurs, oestradiol (E_2) values may help to determine the most appropriate length of time between the hCG administration and oocyte retrieval.¹ Depending on the E_2 levels on the day after hCG administration, a decrease of more than 15% was noted to have more matured oocytes and fewer immatured oocytes compared to the cycles in which E_2 decreased by less than 15% or in which E_2 increased.¹

This paper is an attempt to review the cycles with spontaneous LH surge in superovulatory Malay women, so as to identify the possible factors in determining the outcome of ovum pick up (OPU).

Material and Methods

The NPFDB of Malaysia conducted a preliminary study on IVF-ET and GIFT programmes. The centre accepts women of various ethnic groups in Malaysia, but this paper deals with the data from the Malay women during the two years of preliminary study and with serial hormonal assays during the late follicular phase. Sixteen infertile Malay women between 26 and 39 years of age were accepted into the IVF-ET and GIFT programmes. Before being accepted, preliminary investigations for ovulatory potentials and accessibility of ovaries, as well as monitoring of these stimulated cycles were carried out according to the previous published protocols.⁷ In brief, the procedures before OPU consisted of ovarian stimulation, monitoring of the developing follicles, administration of hCG and laparoscopic timing when the preovulatory oocytes are matured.

Follicular stimulation: In general, stimulation protocols consisted of CC or metrodin (FSH) together with hMG. The hCG containing 5,000 IU was given at the appropriate time for the final maturation of the preovulatory oocytes.

Hormonal Monitoring and laparoscopic timing for ovum pick up. The preovulatory daily serum hormones were analysed from the peripheral venous blood. Preovulatory daily serum E_2 and ovarian ultrasonography were the parameters used for the monitoring of ovarian development. Injection of hCG depends on (1) cycle length (midcycle), (2) plateau or slight decline in E_2 concentration (considered as E_2 peak) and (3) presence of dominant follicles 18mm or larger seen with ultrasound. Laparoscopy was performed for recovery of oocytes 36 hours after the injection of hCG. In the two cycles where spontaneous LH surge were noted, hCG was administered immediately and OPU was performed within 24 hours under hCG injection (the LH concentration twice the base line values was taken as a LH surge²).

The retrieved oocytes were classified into matured preovulatory oocytes and immatured oocytes as reported by Marrs et al.⁸

Analytical Methods of Radioimmunoassay (RIA). Serum E_2 , progesterone (P) and LH were measured using commercial RIA kit (Coat-a-count, Diagnostic Products Corp, Los Angeles, CA). The LH assay has been standardized against the "First International Reference oocytes in the cycle without LH surge (i.e. 4 compared to 4.67 matured oocytes).

A day before the LH surge, although the LH level between the two cycles were similar, i.e. 1.27 and 1.23 times the base line level respectively (Table 2) P level in Case I was far in advance (2.07 times the base line) compared to Case II 1.38 times the base line). On the following day (day 0), 2.07 times the base line level of P on the previous day in Case I was associated with 2.5 times the base line value of LH on day 0; similarly 1.38 times the base line of P the previous day was associated with 1.97 times the LH level in Case II. According to the definition of LH surge, the surge had already occurred in Case I and even slightly in an advance stage, whereas in Case II LH surge was about to occur. The LH surge can be detected as the value that is twice as high as the average base line value and that is followed immediately by another raised value,² in association with a parallel rise in serum P.⁵ On that day (day 0), P levels in Case I and II were 4.5 and 3.8 times the base line values respectively. These observations indicate that double the base line values in the peripheral P concentration might provide information of impending LH surge and about four times the base line values can be associated with LH surge.

Preparation" for human pituitary LH 68/40 of the World Health Organization. The intra- and interassay variation coefficients of the internal quality control pools for E₂, P and LH were all within 6% and 10% respectively.

Statistical Analysis. The data are presented as arithmetic mean \pm SEM. The means of P and LH values are presented as "times the base line values".

Results

Sixteen infertile but spontaneously ovulating Malay women underwent ovarian stimulation for OPU. The outcome of the OPU from the 16 women was reported in the previous published paper.⁷ In brief, a total of 38 matured preovulatory oocytes with the mean (\pm SEM) of 3.45 ± 0.43 were picked up from nine women in 11 stimulated cycles. One woman ovulated spontaneously, and in another six women, it was not possible to retrieve matured oocytes because of poor response to ovarian stimulation.⁷ Two out of 11 cycles had spontaneous LH surge before hCG administration. In another nine cycles, P and LH levels were almost base line before administration of hCG, as shown in Fig. 1. In these cycles without LH surge both the mean serum levels of LH and E₂ were highest on day 0, but occurred only after the hCG administration and decreased on the day of OPU (Fig 1) (the characteristics of cycles, without surge and with LH surge are shown in Table 1).

On the other hand, in the two cycles with LH surge, the mean values of P on the day of LH surge was more than 4 times and the mean value of LH was more than double the base line value before the hCG administration (Fig 1). In these two cycles, the mean serum level of LH and P were similar and almost parallel to one another. Unlike the same serum level of LH in the cycles without LH surge, the LH levels in these two cycles, were higher on the day of OPU compared to the level on day 0 (Fig 1). The day of LH surge was designated as day 0, since hCG was administered immediately. On day 0 of the cycles with LH surge, E₂ levels were at their peak, before administration of hCG, as shown in Fig. 2.

Further observation and comparisons were made on the two cycles with LH surge. The characteristics of these two cycles are shown in Table 2.

Discussion

In our study only two cycles with endogenous LH surge were observed. Therefore to perform

Table 1
The characteristics of the cycles without surge and with LH surge (mean \pm SEM)

Characteristics	Without surge	With surge
1. Age (years)	35.14 \pm 1.65	37.5 \pm 0.5
2. Peak E ₂ /total oocytes retrieved (pg/ml/total oocytes)	259.03 \pm 18.93	214.83 \pm 13.34
3. Mean P value on day 1 (time the base line level)	1	1.75
4. Mean LH value on day 1 (time the base line level)	1	1.25
5. Mean P value on day 0 before hCG administration (time the base line level)	1	4
6. Mean LH value on day 0 before hCG administration (time the base line level)	1	2
7. Total oocytes retrieved	5.63 \pm 0.6	4 \pm 1.9
8. Mean no. of matured oocytes	3.67 \pm 0.44	2.5 \pm 1.5
9. Time between hCG injection and OPU (hours)	36	< 24

1 = base line or almost base line in mean P and LH values.

statistical comparisons on the values of these two cycles are impossible. Nevertheless, one could observe some of the facts and characteristics in these cycles.

The patients experiencing spontaneous LH surge have been reported to be significantly older than the control population.¹ Similarly in our study, the patients with LH surge were found to be slightly older compared to the women without surge. The mean of the highest E₂/total oocytes retrieved in cycles with surge was found to be slightly lower, compared to cycles without surge, and this is in contrast to the findings of Nader et. al.⁴ However, Droesch et. al.¹ reported of no significant difference between the two groups concerning E₂ levels. The mean number of matured prevulatory oocytes picked up from the two cycles with LH surge was lesser than the cycles without surge (which is similar to the previous reports^{1,4}). However, in Case II the number of matured oocytes retrieved was even slightly more, or almost the same as the mean number of Serafini et. al.⁵ have shown that a two-fold rise in the serum P level was associated with a predictability of 64.4% LH surge; four-fold increase in serum P could accurately predict a spontaneous endogenous LH discharge in 93.3% of the cases. Mahadevan et. al.⁹ have also stated that a significant increase in P could trigger a LH surge. Moreover, the P and the LH levels in the cycles with LH surge were almost parallel to one another. This could be taken as one of the evidences in confirming the relationship concerning the surge between the two hormones.

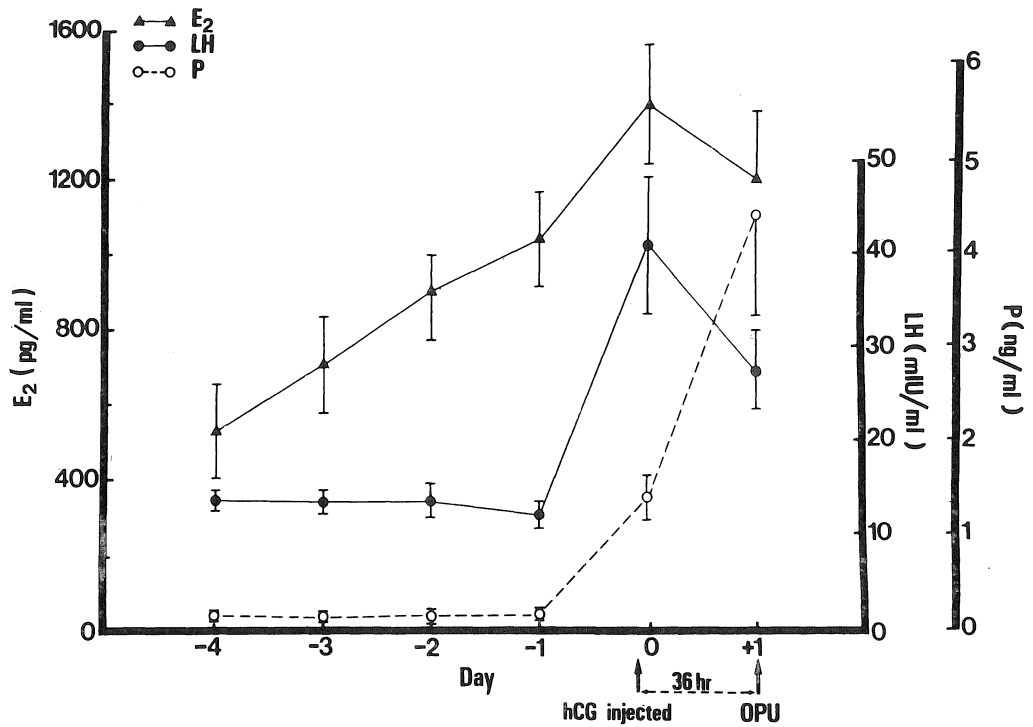


Fig. 1. The mean (\pm SEM) of serum E₂, LH and P levels during the late follicular phase of nine cycles without spontaneous LH surge.

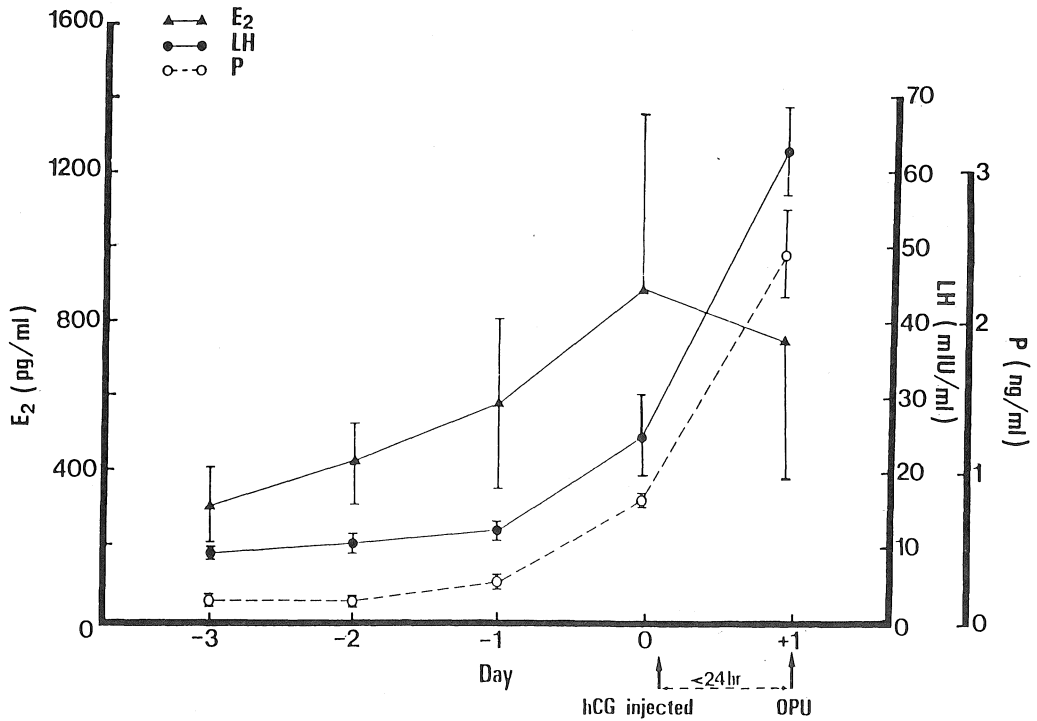


Fig. 2. The mean (\pm SEM) of serum E₂, LH and P levels during the late follicular phase of two cycles with spontaneous LH surge.

Table 2. The characteristics of the two cycles with LH surge

Characteristic	Case I	Case II
1. Maximal E ₂ /total oocytes retrieved (pg/ml/total oocytes)	201.5	228.16
2. LH level on day 1 (times base line level)	1.27	1.23
3. P level on day 1 (times base line level)	2.07	1.38
4. LH level on day 0 (times base line level)	2.5	1.97
5. P level on day 0 (times base line level)	4.5	3.8
6. Time between hCG injection and OPU (hours)	19	22.5
7. Total oocytes retrieved	2	6
8. Number of matured oocytes retrieved	1	4
9. % of matured oocytes per total oocytes retrieved	50.0%	66.6%
10. Treatment regimens	CC	Metrodin
11. Decrease in E ₂ on day after the hCG administration	3.2%	18.99%

There was a slight difference in LH pattern noted between the cycles with LH surge and without surge (Fig 1 and 2). This could be due to the time difference in the hCG injection and the hormonal estimation on the day of OPU, between the two groups.

When the impending LH surge was noted in Case II, LH level was only 1.97 times above the base line level. Thus hCG must have been injected just after the surge, which is in contrast to Case I where the surge was slightly in an advance stage by the time hCG was administered. This might be one of the reasons why four out of six total oocytes in Case II and one out of two total oocytes in Case I were retrieved. Moreover, from the beginning there was failure to recruit multiple follicles in Case I, which is one of the characteristics of the cycles associated with LH surge.⁴ In Case I, not only did the follicles fail to be recruited, but percentage of matured oocytes available from the total number of developing follicles was also slightly less. It seems that Case II benefited from longer time taken between the hCG administration and OPU, as well as the time taken for E₂ concentration to decrease more than 15%. In the two cases with the surge, no differences were noted in the stimulatory regimens. The surge was noted once in each regimen.

In conclusion, similar to the suggestion made by Droesch et. al.¹ each case must be considered individually. A number of parameters should be taken into consideration. Prevention of the endogenous LH surge would be most advantageous in this group of patients. Treatment with a

gonadotropin releasing hormone (GnRH) against before gonadotropin stimulation, while increasing the amount of stimulation required¹⁰ might lead to an improved outcome. As stated by Serafini et. al.,⁵ even daily estimation of P level could be of use in predicting impending LH surge.

However, if LH surge had occurred or if an impending LH is noted, immediate administration of hCG could save the cycle. Furthermore, early detection and appropriate timing of hCG administration and OPU might lead to an improved outcome in these stimulated cycles.

Acknowledgement

We wish to thank the staff of the NPFDB, Mr. K. Raja Sekaran, Miss Margaret Martin, Mrs. S. Suganthi, Mr. Jaafar Ali and Miss Rosliah Harun, the head and the staff of the Department of Physiology, Mr. P.P. Loh and Mr. Sarjit Singh, staff of Medical Illustration Department, UKM and Puan Baridah Hj. Abdullah for typing the manuscript.

References

1. Drosch K, Muasher SJ, Kreiner D, Jones GS, Acosta AA, Rosenwaks Z. Timing of oocyte retrieval in cycles with a spontaneous luteinizing hormone surge in a large in vitro fertilization program, *Fertil Steril* 1988; 50 (3): 451-56.
2. Hillier SG, Afnan AMM, Margara RA, Winston RML. Superovulation strategy before in vitro fertilization. *Clinics in Obstetrics and Gynecology* 1985; 12(3): 687-723.
3. Sopolak VM, Hodgen GD. Blockage of the estrogen-induced luteinizing hormones surge in monkeys: a nonsteroidal, antigenic factor in porcine follicular fluid. *Fertil Steril* 1984; 41(1): 108-113.
4. Nader S, Berkowitz AS, Maklad N, Wolf DP, Held B. Characteristics of patients with and without gonadotropin surges during follicular recruitments in an in vitro fertilization/embryo transfer program. *Fertil Steril* 1986; 45(1): 75-78.
5. Serafini P, Stone B, Kerin J, et al. Occurrence of a spontaneous luteinizing hormone surge in superovulated cycles - predictive value of serum progesterone. *Fertil Steril* 1988; 499 (1): 86-89.
6. Testart J and Frydman R. Minimum time lapse between luteinizing hormone surge or human chorionic gonadotropin administration and follicular rupture. *Fertil Steril* 1982; 37 (1): 50-53.
7. Nwe HH, Wadood HA, Abdullah R, Arshat H. Estradiol levels for ovum pick up in superovulatory Malay women, *Malaysian J Reprod Health* 1989; 7(2): 115-21.
8. Marrs RP, Saito H, Yee B, Sato F and Brown J. Effect of variation of in vitro culture techniques upon oocyte fertilization and embryo development in human in vitro fertilization procedures. *Fertil Steril* 1984; 41(4) 519-23.
9. Mahadevan MM, Fleetham J, Taylor PJ. Effects of progesterone on luteinizing hormone release and estradiol/progesterone ratio in the luteal phase of women superovulated for in vitro fertilization and embryo transfer. *Fertil Steril* 1988; 50(6): 935-37.
10. Neveu S, Hedon B, Bringer J, et al. Ovarian stimulation by a combination of a gonadotropin-releasing hormone agonist- and gonadotropins for in vitro fertilization, *Fertil Steril* 1987; 47 (4): 639-643.