

SINGLE DAILY MONITORING OF PERIOVULATORY ESTRADIOL, PROGESTERONE, AND LUTEINIZING SERA HORMONE LEVELS IN NATURAL CYCLES USEFUL FOR TIMING INTRAUTERINE INSEMINATION

J. H. CHECK
M. PEYMER
P. JAIRAJ

The University of Medicine and Dentistry of New Jersey,
Robert Wood Johnson Medical School at Camden, Cooper Hospital
and University Medical Center, Department of Obstetrics
and Gynecology, Division of Reproductive Endocrinology
and Infertility, Camden, New Jersey, USA

Single daily periovulatory serum levels of luteinizing hormone (LH), estradiol (E_2), and progesterone (P) may be useful to predict the time of ovulation, since serum P is known to rise to 1000 pg/mL about 24 h after the LH surge. Intrauterine insemination (IUI) was performed based on the serum P level at the time of the LH surge; if sera P measures <1000 pg/mL, then IUI is performed 36–40 h later; but if P is ≥ 1000 pg/mL, IUI is performed 12–20 h later. The relationship of sera LH, E_2 , and P levels and follicular diameter were evaluated at the time of the LH surge and the day before in 40 women with cervical factor who conceived following a single IUI. If E_2 levels were obtained the day before and the day of the LH surge and measured <200 or ≥ 200 pg/mL, a shorter interval for the IUI was suggested. The 27% pregnancy rate/cycle suggests that measurement of single daily periovulatory hormonal levels are useful for precise timing of IUI.

Keywords intrauterine insemination, ovulation, periovulatory hormones, progesterone

The timing of ovulation is important for optimum pregnancy rates (PRs) following intrauterine insemination (IUI). Since there are decapacitating factors in both seminal plasma and cervical mucus, the lack of decapacitating factors may allow those sperm capable of fertilization to undergo spontaneous acrosome reaction, and spermatozoa that have already shed the outer membrane can no longer bind to the zona pellucida [5].

When sera samples are measured every 4 h, luteinizing hormone (LH) is seen to rise rapidly. It is impractical, however, under normal clinical conditions, to obtain such frequent sampling in outpatient settings. If daily periovulatory LH levels are obtained, identification of the LH surge may be off by as much as 23 h. Thus, to cover all possibilities, an IUI might be

Address correspondence to Dr. Jerome H. Check, 8002 E. Greentree Commons, Marlton, NJ 08053, USA.

performed 24 h following the surge and then again 12 h later. The second semen specimen may not be as potent as the first and yet may be the one timed more appropriately. Some offices are not set up for evening IUI and it is inconvenient and expensive for patients to have two IUIs performed in the same day.

Since the estradiol (E_2) peak has been found to occur at the time of LH surge and starts dropping sometime thereafter, and progesterone (P) reaches a level of approximately 500 pg/mL at the time of the surge and then reaches 1000 pg/mL 24 h following the surge, we decided to base the timing for IUI on periovulatory LH, E_2 , and P levels [9]. Since previous studies found that when the LH surge was defined as the first level to reach 60 mIU/mL, the prediction of ovulation was earlier (27 h) than other studies (36–38 h). We lowered the level to 50 mIU/mL to define the LH surge and hoped this would be more consistent with a 36-h time [8].

The fertilizing capacity of sperm not in cervical mucus has been estimated to be approximately 4 h and the duration of time that the oocyte can be fertilized once in the fallopian tube is approximately 8 h, so the window of fertilization with IUI may be only 12 h. Unfortunately, it is not practical to obtain sera samples in frequent intervals from patients needing IUI and most centers measure the LH surge by single daily serum or urinary levels. The imprecise timing may explain the relatively poor PRs following IUI in natural cycles. Certain relationships have been previously established with the LH surge and other periovulatory hormonal events, e.g., the E_2 level peak coincides with the LH surge and the serum P approaches 1000 pg/mL approximately 24 h after the surge.

The study presented herein evaluated midcycle follicular dynamics in patients conceiving following a single IUI for cervical factor. The timing of the IUI was not only based on the LH surge, but was also modified by the level of the serum P. Though decisions on when to perform IUI were based on the above parameters, the study also determined whether other parameters could predict the proper timing (timing was assumed to be appropriate since the patients conceived and each couple enlisted had been found not to have any progressively motile sperm on postcoital testing [PCT]). Perhaps, either the follicular diameter or sera E_2 patterns on the day before and day of LH surge could predict ovulation. The possibility also exists that, despite our system of shorter time interval to IUI, if the serum P approached 1000 pg/mL, we might find that the majority of patients conceive only with the longer interval.

MATERIALS AND METHODS

One hundred and fifty cycles of 79 patients were evaluated (a maximum of three cycles per patient) where IUI was performed for cervical factor; no sperm with progressive forward motion had been detected in two cycles. Pregnancies were achieved in 40 (26.7%) of these cycles. The periovulatory hormonal data in 40 cycles were evaluated, since they were considered to be the ones most likely to be accurate, because of pregnancies achieved. Because only one IUI was performed each cycle, we made the assumption that the timing of the IUI is indicative as to whether the window of fertilization was closer to (12–20 h) or further from (36–40 h) the LH surge.

None of the patients in the study received follicle maturing drugs. Sonography confirmed the presence of a mature follicle (>17 mm average diameter) and oocyte release (a 5-mm decrease in follicular size) within 48 h of the LH surge [1, 3]. Sera LH, E_2 , and P were obtained daily beginning when a follicle demonstrated a minimum of 17 mm diameter on ultrasound. Sonography was started 16–17 days before expected menses. The E_2 assay was performed by radioimmunoassay (RIA) (Diag-

nostic Products Inc., Los Angeles, CA); P and LH assays were also performed by RIA (Amersham Inc, Arlington Heights, IL).

Comparison of E_2 and P levels before and on the day of the LH surge was evaluated by paired t test with $p < .05$ as significant. Comparison of change in the E_2 levels before and on the day of the LH surge by level of serum P was performed by comparing change within each group using McNemar's test. Fisher's exact test was used to compare the number of patients whose E_2 level increased versus decreased from the day before to the day of LH surge according to serum P on that same day.

The following assumptions were made to determine the appropriate time to perform IUI: (1) If the serum P was <1000 pg/mL on the day of LH surge, ovulation would be later, therefore IUI would be performed 36–40 h after LH surge to include the possibility of ovulation up to 4 h after the IUI, or the possibility that if ovulation had occurred, the oocyte was not in the fallopian tube more than 8 h. (2) If the serum P was ≥ 1000 pg/mL, the assumption was that ovulation was more imminent and the IUI was performed 12–20 h later. The clinic remains open every day and evening, so only an 8-h time period was not available for performing the IUI. Ultrasonography was performed at the time of IUI. If internal echoes were seen within the follicle, or collapse of the follicle by at least 5 mm was demonstrated, release of oocyte was assumed.

RESULTS

Luteinizing hormone levels increased an average of 76.6 ± 44.5 mIU/mL ($p < .05$) on the day before to the day of LH surge in all patients. Overall, the mean levels of P and E_2 increased significantly from the previous day to the day of LH surge (Table 1). However, based on data refined according to whether the P level reached 1000 pg/mL during the period of investigation, the mean concentration of E_2 on the day of LH surge did not increase significantly in the group where $P < 1000$ pg/mL, but decreased (though not significantly) in the group where $P \geq 1000$ pg/mL. Furthermore, in the group where P remained below 1000 pg/mL, E_2 decreased in only 3 of 24 (12.5%) cycles from the first to the second day vs 11 of 16 (68.8%) in the group where $P \geq 1000$ pg/mL (Fisher's exact test, $p = .001$). In 21 of the 40 (52.5%) patients, initial serum E_2 was <200 pg/mL, and in 16 of the 21 (76.2%) cases those

TABLE 1 Comparison of E_2 and P Levels Before and on Day of LH Surge

Group	Hormone	Mean (1 SD) Before Surge	Mean (1 SD) on Day of Surge	Mean (1 SD) Difference
All cases ($n = 40$)	LH (mIU/mL)	22.1 (15.3)	98.7 (47.9)	76.6 (44.5) ^a
	P (pg/mL)	0.5 (0.2)	1.0 (0.3)	0.4 (0.3) ^a
	E_2 (pg/mL)	197.4 (61.0)	227.9 (84.4)	30.5 (93.4) ^a
$P < 1.0$ ($n = 24$)	LH	23.2 (19.2)	96.7 (51.1)	73.2 (44.1) ^a
	P	0.5 (0.2)	0.7 (0.2)	0.2 (0.2) ^a
	E_2	193.1 (69.8)	267.2 (83.9)	82.9 (82.9%) ^a
$P \geq 1.0$ ($n = 16$)	LH	20.3 (6.4)	101.8 (44.2)	81.5 (46.0) ^a
	P	0.6 (0.2)	1.3 (0.2)	0.7 (0.3) ^a
	E_2	203.9 (46.3)	169.1 (39.2)	34.8 (67.6)

^a $p < .05$ (paired t test).

TABLE 2 Comparison of Change in E_2 Levels Before and on Day of LH Surge by Level of Serum Progesterone on Day of hCG

Group	Pre E_2 and Surge E_2 Both <200 (pg/mL)	Pre E_2 and Surge E_2 Both \geq 200	Pre E_2 <200 Increases to >200	Pre E_2 \geq 200 Decreases to <200
All cases ($n = 40$)	5 (12.5%)	10 (25.0%)	16 (40.0%)	9 (22.5%) ^a
$P < 1.0$ ($n = 24$)	2 (16.7%)	8 (33.3%)	13 (54.2%)	1 (4.2%) ^a
$P > 1.0$ ($n = 16$)	3 (18.7%)	2 (12.5%)	3 (18.7%)	8 (50.0%) ^a

^a $p < .05$, comparing change within each group using McNemar's test.

levels rose to ≥ 200 pg/mL on the day of LH surge (Table 2). None of the 40 patients had a serum $P \geq 1000$ pg/mL in the initial sample. The mean sizes of the follicle in groups 1 and 2 were not significantly different, 21.0 ± 2.2 mm vs 22.0 ± 2.5 mm ($p = .2$).

Note from Table 1 that whether the P level was less than or greater than 1000 pg/mL, the baseline LH on the day before was similar (23.2 vs 20.3 mIU/mL for lower vs higher) and the mean LH the next day, when over 50 mIU/mL (defined as the surge), was also similar (96.7 vs 101.8 mIU/mL). However, based on the serum P levels, the IUI would be timed sooner in the latter groups. This decision was supported by achieved pregnancy, and by the fact that the mean E_2 levels increased from the day before to the day of LH surge in the first group, but E_2 levels dropped in the second group, suggesting that the serum was obtained closer than 36–38 h from ovulation.

Oocyte release by sonography was noted in 35 of these 40 pregnant cycles (87.5%) compared to 78 of 110 (70.9%) of nonconception cycles (Fisher's exact test, $p = .05$). There were 93 cycles where the sera P levels were <1000 pg/mL on the day of LH surge and 57 when the P levels were ≥ 1000 pg/mL. Pregnancy rates according to P level were similar: 25.8% (24 of 93) with lower P levels vs 28.0% (16 of 57) with higher P levels.

DISCUSSION

Hormonal dynamics at midcycle have been established by measuring LH, E_2 and P levels at 2-h intervals, from a heparinized indwelling venous catheter, over a 5-day period, in several normal, regularly menstruating women [9]. Estradiol levels reached a peak at the time of the LH surge. This peak level of E_2 was maintained for the first few hours of the LH surge, when a rapid fall occurred. The P levels exhibited a multiphasic increment, with the doubling phase paralleling the rise in E_2 , then a sudden increase in the second phase (which began 12 h before and continued for 12 h after the onset of the LH surge). A mean P of 550 ± 70 pg/mL was reached at the time of the initiation of the LH surge. In the third phase, a plateau occurred between 14 and 34 h after the LH surge onset and was coincident with the peak phase of the LH surge. The concentration of P remained relatively stable (1190 ± 120 pg/mL) during this time. The plateau was followed by a final phase, which consisted of a rapid rise of P beginning 36 h after the LH surge onset. The mean LH surge duration was found to be 48.7 ± 9.3 h and the mean LH level at the time of initiation of the LH surge was approximately 50 mIU/mL [9].

Testart et al. [11] obtained sera samples of LH four times daily in patients undergoing

laparoscopy for IVF (without hyperstimulation) and concluded that ovulation occurred 36–38 h after the onset of the LH surge. Taymor et al. [19] also performed a study where LH samples were obtained at 3 to 4-h intervals and determined that all corpora lutea were found 38 h or more after the onset of the LH surge; they concluded that ovulation occurs approximately 38 h following the onset of the LH surge [10].

The LH surge has been defined in various ways by different authors. Hoff et al. [9] chose the first LH value exceeding the mean and 2 SD of the six preceding values, whereas Taymor et al. [10] defined the LH surge as the first point rising from baseline followed by a sustained rise. In another study, using 4-h intervals for sampling LH, Garcia et al. [8] selected a level of 50 mIU/mL to define the LH surge, and, using this value, concluded the mean time of ovulation from LH surge to be 27 h and 20 min.

In the present study, we timed the single IUI much earlier than 36 h with LH surge when the serum P was ≥ 1000 pg/mL. We thus hypothesize that, since many physicians give hCG when the follicular diameter is adequate and perform IUI 36 h later, the reduced PRs reported by some groups with IUI in unstimulated cycles may be due to performing the procedure too late; 36.4% of the patients were in the higher serum P group.

Many studies have suggested poor pregnancy results with IUI and natural cycles [7]. Others have reported reasonable PRs when using ovulation-inducing drugs [4, 6]. There are physicians who will give hCG at the time of a mature sized follicle by ultrasound and do the IUI 36 h later. Our data showed no difference in the mean follicle diameters in females with P levels < 1000 pg/mL vs those whose P levels were ≥ 1000 pg/mL. These data demonstrated a high PR (26.6%) with a single timed IUI, based on hormone levels of LH, E_2 , and P, similar to our previous published results of 21.2% per cycle in a randomized study comparing IUI to intercourse to intracervical insemination [2].

The peak E_2 levels occur at around the time of the LH surge. Patients with two E_2 levels > 200 pg/mL and P level < 1000 pg/mL are probably just approaching the LH surge and one could inseminate 40 h later; those with P ≥ 1000 but both sera E_2 levels > 200 pg/mL might be a little more advanced, and earlier IUI of 34 h might be considered. As expected, the majority (80%) of patients in this category have serum P < 1000 pg/mL. The same reasoning and the same timing of IUI could be suggested for those with $E_2 < 200$ then increasing to ≥ 200 pg/mL. As expected in this group, 81.2% had sera P levels < 1000 pg/mL. In contrast, those with a serum $E_2 > 200$ pg/mL dropping to below 200 pg/mL might be expected to be somewhere past the LH surge and be closer to ovulation; as expected, P levels were usually ≥ 1000 pg/mL (24 h past LH surge) in this group.

Thus, the data from Table 2 may be useful for the clinician when only E_2 and LH levels are measured. If the serum E_2 obtained the day before and the day of the surge are both > 200 pg/mL or both < 200 pg/mL, there is an 80% chance that the serum P would be low and a 36 to 40-h IUI should be planned. If the first E_2 level is > 200 pg/mL and the one on the day of LH surge was < 200 pg/mL, there is approximately a 90% chance that serum P will be > 1000 pg/mL and a shorter time interval, e.g., 12–20 h should be considered. The fact that the mean serum E_2 level was lower on the day of the LH surge in women whose sera P levels were ≥ 1000 pg/mL (and thus close to ovulation) than women whose sera P level were < 1000 pg/mL demonstrates the problem of using only sonography and serum E_2 level to determine when to give an hCG injection and then time IUI 36 h later. An E_2 level slightly lower than 200 pg/mL with a mature follicle may suggest to the clinician that the follicle was still immature and to

wait one more day to give hCG and then perform IUI 60 h later than the day of studies, when in fact, the patient might be 12 h from ovulation and IUI should be performed 12–20 h later if the serum P was >1000 pg/mL.

This study, by demonstrating a high PR/cycle following a single IUI for cervical factor, suggests that better prediction of time of ovulation can be made by utilizing single daily periovulatory sera samples of LH, E_2 , and P. The multiple daily sera samples may still be cost-effective by allowing just one IUI per cycle and allowing higher PRs/cycle, thus saving the cost of multiple cycles of IUI. Future prospective randomized studies should determine whether the addition of measuring urinary LH levels every 4 h once a follicle attains sonographic maturity will further improve PRs.

REFERENCES

1. Check JH, Chase JS, Adelson HG, Dietterich C (1986): New approaches to the diagnosis and therapy of the luteinized unruptured follicle syndrome. *Int J Fertil* 30:29–32
2. Check JH, Chase JS, Spirito P (1991): Efficacy of intrauterine insemination versus sexual relations versus intracervical insemination for treatment of cervical factor infertility. *Am J Gynecol Health* 5:11–17
3. Check JH, Goldberg BB, Kurtz A, Adelson HG, Rankin A (1984): Pelvic sonography to help determine the appropriate therapy for luteal phase defects. *Int J Fertil* 29:156–158
4. Corson SL, Batzer FR, Gocial B, Maislin G (1989): Intrauterine insemination and ovulation stimulation as treatment of infertility. *J Reprod Med* 34:397–406
5. Dangott LJ, Garbers DL (1984): Identification and partial characterization of the receptor for speract. *J Biol Chem* 259(22):13712–13716
6. Dodson WC, Whitesides DB, Hughes CL, Easley HA, Haney AF (1987): Superovulation with intrauterine insemination in the treatment of infertility: a possible alternative to gamete intrafallopian transfer and in vitro fertilization. *Fertil Steril* 51:51–57
7. Friedman A, Haas S, Kredentser J, Stewart E, Schiff I (1989): A controlled trial of intrauterine insemination for cervical factor and male factor: a preliminary report. *Int J Fertil* 34:199–203
8. Garcia JE, Jones GS, Wright GL (1981): Prediction of the time of ovulation. *Fertil Steril* 36:308–315
9. Hoff JD, Quigley ME, Yen SSC (1983): Hormonal dynamics at midcycle: a reevaluation. *J Clin Endocrinol Metab* 57:792–795
10. Taymor ML, Seibel MM, Smith D, Levesque L (1983): Ovulation timing by luteinizing hormone assay and follicle puncture. *Obstet Gynecol* 62:191–195
11. Testart J, Frydman R, Feinstein MC, Thebault A, Roger M, Scholler R (1981): Interpretation of plasma luteinizing hormone assay for the collection of mature oocytes from women: definition of a luteinizing hormone surge-initiating rise. *Fertil Steril* 36:50–54