Article

# Premature LH and progesterone rise in intrauterine insemination cycles: analysis of related factors

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## Abstract

Premature LH and progesterone surges are associated with different factors and hormonal modulators. The aim of the present study was (i) to investigate the clinical and laboratory factors and (ii) to highlight the importance of different stimulation protocols in associated premature LH and progesterone surges in intrauterine insemination (IUI) cycles. The study involved a retrospective investigation of 75 patients undergoing IUI for infertility treatment (135 IUI cycles) between 1996 and 2000, with initial serum LH concentrations >10 mIU/ml during ovarian stimulation. Ultrasound characteristics, follicular sizes, serum oestradiol, progesterone and LH concentrations and ovarian stimulation protocols were measured. There was a wide range of oestradiol serum concentrations (93–2245 pg/ml) and follicular size (12–25 mm). In 49.6% of cycles, the dominant follicle was <16.5 mm. Patients with >2 follicles measuring <15 mm had higher oestradiol serum concentrations ($P$ = 0.008). Multiple regression analyses revealed no association between these variables and premature LH/progesterone surge. In conclusion, LH/progesterone surges cannot be predicted utilizing clinical parameters normally employed, e.g. ultrasound serum oestradiol assay or ovarian stimulation protocol. Patients with follicles >14 mm or more and with high numbers of small follicles and high oestradiol are at risk of a spontaneous LH surge. These variables can be used to time the administration of GnRH antagonist administration until better predictive factors are demonstrated.

***Keywords:*** *intrauterine insemination, LH rise, premature luteinization, progesterone increase*

## Introduction

The determination of LH surge and its associated factors is a controversial issue. Published papers differ in studied populations, outcomes and statistical models. Moreover, the modulation of LH surge by oestradiol is disputed by several authors (Eibschitz *et al*., 1986; Kreiner *et al*., 1988; Couzinet and Schaison, 1993; Taylor *et al*., 1995). Others describe an association between maximum follicular size and LH surges (Eissa *et al*., 1986), and propose a role for gonadotrophin surge-attenuating factor (GnSAF) in these events (Fowler *et al*., 1993).

Predicting the initial LH rise during the LH surge is mandatory in IVF to avoid cycle cancellations, which occur in 25% of cases (Eibschitz *et al*., 1986). In addition, this rise is a good predictor of LH surge and ovulation. In stimulated cycles, small follicles may release more oestradiol, increasing the risk of premature LH rise (Testart and Frydman, 1982). Moreover, other factors may be equally important for LH regulation (Loumaye, 1990), since even seasonal and diurnal LH variability cannot be explained by steroid serum concentrations alone (Testart *et al*., 1982). The importance of progesterone support on different stimulation regimes (Macnamee *et al*., 1988) shows the significance of luteal progesterone secretion and ovarian stimulation on results gained with IVF.

Many of these physiological events were determined and studied 20 years ago, using the more limited technologies of ultrasound and hormone assays, when recombinant FSH, gonadotrophin-releasing hormone (GnRH) agonists and antagonists were not available. GnRH antagonists were recently introduced for IVF–embryo transfer, and probably for intrauterine insemination (IUI) also. Two regimens have been described, single- and multiple-dose protocols (Alabano *et al*., 1997; Olivennes *et al*., 1998; Borm and Mannaerts, 2000). Both regimens can be used in a fixed schedule, in which the antagonist is injected on a particular day of the stimulation cycle. A more flexible approach would be possible if the antagonist could be administered only when an LH rise is feared, perhaps diminishing the dose of antagonist or even identifying patients for whom antagonists are unnecessary. To prescribe such protocols, parameters are needed to predict the initial LH rise to screen patients at risk of premature luteinization.

This new understanding of ovarian cycle and follicular development, and the advent of several protocols for ovarian stimulation, including the introduction of recombinant FSH, led to the analysis of the impact of new regimens on LH surges. A reappraisal of common parameters could be useful when combined with new computerized technology for hormonal assays (e.g. chemoluminescence) and the wide utilization of transvaginal ultrasound, with better probes of higher frequency and increased accuracy of diagnostic equipment (De Boever *et al*., 1983; Freimanis and Jones, 1992; Hershlag *et al*., 2000).

The present study thus aims to analyse clinical and laboratory data in different ovarian stimulation protocols in relation to spontaneous elevated serum LH concentrations in patients admitted for IUI.

# Materials and methods

## Design

A retrospective analysis was carried out of all cases of IUI cycles with a spontaneous LH surge.

## Patients

A total of 135 cycles were analysed among 75 patients admitted for IUI at Hôpital Antoine Béclère, Clamart, who showed a spontaneous LH surge during ovarian stimulation or spontaneous cycles between 1996 and 2000.

IUI was used for treatment of infertility, and stimulation protocols were prescribed according to clinical factors. The various protocols included spontaneous cycles using clomiphene, short agonist, human menopausal gonadotrophin (HMG) and recombinant (rec)FSH.

## Measurements

After day 8 of the, cycle, patients were assessed by measuring oestradiol, LH and progesterone in serum, and using transvaginal ultrasound to assess follicular development.

Spontaneous LH surges were considered in patients presenting with serum LH concentrations >10 IU/l during the stimulation protocol, and serum progesterone concentrations >1 ng/ml, according to Fanchin *et al*. (1996).

The number of days required for ovulation induction, together with serum LH, progesterone and oestradiol on the day of the LH rise, were analysed. Oestradiol output per follicle was measured as a marker of oestradiol secretion related to follicular development.

Numbers of follicles >12 mm, diameters of dominant follicles and total numbers of follicles were assessed.

**Table 1.** Comparison of stimulation duration, steroid concentrations and follicle sizes using the different stimulation protocols (medians and ranges).

| | *HMG* (n = *52*) | *Clomiphene* (n = *17*) | *Short agonist* (n = *6*) | *recFSH* (n = *41*) | *Spontaneous* (n = *19*) | P-*value* |
|---|---|---|---|---|---|---|
| Duration of stimulation (days) | 11 (8–17) | 12 (9–17) | 9[a] (9–12) | 11 (9–19) | 12.5 (10–17) | 0.011 |
| Oestradiol concentration (pg/ml) | 584.0 (157–1460) | 461.0 (154–2126) | 1462.0 (210–2048) | 435.0 (192–1575) | 215.5[a] (93–1154) | 0.0001 |
| Progesterone concentration (ng/ml) | 0.40 (0.1–2.6) | 0.40 (0.1–1.2) | 0.70 (0.1–0.9) | 0.54 (0.1–2.0) | 0.48 (0.1–2.7) | 0.874 |
| Ratio oestradiol per follicle | 211.5 (87.2–730.0) | 201.0 (67.5–424.5) | 218.8 (74.7–328.0) | 236.3 (72.5–496.5) | 132.0[a] (77.5–336.0) | 0.040 |
| No. follicles 14–15.9 mm | 1 (0–3) | 1 (0–4) | 1 (0–5) | 1 (0–5) | 0.3[a] (0–1) | 0.0001 |
| No. follicles >16 mm | 1 (0–3) | 1 (0–3) | 3 (0–4) | 1 (0–4) | 1[a] (0–4) | 0.019 |
| Diameter of dominant follicle (mm) | 16.5 (12–25) | 17.0 (14–22) | 18.0 (15–23) | 17.0 (12.5–23) | 16.5 (14–18) | 0.834 |

[a]$P < 0.05$ in comparison with other groups.

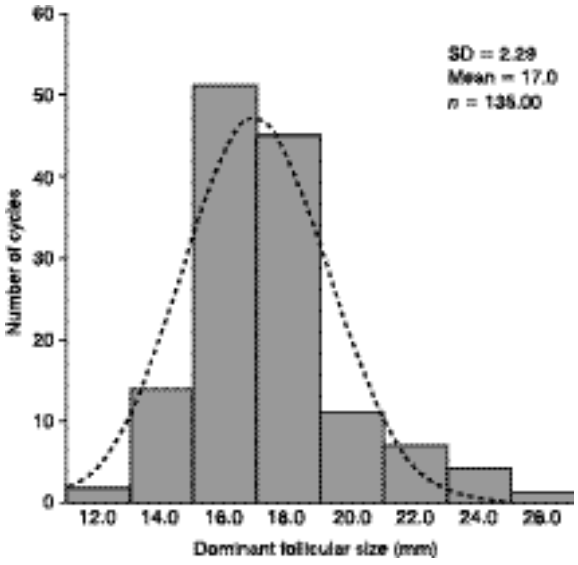


**Figure 1.** Distribution of surges of dominant follicles (mm) and the normal (Gaussian) distribution curve (dotted line).

Patients with >10 follicles at the onset of stimulation protocol were considered to have a polycystic ovary (PCO)-like profile. Follicle diameters were measured by two different investigators, using transvaginal ultrasound (7.5 MHz transvaginal probe; Siemens Elegra®; Siemens SAS, Saint-Denis, France) and calculated as the mean of two perpendicular measures.

Plasma oestradiol, progesterone, LH and FSH concentrations were determined by automated and direct chemoluminescent methods (ACS:180; Chiron Diagnostics Corp., USA). Sensitivity (minimum detectable concentration) was 10 pg/ml for oestradiol (conversion factor to SI units, 3.671), 0.1 ng/ml for progesterone (conversion factor 3.180), 0.1 mIU/ml for LH (conversion factor 1.00) and 0.3 mIU/ml for FSH (conversion factor 1.00). Intra- and inter-assay coefficients of variation over the concentration range were <7% for oestradiol, <10% for progesterone and <5% for both LH and FSH.

## Statistical analysis

The groups were divided according the prescribed stimulation protocol. To compare studied variables among the groups, Kruskall–Wallis or Mann–Whitney *U*-tests were used, since the distribution of data was not parametric.

The effect on premature luteinization as the dependent variable, assessed by serum LH concentrations >10 IU/l and progesterone >1 ng/ml, was measured using multiple regressions in two models. The first model assessed the impact of the stimulation protocol to elicit premature luteinization. The second model utilized age, dose of gonadotrophins (IU), total number of follicles >12 mm, serum oestradiol concentrations, size of dominant follicle and PCO-like ovarian profile as independent variables. All variables are expressed as medians and ranges. The significance level was 5%.

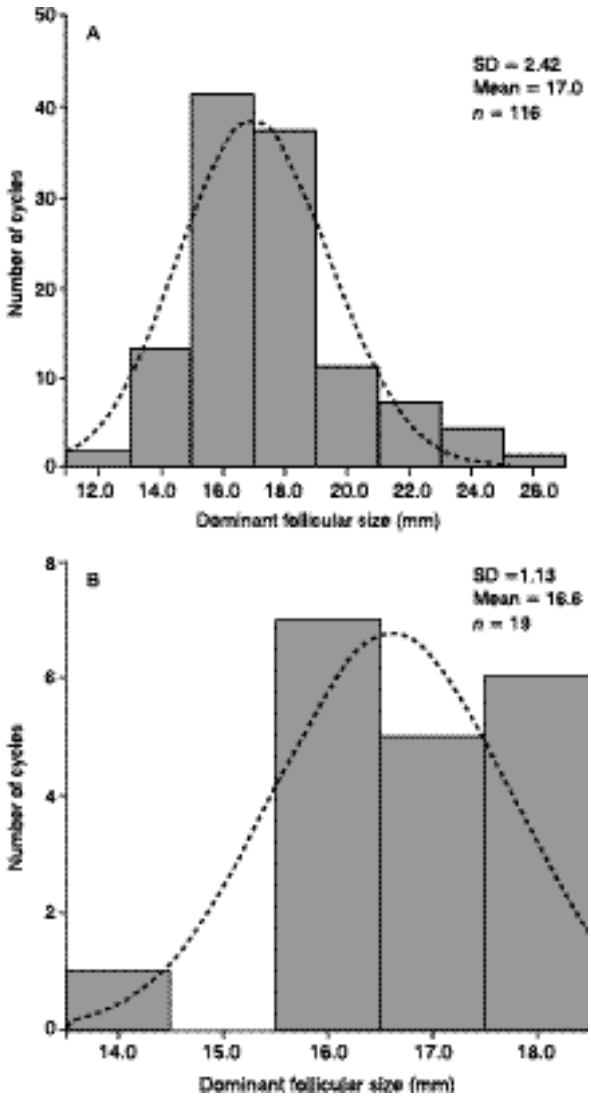


**Figure 2.** Distribution of sizes of dominant follicles size (mm) and normal (Gaussian) distribution curve (dotted line) in patients with stimulated cycles (**A**) and spontaneous cycles (**B**), $P > 0.05$.

# Results

**Table 1** shows the results for ovarian stimulation with different protocols. A total of 75 patients (median age 33 years; range 25–41) were studied in 135 cycles. The most important findings included the wide range observed for oestradiol serum concentrations (median: 437.50 pg/ml; range: 93–2245 pg/ml) and dominant follicular size (median and mean: 17.00 mm; range: 12–25 mm; SD: 2.29, coefficient of variability: 14.67%).

Patients on spontaneous cycles showed an LH surge at lower serum oestradiol concentrations than in stimulated patients, and follicles were fewer.

Distributions in the sizes of dominant follicles are shown in **Figures 1** and **2**. Dominant follicles in 67 patients (49.6%)

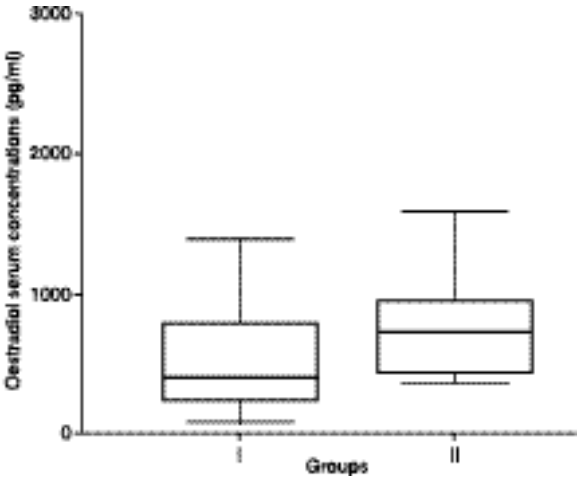


**Figure 3.** Distribution of serum oestradiol concentrations (pg/ml). The box represents the interquartile range which contains 50% of the values. The whiskers are lines extending from the box to the highest and lowest values, excluding outliers. A line across the box indicates the median. Group I comprises patients with follicles >15 mm ($n$ = 114) and group II those with >2 follicles <15 mm ($n$ = 21) ($P$ = 0.008).

**Table 2.** Multiple regression analysis (dependent variable: serum progesterone concentration >1 ng/ml).

*Model 1: independent variable: ovarian stimulation protocols*

| Protocol | B | 95%CI | P-value |
|---|---|---|---|
| HMG | 2.31 | 0.26–20.57 | 0.45 |
| Clomiphene | 0.78 | 0.04–14.03 | 0.87 |
| Short agonist | 0.01 | 0.00–1.63 | 0.85 |
| recFSH | 2.20 | 0.23–20.40 | 0.49 |

*Model 2: independent variable: clinical and laboratory data.*

| | B | 95%CI | P-value |
|---|---|---|---|
| Age (years) | –4.41 | –0.29–0.21 | 0.728 |
| Gonadotrophins (IU) | 1.92 | –0.01–1.00 | 0.213 |
| Oestradiol | –6.89 | –0.01–1.00 | 0.574 |
| Follicles (12–13.9) | 2.74 | –0.03–0.09 | 0.376 |
| Follicles (14–15.9) | 3.40 | –0.06–0.13 | 0.468 |
| Follicles >16 mm | 2.84 | –0.05–0.12 | 0.502 |
| Dominant follicular size | 2.31 | –0.01–0.06 | 0.181 |
| PCO-like appearance | 9.88 | 0.13–0.15 | 0.148 |

were <16.5 mm. Normal distribution curves of dominant follicles are indicated (red line).

Overall differences between the sizes of dominant follicles in spontaneous and stimulated cycles did not differ (**Figure 2**).

Multiple logistic regression analyses were applied to investigate possible factors associated with precocious rises in progesterone concentrations. Serum progesterone concentrations >1 ng/ml were the dependent variable (premature luteinization) in two different models. In model 1, five different ovarian stimulation protocols were investigated as the independent variable. In model 2, independent variables included age, dose of gonadotrophins (IU), serum oestradiol concentrations, PCO-like profiles, total number of follicles and size of the dominant follicle (**Table 2**). The occurrence of premature luteinization was not associated with any of these independent variables.

The impact of small follicles on hormonal serum concentrations was assessed by comparing cycles with >2 follicles measuring <15 mm [group I ($n$ = 21)] versus those with follicles measuring >15 mm [group II ($n$ = 114)]. This analysis shows that patients with >2 follicles measuring <15 mm had higher oestradiol serum concentrations ($P$ = 0.008, **Figure 3**).

## Discussion

The present data show how serum LH concentrations in infertile patients undergoing IUI with spontaneous LH surge (>10 mIU/ml) and premature luteinization (serum progesterone concentrations >1 ng/ml) were not correlated with various characteristics of the treatment. These included the ovarian stimulation protocol, oestradiol serum concentrations, dominant follicular size and total number of follicles. Moreover, during spontaneous cycles, the initial LH rise is elicited in a different manner than in stimulated cycles.

In addition, a particular group of patients with a large number of follicles <15 mm in diameter had higher concentrations of serum oestradiol and were at risk of premature luteinization.

Oestradiol is essential for oocyte development and the LH surge. However, the critical limit when serum oestradiol concentrations elicit these rapid LH releases is controversial (Kreiner *et al*., 1988; Cahill *et al*., 1998, 2000). Some authors propose that rapid rises in concentrations of oestradiol are more important than serum concentrations *per se* (Kreiner *et al*., 1988). Others show how only 1% of infertile patients with tubal occlusion begin their LH surge with serum oestradiol concentrations <600 pmol/l (Cahill *et al*., 1998).

Our multiple regression analysis clearly confirms that LH secretion is not solely under the influence of dominant follicular size, numbers of follicles or concentrations of steroids in serum, as found by other groups (Loumaye, 1990; Kagawa *et al*., 1992; Couzinet and Schaison, 1993; Fowler *et al*., 1993; Costello *et al*., 1998; McCartney *et al*., 2002).

Other ovarian factors may be necessary to modulate hypothalamic and pituitary response in terms of LH and progesterone surge. Patients receiving physiological doses of oestradiol and progesterone were shown to have a mid-cycle FSH increase resembling that occurring in the natural cycle. However, the LH surge was of lower amplitude, confirming the hypothesis that independent controls regulate FSH and LH secretion (Taylor *et al*., 1995). It was also demonstrated that different formulations of gonadotrophins used for ovarian stimulation (HMG or recFSH) did not alter the nature of the LH surge or premature luteinization. Nor was the size of the dominant follicule in stimulated cycles any different to that

occurring during natural cycles. Modulation of LH surge is probably very complex, and associated with different pathways.

The LH surge is naturally modulated by GnRH, and other hormones and peptides, e.g. GnSAF, which may induce it under special hormonal and metabolic environments (Loumaye, 1990; Fowler *et al*., 1993).

Recently, small follicles were shown to contain high concentrations of GnSAF, which could prevent premature LH surges during non-stimulated cycles (Fowler *et al*., 2001). The present results are in agreement, and show how the premature LH rise cannot be predicted. Similar conclusions have emerged for progesterone increments, based only on clinical and laboratory parameters.

The clinical impact of premature LH surges during IUI on subsequent pregnancy rates was evaluated in patients given human chorionic gonadotrophin (HCG) before the onset of their LH surge (Fuh *et al*., 1997). However, the authors did not measure serum progesterone concentrations, so the impact of premature luteinization in relation to the clinical outcome of the patients could not be measured. Clinical outcomes were not reported in this study, because several confounding biases can interfere with a specific clinical trial, and numbers of patients in each group were low.

Because the study design was retrospective and two different investigators measured the follicular diameters, the coefficient of variability (<15%) was extremely low, excluding the possibility of significant bias. Nevertheless, a larger prospective trial is needed to clarify the exact nature of factors determining rising progesterone concentrations and premature LH surges.

The advent of the third generation of GnRH antagonist opens a new horizon in controlled ovarian stimulation. This compound can induce rapid and effective declines in LH secretion, even as the initial LH rise is in progress (Christin-Maitre *et al*., 2000). However, a premature LH surge, or the selection of patients at risk, cannot be predicted from their clinical or laboratory characteristics, due to the variability and complexity of associated variables. The scheduled administration of GnRH antagonists in patients developing a premature LH surge offers an interesting alternative, but requires a large randomized trial to evaluate reproductive outcomes using this strategy.

Patients with >2 follicles and higher oestradiol secretion should be managed with special care, to prevent a premature LH rise, compared with those having only one follicle. In addition, spontaneous cycles demonstrate an important hormonal profile, with premature LH surges occurring when the dominant follicle reaches 17 mm (15.5–18). This differs from classically described values of 20 mm (range 18–26 mm). This discrepancy can be explained by the different technologies now employed for follicular measurement (Gougeon and Lefèvre, 1983; Loumaye, 1990).

In conclusion, the study and prediction of premature LH and progesterone surges are important in assisted reproduction. The data presented in this study show that the initial LH rise cannot be predicted utilizing the clinical parameters normally employed (ultrasound and serum oestradiol assay). New prospective studies are essential to confirm and extend available data. Nevertheless, patients with follicles >14 mm, or patients with many small follicles and high oestradiol, are at risk of a spontaneous LH surge. In assisted reproduction cycles, these criteria could be used to time the administration of GnRH antagonists until better predictive factors are demonstrated. New studies will be welcome to isolate new peptides offering better predictions of premature LH rise, investigating the role of inhibins and activins, and offering prospective studies designed to elucidate follicular dynamics during the initial LH surge.

## Acknowledgements

JS Cunha-Filho was supported by a CAPES post-doctoral grant.

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*Received 20 February 2003; refereed 3 April 2003; accepted 18 April 2003.*