PHYSIOLOGY

Effectiveness of Low Dose of Gonadotropin Releasing Hormone Agonist on Hormonal Flare-Up

BETTINA BSTÄNDIG,^{1,2} ISABELLE CÉDRIN-DURNERIN,¹ and JEAN NOËL HUGUES¹

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Purpose: The hormonal response (flare-up) following administration of a standard dose (100 µg) or a low dose (25 µg) of gonadotropin releasing hormone agonist (GnRH-a) (Triptorelin) was compared in patients prior to an in vitro fertilization (IVF) cycle and during the early follicular phase of a short-term IVF protocol.

Methods: The gonadotroph (FSH, LH) and steroid [estradiol (E₂) and progesterone (P)] flare-up was studied on two consecutive cycles in 30 normo-ovulatory women. Patients were randomized to receive either 25 or 100 µg of triptorelin for three days at the beginning of the first cycle. Then doses were switched according to a crossing over design in the second cycle.

Results: No significant difference in the magnitude of FSH and E₂ release could be observed following administration of the two doses of agonist whereas maximal plasma LH level was significantly reduced after injection of 25 µg of triptorelin.

Conclusions: As compared to a standard dose, using a low dose of GnRH-a induces an hormonal flare-up which seems adequate for an optimal follicular recruitment.

KEY WORDS: flare-up; gonadotropin releasing hormone agonist; low dose; short-term IVF protocol.

INTRODUCTION

Gonadotropin releasing hormone agonists (GnRH-a) have been extensively used in controlled ovarian hyp-

erstimulation (COH) over the last decade. First, they were introduced in in vitro fertilization (IVF) protocols to prevent endogenous luteinizing hormone (LH) surge through pituitary desensitization. As inducing desensitization prior to the initiation of ovarian stimulation proved to markedly improve the IVF outcome (1), long-term protocols have been widely used. Conversely, other investigators tried to take advantage of the initial endogenous gonadotropin flare induced by agonists to improve follicular recruitment in the so-called short-term protocols. Meanwhile, other clinical studies pointed out some adverse effects of the agonist on follicular recruitment, assessed by a higher need of exogenous gonadotropins (2) or on oocyte maturation and embryo development (3). Thus, it was suggested to reduce the dosage of analogs particularly for women with poor ovarian response to COH treated with long-term IVF protocols (4,5). However, this approach was proved effective in short-term protocols with a significant improvement in the ovarian response to gonadotropins in patients pretreated with oral contraceptive pills (6–8). As many confounding factors may be involved in a poor ovarian response to COH, it is still unclear whether the reduction of GnRH-a dose per se is a major contributive factor in the improvement of ovarian response. Moreover, reducing the dose of GnRH-a may interfere with several effects of the agonist at the pituitary level. On one hand, consequences of such a reduction on both hypophyseal desensitization and recovery periods have been extensively assessed (9–11) and it is clear that even very low doses of analogs may adequately suppress pituitary secretion and prevent any premature LH surges. On the other hand, it is still unclear whether such a reduction in

¹ Reproductive Medicine Unit, Department of Gynaecology and Obstetrics, Jean Verdier Hospital, University Paris XIII, Bondy 93143, France.

² To whom correspondence should be addressed.

Treatment Protocol

GnRH-a dose may adversely affect the hypophyseal and ovarian hormonal secretion during the flare-up period.

Thus, the purpose of this study was to prospectively compare the endogenous gonadotropin (FSH, LH) and steroid (estradiol and progesterone) responses observed following administration of a standard dose (100 µg) or a low dose (25 µg) of triptorelin in normo-ovulatory women.

MATERIALS AND METHODS

Patients

Thirty patients entering an IVF program for tubal disease, male factor, endometriosis, or unexplained infertility were included in this study. The mean age was 33.3 ± 4.5 years (range 25–38 years). All women had regular and ovulatory cycles with basal serum FSH level within the normal range for our laboratory (<12 mIU/ml on Day 3). Thus, they were considered as normoresponders.

For each patient, the hormonal flare-up was evaluated following subcutaneous administration of triptorelin (Decapeptyl; Ipsen-Biotech, Paris, France) at two separate doses (25 and 100 µg/day) in two consecutive cycles (Fig. 1). The first flare-up resulted from agonist injection during the first 3 days of the cycle preliminary to IVF while the second one was studied in the early follicular phase of a short-term IVF protocol with agonist administration being maintained until hCG administration.

Patients were randomized to receive either 25 µg or 100 µg of triptorelin in the first cycle and doses

were switched in the second cycle according to a crossing over design.

A progestogen pretreatment (norethisterone; Norlutin; Smith-Kline-French, Nanterre, France: 10mg/day for 10–15 days starting at mid-cycle) is currently used in our unit in order to program IVF cycles and to avoid oocyte retrieval during the weekend. As progestogens are known to modify the hormonal response to GnRH agonist (12), a similar administration of progestogen was performed in both cycles before administration of the agonist.

Blood samples were obtained every morning before each injection of triptorelin from Day 1 to Day 3 of the cycle and were kept frozen until subsequent determination of plasma FSH, LH, estradiol, and progesterone levels.

Hormonal Assays

Radioimmuno-assays were used to measure serum estradiol and progesterone (CoatRIA, Biomerieux, Lyon, France). The lower limit of sensitivity was 7 pg/ml for the estradiol assay and 0.05 ng/ml for the progesterone assay. The interassay coefficients of variation (CV max) were 12 and 8.5%, respectively. Interassay variabilities were 16 and 11%, respectively.

FSH and LH were measured by enzyme immunoassay (MEIA, Abbott, Rungis, France). The FSH assay had an intra- and interassay CV max of 6% with a minimum detectable value of 0.37 IU/ml. The LH assay had an intra- and interassay CV max of 8% with a minimum detectable value of 0.5 IU/ml.

Statistical Analysis

FSH, LH, estradiol, and progesterone values were analyzed by a paired Student *t* test. A *P* value < 0.05 was considered significant. Results are expressed as mean $\pm$ SEM.

RESULTS

Cycle to Cycle Individual Variability

Serum LH and FSH values on Day 1 were similar in both cycles. A slight but significant difference between E_2 values was observed on Day 1 of two cycles (24.2 ± 1.9 pg/ml vs. 19.9 ± 2 pg/ml, *P* < 0.05). This may be related to intercycle variability in the onset of menses following cessation of progestogen pretreatment.

Fig. 1. Treatment protocol. Each arrow corresponds to a triptorelin subcutaneous injection (25 µg or 100 µg according to randomization).

Endogenous Gonadotropin Release

The peak serum values of LH and FSH were constantly observed on Day 2. As shown on Fig. 2b, LH release was significantly higher following administration of 100 μ g of Triptorelin than after injection of 25 μ g; maximal values for LH are 22 ± 2 mUI/ml vs 16.3 ± 1.6 mUI/ml ($P < 0.005$) and relative maximal

Fig. 2. Hormonal flare-up after administration of triptorelin. This figure shows the pattern of FSH (a), LH (b), and oestradiol (c) serum concentration following administration of 100 μ g (—●—) or 25 μ g (---■---) of triptorelin at Day 1 and 2 of the cycle. Results are expressed as mean $\pm$ SEM. * $P < 0.05$

increases (Δ LH/basal LH) are $492 \pm 70\%$ vs. $285 \pm 40\%$, respectively ($P < 0.005$) (Fig. 2b). Conversely, FSH release was not significantly different following administration of both dosages (maximal FSH values are 12.3 ± 2.4 mUI/ml vs 15.2 ± 2 mUI/ml and Δ FSH / basal FSH $100 \pm 15\%$ vs. $119 \pm 20\%$ after 100 μ g and 25 μ g injection, respectively) (Fig. 2a).

Ovarian Response

Maximum E_2 concentrations were observed on Day 2 (24 hr after the first GnRH-a injection) in about 80% of cycles. The ovarian response assessed by serum E_2 increments was not significantly different following administration of a standard or a low dose of triptorelin (E_2 maximal serum value, 38 ± 9 pg/ml vs. 48 ± 15 pg/ml and ΔE_2 /basal E_2 , $113 \pm 28\%$ vs. $115 \pm 21\%$ after 100 μ g and 25 μ g respectively) (Fig. 2c).

Progesterone values were below 0.9 ng/ml in all samples. No significant rise was observed following triptorelin administration whatever the dose of GnRH-a used (data not shown).

DISCUSSION

In a normal menstrual cycle, initiation of follicular growth is strongly dependent on FSH secretion. Similarly, the gonadotropin flare-up induced by GnRH-a administration at the early follicular phase of short-term GnRH-a protocols participates in the follicular recruitment. It was recently suggested that diminishing the doses of GnRH agonists in IVF programs could be relevant to reducing the potential adverse effects of GnRH-a on ovarian parameters (2,3) and thus to improve ovarian responsiveness to exogenous gonadotropins. Such a reduction in GnRH-a doses proved to be helpful for poor responders treated with long- or short-term GnRH-a protocols (4–8). The improvement in ovarian response was attributed to a lower negative effect of the agonist at the ovarian level but it remains to assess whether low doses of GnRH-a may quantitatively and qualitatively modify the hormonal flare-up of the agonist.

In our study, we observed in all patients a significant increase in serum levels of both gonadotropins within 24 hr following the first administration of the GnRH-a, whatever the dose used. This observation is in good accordance with previous reports that low doses of other agonists (13–15) are effective in inducing a significant gonadotroph flare-up. However, as previously shown by Lemay *et al.* (13), lowering GnRH-a dosage

may have separate effects on pituitary FSH and LH release. Our results show that, in contrast with the dose-dependent LH response to GnRH-a, FSH release following administration of each dose was similar. These observations that an optimal FSH secretion could be achieved with a low dose of triptorelin, may be clinically relevant for short-term IVF protocols.

As far as LH secretion is concerned, previous study in nonhuman primates indicated a GnRH-a dose-dependent increase of serum LH (16) and our data are in line with this report. However, the actual role of LH in the follicular development induced by the flare-up is still unclear. As follicular development is directly dependent on FSH secretion, LH mainly contributes to the estradiol production by providing androgen substrates as also shown by the positive correlation observed between serum LH and estradiol values during the flare-up period (17). Thus it is likely that the contribution of LH to human follicular development is mediated by local steroid production: estradiol involved in the stimulation of FSH receptor synthesis and androgens acting as a strong stimulator of granulosa cell proliferation in the early follicular phase (18). In that respect, LH appears to be an amplifying factor of the FSH-mediated follicular growth.

However, the optimal level of LH secretion during the flare-up remains to be determined. It may be minimal, as suggested by the effectiveness of FSH stimulation in profoundly LH-suppressed women following a long-term administration of GnRH-a (19). Conversely, high serum LH levels may be detrimental to oocyte quality and embryo development by overstimulating thecal cell androgen synthesis (20–21). In that respect, the smaller increase in serum LH induced by low dose of GnRH-a as compared to standard dose may be considered as a beneficial effect.

Finally, the estradiol response to gonadotroph flare-up is similar following administration of both doses of GnRH analogs. Thus, it may be presumed that the LH threshold required to induce steroid synthesis is achieved with this low dose of GnRH agonist and that the prognostic value of estradiol response to a standard dose of analog as an indicator of the ovarian sensitivity (12) is preserved when using 25 µg of triptorelin.

In conclusion, this study shows that the use of low dose of GnRH analog in a short-term protocol does not significantly affect the FSH and estradiol initial flare-up while the LH response is reduced. Although comparison of only two doses of GnRH-a preclude any definitive conclusions, our data would suggest that a lower dose than the standard regimen of GnRH-a is sufficient to adequately stimulate folliculogenesis in

the early follicular phase of a short-term protocol. This observation may be relevant specially for women whose poor ovarian response has been related to a potential negative effect of GnRH agonist at the ovarian level. Other studies are needed to confirm these observations by measuring clinical endpoints.

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