

# Lower levels of inhibin A and pro- $\alpha$ C during the luteal phase after triggering oocyte maturation with a gonadotropin-releasing hormone agonist versus human chorionic gonadotropin

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**Objective:** To investigate the effect of triggering oocyte maturation with GnRH agonist on corpus luteum function by measuring luteal phase levels of inhibin A and pro- $\alpha$ C.

**Design:** Prospective randomized trial.

**Setting:** In vitro fertilization (IVF) program at a university hospital.

**Patient(s):** Infertile women undergoing IVF-ET treatment.

**Intervention(s):** Controlled ovarian hyperstimulation with FSH and GnRH antagonist, triggering of final oocyte maturation with either hCG ( $n = 8$ ) or GnRH agonist ( $n = 8$ ), IVF-ET, and collection of blood samples every 2–3 days during the luteal phase.

**Main Outcome Measure(s):** Luteal phase serum levels of inhibin A and pro- $\alpha$ C, P, and  $E_2$ .

**Result(s):** Levels of inhibin A, pro- $\alpha$ C, estrogen, and P were significantly lower from day 4 to day 14 after triggering final oocyte maturation by GnRH agonist compared with hCG. Maximal luteal serum inhibin A and pro- $\alpha$ C levels were  $91.5 \pm 23.6$  and  $184.1 \pm 23.5$  pg/mL in the GnRH agonist-treated women compared with  $464.7 \pm 209.1$  and  $7,351.6 \pm 934.3$  pg/mL in women treated with hCG.

**Conclusion(s):** Triggering final oocyte maturation with GnRH agonist instead of hCG in IVF cycles dramatically decreases luteal levels of inhibins, reflecting significant inhibition of the corpus luteum function. This effect may explain, at least in part, the mechanism of ovarian hyperstimulation syndrome prevention by the use of GnRH agonist. (Fertil Steril® 2003;79:1123–8. ©2003 by American Society for Reproductive Medicine.)

**Key Words:** GnRH agonist, GnRH antagonist, inhibin A, pro- $\alpha$ C, IVF, luteal phase, OHSS

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Ovarian hyperstimulation syndrome (OHSS) is a major complication of ovulation induction and IVF-ET programs (1). OHSS is characterized by enlarged ovaries full of corpora lutea that secrete high levels of steroid hormones and other vasoactive factors (1, 2), causing increased vascular permeability, third space fluid accumulation, hemoconcentration, and oliguria. One way of preventing OHSS is to use GnRH agonist (GnRH-a) instead of hCG for the induction of final oocyte maturation and ovulation in stimulated cycles (3–5).

Gonadotropin-releasing hormone-a (GnRH-a) induces a sustained release of LH that lasts for

approximately 24 hours (4, 6). This initial LH-FSH surge is followed by an apparently normal follicular-luteal shift in ovarian steroidogenesis (4). However, luteal levels of  $E_2$  and P are lower compared with luteal levels after hCG triggering, and some patients have early luteolysis and a short luteal phase (4, 7). The incidence of luteal phase dysfunction is increased in patients who undergo ovulation induction, and oocyte maturation is triggered by hCG, especially when GnRH-a is used to prevent ovulation (8). The administration of GnRH antagonist in stimulated cycles does not alter the luteal phase profile (9), while a short

luteal phase and low E<sub>2</sub> and P concentrations were reported in IVF patients (10).

The effect of GnRH-a (when used for oocyte maturation triggering) on the luteal phase may be explained by two possible mechanisms: [1] prolonged down-regulation of pituitary GnRH receptors after a midcycle injection of high-dose GnRH-a results in reduced LH support of the corpus luteum; [2] direct effect on the ovarian GnRH receptors (6, 11–13).

The corpus luteum is recognized as the major source of inhibin A and pro- $\alpha$ C production during the luteal phase (14, 15) and during early pregnancy (16). The number of follicles and corpora lutea is correlated with inhibin A and pro- $\alpha$ C levels (17–19). Previous studies have also shown that inhibin A, but not pro- $\alpha$ C, is produced by the fetoplacental unit during early pregnancy (20, 21). Inhibin pro- $\alpha$ C is secreted in excess of the inhibin A dimer (22). Inhibin A has a paracrine effect of increasing the production of androgens by theca cells and may be involved in the gradual release of ovarian negative feedback on FSH secretion during the luteal-follicular transition (23). The physiological effect of pro- $\alpha$ C is less clear.

A high level of pro- $\alpha$ C is considered to be an early sign of impending OHSS (22). Inhibin A concentrations are elevated after OHSS onset (24). Therefore, inhibin A and pro- $\alpha$ C can be seen as peptide markers of luteal activity, with particular relevance to the risk of OHSS development.

The present study investigates luteal phase inhibins (inhibin A and pro- $\alpha$ C) after two different protocols for IVF treatment: ovarian stimulation with daily FSH and GnRH antagonist for the prevention of LH surge and GnRH-a or hCG for final oocyte maturation triggering. Our hypothesis was that the elimination of OHSS with the former protocol is associated with corpora lutea inhibition, which would be reflected by decreased inhibin levels. Both inhibins were measured to exclude the possible effect of early pregnancy on inhibin A levels.

## MATERIALS AND METHODS

### Study Population

IVF patients were part of a larger multicenter, open-label, randomized, three-armed clinical trial on the efficacy of recombinant FSH (rFSH) and GnRH antagonist for ovarian stimulation and hCG (hCG group) or GnRH-a (agonist group) for inducing oocyte maturation in IVF-ET cycles (25). Subjects were between 18 and 39 years old, had regular ovulatory cycles (24–35 days), normal ovarian morphology, and normal body weight (body mass index, 18–29 kg/m<sup>2</sup>). The first eight subjects in each treatment group who had full consecutive serum samples were included. Written informed consent for the study, which was approved by the local ethics committee, was obtained from the subjects.

### Treatment Protocols

Ovarian stimulation using rFSH (Puregon; Organon, Oss, the Netherlands) was commenced on day 2 or 3 of the cycle at a daily dose (150–225 IU) determined for each patient on the basis of age, weight, gonadotropin level, and response to previous treatments. After the first 5 treatment days, the daily dose could be adjusted based on the follicular development as observed by ultrasound and E<sub>2</sub> levels.

GnRH antagonist was commenced on day 6 of the treatment using ganirelix (0.25 mg Orgalutran; Organon) administered by an SC injection once daily until the day of triggering final oocyte maturation. Final oocyte maturation was triggered when at least three follicles with a diameter of at least 17 mm were observed. On that day, patients were randomized to treatment with hCG 10,000 IU (Pregnyl; Organon) administered as a single IM injection (hCG group) or GnRH-a (randomized as triptorelin 0.2 mg; Decapeptyl, Ferring Pharmaceuticals Ltd., Copenhagen, Denmark; or leuprorelin 0.5 mg; Lupron, TAP Pharmaceuticals, Inc., Lake Forest, IL) administered as a single SC injection (agonist group).

Oocytes were collected 36 hours later, using transvaginal ultrasound-guided follicle aspiration. Luteal support in all protocols was by IM injection of P 50 mg once a day administered after ET and E<sub>2</sub> 4 mg daily (Estrofem, Novo Nordisk, Denmark) in the agonist group.

### Luteal Phase Serum Samples

Blood was collected from the day of ovulation triggering (hCG or GnRH-a, day 0) and every 2–3 days until day 16–17. Blood was immediately separated and serum samples were frozen at –20°C until assayed for inhibin A and pro- $\alpha$ C. Serum levels of E<sub>2</sub>, P, and LH were determined on the day of blood collection.

### Assays

Serum concentrations of estrogen and P were measured using a direct chemiluminescent immunoassay (ADVIA Centaur, Bayer, Leverkusen, Germany), with a sensitivity of 36.7 pmol/L and 0.48 nmol/L, respectively. The inter- and intra-assay coefficients of variation were, respectively, 5.2% and 5.5% for E<sub>2</sub> and 5.7% and 7.2% for P. Inhibin A was measured by the commercial two sites ELISA kit (Oxford Bio-Innovations, Oxford, UK), with a sensitivity of 4 pg/mL. Intra- and interplate coefficients of variation were 9% and 10%, respectively. Pro- $\alpha$ C was measured by the commercial ELISA assay kit (Serotec, Oxford, UK) with a sensitivity of 1.56 pg/mL. Intra- and interplate coefficients of variation were 8% and 8.5%, respectively.

### Midluteal Ovarian Size

Transvaginal ultrasound was performed on day 10 after oocyte maturation triggering, and ovarian size was measured as a rough clinical correlation to corpora lutea function.

TABLE 1

Clinical characteristics (mean  $\pm$  SD) of a comparison between two IVF treatment protocols groups—GnRH antagonist for ovulation prevention and hCG (hCG group) or GnRH agonist (agonist group) for oocyte maturation triggering.

	Agonist (n = 8)	hCG <sup>a</sup> (n = 8)
Age (years)	28.7 $\pm$ 5.4	30.6 $\pm$ 4.4
Duration of infertility (years)	4.3 $\pm$ 1.9	5.9 $\pm$ 4.2
No. of patients with primary infertility	7	6
Cause of infertility (no.):		
Male factor	6	6
Unexplained	1	2
Mechanical	1	
Treatment cycle no.	1.62 $\pm$ 0.9	1.75 $\pm$ 0.8
Duration of FSH treatment (days)	9 $\pm$ 1.2	9.5 $\pm$ 1.6
No. of follicles $\geq$ 11 mm at day 0	11.75 $\pm$ 3.3	15 $\pm$ 4.8
No. of oocytes retrieved	9.25 $\pm$ 3.8	11 $\pm$ 5.5
No. of clinical pregnancies	4	4

<sup>a</sup> P = NS for all characteristics.

Nevo. Low luteal inhibins after GnRH agonist. *Fertil Steril* 2003.

## Statistical Analyses

Results are presented as mean  $\pm$  SE. Inhibin A measurements below the detection limit of 4 pg/mL were calculated as 4 pg/mL for statistical analysis. Daily differences of hormone levels between the groups were evaluated by the Mann-Whitney test. Friedman's test for changes of repeated measures over time was applied in each group. Clinical data were tested by the Mann-Whitney test. Statistical significance was considered as  $P < .05$ .

## RESULTS

### Clinical Data

Both groups were comparable in demographic and infertility characteristics (Table 1). The number of follicles and oocytes was not significantly different between the agonist and hCG groups.

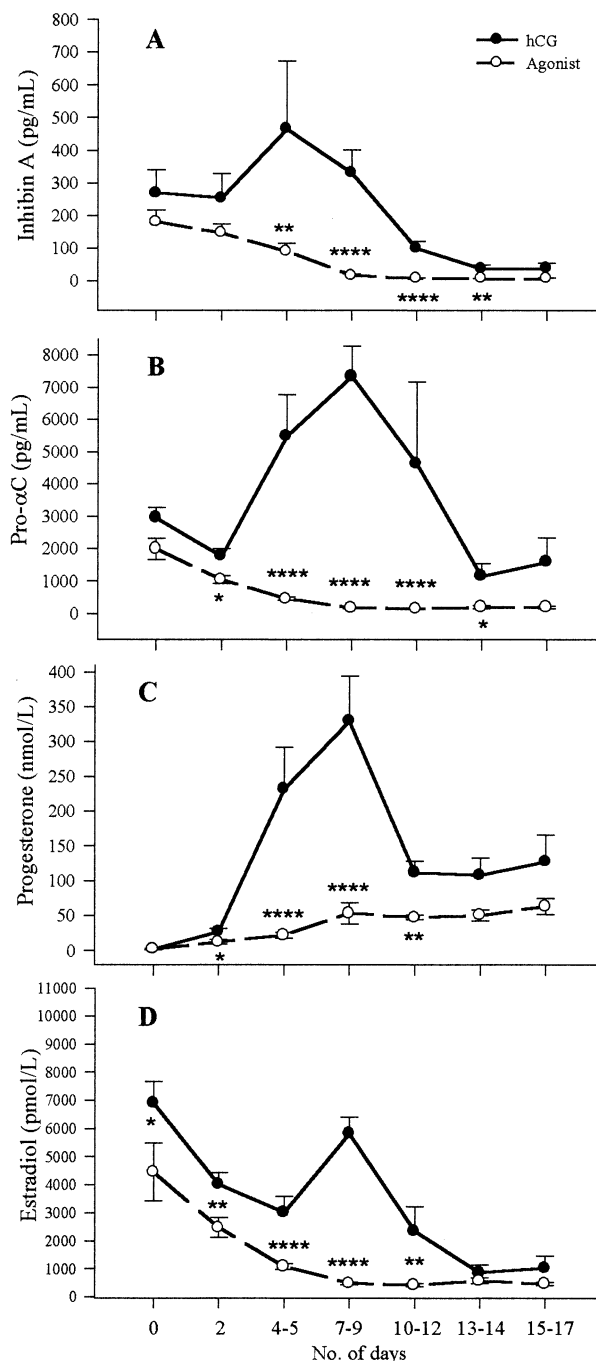
### Inhibin A

In the hCG group, serum inhibin A levels increased significantly from day 2 (254.1  $\pm$  75.1) to day 4–5 (464.7  $\pm$  209.1 pg/mL) (Fig. 1). In contrast, in the agonist group, the levels decreased and were 5 times lower on day 4 (91.5  $\pm$  23.6 pg/mL) and 18 times lower on days 7–9 (17.7  $\pm$  4.4 pg/mL) than those in the hCG group. Moreover, they continued to decrease and were almost undetectable from days 7–9 to days 15–17.

Serum inhibin A levels were significantly lower ( $P < .01$ – $0.0001$ ) throughout the luteal phase among women treated with GnRH-a as compared with those treated with hCG. To negate the possibility that higher ovarian response to FSH

FIGURE 1

Luteal phase serum concentrations (mean  $\pm$  SE) of inhibin A (A), pro- $\alpha$ C (B), P (C), and E<sub>2</sub> (D) in two IVF protocols: GnRH antagonist for ovulation prevention and hCG (hCG group) or GnRH-a (agonist group) for oocyte maturation triggering. Time is represented as days relative to oocyte maturation triggering (day 0). The changes in the levels of all four hormones in both groups were significant over time.  $P < .0001$  (Friedman test). \* $P < .05$ ; \*\* $P < .01$ ; \*\*\* $P < .001$ ; \*\*\*\* $P < .0001$ .



Nevo. Low luteal inhibins after GnRH agonist. *Fertil Steril* 2003.

stimulation in the hCG group was the cause for higher luteal inhibin levels, we calculated inhibin levels per follicle in both groups. Dividing the inhibin A level by the number of follicles (as counted on day 0) yielded similar results (Fig. 2).

Importantly, the four pregnant subjects in the agonist group did not differ from those who did not become pregnant with regard to inhibin A levels on days 10–17 (5.1 compared with 9.6 pg/mL, respectively).

### Pro- $\alpha$ C

In both groups, serum inhibin pro- $\alpha$ C decreased from day 0 to day 2 (Fig. 1). However, while in the hCG group the levels increased extensively (3-fold) from day 2 (1,769.3  $\pm$  234.4) to days 4–5 (5,480.4  $\pm$  1,300.9 pg/mL) and continued to increase (4-fold) on the next 2–3 days (7,351.6  $\pm$  934.3 pg/mL), in the agonist group, the levels continued to decrease from day 2 throughout the luteal phase. Midluteal levels were 40 times lower in the agonist group (184.1  $\pm$  23.5) compared with the hCG group (7,351.6  $\pm$  934.3 pg/mL). Serum levels of pro- $\alpha$ C were significantly lower ( $P < .05$ – $0.0001$ ) in the agonist group compared with the hCG group from day 2 throughout the luteal phase (Fig. 1). Pro- $\alpha$ C per follicle yielded the same results (Fig. 2).

### Progesterone

Midluteal levels in the agonist group were significantly lower compared with the hCG group (Fig. 1). On days 7–9 (maximal serum concentration), P levels were 6 times higher in the hCG group compared with the agonist group (330.0  $\pm$  64.0 compared with 53.3  $\pm$  15.6 nmol/L). The P level per follicle yielded similar results with a significant difference on days 4–9 (Fig. 2).

### Estradiol

Serum levels increased from day 4–5 to day 7–9 in the hCG group, while in the agonist group the levels decreased (Fig. 1). GnRH-a-treated women had significantly ( $P < .05$ – $0.0001$ ) lower levels of  $E_2$  (Fig. 1). Levels of  $E_2$  per follicle showed significant differences on days 4–9 (Fig. 2).

### Midluteal Ovarian Size

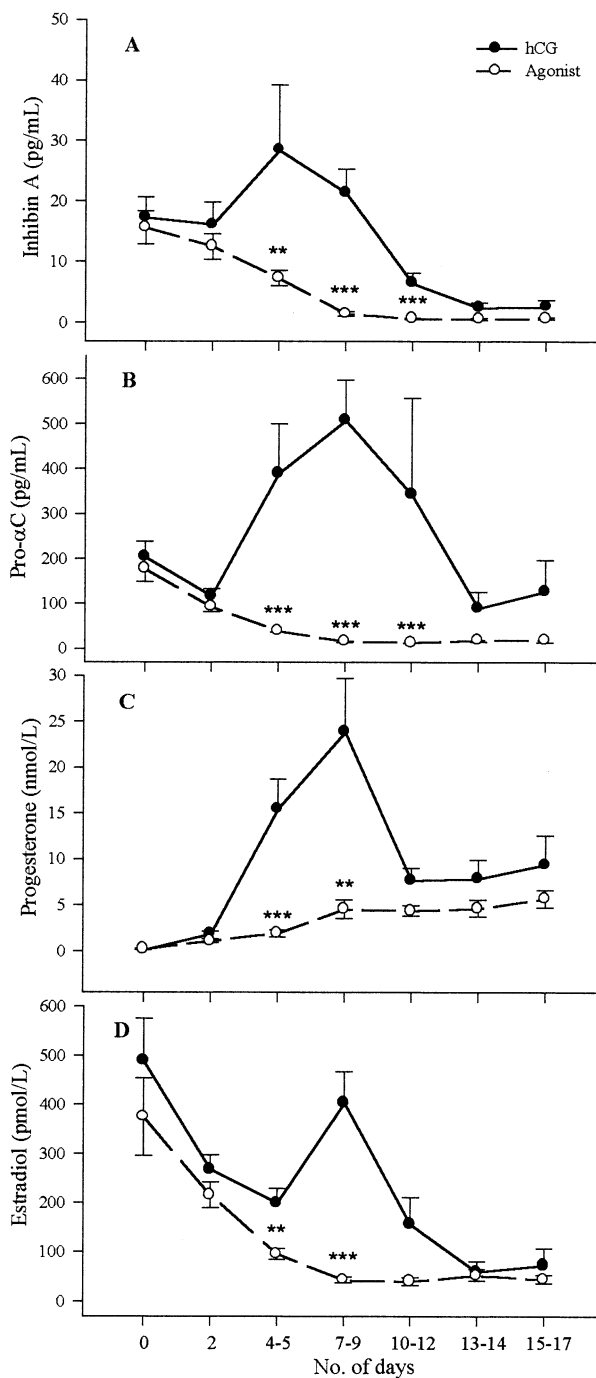
Midluteal ovarian size, which was measured as a rough clinical correlate to corpora lutea function, was significantly smaller in the agonist group (3.3  $\pm$  0.32 compared with 5.91  $\pm$  0.52 cm;  $P < .01$ ).

## DISCUSSION

To the best of our knowledge, this is the first report on the effect of oocyte maturation triggering with GnRH-a on luteal phase serum inhibin A and pro- $\alpha$ C levels. Despite the low number of subjects, we show highly significant differences in serum levels (compared with the hCG protocol), suggesting that luteal function inhibition is the mechanism by which GnRH-a (given for oocyte maturation triggering) prevents OHSS (3, 6).

**FIGURE 2**

Luteal phase serum concentrations (mean  $\pm$  SE) of inhibin A (A), pro- $\alpha$ C (B), P (C), and  $E_2$  (D) per follicle in two IVF protocols: GnRH antagonist for ovulation prevention and hCG (hCG group) or GnRH-a (agonist group) for oocyte maturation triggering. Time is represented as days relative to oocyte maturation triggering (day 0). \*\* $P < .01$ ; \*\*\* $P < .001$ .



Nevo. Low luteal inhibins after GnRH agonist. *Fertil Steril* 2003.

Our patients were part of a larger randomized trial that compared endocrine profiles after triggering final oocyte maturation with GnRH-a or hCG (25). The results of that study (25) indicate that an adequate and comparable pituitary response in terms of a rise in endogenous LH and FSH was observed after the administration of either 0.2 mg triptorelin or 0.5 mg leuporelin and that luteal phase steroids levels were closer to the physiological range. The duration of the LH surge appeared to be shorter compared with that in the natural cycle (24 vs. 36–48 hours, respectively). This phenomenon may be explained by the immediate pituitary desensitization after the initial flare effect. Serum E<sub>2</sub> and P levels were comparable for all treatment groups up to the day of oocyte retrieval. However, thereafter, both E<sub>2</sub> and P levels were higher in hCG-treated subjects, which is in agreement with our current results.

OHSS is a major complication of ovulation induction. The condition worsens when hCG is given for luteal support or when pregnancy commences and endogenous hCG stimulates the corpora lutea. Although the number of pregnant women in the agonist group was small (n = 4), they showed a low inhibin level on day 16, when endogenous hCG is available. In fact, pregnancy had no effect on the late-luteal levels of inhibin A and pro- $\alpha$ C. This remarkable phenomenon suggests that the late rise in hCG cannot reverse the luteal phase inhibition that characterizes this protocol.

Previous reports (3, 4) on OHSS prevention focused on luteal levels of E<sub>2</sub> and P. Since luteal support is mandatory, the exact contribution of endogenously produced steroids cannot be directly measured and can only be roughly estimated. Using inhibin A and pro- $\alpha$ C as nonsteroidal markers of luteal function, we have directly demonstrated in the present study that the luteal phase bioactivity in the agonist group is inhibited compared with the hCG group. A direct correlation between luteal secretion of steroidal hormones and inhibin A and pro- $\alpha$ C has been previously reported (21, 26), which is in line with our findings, even though luteal support was given.

In contrast to the high levels of luteal inhibin A and pro- $\alpha$ C after hCG, the low levels after GnRH-a are closer to the physiological level described elsewhere (21, 22), although somewhat lower. Mean inhibin A level on days 7–9 after oocyte maturation triggering by GnRH-a was  $17.7 \pm 12$  pg/mL compared with  $75 \pm 25$  pg/mL in a natural cycle (22). Mean pro- $\alpha$ C level on days 7–9 was  $184 \pm 66$  pg/mL in the agonist group compared with  $750 \pm 200$  pg/mL during a natural cycle (21).

In summary, a GnRH antagonist-based protocol for ovulation induction and IVF enables the use of GnRH-a in the final stage of oocyte maturation. The lower levels of steroidal and nonsteroidal hormones, which are secreted by corpora lutea, are closer to the physiological range in patients treated with this protocol (in comparison with the hCG-

treated patients) and may explain, at least in part, the mechanism of OHSS prevention by GnRH-a.

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