

# Follicular and luteal phase characteristics following early cessation of gonadotrophin-releasing hormone agonist during ovarian stimulation for in-vitro fertilization\*

N.G.M.Beckers<sup>1</sup>, J.S.E.Laven<sup>1</sup>, M.J.C.Eijkemans<sup>2</sup> and B.C.J.M.Fauser<sup>1,3</sup>

<sup>1</sup>Division of Reproductive Medicine, Department of Obstetrics and Gynaecology and <sup>2</sup>Department of Public Health, Erasmus University Medical Center Rotterdam, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands

<sup>3</sup>To whom correspondence should be addressed

**Gonadotrophin-releasing hormone agonists (GnRHa) are widely used in in-vitro fertilization (IVF) for the prevention of a premature rise in luteinizing hormone (LH) concentrations. However, the administration of GnRHa during the follicular phase may also impair subsequent luteal function due to retarded recovery of pituitary gonadotrophin secretion. Therefore, luteal supplementation is generally applied. The present study was designed to determine whether a premature LH surge would still be prevented after early cessation of GnRHa during ovarian stimulation and whether subsequent luteal phase LH production would be sufficient to support progesterone synthesis by the corpus luteum. Sixty patients were randomized for three groups: (i) A long GnRHa/human menopausal gonadotrophin (HMG) protocol with luteal support by repeated human chorionic gonadotrophin (HCG) ( $n = 20$ ), (ii) early follicular phase cessation of GnRHa without luteal support ( $n = 20$ ), and (iii) a long GnRHa protocol without luteal support ( $n = 20$ ). Frequent ultrasound and blood sampling was performed during the entire IVF cycle. Forty normo-ovulatory women served as controls. No premature LH surges were found after early cessation of GnRHa. In this group, some pituitary recovery occurred during the late luteal phase, but this did not affect corpus luteum function. Progesterone concentrations were shown to be dependent on disappearance of the pre-ovulatory bolus of HCG. Pregnancies occurred in all three groups. In conclusion, early follicular phase cessation of GnRHa is still effective in the prevention of a premature rise in LH. Although some pituitary recovery was observed thereafter, corpus luteum function is still abnormal due to early luteolysis.**

*Key words:* endocrine profiles/GnRHa/IVF protocols

## Introduction

The use of a gonadotrophin-releasing hormone agonist (GnRHa) to prevent a premature rise in serum luteinizing

hormone (LH) concentrations in in-vitro fertilization (IVF) cycles was first described in 1984. Next to exogenous gonadotrophins, GnRHa was applied to induce a reversible hypogonadotrophic state by means of pituitary desensitization (Porter *et al.*, 1984). Consequently, ovarian stimulation with gonadotrophins could be continued for an extended period of time and more oocytes could be obtained. Clinical pregnancy rates per cycle and per embryo transfer were reported to increase with the routine use of GnRHa for IVF (Hughes *et al.*, 1992). However, the use of GnRHa during the follicular phase also impairs corpus luteum function, introducing the need for luteal phase supplementation (Smitz *et al.*, 1987). Defective function of the corpus luteum after cessation of GnRHa may be caused by prolonged blockage of pituitary gonadotrophin release during the luteal phase (Smitz *et al.*, 1988). It was suggested that luteal supplementation would improve endometrial quality and pregnancy rates (Smitz *et al.*, 1988). A meta-analysis comparing pregnancy rates with and without luteal support following ovarian stimulation with gonadotrophins combined with GnRHa suggested indeed that luteal support was beneficial (Soliman *et al.*, 1994).

Several authors have shown extremely low endogenous LH concentrations until 10–14 days after discontinuation of the GnRHa (Smitz *et al.*, 1992a; Donderwinkel *et al.*, 1993; Sungurtekin and Jansen, 1995). It may therefore be postulated that GnRHa could be stopped earlier in the stimulation cycle, allowing pituitary recovery to occur during the luteal phase providing endogenous support of the corpus luteum. Preliminary observations indeed suggest that no premature rises in LH and progesterone concentrations took place in patients in which GnRHa was stopped earlier (Pantos *et al.*, 1994). The objective of the present prospective randomized controlled study was to assess whether early follicular phase cessation of GnRHa still avoids a premature rise in serum LH and to study luteal phase LH and progesterone concentrations without exogenous support of the corpus luteum.

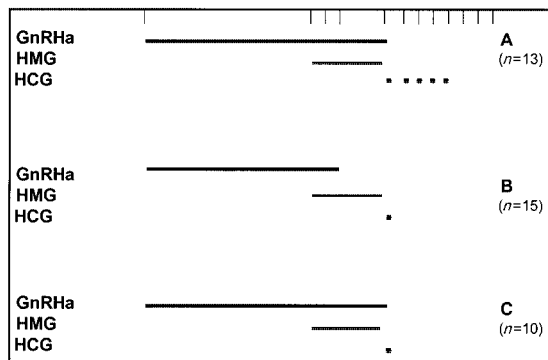
## Materials and methods

### Patients

The study was approved by the local ethics review committee and a signed informed consent was obtained from all patients. Sixty IVF patients less than 39 years of age were included in the present study. All were having regular menstrual cycles (between 25 and 32 days), and had no known hormonal abnormalities. Indications for IVF included tubal pathology and male factor.

Forty paid volunteers aged 20–34 years with a normal regular menstrual cycle (i.e. 26–30 days), normal body weight (body mass index 19–24 kg/m<sup>2</sup>) and no history of infertility or any endocrine

\*Presented during the 14th Annual ESHRE Meeting, Göteborg, Sweden, 1998



**Figure 1.** Schematic representation of the three different treatment protocols. A routine long gonadotrophin-releasing hormone agonist (GnRHa)/human menopausal gonadotrophin (HMG) protocol with luteal support (group A), early cessation of GnRHa without luteal support (group B), and long use of GnRHa without luteal support (group C). The small vertical lines at the top indicate the days on which blood samples were taken. HCG = human chorionic gonadotrophin.

abnormalities served as controls. Daily blood sampling and transvaginal ultrasound was performed, as published previously (Schipper *et al.*, 1998).

#### Study protocol

All patients were treated with the so called long protocol. The GnRHa Decapeptyl® (Ferring Nederland B.V., Hoofddorp, The Netherlands) 0.1 mg s.c. daily injections were initiated on cycle day 1. Patients were randomized on the same day (i.e. day 1 of the treatment cycle) by means of sealed envelopes for one of the three treatment groups A, B or C (20 patients each). Down-regulation was confirmed (reflected by serum oestradiol concentration <150 pmol/l) after 3 weeks GnRHa use, and ovarian stimulation was initiated using human menopausal gonadotrophin (HMG) (Humegon®; N.V. Organon, Oss, The Netherlands) 3 amp/day (=225 IU) i.m. Ultrasound examination was performed from stimulation day 6 onwards every other day until the leading follicle reached a diameter of at least 15 mm. From that day onwards ultrasound examinations were performed daily. Blood samples were drawn on the first day of GnRHa, on the first day of administration of HMG, on the third day of HMG, on each day the patient visited the outpatient clinic for ovarian response monitoring, on the day of human chorionic gonadotrophin (HCG), on the day of oocyte retrieval, and every other day thereafter. Rapid serum oestradiol measurements were performed on day 21 (after 3 weeks use of GnRHa) and following every ultrasound examination. HCG (Pregnyl®; N.V. Organon) 10 000 IU was administered i.m. as a single bolus on the day the diameter of the leading follicle was at least 18 mm and at least 3 follicles >10 mm were present. Oocyte retrieval was performed 35 h later and blood samples were drawn immediately prior to this. Embryos were transferred after 4 days of culture.

Patients in group A received GnRHa until the day of HCG, and subsequent luteal support; HCG 1500 IU i.m. on the day of oocyte retrieval and 2, 4 and 6 days thereafter (conventional long protocol). Patients in group B stopped GnRHa on the third day of HMG stimulation and received no luteal support (see Figure 1). Group C used GnRHa until the day of HCG and received no luteal support. To diminish the risk of ovarian hyperstimulation syndrome (OHSS), only patients with oestradiol concentrations below 8000 pmol/l on the day of HCG received luteal support with HCG in group A. In case oestradiol concentrations were above 8000 pmol/l, micronized progesterone (600 mg/day intravaginally) was given and patients were excluded from further analysis. The oestradiol threshold of

8000 pmol/l was arbitrarily chosen. This was based on our own unpublished observations. It has previously been suggested that when a patient is considered to be at particular risk of developing OHSS, it is recommended that progesterone rather than HCG should be used for luteal support (Akande *et al.*, 1996). However, no absolute threshold for oestradiol could be found to predict an OHSS risk (Mathur *et al.*, 1996).

#### Hormone assays

Blood samples were centrifuged at 1000 g for 15 min and serum was frozen and stored at -20°C. Serum was assayed for follicle stimulating hormone (FSH), LH, oestradiol, and progesterone concentrations. In addition, HCG concentrations were assayed during the luteal phase. From each patient, hormone assays were performed in the same run. LH and FSH concentrations were measured by immunofluorometric assay (Amerlite, Orto-Clinical Diagnostic, Amersham, UK) as published previously (Schipper *et al.*, 1998). Oestradiol and HCG concentrations were measured by Coat-A-Count radioimmunoassay (Diagnostic Products Corp. Los Angeles, CA, USA). Progesterone concentrations were measured by radioimmunoassay kits as described previously (de Jong *et al.*, 1974). Intra- and inter-assay variation was less than 3 and 6% for LH, less than 5 and 7% for FSH, less than 11 and 15% for oestradiol, less than 6 and 7% for HCG, and less than 11 and 12% for progesterone. Lower limit of assay sensitivity was 0.09 IU/l for LH, 0.24 IU/l for FSH and 0.5 nmol/l for progesterone. Cross-reaction of the LH and FSH assay with the HCG injected was <0.1%.

#### Statistical analysis

Potential differences in patients' ages and oestradiol concentrations on the day of HCG were tested using the Kruskal-Wallis test. To determine differences in luteal phase hormone profiles the mean area under the curve (AUC) was calculated for LH, FSH, and progesterone during the luteal phase. *P* values below 0.05 were considered to indicate significant differences. Statistical analysis was performed using commercially available software packages (GraphPad prism; Statistical Package for Social Sciences, SPSS, SPSS inc. Chicago, IL, USA).

#### Results

After randomization, 20 patients were included in all three groups. In group A 13 patients were analysed, in group B 15, and in group C 10. The major reason for exclusion was oestradiol concentrations above 8000 pmol/l on the day of HCG (*n* = 5, 3 and 7 for groups A, B and C respectively). Other reasons were treatment cancellation due to poor response (*n* = 1, 1 and 0 for groups A, B and C respectively), a sudden decrease in husband's sperm count after illness (*n* = 1, 0 and 1 for groups A, B and C respectively), and spontaneous ovulation before oocyte retrieval (one patient, group C). In addition, one cycle was cancelled for patient's private reasons (group B), and one patient accidentally injected the HCG bolus 24 h too late (group C). After exclusion no significant differences were found comparing the three groups regarding patients' age, duration of stimulation, number of follicles on the day of HCG, and number of oocytes obtained after retrieval (Table I). As a fixed dose of HMG (i.e. 225 IU/day) was used, the total amount of HMG used was directly related to the duration of stimulation. As a relatively large number of patients was considered to be a drop-out due to oestradiol concentration

**Table 1.** Patients and follicular and luteal phase characteristics in 38 women undergoing in-vitro fertilization (IVF) receiving long GnRHa/HMG stimulation plus luteal support by repeated HCG (group A), early GnRHa cessation without luteal support (group B), and long GnRHa/HMG without luteal support (median and range)

	Group A (n = 13) <sup>a</sup>	Group B (n = 15) <sup>a</sup>	Group C (n = 10) <sup>a</sup>
Age (years)	32 (29–38)	32 (26–36)	33 (28–38)
Duration of HMG stimulation (days)	10 (7–13)	10 (8–22)	9 (7–12)
Follicles <sup>b</sup> (n)	11 (7–16)	10 (3–17)	11 (4–13)
Oocytes retrieved (n)	10 (8–19)	13 (3–19)	7 (4–19)
Embryos (n)	5 (0–11)	2 (0–15)	5 (0–10)
Pregnancies <sup>c</sup>	4 (5)	3 (3)	0 (1)

<sup>a</sup>Number of patients in each group after exclusion due to ovarian hyper-response (oestradiol >8000 pmol/l).

<sup>b</sup>Values reflect number of follicles ≥10 mm on the day of HCG.

<sup>c</sup>Number of pregnancies (positive pregnancy test) after exclusion (and before).

GnRHa = gonadotrophin-releasing hormone agonist.

HMG = human menopausal gonadotrophin.

HCG = human chorionic gonadotrophin.

higher than 8000 pmol/l, potentially a post-randomization bias could have been introduced. For this reason patients' characteristics were also analysed, including those patients showing ovarian hyper-response. Again, no differences were found regarding patients' age, duration of stimulation, number of follicles on the day of HCG, and number of oocytes obtained after retrieval (data not shown). It was concluded that although the drop-out rate was high, no obvious post-randomization bias was introduced.

In group A, five patients became pregnant (defined as a positive urinary pregnancy test 17 days after oocyte retrieval), one of them was not analysed due to an oestradiol concentration above 8000 pmol/l on the day of HCG. In group B, three patients became pregnant of whom all were analysed. In group C, one patient became pregnant (not analysed due to high oestradiol concentrations). In the analysed patients, six pregnancies were ongoing. Ongoing pregnancies (n = 6) ended in the birth of four healthy singletons and two healthy twins. The remaining pregnancy was a singleton pregnancy that ended in an early abortion. This patient was in group A.

LH and FSH concentrations are depicted in Figure 2. Median LH concentration on the day of HCG were 0.6 IU/l (range <0.09–1.5), 0.4 IU/l (range <0.09–2.5) and 0.7 IU/l (range 0.4–1.0) for groups A, B and C respectively [ $P = 0.37$  not significant (NS)]. In the control group median LH concentration on the day before LH surge was 4.8 IU/l (range 1.3–10.4). No premature LH rises (defined as LH concentrations above 5 IU/l) could be observed in group B patients who stopped GnRHa earlier. The median duration between GnRHa cessation and day of HCG was 7 days (range 4–10). Extremely low LH concentration were found in the luteal phase in groups A and C (most concentrations below assay sensitivity). The mean area under the curve (AUC) in group B (4.8) was significantly higher compared to groups A or C (0.4, and 0.3 respectively) ( $P = 0.01$  and  $0.02$  respectively) but significantly lower ( $P < 0.001$ ) versus controls (39.5). Median FSH concentrations on the day of HCG were 6.9 IU/l (range 5.6–10.4), 6.0 IU/l

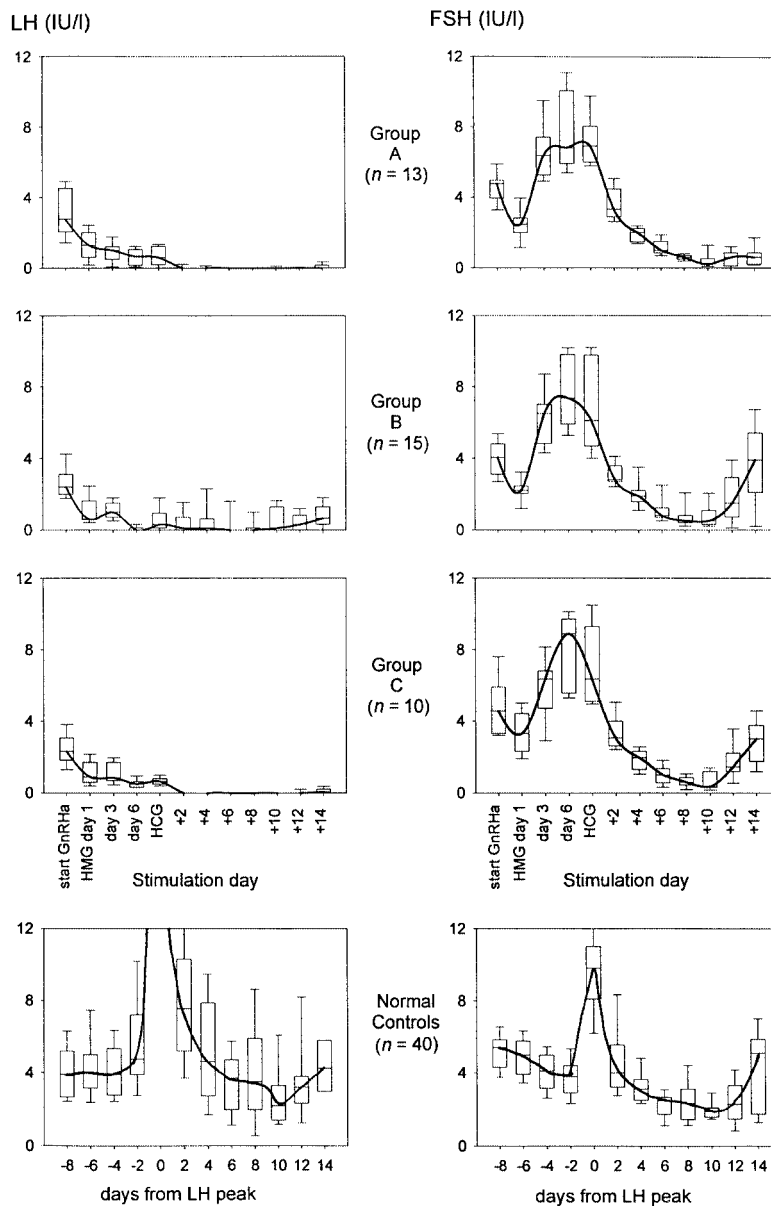
(range 3.9–10.2), 7.2 IU/l (range 4.8–10.8), and 3.8 IU/l (range 1.8–6.3) for groups A, B, C, and controls respectively. Compared to the control group, luteal phase FSH concentrations were significantly lower in group A ( $P = 0.001$ ) but similar for groups B and C. In groups B and C (and in controls) a rise in serum FSH concentrations was observed from day 10 following HCG onwards. This rise was absent in group A. The median rise in FSH concentrations between day 10 and 14 was 0.0, 2.6, 2.6 and 0.5 IU/l for groups A, B, C and controls respectively. This was significantly different ( $P = 0.005$ ) between groups.

Figure 3 depicts oestradiol and progesterone concentrations in different groups. There was no indication of a premature progesterone rise in the late follicular phase. Luteal phase progesterone concentrations as reflected by the mean AUC were higher in group A (2.4) as compared to groups B and C (1.2 and 1.2 respectively) ( $P < 0.001$ ). There was a significantly higher maximum luteal progesterone concentration in group A [358.6 nmol/l (range 183.6–490.0)] compared to group B [182.3 nmol/l (range 48.2–460.9)] and C [200.3 nmol/l (range 114.0–406.2)] ( $P = 0.02$ ). AUC and maximum concentrations for progesterone were higher in groups B and C versus controls ( $P < 0.001$ ). Moreover, progesterone decrease started significantly later in group A [day 10 (range 8–10 after HCG)] versus B and C [both groups day 8 (range 6–8 after HCG)] ( $P = 0.0005$ ). Progesterone concentrations and mean AUC were similar for groups B and C. Progesterone serum concentration decreased more gradually in controls (due to reduced maximum concentrations) and this decrease started between days 6 and 8 following the LH surge (i.e. 4–6 days after ovulation). Similarly, luteal phase oestradiol concentrations in group A were significantly higher ( $P < 0.0001$ ) as compared to groups B and C. In turn, oestradiol concentrations in groups B and C were higher as compared to controls ( $P = 0.005$ , and  $P = 0.002$  respectively). Late luteal oestradiol changes were similar to changes in progesterone. Oestradiol serum concentrations also decreased later in group A compared to groups B and C.

HCG concentrations are shown in Figure 4. Mean AUC for groups A, B and C were 554, 347 and 290 respectively, this is significantly different between groups ( $P = 0.005$ ). In group A, HCG was administered during the luteal phase. The relationship between serum HCG and progesterone concentrations during the luteal phase is described in Figure 5, separately for all three groups. In pregnant patients, HCG concentrations started to rise between days 12 and 14 after HCG.

## Discussion

The co-administration of GnRHa in IVF has improved overall treatment outcome (Hughes *et al.*, 1992). However, with the use of GnRHa the late luteal phase progesterone production was inadequate in women not receiving luteal support (Smitz *et al.*, 1988; Smith *et al.*, 1989). GnRHa is routinely continued until oocyte retrieval criteria are met, but pituitary suppression continues after stopping GnRHa (Sungurtekin and Jansen, 1995). Three studies were previously conducted in which GnRHa was stopped earlier (Smitz *et al.*, 1992b; Pantos *et al.*,

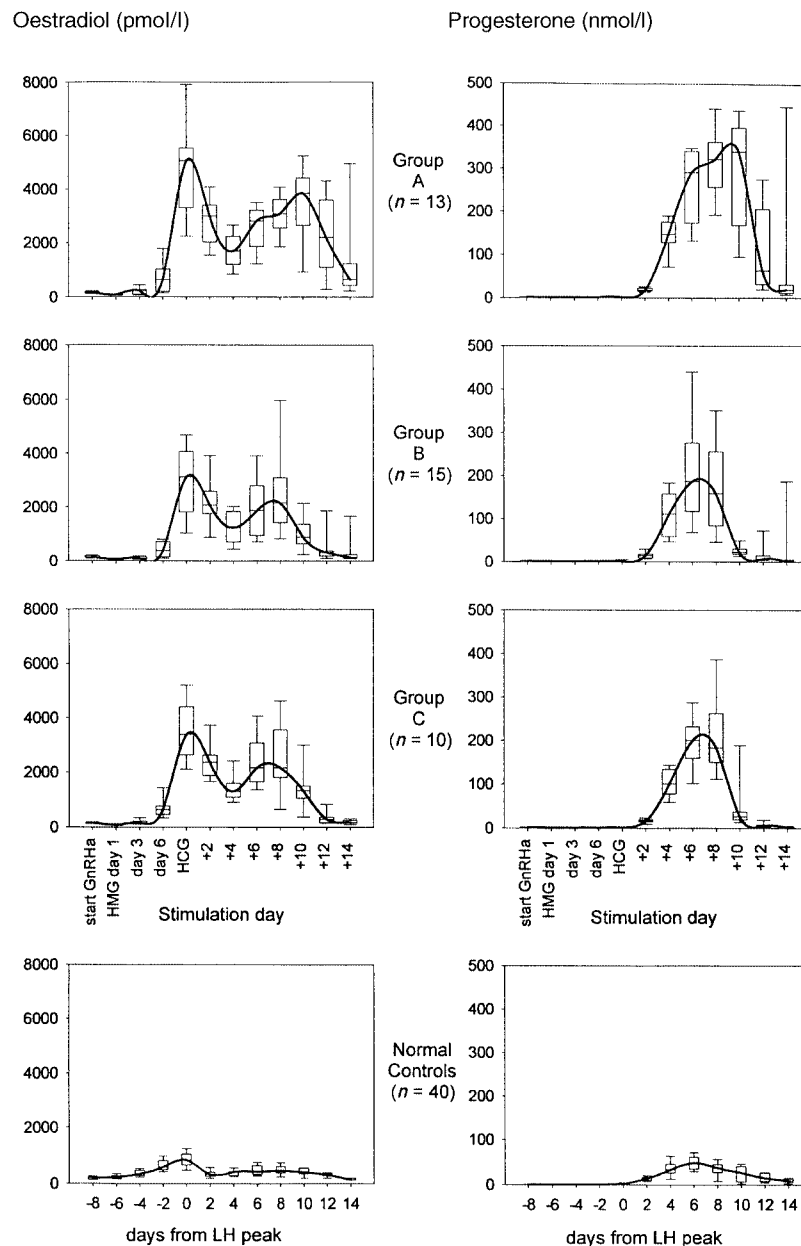


**Figure 2.** Box and whisker plots representing median values and 25th and 75th percentiles of luteinizing hormone (LH) and follicle stimulating hormone (FSH) serum concentrations in three different in-vitro fertilization (IVF) treatment protocols and normal ovulatory controls. Long GnRHa/HMG protocol with luteal support (group A), early cessation of GnRHa without luteal support (group B), and long use of GnRHa without luteal support (group C).

1994; Faber *et al.*, 1998). However, luteal support was included in these studies and hormonal measurements were performed on the day of HCG administration only. It was observed that pituitary down-regulation continues following cessation of GnRHa early during ovarian stimulation for IVF. Indeed, pituitary recovery takes an extended period of time, as was also shown following HMG ovulation induction combined with GnRHa in polycystic ovarian syndrome patients (Donderwinkel *et al.*, 1993).

If impaired luteal progesterone production were caused by prolonged pituitary suppression after GnRHa use, this may normalize if pituitary recovery took place earlier in the luteal phase. In the present prospective, randomized study, it was investigated whether pituitary recovery and endogenous corpus luteum support would occur if GnRHa were stopped early

during ovarian stimulation. The entire follicular and luteal phase was studied and normo-ovulatory women served as controls. Follicular-phase characteristics were not different in the three different treatment groups (except for a minor unexplained difference in oestradiol concentrations). Hence, cessation of GnRHa early in the follicular phase did not affect ovarian stimulation by exogenous gonadotrophins. LH or progesterone rises were not observed in any patients prior to HCG. These results confirm previously published observations (Pantos *et al.*, 1994). After continuation of GnRHa until HCG, LH concentrations were extremely low during the subsequent luteal phase both with or without luteal support. Several authors also found early and late luteal LH serum concentrations below 1 IU/l after conventional GnRHa use until HCG (Smitz *et al.*, 1988; Urbancek *et al.*, 1990; Valbuena *et al.*, 1997). LH

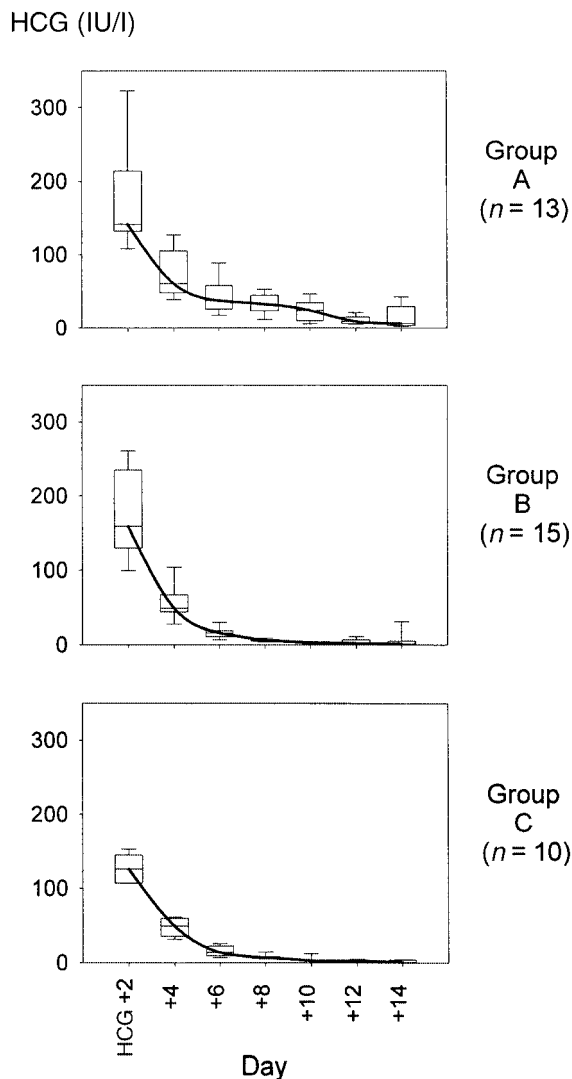


**Figure 3.** Box and whisker plots representing median values and 25th and 75th percentiles of oestradiol and progesterone serum concentrations in three different IVF treatment protocols and normal ovulatory controls. Long GnRH $\alpha$ /HMG protocol with luteal support (group A), early cessation of GnRH $\alpha$  without luteal support (group B), and long use of GnRH $\alpha$  without luteal support (group C).

concentrations remain extremely low for at least 14 days after discontinuation of GnRH $\alpha$  (Donderwinkel *et al.*, 1993). This was confirmed in the present study. However, the present study shows for the first time that after stopping GnRH $\alpha$  earlier in the follicular phase, LH concentrations in the late luteal phase partially recover. Smitz *et al.* found an earlier increase in LH concentration in the day of HCG after early cessation of GnRH $\alpha$  but clearly no LH rise or progesterone rise (Smitz *et al.*, 1992b). In the present study, from day 8 after HCG, a slight increase in LH concentrations was found, but these levels did not reach concentrations as measured in regularly cycling controls. In the patients who stopped GnRH $\alpha$  on stimulation day 3 the interval between cessation of GnRH $\alpha$  and HCG was 7–13 days. Hence, the observation period from the day of discontinuation of the GnRH $\alpha$  until the 12th

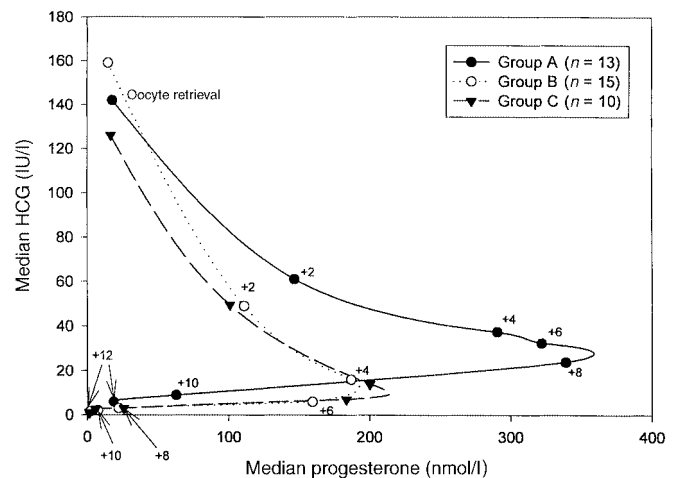
day after oocyte retrieval varied from 16 to 22 days in this group. It can be concluded that some pituitary recovery occurs 16–22 days after GnRH $\alpha$  cessation. However, LH concentrations were still below the physiological range (<0.09–1.9 IU/l). Compared to regular cycling controls, in all three study groups more corpora lutea were present to produce steroids in the luteal phase. The higher concentrations of oestradiol and progesterone itself could cause extremely low LH concentrations in the luteal phase by a strong negative feedback mechanism (Gibson *et al.*, 1991). FSH concentrations rose in the late luteal phase in both groups without luteal support. This rise may have been secondary to reduced negative feedback associated with decreased progesterone concentrations.

Progesterone production occurred in both groups without



**Figure 4.** Box and whisker plots representing median values and 25th and 75th percentiles of HCG serum concentrations during the luteal phase of three different IVF treatment protocols: long GnRHa/HMG protocol with luteal support (group A), early cessation of GnRHa without luteal support (group B), and long use of GnRHa without luteal support (group C).

luteal support. However, distinctly lower concentrations were reached and an earlier decrease in progesterone production was noted compared to the group with luteal support. This latter observation is in agreement with previous reports (Smits *et al.*, 1987; Valbuena *et al.*, 1997). Surprisingly, no difference in progesterone production was found comparing shorter or longer GnRHa use, despite the partial recovery of LH concentrations when GnRHa was stopped earlier. This may be due to a discrepancy between bioactive and immunoassayable LH following GnRHa use (Meldrum *et al.*, 1984) or irreversible luteolysis before the onset of stimulation by endogenous LH. It seems likely that during the early luteal phase the corpus luteum is supported by the HCG bolus (10 000 IU) administered 35 h before oocyte retrieval. This effect lasted for approximately 1 week as can be seen from the relationship between decreasing HCG concentrations and the duration of progesterone production (Figure 5). In patients receiving luteal



**Figure 5.** Median HCG serum concentrations in relation to median progesterone levels separately, for three different treatment protocols: long GnRHa/HMG protocol with luteal support (group A), early cessation of GnRHa without luteal support (group B), and long use of GnRHa without luteal support (group C). Starting point is the day of oocyte retrieval. Numbers in graph indicate number of days following HCG bolus dose.

support by HCG, progesterone concentrations reached a higher maximum as compared to patients without luteal support or normal regularly cycling controls. Furthermore, the increase in progesterone concentrations lasted longer while the decrease started later. This was probably due to higher HCG concentrations in the luteal phase due to HCG supplementation. Progesterone concentrations decreased the moment that HCG levels fell below approximately 30 IU/l in all three groups.

In natural cycles luteal progesterone concentrations increase to a maximum of 70 nmol/l. In all groups, concentrations substantially higher than these were found. The effects of high progesterone concentrations on implantation chances in stimulated cycles are unclear (Pellicer *et al.*, 1996). Since pregnancies occurred in all three groups, it seems fair to conclude that the described hormonal features did not preclude implantation. Due to the small sample size, the possibility that implantation chances are impaired cannot be excluded. Withholding luteal support resulted in an earlier decrease in progesterone concentrations. Effects on implantation chances remain unclear. Progesterone concentrations in the control group were at a more steady level. It is unknown whether a sharp decrease in progesterone concentration could be detrimental despite preceding supraphysiological progesterone levels.

In conclusion, this study shows that after cessation of GnRHa earlier in the follicular phase, a premature rise in LH or progesterone concentration is still prevented. In addition, it was found that after earlier cessation of GnRHa, luteal immunoassayable LH concentrations recovered partially. However, no effect on luteal progesterone production could be observed. Progesterone production in IVF patients without luteal support was higher as compared to the natural cycle, but lower compared to patients with luteal support. As progesterone profiles in the luteal phase were not different after earlier discontinuation of GnRHa compared to continuation until HCG, it is concluded that endogenous support of corpus luteum

function in the second half of the luteal phase remains insufficient. IVF treatment without luteal support might become a reality when GnRH antagonists become available in the near future. However, other factors potentially involved in impaired luteal phase gonadotrophin secretion such as the preceding bolus injection of HCG or supraphysiological luteal phase steroid feedback should also be considered.

### Acknowledgements

This study was financially supported by the 'Stichting Voortplantings-geneeskunde Rotterdam' and by an unrestricted research grant from Ferring Nederland BV.

### References

- Akande, A.V., Mathur, R.S., Keay, S.D. *et al.* (1996) The choice of luteal support following pituitary downregulation, controlled ovarian hyperstimulation and *in vitro* fertilisation. *Br. J. Obstet. Gynaecol.*, **103**, 963–966.
- De Jong, F.H., Baird, D.T. and Van der Molen, H.J. (1974) Ovarian secretion of oestrogens, androgens and progesterone in normal women and in women with persistent ovary follicles. *Acta Endocrinol. (Copenh.)*, **77**, 575–581.
- Donderwinkel, P.F.J., Schoot, D.C., Pache, T.D. *et al.* (1993) Luteal function following ovulation induction in polycystic ovary syndrome patients using exogenous gonadotrophins in combination with a gonadotrophin-releasing hormone agonist. *Hum. Reprod.*, **8**, 2027–2032.
- Faber, B.M., Mayer, J., Cox, B. *et al.* (1998) Cessation of gonadotrophin-releasing hormone agonist therapy combined with high-dose gonadotrophin stimulation yields favorable pregnancy results in low responders. *Fertil. Steril.*, **69**, 826–830.
- Gibson, M., Nakajima, S.T. and McAuliffe, T.L. (1991) Short-term modulation of gonadotropin secretion by progesterone during the luteal phase. *Fertil. Steril.*, **55**, 522–528.
- Hughes, E.G., Fedorkow, D.M., Daya, S. *et al.* (1992) The routine use of gonadotrophin-releasing hormone agonists prior to *in vitro* fertilization and gamete intrafallopian transfer: a meta analysis of randomized controlled trials. *Fertil. Steril.*, **58**, 888–896.
- Mathur, R.S., Joels, L.A., Akande, A.V. *et al.* (1996) The prevention of ovarian hyperstimulation syndrome. *Br. J. Obstet. Gynaecol.*, **103**, 740–746.
- Meldrum, D.R., Tsao, Z., Monroe, S.J. *et al.* (1984) Stimulation of LH fragments with reduced bioactivity following GnRH agonist administration in women. *J. Clin. Endocrinol. Metab.*, **58**, 755–757.
- Pantos, K., Meimeth-Damianaki, T., Vaxevanoglou, E. *et al.* (1994) Prospective study of a modified gonadotrophin-releasing hormone agonist long protocol in an *in vitro* fertilization program. *Fertil. Steril.*, **61**, 707–713.
- Pellicer, A., Valbuena, D., Cano, F. *et al.* (1996) Lower implantation rates in high responders: evidence for an altered endocrine milieu during the preimplantation period. *Fertil. Steril.*, **65**, 1190–1195.
- Porter, R.N., Smith, W., Craft, I.L. *et al.* (1984) Induction of ovulation for *in-vitro* fertilisation using buserelin and gonadotrophins. *Lancet*, **ii**, 1284–1285.
- Schipper, I., de Jong, F.H. and Fauser, B.C.J.M. (1998) Lack of correlation between maximum early follicular phase serum follicle stimulating hormone concentrations and menstrual cycle characteristics in women under the age of 35 years. *Hum. Reprod.*, **13**, 1442–1448.
- Smith, E.M., Anthony, F.W., Gadd, S.C. *et al.* (1989) Trial of support treatment with human chorionic gonadotrophin in the luteal phase after treatment with buserelin and human menopausal gonadotrophin in women taking part in an *in vitro* fertilisation programme. *Br. Med. J.*, **289**, 1483–1486.
- Smitz, J., Devroey, P., Braeckmans, P. *et al.* (1987) Management of failed cycles in an IVF/GIFT program with the combination of a GnRH analogue and HMG. *Hum. Reprod.*, **2**, 309–314.
- Smitz, J., Devroey, P., Camus, M. *et al.* (1988) The luteal phase and early pregnancy after combined GnRH agonist/HMG treatment for superovulation in IVF or GIFT. *Hum. Reprod.*, **3**, 585–590.
- Smitz, J., Erard, P., Camus, M. *et al.* (1992a) Pituitary gonadotrophin secretory capacity during the luteal phase in superovulation using GnRH-agonists and HMG in a desensitization or flare-up protocol. *Hum. Reprod.*, **7**, 1225–1229.
- Smitz, J., Van Den Abbeel, E., Bollen, N. *et al.* (1992b) The effect of gonadotrophin-releasing hormone (GnRH) agonist in the follicular phase on *in-vitro* fertilization outcome in normo-ovulatory women. *Hum. Reprod.*, **7**, 1098–1102.
- Soliman, S., Daya, S., Collins, J. *et al.* (1994) The role of luteal phase support in infertility treatment: a meta-analysis of randomized trials. *Fertil. Steril.*, **61**, 1068–1076.
- Sungurtekin, U., Jansen, R.P.S. (1995) Profound luteinizing hormone suppression after stopping the gonadotrophin releasing hormone-agonist leuprolide acetate. *Fertil. Steril.*, **3**, 663–665.
- Urbancek, J., Rabe, T., Grunwald, K. *et al.* (1990) Analysis of hormonal changes during combined buserelin/HMG treatment. *Hum. Reprod.*, **5**, 675–681.
- Valbuena, D., Pellicer, A., Guanes, P.P. *et al.* (1997) Effect of disruption versus continuation of gonadotrophin-releasing agonist after human chorionic gonadotrophin administration on corpus luteum function in patients undergoing ovulation induction for *in-vitro* fertilization. *Hum. Reprod.*, **12**, 2118–2121.

Received on June 9, 1999; accepted on September 17, 1999