Article

Luteal hormonal profile of oocyte donors stimulated with a GnRH antagonist compared with natural cycles

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Abstract

The effect of gonadotrophin-releasing hormone (GnRH) antagonist treatment on luteal phase hormonal profile has not yet been fully investigated. Cycle characteristics of 23 fertile donors stimulated with recombinant FSH and the GnRH antagonist, ganirelix 0.25, for IVF and receiving no kind of luteal supplementation were compared with control, natural cycles. Luteal luteinizing hormone (LH) serum concentrations as well area under the curve (AUC) for LH were significantly higher in natural cycles. In addition, luteal phase length was longer in natural cycles compared with donor cycles. Luteinizing hormone values dropped in the luteal phase of the stimulated cycles, with the lowest values being observed in the mid-luteal phase. AUC for progesterone in the luteal phase was significantly higher in the stimulated cycles compared with natural cycles ($P < 0.001$). Low LH serum concentrations and shortened luteal phase indicate the need for luteal phase supplementation in GnRH antagonist IVF cycles.

Keywords: *ganirelix, gonadotrophin-releasing hormone antagonist, IVF, luteal phase, luteinizing hormone, oocyte donors*

Introduction

Luteal phase supplementation with the association of a gonadotrophin-releasing hormone (GnRH) agonist is mandatory in ovarian stimulation protocols, otherwise luteal phase is shortened and pregnancy rates are low (Smitz *et al*., 1988). In GnRH agonist cycles, this abnormal luteal phase was attributed to a prolonged pituitary suppression from the analogue (Smitz *et al*., 1992).

In contradiction to GnRH agonists, adenohypophysis maintains its responsiveness to endogenous GnRH stimulus after antagonist treatment (Felberbaum *et al*., 1995) and gonadotrophin concentrations recover within 2 days after GnRH antagonist discontinuation (Oberye *et al*., 1999). Normal corpus luteum function is also preserved after mid-follicular antagonist administration (Mais *et al*., 1986). Therefore, it was postulated that GnRH antagonists might not be in need of luteal phase supplementation. However, luteal phase was shortened and no pregnancy occurred in IVF cycles after co-treatment with the GnRH antagonist cetrorelix 0.5 mg (Albano *et al*., 1998).

Despite the wide introduction of GnRH antagonists for ovarian stimulation, studies analysing the luteal phase of unsupplemented GnRH antagonist cycles are lacking, mainly, because of the difficulty of conducting such a trial. Recently, a similar study was prematurely discontinued for ethical reasons because of extremely low pregnancy rates (Beckers *et al*., 2003). As a result, there are only a few reports in the literature, and in most of them the number of participating subjects is fairly low. Oocyte donors provide the means to investigate the hormonal parameters of the luteal phase, there being no need for luteal

phase supplementation. In addition, studies on the luteal phase are mandatory as it is during that period of time that embryonic implantation takes place and low pregnancy rates have been associated with an abnormal luteal phase profile. The aim of the present study was, therefore, to investigate the luteal phase hormonal profile of unsupplemented in-vitro fertilization cycles of oocyte donors stimulated in association with rFSH and a GnRH antagonist, ganirelix 0.25 mg, compared with control, natural cycles.

Materials and methods

Two cohorts of subjects were analysed in this retrospective study. The first cohort consisted of 23 oocyte donors and the second cohort of 25 subjects followed in a natural cycle (controls) (Tavaniotou and Devroey, 2003). Donors were fertile women undergoing ovarian stimulation. Natural cycle controls were infertile women followed during a natural cycle by means of daily blood samples for intrauterine insemination or intercourse. Both groups consisted of patients between 26 and 38 years of age, with a normal cycle length, a day-3 FSH concentration of less than 10 IU/l, no endocrinopathies, no polycystic ovarian syndrome, and a body mass index <28. In the control natural cycle group, only strictly monitored ovulatory cycles were included in the study.

Ovarian stimulation was commenced on day 2 of the menstrual cycle with 150 or 200 U of rFSH (Puregon; Organon, Oss, The Netherlands). The dose of gonadotrophins was adjusted individually from day 7 of the menstrual cycle according to oestradiol values and ultrasonographic follicular measurements. From day 7 of the cycle (day 6 of the treatment), 0.25 mg of the GnRH antagonist ganirelix 0.25 (Orgalutran; Organon) was also administered daily s.c. in the anterior abdominal wall, up to and including the last day of rFSH administration. Ovulation was induced when at least three follicles were ≥17 mm in mean diameter by the injection of 10,000 IU of human chorionic gonadotrophin (HCG; Pregnyl; Organon).

In the group of natural cycles no intervention was carried out. Patients received no kind of luteal phase supplementation in any of the study groups. In both groups, patients were intensively monitored through daily blood samples during the pre-ovulatory and peri-ovulatory period. Patients were also asked to give a blood sample in the early, mid and late luteal phase, until menstruation.

Serum gonadotrophins were measured by specific monoclonal immunoradiometric assays (IRMA) for FSH and luteinizing hormone (LH), and expressed in IU/l as previously described in detail (Tavaniotou *et al.*, 2003). Steroid serum concentrations were expressed in ng/l for oestradiol and µg/l for progesterone. Values are expressed as means ± SD. Comparison between groups was done by means of Wilcoxon rank sum test and a *P*-value of less <0.05 was considered as statistically significant.

Results

Mean age of the donors was 31.9 ± 4 and mean age of the subjects followed in the natural cycle was 32.5 ± 3.9 years. Luteal phase length was significantly shorter in the donors' group (11.9 ± 1.9 days versus 13.3 ± 2.5 days $P < 0.05$). Cycle characteristics of the donors are presented in **Table 1**. All patients underwent oocyte retrieval, and in all patients there were more than two retrieved cumulus-oocyte complexes.

On the day of HCG injection, oestradiol serum concentrations were 1519 ± 850 ng/l in the stimulated cycles. On the day of LH surge, oestradiol serum values were 240 ± 107 ng/l during the natural cycles. Luteinizing hormone serum concentrations were significantly higher in natural cycles compared with donor cycles on all the studied days (**Figure 1**). Cumulative exposure to LH, measured by area under the curve (AUC), was also significantly higher in natural cycles compared with stimulated cycles during the whole cycle length, during the follicular as well as the luteal phase ($P < 0.0001$) (**Figure 1**). On the contrary, cumulative exposure to oestradiol was significantly higher in donor cycles (**Figure 2**).

AUC for progesterone was similar between natural cycles and stimulated cycles in the follicular phase. On the day of HCG injection progesterone serum concentrations were significantly higher in the stimulated cycles than the corresponding day in natural cycles (1.3 ± 0.8 µg/l versus 0.5 ± 0.2 µg/l). Serum progesterone concentrations were significantly higher during the early luteal and mid-luteal phase ($P < 0.001$) but not in the late luteal phase in stimulated cycles (**Figure 3**). AUC for progesterone was also significantly higher in stimulated cycles compared with natural cycles in the luteal phase of the cycle ($P < 0.001$).

GnRH antagonist administration decreased LH concentrations in the follicular phase (from 2.9 ± 3.2 IU/l to 1 ± 0.9 IU/l). On the day of HCG injection, LH serum concentration was 1 ± 1.1 IU/l in the donor cycles. Luteinizing hormone values dropped in the luteal phase, with the lowest values being observed in the mid-luteal phase of the cycle 0.39 ± 0.55 IU/l (**Figure 1**). Luteinizing hormone serum concentrations started to increase again in the late luteal phase of the donor cycles.

Table 1. Cycle characteristics of donors stimulated with the association of recombinant FSH and the gonadotrophin-releasing hormone (GnRH) antagonist ganirelix 0.25 mg.

Cycle characteristic	*Value*
Duration of follicular phase (days)	11 ± 1.7
Duration of luteal phase (days)	11.9 ± 1.9
Days of gonadotrophin injection	10.4 ± 1.8
Days of GnRH antagonist administration	5.4 ± 2.1
Mean number of gonadotrophin units	2115 ± 442
Oestradiol on the day of HCG (ng/l)	1519 ± 850
Cumulus–oocyte complexes	12.7 ± 7.2

HCG = human chorionic gonadotrophin.

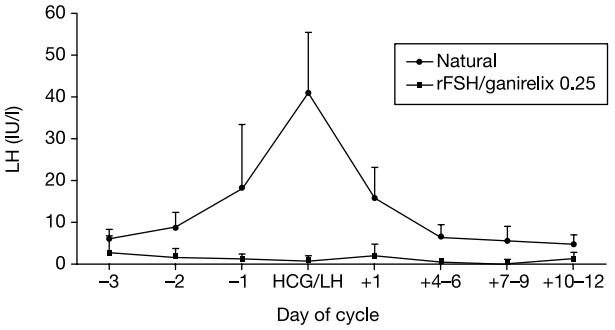

Figure 1. Luteinizing hormone values in gonadotrophin-releasing hormone (GnRH) antagonist cycles compared with natural cycles. Luteinizing hormone values were significantly higher ($P < 0.05$) in natural cycles on all the studied days. HCG = human chorionic gonadotrophin; LH = luteinizing hormone; rFSH = recombinant FSH.

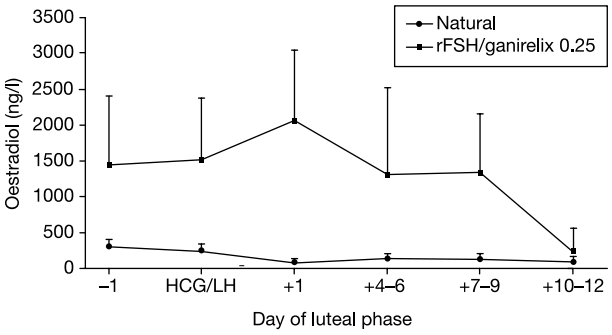

Figure 2. Oestradiol serum values in the two groups. Oestradiol concentrations were significantly higher ($P < 0.05$) on all the studied days in stimulated cycles. HCG = human chorionic gonadotrophin; LH = luteinizing hormone; rFSH = recombinant FSH.

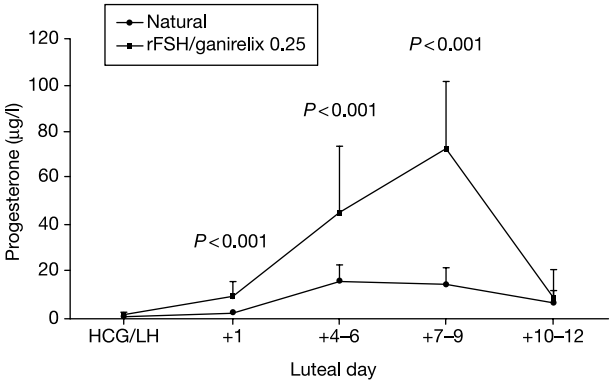

Figure 3. Progesterone serum concentrations in the two study groups. HCG = human chorionic gonadotrophin; LH = luteinizing hormone; rFSH = recombinant FSH.

Discussion

In all the different protocols used for IVF, an abnormal hormonal profile in the luteal phase was demonstrated (Edwards *et al.*, 1980; Smitz *et al.*, 1988; Albano *et al.*, 1998). GnRH antagonist administration during the follicular phase in natural as well as stimulated cycles was proven to be effective in blocking the endogenous LH surge (Albano *et al.*, 1998; Trokoudes *et al.*, 2005). However, luteal GnRH antagonist administration induced luteolysis (Humaidan *et al.*, 2005). Luteinizing hormone has a versatile role during the menstrual cycle. During the follicular phase, LH is responsible for the androgen secretion from the theca cells that provide the substrate for oestradiol production. During the LH surge, LH induces final oocyte maturation. It has been demonstrated in humans as well as in primates that LH support during the luteal phase is totally responsible for the maintenance and the steroidogenic activity of the corpus luteum (Casper and Yen, 1979). As a result, withdrawal of LH induces premature luteolysis (Duffy *et al.*, 1999).

In GnRH agonist cycles, pituitary recovery is retarded in the luteal phase because of the analogue administration in the preceding follicular phase and LH concentrations are extremely low (Smitz *et al.*, 1988). Treatment with the combination of human menopausal gonadotrophin (HMG) and cetrorelix 0.25 or 0.5 mg for ovarian stimulation reduced LH serum concentrations to almost undetectable concentrations for the whole length of the luteal phase in cycles supplemented with HCG (Albano *et al.*, 1999). Low LH concentration were also observed in supplemented IVF cycles stimulated with HMG as well as in cycles stimulated with HMG and the GnRH antagonist cetrorelix 0.25 mg (Tavaniotou *et al.*, 2001), indicating a common mechanism. In the present study, LH concentrations were extremely low in the luteal phase of the donor cycles, reaching their lowest values in the mid-luteal phase, which demonstrates an abnormal luteal hormonal function.

The reasons for the abnormal corpus luteum function in GnRH antagonist cycles are only speculative. Low LH concentration

might be attributed to the ovulatory HCG injection via a short-loop feedback mechanism (Miyake *et al*., 1979). However, in natural cycles with and without HCG injection, serum LH concentrations were similar, indicating that ovulatory HCG does not reduce LH concentrations (Tavaniotou and Devroey, 2003).

In ovarian stimulation, multiple follicular development results in higher steroid serum concentrations than those observed in the natural cycles. Supraphysiological steroid serum concentrations might reduce LH secretion by a possible action at the hypothalamic level (Steele and Judd, 1988; Nippoldt *et al*., 1989). In the present study, luteal LH serum concentrations were extremely low in the stimulated cycles compared with natural cycles. On the contrary, oestradiol and progesterone serum concentrations were significantly higher in the stimulated cycles. In addition, the lowest LH values were observed in the mid-luteal phase of the stimulated cycles, when peak values of oestrogen and progesterone were attained. It may be postulated, therefore, that high steroid serum concentrations may reduce LH values and that low LH concentration may, in turn, induce premature luteolysis. This is further supported by a previous report in which luteal phase length was normal in intrauterine insemination cycles stimulated in association with a GnRH antagonist. Compared with IVF cycles, lower gonadotrophin doses are used for intrauterine insemination and, as a result, formation of corpus luteum and steroid serum concentrations may also be restricted (Ragni *et al*., 2001). Furthermore, in these results, suppression of LH had already started from the follicular phase (**Figure 1**) due to the antagonist, but also as a general suppression of the pituitary by the ovulation induction process, as it has been previously suggested (Messinis *et al*., 1998).

In addition, in cycles stimulated with FSH, lower early serum LH concentrations have been detected compared with natural cycles (Messinis and Templeton, 1987). In these results, LH concentration started to rise again in the late luteal phase, when oestradiol and progesterone concentration started to decrease. Indirect evidence that high steroid values are associated with the abnormal luteal phase was gathered from the observation that higher oestradiol concentrations were detected in patients with shorter luteal phase length (Beckers *et al*., 2003). Oestradiol was also found to be suppressive on pituitary LH release (Messinis and Templeton, 1987). In stimulated cycles, the ovulatory dose of HCG supports corpus luteum for 7–10 days, until it is cleared from the circulation. (Damewood *et al*., 1989; Mannaerts *et al*., 1998). After this period, the corpus luteum is totally dependent on endogenous LH secretion. These low LH serum concentrations may not be able to support corpus luteum function, which results in premature luteolysis

In conclusion, luteal LH serum concentrations are low in unsupplemented IVF cycles stimulated in association with a GnRH antagonist, which indicates the need for luteal phase support.

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