Is there a difference in the function of granulosa–luteal cells in patients undergoing in-vitro fertilization either with gonadotrophin-releasing hormone agonist or gonadotrophin-releasing hormone antagonist?

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Gonadotrophin-releasing hormone (GnRH) regulates gonadotrophin release. It has been shown that GnRH may have a direct effect on the ovary, as the addition of GnRH to granulosa cell cultures inhibits the production of progesterone and oestradiol. Specific GnRH receptors have been found to be present in rat and human granulosa cells. Desensitization of the pituitary by GnRH agonist has become common in in-vitro fertilization (IVF) treatment, usually by a long protocol of 2–3 weeks. With the introduction of GnRH antagonists, which produce an immediate blockage of the GnRH receptors, a much shorter exposure is needed of 3–6 days. The aim of this study was to evaluate the effect of a GnRH agonist (buserelin) and a GnRH antagonist (cetrorelix) on the function of granulosa cells cultured in vitro from IVF patients. Women were treated by IVF randomized either to have buserelin nasal spray from the luteal phase in the previous cycle or cetrorelix from day 6 of the cycle. Both groups had ovarian stimulation with human menopausal gonadotrophin (HMG) 150 IU daily, i.e. HCG was administered when the leading follicle(s) reached 20 mm and ovum retrieval was performed 36–38 h later using a transvaginal ultrasound-guided procedure. The progesterone and oestradiol concentrations in the culture medium were measured by microprotein assay. The results showed that granulosa cells from women treated with GnRH antagonist (cetrorelix) responded earlier to the in-vitro hormone stimulation in terms of progesterone accumulation than women treated with the GnRH agonist (buserelin). This may have been due to difference in time of exposure to the analogue. The results may indicate that the luteal function is less impaired in GnRH antagonist treatment than in GnRH agonist treatment.

Key words: GnRH agonist/GnRH antagonist/granulosa cells/IVF/steroidogenesis

Introduction

With progress in clinical management of infertility, gonadotrophin-releasing hormone (GnRH) and its analogues have been widely used to prevent the spontaneous luteinizing hormone (LH) surge in the in-vitro fertilization (IVF) procedure. Suppression of gonadotrophin secretion can be achieved with either GnRH agonists or GnRH antagonists. GnRH agonists induce desensitization of the gonadotrophic cells by reducing the number of LH-releasing hormone (LHRH) receptors on the cell membrane, so-called down-regulation. Conversely, GnRH antagonists achieve suppression of gonadotrophin secretion by the competitive blockade of the GnRH receptors. This leads to a rapid decline of gonadotrophin secretion (Borghi et al., 1983; Rivier et al., 1986). The duration of the treatment with a GnRH antagonist can therefore be reduced in comparison to that of the agonist. The newly developed GnRH antagonist cetrorelix has been available for clinical trials and found to be effective in suppressing premature LH surges (Diedrich et al., 1994; Olivennes et al., 1994; Felberbaum et al., 1995). Little is known whether this antagonist has any direct action on the ovarian cells. This is of particular interest, since it has been shown that GnRH analogues may also have a direct effect on the ovary and specific GnRH receptors have been demonstrated in rat and human granulosa cells (Latouche et al., 1989; Bauer-Dantoin and Jameson, 1995), although the function of these receptors is unknown. The aim of this study was to evaluate the function of granulosa–luteal cells (GLC) isolated from women undergoing IVF, who had been treated with human menopausal gonadotrophin (HMG) and either with the GnRH agonist buserelin or the GnRH antagonist cetrorelix.

Materials and methods

GLC were obtained from women undergoing IVF with treatment either by the GnRH agonist buserelin (n = 12) or the GnRH antagonist cetrorelix (n = 13). Patient characteristics are shown in Table I. Women in the agonist group were down-regulated by buserelin (Suprecur; Hoechst AG, Frankfurt, Germany) nasal spray 150 µg four times daily from day 19–21 of the previous cycle. After down-regulation, HMG (Humegon®; Organon, Oss, The Netherlands) 150 IU daily was administered and the dose was adjusted according to the ovarian response. Women in the antagonist group started the stimulation with HMG 150 IU daily on cycle day 2. Cetrorelix (ASTA Medica AG, Frankfurt Main, Germany) 0.25 mg daily s.c. was administered from cycle day 6. In both groups of women human chorionic gonadotrophin (HCG) (Pregnyl®; Organon) 10 000 IU was administered when the leading follicle(s) reached 20 mm and ovum retrieval was performed 36–38 h later using a transvaginal ultrasound-guided procedure.
Table I. Patient characteristics. Values shown are means ± SEM. Serum oestradiol, progesterone and LH were analysed on the day of HCG administration. No significant difference was found between the two groups.

<table>
<thead>
<tr>
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<th>Agonist group</th>
<th>Antagonist group</th>
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<tbody>
<tr>
<td>No. of patients</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Age (years)</td>
<td>31.1 ± 1.2</td>
<td>30.2 ± 1.0</td>
</tr>
<tr>
<td>Serum oestradiol (pmol/l)</td>
<td>7012 ± 1178</td>
<td>5483 ± 1389</td>
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<tr>
<td>Serum progesterone (nmol/l)</td>
<td>3.57 ± 0.53</td>
<td>4.16 ± 0.81</td>
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<tr>
<td>Serum LH</td>
<td>2.37 ± 0.74</td>
<td>2.80 ± 1.60</td>
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When the oocytes had been recovered from the follicular fluid, the GLC clumps were collected under the microscope from the fluid of follicles >18 mm in diameter. The cell clumps were then washed repeatedly in pre-warmed 37°C Earle’s balanced salt solution (Sigma, St Louis, MO, USA) to remove red blood cells. The washed cell clumps were dispersed by finely drawn Pasteur pipettes. Cell number was counted using a haemocytometer and cell viability (50–87%) was determined by Trypan blue (Sigma) exclusion.

Between 2 and 6 × 10^4 viable cells per well were cultured in 24-well dishes (Nunc, Copenhagen, Denmark). The medium was Medium 199 (Sigma) with 25 mmol/l sodium bicarbonate, 1% heat inactivated fetal bovine serum (Sigma), and 50 IU/ml penicillin and 50 µg/ml streptomycin (Sigma). The cells were pre-cultured in 0.5 ml medium without any hormone at 37°C, 5% CO₂ and 100% humidity in air for 2 days to regain gonadotrophin sensitivity (Hillensjo et al., 1985). After the pre-culture, the medium was removed and stored at –20°C until analysis. The cells were washed with fresh culture medium and then were cultured in the same conditions with hormone additions and testosterone (5 × 10⁻⁶ mol/l) but without antibiotics in the medium for 4 days, with medium changed on alternate days. Hormones included were human chorionic gonadotrophin (HCG) 0.05–2 IU/ml (Profasi®, Serono, Geneva, Switzerland) or dibutyryl cyclic AMP 0.1–2 mmol/l (Sigma). The culture media were collected and kept frozen at –20°C until the hormone assay. The cells were also kept at –20°C for the cellular protein determination. Each control and experimental group consisted of duplicate or triplicate culture wells.

Progesterone and oestradiol content of the media were measured by the commercially supplied immunoassay instrument Immulite® and kit (DPC, CA, USA). The interassay coefficient of variation (CV) was 5.1% for progesterone and 3.7% for oestradiol. The sensitivity of progesterone analysis was 0.7 nmol/l and oestradiol 73 pmol/l. Cellular protein was determined by the bicinchoninic acid (BCA) method (Smith et al., 1985) using BCA Kit (Sigma). The sensitivity of the assay was 10 µg/ml. The intra-assay CV was <5%.

Statistics
The results are expressed as the mean ± SEM. Statistical difference between groups was calculated by Student’s t-test. A P-value <0.05 was considered significant.

Results
In order to establish the effect of incubation time, time-course experiments were performed in both groups of patients. Accumulation of progesterone and oestradiol were stimulated by HCG and cAMP in a linear fashion between 12 and 48 h (data not shown). Since the clearest effects were seen after 48 h, this incubation time was chosen for the remaining experiments.

The basal concentrations of progesterone and oestradiol of the pre-culture media were analysed in some of the experiments and no difference was found between the agonist and antagonist group (Table II).

Progesterone accumulation in granulosa–luteal cells obtained from patients treated with buserelin (open bars; agonist group) or cetrorelix (hatched bars; antagonist group) during days 2–4 (top) or days 4–6 (bottom) of culture in the presence of HCG (0.1–1.0 IU/ml) or dibutyryl cAMP (0.01–1.0 mmol/l). Means ± SEM of 12 (buserelin) or 13 (cetrorelix) patients are shown. *P < 0.05 versus respective control.
and cAMP caused a significant stimulation of progesterone accumulation during days 2–4 and 4–6. The GLC from the antagonist group showed a seemingly stronger effect of hormone stimulation, but when directly comparing the concentrations of progesterone in the agonist and antagonist groups these differences were not significant.

The accumulation of oestradiol in the presence of testosterone in GLC from both groups of women is shown in Figure 2. No significant effect was found in cells from the agonist group during culture days 2–4 or days 4–6. In cells from the antagonist group, HCG (0.1–1 IU/ml) and dibutyryl cAMP (0.01–1.0 mmol/l) produced significant stimulation on oestradiol accumulation during days 2–4 (top) or days 4–6 (bottom) of culture in the presence of HCG (0.1–1.0 IU/ml) or dibutyryl cAMP (0.01–1.0 mmol/l). Means ± SEM of 12 (buserelin) or 13 (cetrorelix) patients are shown. *P < 0.05 versus respective control.

**Figure 2.** Oestradiol accumulation in granulosa–luteal cells obtained from patients treated with buserelin (open bars; agonist group) or cetrorelix (hatched bars; antagonist group) during days 2–4 (top) or days 4–6 (bottom) of culture in the presence of HCG (0.1–1.0 IU/ml) or dibutyryl cAMP (0.01–1.0 mmol/l). Means ± SEM of 12 (buserelin) or 13 (cetrorelix) patients are shown. *P < 0.05 versus respective control.

Discussion

To our knowledge, there has only been one previous study in which human GLC have been examined in women receiving a GnRH antagonist. Minaretzis et al. (1995) compared the GLC steroidogenesis in vitro from Nal-Glu and leupropride acetate-treated women respectively. They reported that basal and gonadotrophin-stimulated progesterone secretion was similar in the two treatment groups, but that GLC aromatase activity 6 h after cell isolation was lower in the antagonist-treated patients. These results differ from the present study in which we found that basal progesterone and oestradiol production of the GLC during the pre-culture period was similar between the agonist-treated and antagonist-treated women. Furthermore, we found that GLC from antagonist-treated women responded to hormone-stimulated progesterone production earlier and were more sensitive to stimulation than the cells from agonist treated women. There are several possible explanations for the discrepancy between our study and that of Minaretzis et al., (1995). The antagonists employed differed, as did the culture conditions and the number of patients included, being 25 in this study versus 12 in the earlier study.

Whether the GnRH antagonists have any direct effect on the ovarian cells is not known. Many studies have investigated the direct effects of pharmacological doses of GnRH agonists on ovarian function. In the rat specific high-affinity GnRH receptors are present in the granulosa cells (Harwood et al., 1980) and activation of these receptors causes stimulatory effects on oocyte meiosis and ovulation (Hillenso and LeMaire, 1980; Ekholm et al., 1981), or long-term inhibitory effects on follicular development and steroid production (Rippel and Johnson, 1976; Hsueh and Erickson, 1979). Similar direct GnRH effects have been considered for the human ovary, but the results have been contradictory (e.g. Casper et al., 1982; Tureck et al., 1982; Bussenot et al., 1993). Since we examined the effect of cetrorelix and buserelin exposure in vitro, it is impossible to draw conclusions regarding any direct effects on the granulosa cells in vivo.

There is some evidence that GnRH agonist exposure in vivo may affect human GLC function in vitro. Pellicer and Miro (1990) compared the progesterone accumulation of GLC in culture between patients treated with clomiphene citrate/gonadotrophin and GnRH agonist/gonadotrophin. Granulosa cells obtained from patients treated with the GnRH agonist had lower progesterone production and a higher accumulation of 20 α-hydroxy-progesterone than cells isolated from women treated with clomiphene citrate/gonadotrophin. Pellicer and Miro (1990) compared the progesterone accumulation of GLC in culture between patients treated with clomiphene citrate/gonadotrophin and GnRH agonist/gonadotrophin. Granulosa cells obtained from patients treated with the GnRH agonist had lower progesterone production and a higher accumulation of 20 α-hydroxy-progesterone than cells isolated from women treated with clomiphene citrate/gonadotrophin.

One possible explanation for the difference we have observed between steroidogenesis in GLC from antagonist as compared to agonist treated women might be that the cells from the antagonist group were exposed to the GnRH analogue in vivo during a much shorter period than the GLC from the agonist group. Secondly, there is some evidence from porcine granulosa cell cultures that GnRH antagonist combined with follicular fluid can enhance LH-stimulated progesterone secretion (Ledwitz-Rigby, 1989). It should be realized, however, that the GLC obtained from follicle aspirates are heterogeneous and that the response in vitro may be highly variable depending on patient and stimulation protocol as well as preparation and culture techniques (Figenschau et al., 1997).

To summarize, the cultured GLC from the cetrorelix-treated women responded earlier to the in-vitro hormone stimulation in terms of progesterone accumulation than those of the buserelin-treated women. This might have been due to the
cells of the cetrorelix group being exposed to the GnRH analogue during a shorter period in vivo. These results are compatible with the view that the GnRH antagonist cetrorelix would cause minimal impairment of luteal function.

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References


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