Gonadotropin-releasing hormone antagonists do not influence the secretion of steroid hormones but affect the secretion of vascular endothelial growth factor from human granulosa luteinized cell cultures

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Objective: To evaluate the secretion of E_2, P, and vascular endothelial growth factor (VEGF) in human granulosa luteinized cell cultures with the presence of a gonadotropin-releasing hormone (GnRH) agonist or antagonist.
Design: In vitro cell culture study.
Setting: Research laboratory of a university hospital.
Patients: Granulosa luteinized cells were obtained from 24 patients undergoing ovarian stimulation for IVF treatment.
Interventions: Granulosa cells were cultured for 48 hours with 1 nM of cetrorelix or leuprorelide. For a further 48 hours, granulosa cells were cultured with or without the combination of cetrorelix plus leuprorelide.
Main Outcomes: At the end of each culturing period, the concentrations of E_2, P, and VEGF were measured in culture supernatants by immunoassays.
Results: Estradiol and P concentrations were similar between the culture supernatants from controls and treatment groups. The VEGF concentrations in supernatants from cultures with cetrorelix (2,315.1 ± 1,565.5 pg/mL) were moderately, but significantly, lower than in controls (2,604.3 ± 1,907.1 pg/mL) or cultures with leuprorelide (2,558.8 ± 1,403.1 pg/mL).
Conclusions: The GnRH analogues do not affect steroidogenesis in human granulosa luteinized cell cultures. The GnRH antagonists moderately affect the secretion of VEGF from human granulosa luteinized cells. (Fertil Steril® 2006;86:636–41. ©2006 by American Society for Reproductive Medicine.)

Key Words: GnRH analogues, estradiol, progesterone, VEGF, granulosa cells

Granulosa cells support and regulate the development of the oocyte by the production not only of steroid hormones but also a plethora of growth factors. The function of granulosa cells is mainly under the influence of gonadotropins that sustain the growth, proliferation, and secretion of this type of cells. The ovulation-inducing LH surge, discharged by the pituitary gland, reprograms granulosa cell function, leading to their terminal differentiation. The LH surge orchestrates profound changes in the production and secretion of sex steroid hormones and cytokines from granulosa cells.

Among the growth factors secreted from granulosa cells, vascular endothelial growth factor (VEGF) is of particular interest. The VEGF is responsible for the vascularization of the follicle and the regulation of vascular permeability, thus participating in the supply of the oocyte with oxygen, nutrients, and regulatory molecules. The role of VEGF is essential for follicular selection and development, as well as for the formation of corpus luteum and its function (1–4). Particularly after LH surge, VEGF production is augmented to enhance the microvascular network of the follicle, and consequently, it contributes to the formation of a functional corpus luteum (5–7). The VEGF is also implicated in various pathophysiological conditions of the ovary, including the pathogenesis of ovarian hyperstimulation syndrome (OHSS), a potentially lethal complication of ovarian stimulation (8).

During controlled ovarian hyperstimulation (COH), which is used for the preparation of women participating in IVF treatment, granulosa cells function under a different environment than in natural cycles. Apart from the supraphysiological doses of exogenously administered gonadotropins, during COH there is a continuous presence of molecules used to suppress the secretion of endogenous gonadotropins from the pituitary gland: gonadotropin-releasing hormone (GnRH) analogues (agonists or antagonists).

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The GnRH analogues are broadly used in IVF to suppress the secretion of the pituitary gland, thus preventing premature LH surges. The binding of GnRH-agonists to GnRH receptors of gonadotroph pituitary cells initially results in a stimulatory (flare-up) effect, further leading to their desensitization and to a reduction in the number of GnRH receptors. On the other hand, GnRH-antagonists directly inhibit gonadotropin secretion through a competitive binding to GnRH receptors.

The GnRH-antagonists in COH protocols have a number of advantages compared with GnRH-agonists: avoidance of the flare-up effect, reduction of the length of stimulation, and a lower incidence of the OHSS (9–14). However, GnRH-antagonist protocols appear to be associated with lower E_2 serum concentrations on the day of hCG administration and slightly lower pregnancy rates than GnRH-agonist protocols (14–15). This fact, along with the presence of GnRH receptors in the ovary, has fueled an ongoing debate on the ovarian actions of GnRH analogues. Several researchers who studied the direct effects of GnRH analogues on steroidogenesis in granulosa luteinized cell cultures have come to contradictory results. At the same time, it was hypothesized that GnRH-antagonists negatively affect the biosynthesis and/or the secretion of growth factors produced locally by normal ovarian cells (16).

In this study, human granulosa luteinized cells (GLCs) were cultured with or without a GnRH-agonist (leuprorelide), a GnRH-antagonist (cetrorelix), or their combination. The aim was to investigate the secretion of E_2, P, and VEGF (namely $VEGF_{165}$, which is the predominant isoform) by measuring their levels in culture supernatants. In addition, the secretion of the soluble receptor 1 of VEGF (sVEGFR1), which is an important regulator of VEGF activity in vivo, was studied.

MATERIALS AND METHODS

The GLCs were obtained at the time of oocyte retrieval from 24 women (29–39 years old) following COH for IVF treatment because of male factor infertility in the Clinic of Obstetrics and Gynecology, University of Schleswig-Holstein, Campus Lübeck. The patients gave their verbal consent and did not receive any monetary compensation. Approval from the institutional review board was not required because GLCs are normally discarded after oocyte recovery.

The COH followed the multidose antagonist protocol (Lübeck protocol) (9–11). Briefly, the ovarian stimulation was made with human menopausal gonadotropins (Menogon; Ferring Arzneimittel GmbH, Kiel, Germany) and recombinant FSH (Gonal-F; Serono International S.A., Geneva, Switzerland). Pituitary suppression was achieved with a daily dose of 0.25 mg of cetrorelix (Cetrotide; Serono International S.A., Geneva, Switzerland) from the sixth day of ovarian stimulation until the day of the induction of ovulation. The ovulation was induced with 10,000 IU of hCG (Choragon; Ferring Arzneimittel GmbH, Kiel, Germany).

Following oocyte retrieval, intracytoplasmic sperm injection was performed, with the exception of two cases where conventional IVF was applied. Embryo transfers were made at day 2. Up to three cleaving embryos were transferred according to the German Embryo Protection Law. Clinical pregnancy was defined by the presence of positive fetal heartbeat.

Cell Preparation

After harvesting the cumulus-oocyte complex for IVF, granulosa cells were isolated from follicular fluids through repeated centrifugation and washing with phosphate-buffered saline or culture medium. They were plated into 24-well culture dishes at a density of 100,000 cells per well with the addition of 1 mL Dulbecco's Eagle Modified Medium F12 (Gibco, Grand Island, NY) supplemented with 10% Ultroser G (Ciphergen BioSystems Inc., Cergy-Saint-Christophe, France), 100 IU/mL penicillin, and streptomycin [Dulbecco's Eagle Modified Medium F12 (Gibco) + 10% Ultroser G (Ciphergen BioSystems) + penicillin + streptomycin (DMEM/U/PS)].

An overnight incubation (37°C, humidified atmosphere, 5% CO_2) followed to allow attachment of the cells to the bottom of the plates and recovery from any effect of the in vivo exposure to GnRH analogues and gonadotropins during COH. Twenty-four hours later, the incubation medium was replaced.

Experiment 1

The cultures derived from each patient were divided into controls (group 1) and treated cultures. Treated cultures were divided into those treated with cetrorelix (group 2) and those treated with leuprorelide (group 3). Each patient contributed at least two cultures to each group; consequently, the three groups were interdependent. With this experimental design, comparisons were made between cultures belonging to different groups but derived from the same patient.

During the next 48 hours, group 1 control cultures were incubated in 1 mL of DMEM/U/PS, group 2 cultures in 1 mL of DMEM/U/PS plus 1 nM of cetrorelix, and group 3 cultures in 1 mL of DMEM/U/PS plus 1 nM of leuprorelide. The cultures were incubated at 37°C in a humidified atmosphere with 5% CO_2. After this period (day 3), the medium samples were collected and stored at −20°C.

Experiment 2

After experiment 1, another 48-hour culturing period ensued, in which the group 1 controls were incubated under the same conditions (1 mL of DMEM/U/PS), while group 2 and group 3 were incubated in 1 mL of DMEM/U/PS supplemented with 1 nM of cetrorelix plus 1 nM of leuprorelide. At the end of this period (day 5), the medium samples were stored at −20°C. For technical reasons, experiment 2 was performed with GLC cultures only from nine of the patients.

Measurements

All measurements were made with commercial ELISA kits as follows:

E_2: DSL-10-4300 (DSL, Webster, Texas); sensitivity: 7 pg/mL; intraassay coefficient of variation (CV): 3.3%–4.8%; interassay CV: 6.5%–8.2%.