Influence of gonadotrophin releasing hormone (GnRH) and a GnRH-agonist on granulosa cell steroidogenesis

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Summary: The influence of gonadotropin releasing hormone and Decapeptyl® on steroidogenic activity was evaluated at concentrations of 0.1 ng/ml, 1 ng/ml and 10 ng/ml culture medium. In the granulosa cell cultures of 9 out of 18 patients addition of Decapeptyl® or gonadotrophin releasing hormone to the culture media caused dose-dependent inhibition of progesterone and öestradiol secretion in vitro. The steroidogenic activity in vitro of the granulosa cells of 84 IVF cycles was correlated to the maximal serum E_2 levels of the patients at induction of ovulation. The granulosa cells of patients who were stimulated including Decapeptyl® produced on average higher E_2 levels in vitro as compared to granulosa cells from hMG stimulation cycles. This may be regarded as an indirect clue to an inhibitory effect of Decapeptyl® on oestradiol synthesis in vivo.

Key words: Granulosa cells; Steroidogenesis; GnRH/GnRH-agonist.

INTRODUCTION

Agonists of gonadotrophin releasing hormone (GnRH) are used as part of ovarian hyperstimulation regimes in IVF-programs. Reports in the literature about the effects of GnRH-agonists on steroidogenic activity of granulosa cells in vitro are contradictory. Hsueh *et al.* ([1]), Knecht *et al.* ([2]) and Ledwitz-Rigby ([3]) found an inhibitory mode of action while Hillensjö *et al.* ([4]) reported a stimulating effect. Pari-naud *et al.* ([5]) interpreted these observations as effects related to the concentration of GnRH in the culture medium, and Olson *et al.* ([6]) found correlations with the cycle phase at granulosa cell retrieval. However, it should be noted that the effects which have been observed in vitro were studied at concentrations which are much higher compared to serum GnRH levels: whereas intramuscular depot application of the GnRH-agonist Decapeptyl ® results in serum concentrations comparable to the concentration used in our in vitro studies ([7]).

There is evidence for GnRH-receptors in granulosa cells ([8]) and in luteal cells ([9]). Also, GnRH-like peptides have been isolated from the ovaries ([10]). These observations may support the concept of a pa-

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racrine regulation of ovarian function by GnRH. Therefore, the therapeutic effects of GnRH-agonists may not only be due to suppression of the hypothalamic-hypophyseal-axis, but GnRH-agonists may also have a direct effect on ovarian function. In this study, granulosa cells of IVF-patients were cultured, and their steroidogenic activity was analysed.

MATERIALS AND METHODS

Patients

The causes of infertility in our IVF-program included dyspermia, tubal occlusion, endometriosis, and hormonal as well as immunological factors. Ovarian hyperstimulation was induced on an individualized basis using human menopausal gonadotrophin (hMG). In patients with a history of recurrent ovarian cysts and with premature LH-rise in a previous IVF trial a GnRH-agonist (Decapeptyl®; Ferring, Kiel, Germany) was added to hMG-stimulation. The time of administration of human chorionic gonadotrophin (hGC) for induction of ovulation was determined by using serial vaginal ultrasound examinations and monitoring of oestradiol (E_2) and luteinising hormone (LH). Vaginosonographically guided aspiration of the follicles was carried out 36 h after injection of 10.000 I.U. hCG.

Granulosa cell culture

The follicular aspirates from a total of 84 patients were centrifuged for 10 minutes at 175 g. The cell pellets were resuspended in cold phosphate buffered saline (PBS), layered on a 45% Percoll® (Pharmacia, Uppsala, Sweden) density gradient in PBS, and recentrifuged for 20 minutes at 700 g.

Red blood cells pelleted at the tip of the tubes while granulosa cells settled at the interphase between the supernatant and the Percoll®. The granulosa cells were washed twice in PBS and counted in a Neubauer's cell chamber using trypan blue dye exclusion test. 10^5 viable cells were plated in 1 ml of Ham's F10 medium (Boehringer Mannheim, Mannheim, Germany) with 10% fetal calf serum, 100 I.U. penicillin, and 100 µg streptomycin. GnRH (Ferring, Kiel, Germany) and Decapeptyl® (Ferring, Kiel, Germany) were added to the granulosa cell cultures of 18 patients in concentrations of 0.1 ng, 1 ng and 10 ng per ml medium beginning at day 1 of culture. The cells

were incubated for 8 days at 37 °C in a 5% CO_2-gas atmosphere. The culture media were changed daily.

Hormonal assays

Serum oestradiol (E_2) levels were measured using a competitive enzyme immunoassay, based on enhanced luminescence (Amersham, England). E_2 and progesterone (P) concentrations in the culture media were evaluated by a radioimmunoassay using commercial kits (Coat-A-Count® Estradiol and Coat-A-Count® Progesterone, Diagnostic Product Corperation, Los Angeles, USA).

Statistical evaluation

The correlation of the maximal serum E_2 concentrations and the maximal E_2 concentrations in the granulosa cell culture media was evaluated for significance using Spearman's correlation coefficent test. The inhibition of steroid secretion by Decapeptyl® and GnRH was evaluated for significance using t-test.

RESULTS

Granulosa cell inhibition in vitro by GnRH and Decapeptyl®

The effect of GnRH and Decapeptyl® on the steroidogenic activity of the granulosa cells of 18 IVF-patients was investigated in vitro. In the granulosa cell cultures of 9 patients dose-dependent inhibition of progesterone (significant at day 1 to 8) and estradiol secretion (significant at day 1) secretion was caused by each of the substances. Figures 1 and 2 show the inhibitory effect of GnRH and Decapeptyl® on the progesterone and estradiol production of granulosa cells of this patients exemplary at days 1 and 4 of culture. In the granulosa cell cultures of the remaining 9 cases neither GnRH nor the agonist had an effect.

The applied stimulation regime received by the patients did not influence the reaction of the granulosa cells to GnRH or Decapeptyl® in vitro.

Stimulation regimes and E_2 secretion of granulosa cells in vitro

The maximal serum E_2 levels in 84 IVF-clyces were plotted against the E_2 levels in the corresponding granulosa cell culture media after 24 h. Significant correlation was found in the hMG stimulation group ($p \leq 0.01$). Granulosa cells from patients who were stimulated with additional Decapeptyl® produced on average comparatively higher levels of E_2 in vitro as shown in the regression lines in Figures 3 and 4.
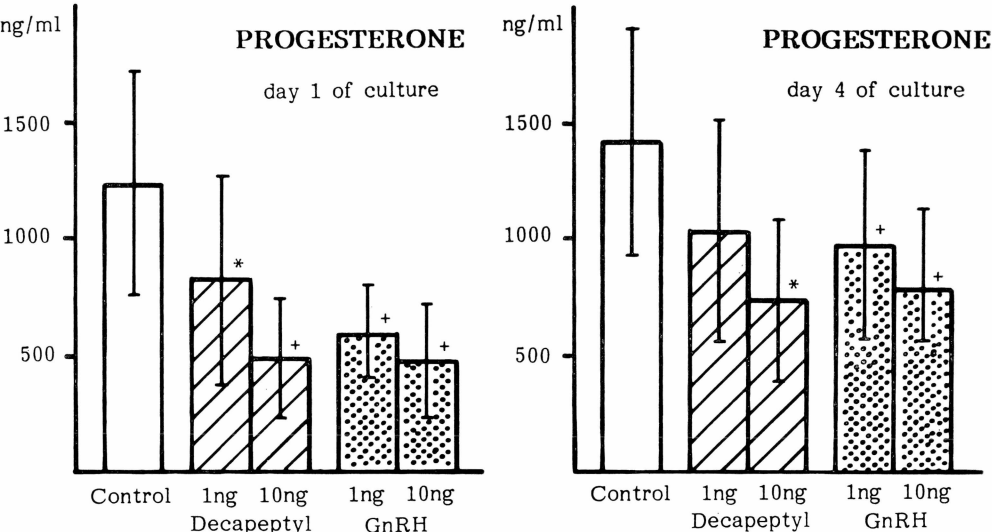
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Fig. 1. — Inhibitory influence of GnRH and Decapeptyl® (DP) on the secretion of progesterone (P) of cultured human granulosa cells (10^5 cells/ml). * $p \leq 0.01$ and + $p \leq 0.05$ vs. control.
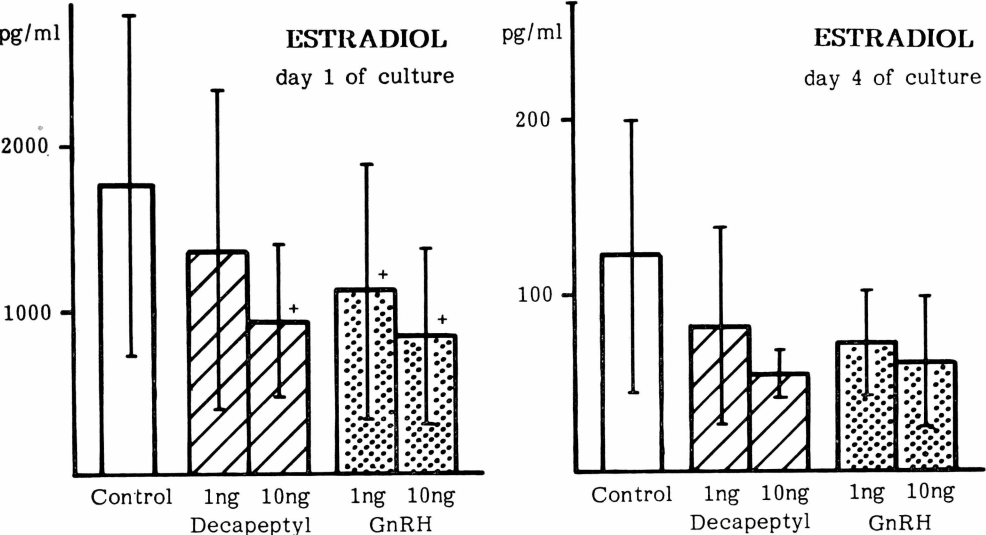

Fig. 2. — Inhibitory influence of GnRH and Decapeptyl® on the secretion of oestradiol (E_2) of cultured human granulosa cells (10^5 cells/ml). * $p \leq 0.01$ and + $p \leq 0.05$ vs. control.
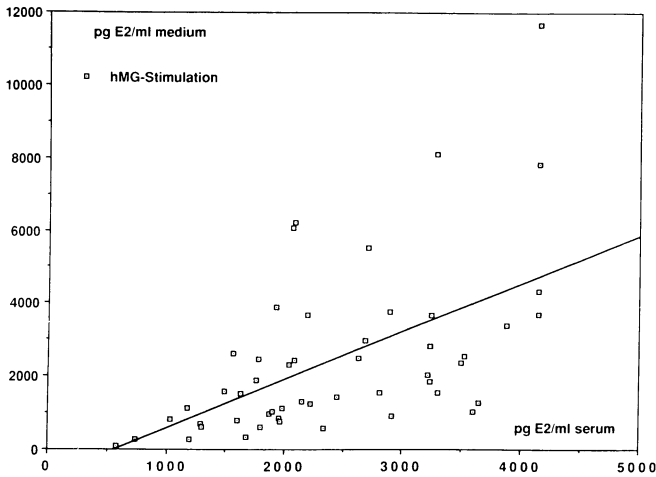
Fig. 3. — Plots of the oestradiol (E_2) levels in granulosa cells culture media after 24 h (10^5 cells/ml) versus maximal E^2 serum levels of the same patients at ovulation induction (n = 51, r = 0.55). Each dot represents the mean of 2 to 6 wells of an individual patient.

DISCUSSION

In IVF-cycles stimulated with hMG and a GnRH-agonist Neveu *et al.* [11] found relatively lower urinary oestrogen levels on the day of hCG administration then in hMG cycles. In individual patients who were stimulated with different regimes we also observed lower E_2 serum levels in stimulation cycles with Decapeptyl® downregulation as compared to hMG stimulation only (unpublished data).
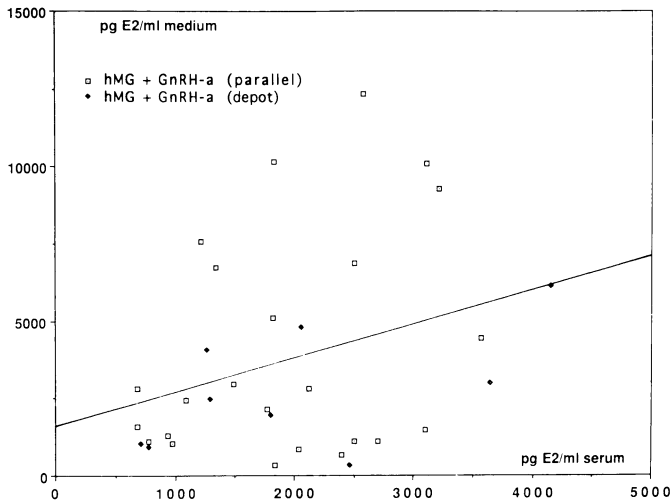

Fig. 4. — Plots of the oestradiol (E^2) levels in granulosa cells culture media after 24 h (10^5 cells/ml) versus maximal E_2 serum levels of the same patients at ovulation induction (n= 33, r = 0.30). Each dot represents the mean of 2 to 6 wells of an individual patient.

Therefore, it may be suspected that Decapeptyl® may have an inhibitory influence on ovarian function. In our in vitro studies both GnRH and Decapeptyl® had a suppressing effect on steroid secretion of the granulosa cells in half of the patients. This phenomenon may be due to different grades of luteinisation of the granulosa cells or other individual factors. Bramley *et al.* ([9]) described higher GnRH-agonist binding in granulosa-luteal cells when compared to granulosa cells of pre-ovulatory follicles.

The granulosa cells were retrieved after in vivo exposure to high doses of hMG, which is a combination of LH and FSH, and of hCG, which has a biological LH effect. Behrman *et al.* ([12]) found in their in vitro studies that high levels of LH override the inhibitory effect of GnRH on progesterone secretion. This may explain the lack of a suppressing effect on steroid secretion in half of our in vitro experiments. The stimulation regime may be without influence on the effect of GnRH Decapeptyl® on steroidogenesis of granulosa cell in vitro. In the "non-re-sponder" and the "responder" group 3 patients received hMG + Decapeptyl® and 6 patients pure hMG stimulation, respectively.

The correlations of maximal serum E_2 concentrations with E_2 secretion of granulosa cell cultures in the first 24 h indicate that the granulosa cells from hMG + Decapeptyl® stimulation cycles may produce in vitro comparatively larger amounts of E_2 as compared to granulosa cells from hMG stimulation cycles. This may be supposed as an indirect clue to Decapeptyl® inhibition of E_2 synthesis of granulosa cells in vivo as external influence of Decapeptyl® was withdrawn from the cells in culture.

There is evidence that cumulus cells influence the maturation of oocytes ([13]). Therefore, an inhibitory effect of Decapeptyl® on granulosa cells may lead to indirect effects on the development of oocytes. Meiotic maturation of follicle enclosed rat oocytes was stimulated by GnRH and resulted in parthenogenesis and fragmentation ([14]). The question may be posed as to whether an inhibitory effect of Decapeptyl® on granulosa cell function may influence IVF-outcome.

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