Human granulosa cells in vitro: Influence of GnRH/GnRH-agonist on steroidogenesis and on IVF outcome

A F G Stevenson*
Institute for Toxicology, University of Kiel, Brunswick Str. 10, D-24105 Kiel, Germany

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Steroidogenic activities of the granulosa cells (GCs) from 84 IVF trials were evaluated with respect to a set of ovarian stimulation regimens. Oestradiol (E2) synthesis of the GCs in vitro (obtained at oocyte retrieval) was compared to the maximal serum E2 levels of the same patients at induction of ovulation. Three stimulation regimens were employed: human post-menopausal gonadotrophin (hMG) alone; hMG accompanied by daily doses of a gonadotrophin releasing hormone agonist (GnRH-a); hMG preceded by a single depot application of the GnRH-a. Plots of E2 synthesis in vitro against serum E2 levels indicated that the GnRH-a directly inhibited E2 synthesis in the granulosa cells. This was confirmed in vitro by adding the agonist to the culture medium: both progesterone (P) and E2 syntheses were reduced in the presence of GnRH-a. Despite this drawback, the success of in vitro fertilisation (IVF), as gauged by pregnancies achieved, was best for the group which received the GnRH-a as a single depot dose during the previous menstrual cycle, prior to the commencement of stimulation. This success is attributed to the lower incidence of cancellations because of premature luteinising hormone (LH) surges which happen sometimes during ovarian stimulation. The implications of a direct influence of GnRH-a on E2 synthesis need to be further investigated.

Agonists of gonadotrophin releasing hormone (GnRH-a) are used in assisted human reproduction to induce a temporary state of hypogonadotrophic hypogonadism, before ovarian stimulation with follicle stimulating hormone (FSH) or human menopausal gonadotrophin (hMG). In this way, the possibility of an inappropriate premature surge of luteinising hormone (LH), which would cause cancellation of the procedure and repetition, could be averted. The paradoxical inhibitory effect of GnRH-a has been attributed to the failure of exogenous GnRH-a at maintaining the rhythmicity of natural GnRH. In addition to the hypophysological effects, peripheral effects on the gonads, in particular on granulosa cells (GCs), have been the subject of several papers. In vitro studies on the steroidogenic activity of GCs in the presence of various GnRH-a have reported inhibitory as well as stimulatory effects. In any case, receptors for GnRH have been found in GCs and in luteal cells. In this study the influence of a GnRH-a on the production of oestradiol (E2) by GCs in vivo - in patients’ sera just before oocyte retrieval for in vitro fertilisation (IVF) - was correlated with the respective E2 values ex vivo, obtained upon culturing the GCs and measuring E2 concentrations in the culture medium. Further, the GnRH-a used in this study to induce hormonal hypogonadism was added to the culture medium of the GCs to observe purported direct effects. Natural GnRH was also tested. The cell physiological implications of these laboratory findings are discussed in connection with a retrospective analysis of clinical results in terms of pregnancies achieved through the application of this GnRH agonist in varied stimulation protocols.

Materials and Methods

Patients and stimulation regimens—The GCs for this study were obtained from patients who opted for assisted reproduction at the Gynaecological Clinics of the university. Ovarian hyperstimulation was given on an individual basis by injection of hMG. Patients having a history of recurrent ovarian cysts, who had premature LH rise in a previous IVF trial, received GnRH-a (Decapeptyl from Ferring, Kiel, Germany) either as an intramuscular depot injection of 3.75 mg during the luteal phase of the preceding cycle or, alternatively, as daily subcutaneous injections of 0.5 mg from day 2 to 6 of the cycle, followed by 0.1 mg daily until induction of ovulation. Ovulation was induced by injection of 10,000 IU human chorionic gonadotrophin (hCG) based on ultrasonographic judgement of follicular maturity.
Granulosa cell culture—As details of procedures have been given in a preceding communication, description of methodology will be minimised. GCs were pelleted from the follicular aspirates, resuspended in PBS and separated on 45% Percoll. Viable cells were counted in a cytometer and plated into multiwell (1.5 cm² growth surface) culture dishes. Basing on the afore-mentioned earlier study, Ham's F10 culture medium supplemented with 10% foetal calf serum was used. Culture medium was replaced daily, the spent medium being stored at 4°C until hormone measurement.

The influence of GnRH-a (Decapeptyl) and natural GnRH (Gonadorelin) were tested in culture at concentrations of 1 and 10 ng/ml, respectively.

Hormone measurement—Serum oestradiol (E₂) levels were measured by a competitive enzyme immunoassay, based on enhanced luminescence (Amersham, England). E₂ and progesterone (P) concentrations in culture medium were estimated by radioimmunoassay using commercial kits (Coat-A-Count, Diagnostic Product Corporation, Los Angeles, USA).

The correlation of serum and culture E₂ concentrations was evaluated for statistical significance using Spearman's correlation coefficient test.

Results

Serum E₂ vs E₂ in culture medium—The maximal serum E₂ levels from a total of 84 IVF cycles were compared to the corresponding E₂ concentrations in culture medium after 24 hr culture of the GCs, by regression analysis. Since the present purpose was to investigate the possibility of direct effects of GnRH-a on the steroidogenic activity of GCs, the E₂ data from the groups that received GnRH-a in depot form and as daily injections were pooled. Fig. 1 (lower line) shows the regression line obtained after plotting E₂ values from serum against E₂ from culture medium for patients who received hMG stimulation without GnRH-a application (reference group). The same pooled values for the GnRH-a treated groups is represented in the upper line. The linear correlation between the plotted values are statistically significant (P ≤ 0.01 for hMG and P = 0.05 for GnRH-a).

Although the difference between the two lines is statistically not significant, the data from Fig. 1 can certainly be interpreted to indicate that GCs from patients treated with GnRH-a synthesised more hormone in vitro, implying a release from inhibition by GnRH-a which was present in the circulation.

An inhibitory effect of GnRH-a on hormone production is affirmed by the experiments in which GnRH-a and GnRH, respectively, were added directly to culture medium. This notion is supported by current concepts on GnRH action which will be discussed.

Steroidogenesis in vitro—The kinetics of production of P and E₂ by the same patients' GCs in culture are shown in Figs 2 and 3, respectively. The pattern of
secretion over the course of 8 days is typical and has already been discussed in two preceding papers. The results clearly indicate that both GnRH-a and natural GnRH suppress P and E2 secretion in a dose-dependent way, the effect of GnRH-a being stronger than that of natural GnRH. This is easily seen in Fig. 2 in which the level of P production continues over a longer period than that of E2 (Fig. 3).

Influence on fecundity—The results from in vitro fertilisation and embryo transfer (IVF-ET) are summarised from clinical records in the Table 1. The results indicate that patients who received treatment with GnRH-a had lowered pregnancy rates when compared to the reference group (hMG alone). However, the chances for pregnancy were almost normal when the GnRH-a was given in depot form in the luteal phase of the preceding cycle. An explanation for this difference between the form of administration of the GnRH-a can at the moment be only speculative.

Table 1—Summary of clinical data on pregnancy rates achieved after employing different stimulation regimens in which GnRH-a was applied either as a single depot injection in the preceding cycle or as daily injections parallel to stimulation with hMG. Patients who received hMG alone formed the reference group.

<table>
<thead>
<tr>
<th>Stimulation regimen</th>
<th>Embryo transfer (n)</th>
<th>Preganacies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hMG+GnRH-a Depot</td>
<td>30</td>
<td>7</td>
</tr>
<tr>
<td>hMG+GnRH-a Daily</td>
<td>197</td>
<td>33</td>
</tr>
<tr>
<td>GnRH-a (Pooled)</td>
<td>227</td>
<td>40</td>
</tr>
<tr>
<td>hMG alone</td>
<td>397</td>
<td>103</td>
</tr>
</tbody>
</table>

Discussion

GnRH is generally thought to act on the anterior pituitary gland, like all other Releasing Hormones, with no other site of action. However, recent investigations have generated evidence which support the notion of direct GnRH action on the ovaries viz. on granulosa14-15 and luteal cells14,15. These studies have demonstrated the presence of GnRH/GnRH-a receptors, the implications and consequences of which are at present only speculative. Nonetheless, it seems reasonable to think that GnRH might be directly involved in the modulation of paracrine regulatory functions which not only control the production of gonadal steroid hormones but also trigger the resumption of oocyte meiosis - which is arrested at a specific stage during foetal development - to render mature fertilisable oocytes.

Administration of GnRH-a can, therefore, perturb the subtle cell physiological processes involved in follicle development and even oocyte maturation via effects on the cumulus oophorus which is the population of GCs intimately bound to the oocyte. The significantly lower pregnancy rate achieved after GnRH-a application, particularly when it was given on an arbitrary daily basis, is probably none other than an affirmation to this effect. On the other hand, how come the group which received GnRH-a in depot form achieved a pregnancy rate which was close to that of the reference group cannot be explained but only speculated about on the basis of what is known about the mode of action of natural GnRH which is rhythmic. In ultimate analysis, the difference between the two GnRH-a groups is one of dose rate: the depot form ensuring constancy in blood plasma level of the substance, while daily applications introduced sudden spikes in the blood levels. This could have caused inadequacies in the maturing of the oocytes, which leads to reduced viability.

Follicle development which includes the whole process of follicle and oocyte maturation is closely correlated with the steroidogenic activity of the GCs, as seen from the finding that GnRH-a caused an inhibition of E2 synthesis in vivo. Culture of these cells automatically led to higher E2 secretion, thus, indicating the elimination of an inhibitory effect. Therefore, judging from the hormonal values, poorer performance in terms of acquired pregnancies could be expected from the GnRH-a treated groups. The inhibitory effect was confirmed in vitro by direct addition of GnRH-a and natural GnRH to the
cultures. The stronger action of GnRH-a is consistent with the definition of an agonist, being the pharmacologically more potent analogue of the natural substance.

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References