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Progesterone serum levels during the follicular phase of the menstrual cycle originate from the crosstalk between the ovaries and the adrenal cortex

Christian De Geyter[1,4], Maria De Geyter[1], Peter R.Huber[2], Eberhard Nieschlag[3] and Wolfgang Holzgreve[1]

[1]University Women's Hospital of Basel, Schanzenstrasse 49, CH-4031 Basel, [2]Hormone Laboratory of the University of Basel, Switzerland and [3]Institute of Reproductive Medicine, University of Münster, Domagkstrasse 11, D-48129 Münster, Germany

[4]To whom correspondence should be addressed at: Division Gyn. Endocrinology & Reproductive Medicine, Schanzenstrasse 46, CH-4031 Basel, Switzerland. E-mail: cdegeyter@uhbs.ch

BACKGROUND: The preovulatory rise of progesterone is important for ovulation, but both its regulation and its origin are controversial. Three experiments were performed to determine whether follicular phase progesterone arises from the ovary, the adrenal cortex or both. METHODS: The first study was performed in patients scheduled for assisted reproduction, who received a long-acting GnRH agonist either during intake of an oral contraceptive or during the luteal phase of an otherwise untreated menstrual cycle. The second study was also performed during down-regulation with a GnRH agonist: some patients with elevated progesterone levels received dexamethasone (DXM). Others with similarly elevated basal progesterone levels and those with low progesterone levels were not treated with DXM and served as controls. Finally, adrenocorticotrophic hormone (ACTH) tests were performed in normocyclic volunteers both during early and late follicular phase and during intake of a contraceptive pill. RESULTS: During the suppression of endogenous gonadotrophin secretion progesterone levels rose after the administration of ACTH, but not of GnRH. DXM did not prevent the preovulatory rise of the serum progesterone concentration. The ACTH-stimulated concentration of progesterone and of 17α-hydroxyprogesterone were significantly reduced during intake of ethinyl estradiol. CONCLUSIONS: Progesterone arises in the adrenal cortex during most of the follicular phase, whereby its function is modulated by an unknown ovarian factor, which is suppressed by ethinyl estradiol. The source of progesterone shifts towards the ovaries prior to ovulation.

Key words: 17α-hydroxyprogesterone/adrenal cortex/granulosa/progesterone/theca interna

Introduction

The rise of the serum concentrations of progesterone and 17α-hydroxyprogesterone is considered to be the first sign of an imminent ovulation, preceding the LH surge by ~12 h (Hoff *et al.*, 1983). In many species, including the human, successful ovulation can be prevented with either progesterone antagonists or inhibitors of progesterone synthesis (Collins and Hodgen, 1986; Liu *et al.*, 1987; Shoupe *et al.*, 1987; Batista *et al.*, 1992; Hibbert *et al.*, 1996). Homozygous transgenic mice lacking the progesterone receptor are unable to ovulate, so that their oocytes remain trapped within the unruptured follicles (Lydon *et al.*, 1995). Despite this experimental evidence, the progesterone receptor was only faintly and temporarily expressed in the granulosa of mature Graafian follicles (Iwai *et al.*, 1990; Park and Mayo, 1991). Progesterone alone can not induce ovulation, but seems to mediate the effects of LH on the Graafian follicle prior to and during ovulation (Brännström and Janson, 1989).

Despite the evident role of progesterone in the process of ovulation, its source during this period of the menstrual cycle has not been defined. The enzyme responsible for the synthesis of progesterone, 3β-hydroxysteroid dehydrogenase (3β-HSD), is expressed in the adrenal cortex, the theca interna and, at least in some species, also in the granulosa of the Graafian follicle (Dupont *et al.*, 1990). While some authors argue that the adrenals contribute significantly to preovulatory progesterone (Eldar-Geva *et al.*, 1998), others consider the ovary as the main source (Fanchin *et al.*, 1997; Urman *et al.*, 1999). In addition to these uncertainties, nothing is known about the regulatory signals that induce the rise of progesterone at the end of follicular development.

This communication summarizes the results of three prospective experiments, which were carried out to determine the origin and regulation of follicular phase progesterone secretion in regularly menstruating women. Two sets of experiments were performed during desensitization with a long-acting GnRH agonist in patients treated with IVF or ICSI, because this treatment effectively reduces the contribution of ovarian

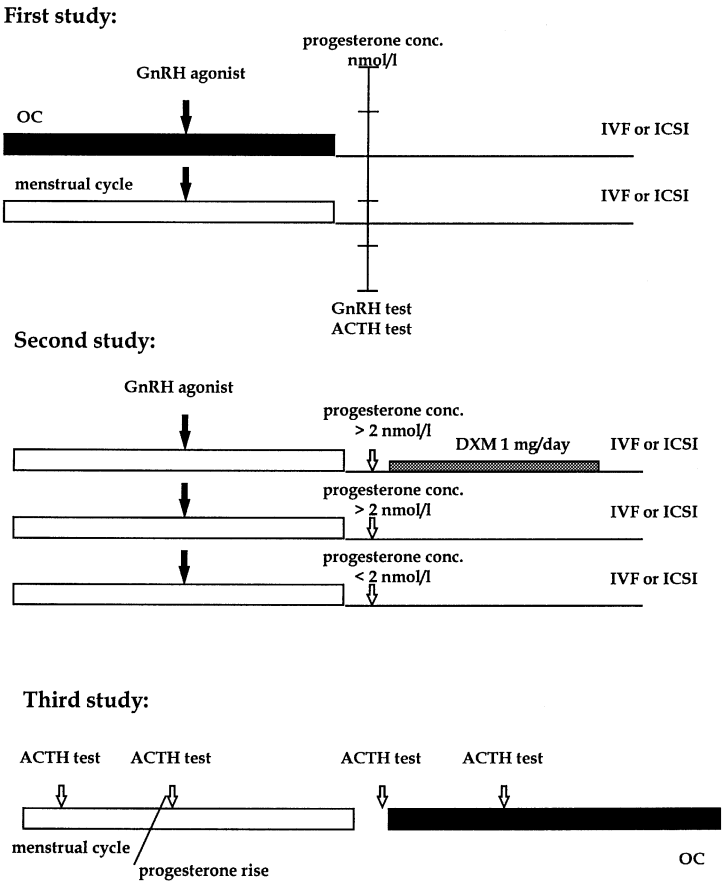


Figure 1. Diagrams of the three experimental protocols.

steroidogenesis. The third experiment was performed in normally menstruating volunteers during their untreated, natural cycle and, subsequently, during intake of a contraceptive pill.

Material and methods

The first study was designed to study the effect of any residual hormonal activity from the preceding menstrual cycle on circulating progesterone levels during the subsequent cycle. For this purpose, patients were treated with ethinyl estradiol and desogestrel during the administration of a long-acting GnRH agonist. The second study was designed to evaluate the role of the adrenal cortex on progesterone levels during the follicular phase by using a suppressor of endogenous adrenocortical steroid activity, dexamethasone (DXM), in patients during desensitization with a long-acting GnRH agonist. Finally, in the third study, a stimulatory agent of adrenocortical steroidogenesis, adenocorticotrophic hormone (ACTH), was given to women during a natural menstrual cycle and, subsequently, during intake of an oral contraceptive containing ethinyl estradiol and desogestrel. The three experimental protocols are visualized in Figure 1. The first and the second clinical studies presented below were carried out in the Institute of Reproductive Medicine at the University of Münster, Germany, whereas the third study was performed in the University Women's Hospital of Basel, Switzerland. All studies were presented to the local Ethic's Committees and the participants signed an informed consent form.

First study: the source of progesterone during suppression with a long-acting GnRH agonist

In a randomized prospective setting, 23 patients were treated with a hormonal contraceptive pill containing 50 µg ethinyl estradiol and 0.125 mg desogestrel given sequentially (Ovidol, Nourypharma, Oss, The Netherlands) during the menstrual cycle preceding the treatment with gonadotrophins. During treatment with the oral contaceptive a long-acting GnRH agonist was given i.m. (triptoreline acetate, Decapeptyl Retard; Ferring, Wallisellen, Switzerland) for later treatment with IVF or ICSI. Another group of 32 women received triptoreline acetate during the luteal phase of the untreated menstrual cycle. All patients included had normal, ovulatory menstrual cycles and none had polycystic ovarian syndrome (PCOS) or any other endocrine abnormality affecting the ovaries or the adrenals. Randomization was based on the women's birth month; those with an even month received the hormonal contraceptive, and those with an uneven month received no medication. Eight patients refused to participate in the prospective study. After menstruation ovarian hyperstimulation with gonadotrophins was initiated and monitored with repeated measurements of serum concentrations of 17β-estradiol and progesterone together with vaginal ultrasound examinations as described previously (De Geyter *et al.*, 1994). Each couple was included into the study only once.

Additionally, in 11 women a GnRH-test, consisting of a bolus injection of 100 µg of GnRH (Ferring), and in 18 women an ACTH test, consisting of a bolus i.v. injection with 250 µg ACTH (Synacthen; Ferring), were performed to evaluate the activity of the pituitary–ovarian and of the pituitary–adrenal axis respectively during desensitization with triptoreline acetate. The LH response in the

Table I. Clinical characteristics of the patients pretreated with 50 μg ethinyl estradiol together with 0.125 mg desogestrel and GnRH agonist as compared with those who received the GnRH agonist alone during their preceding natural menstrual cycle

	Natural cycle	Pretreated
No. of patients	32	23
Patient's age (years)	33.9 ± 1.7	32.9 ± 2.1[a]
Husband's age (years)	36.4 ± 2.3	35.7 ± 2.5[a]
Duration of infertility (years)	6.6 ± 1.4	5.6 ± 1.1[a]
Initial progesterone concentration (nmol/l)	1.72 ± 0.64	1.16 ± 0.30[a]
Progesterone at HCG administration (nmol/l)	4.53 ± 1.22	4.58 ± 1.34[a]
Estradiol at HCG administration (nmol/l)	6.14 ± 0.41	6.19 ± 0.84[a]
Oocytes fertilized/inseminated (%)	81/155 (52.3 %)	77/105 (73.3 %)[b]
Pregnancies achieved (%)	7 (21.9 %)	6 (26.1 %)[b]

[a]None of the differences were statistically significant (Mann–Whitney *U*-test).
[b]None of the differences were statistically significant (χ^2-test).

GnRH challenge test is considered optimal for the assessment of pituitary desensitization during treatment with GnRH agonists (Scheele *et al.*, 1996). Both the GnRH and the ACTH tests were performed before the start of ovarian stimulation with gonadotrophins. All serum samples were stored frozen at –20°C until assay.

Second study: suppression of follicular phase progesterone serum levels with DXM

DXM was used to suppress the adrenal production of progesterone and to observe the ovarian contribution to the progesterone levels during the follicular phase. Patients with regular menstrual cycles and a normal ovarian reserve (as assessed by day 3 cycle FSH level <9 IU/l), but suffering from tubal infertility or male immunological infertility, were recruited prior to their first treatment with IVF. All participants had normal, ovulatory menstrual cycles and none was suffering from the PCOS or any apparent endocrine abnormality of the ovaries or adrenals. The serum progesterone concentration was determined in three serum samples, taken every 10 min, during suppression of endogenous gonadotrophin secretion, which was achieved with triptoreline acetate 2–3 weeks earlier (Decapeptyl Retard; Ferring). Patients with all three basal serum progesterone concentrations uniformly >2 nmol/l during suppression of the ovaries were treated with DXM (1 mg daily, taken orally) to suppress the secretion of adrenal progesterone throughout ovarian stimulation with gonadotrophins. One control group of patients with three subsequent serum progesterone concentrations >2 nmol/l was not treated with DXM during ovarian stimulation. The patients were randomized based on their birth month (those with an even birth month were treated with DXM, and those with an uneven birth month were not). Patients with three subsequent serum concentrations of progesterone <2 nmol/l and treated with IVF for the first time during the same period constituted a further control group. Each couple was included into the study only once. The serum samples were taken prior to ovarian stimulation and on the day of ovulation induction with HCG (Profasi; Serono, Zug, Switzerland). These serum samples were stored frozen at –20°C until assay. The clinical data of the patients participating in this study and the results of treatment with DXM are summarized in Table II.

Third study: origin of progesterone during the late follicular phase

Stimulatory tests of the adrenals with ACTH were performed in nine normocyclic volunteers to test their adrenocortical function during a natural menstrual cycle or during intake of 50 μg ethinyl estradiol (Ovidol; Nourypharma). All participants had regular menstrual cycles and did not take any other medication. Their mean age was 28 years (range 22–39).

All ACTH tests were performed during early morning (8.00–10.00 a.m.) and the nine participants were permitted to rest, lying throughout the procedure. Bolus i.v. injections of ACTH (250 μg bolus i.v.) were given on day 3 of an untreated menstrual cycle. Fasting serum samples were taken during morning hours 30 and 0 min prior to, and 30 and 60 min after, bolus administration of ACTH. The second ACTH test was performed during the preovulatory rise of progesterone, which was determined by measuring daily the early morning (e.g. 7.00–9.00 a.m.) serum progesterone concentration starting on day 12 of the untreated menstrual cycle. An additional ACTH test was performed on day 3 of the next menstrual cycle, after which the participants started taking 50 μg ethinyl estradiol for 7 days followed by 50 μg ethinyl estradiol and 0.125 mg desogestrel for an additional 15 days (Ovidol; Nourypharma). A fourth ACTH test was performed after 7 days of treatment with 50 μg ethinyl estradiol daily, before taking desogestrel. The serum samples were stored frozen at –20°C until assay.

Hormone concentration measurements

All hormone concentrations were measured with commercially available assay kits. Measuring the progesterone levels reliably during the follicular phase of the menstrual cycle warrants assay systems with high accuracy at the lower end of the standard curve. For the progesterone measurements, two sensitive assay systems were used: SR1 (ImmunoChem, Freiburg, Germany) and Elecsys (Roche Diagnostics, Basel, Switzerland), both with an analytical sensitivity of 0.48 nmol/l and a functional sensitivity of 1.43 nmol/l, i.e. the lowest concentration that can be measured reproducibly with an inter-assay coefficient of variation of 20%. During initial testing, the results of the SR1 enzyme-linked immunosorbent assay (ELISA) kit for the measurement of low progesterone concentrations were compared with a commerically available radioimmunoassay (RIA; Biermann, Bad Nauheim, Germany) and the correlation was found to be highly significant (0.811, $P < 0.00001$). The inter-assay and intra-assay coefficients of variations for the progesterone, estradiol and LH measurements were below 10.1, 5.7 and 9.0% respectively, determined with control solutions with concentrations set at 5 nmol/l, 196 pmol/l and 15 IU/l respectively. The concentration of androstenedione was measured with an RIA from Diagnostic Systems Laboratories I7nc. (DSL, Webster, TX, USA). The concentration of 17β-hydroxyprogesterone was measured with a RIA from Diagnostic Products Corporation (DPC, Los Angeles, CA, USA). For androstenedione and 17α-hydroxyprogesterone, the inter- and intra-assay coefficients of variation were below 7.1 and 8.4% respectively, determined with low concentration solutions at 3.3 and 2.6 nmol/l respectively.

Statistical analysis

Data were analysed with Mann–Whitney *U*-test, Kruskal Wallis or χ^2-test as appropriate using the Statgraphics statistical software package (Manugistics Inc., Rockville, MA, USA). The data were presented by the mean values together with their 95% confidence interval in all instances. The level of statistical significance was set at 5%.

Results

Origin of progesterone during the early follicular phase

The clinical data of the patients participating in this part of the study and the results achieved with IVF or ICSI are

Table II. Effect of co-treatment with DXM (taken orally, 1 mg daily) during ovarian stimulation with gonadotrophins on the serum progesterone concentration during follicular development

Progesterone concentration	<2 nmol/l	>2 nmol/l with DXM	>2 nmol/l without DXM
No. of patients	21	12	8
Duration of infertility (years)	6.0 ± 0.7	6.8 ± 0.9	5.6 ± 0.5
Patient′s age (years)	32.0 ± 0.9	31.7 ± 1.5	32.1 ± 1.7
Husband′s age (years)	35.0 ± 1.1	35.0 ± 2.7	35.9 ± 2.3
Initial progesterone (nmol/l)	1.05 ± 0.10	3.28 ± 0.19[a]	3.72 ± 0.76[a]
Progesterone at HCG administration (nmol/)	4.23 ± 0.29	3.47 ± 0.57	7.38 ± 1.49[b]
Estradiol at HCG administration (nmol/l)	7.34 ± 1.47	7.71 ± 1.10	9.54 ± 2.20
Duration of follicular phase (days)	12.9 ± 0.4	12.6 ± 0.4	12.7 ± 0.5
FSH administered (IU)	4443 ± 348	4437 ± 530	3919 ± 556
Oocytes fertilized/inseminated (%)	66/102 (64.7)	44/55 (80.0)	34/40 (85.0)
Pregnancies achieved (%)	11 (52.4)	7 (58.3)	6 (75.0)

[a]Statistically significant difference (Mann–Whitney *U*-test, $P < 0.001$).
[b]Statistically significant difference as compared with both other values (Mann–Whitney *U*-test, $P < 0.0001$).

summarized in Table I. The observed serum concentrations of progesterone during the initial phase of the follicular phase were lower after pretreatment with ethinyl estradiol and desogestrel than in the cycles without pretreatment, but the differences among both groups did not reach statistical significance. The Kolmogorov–Smirnov statistic, which was used to analyse the distribution of the progesterone concentrations in both groups, was significantly lower in the cycles pretreated with ethinyl estradiol and desogestrel (0.87 versus 1.75, $P < 0.01$), indicating a lower variance of the progesterone concentrations after pretreatment with an oral contraceptive pill, during the final stages of the follicular development. However, no difference in the serum concentrations of progesterone nor in their distributions could be noted among both groups.

No change in the circulating serum concentrations of progesterone was provoked by a dose of GnRH of 100 μg during desensitization with triptoreline acetate (Figure 2A), whereas 250 μg of ACTH induced an immediate and significant rise in the serum levels of progesterone (Figure 2B).

Effect of DXM on the serum concentration of progesterone

The clinical data of the patients involved and their treatment results are summarized in Table II. DXM was administered orally throughout ovarian stimulation (1 mg daily) to examine the effect of suppression of adrenocortical steroidogenesis on the serum levels of progesterone.

DXM effectively lowered the serum progesterone concentration during follicular development in patients with initially elevated progesterone concentrations, but the pregnancy rate among the patients treated with DXM was not different from those with elevated basal serum levels of progesterone left untreated and of patients with low basal serum levels of progesterone. The serum concentration of progesterone on the day of HCG administration—in patients left untreated with DXM despite an elevated initial progesterone serum concentration—rose significantly higher than that of patients treated with DXM ($P < 0.001$).

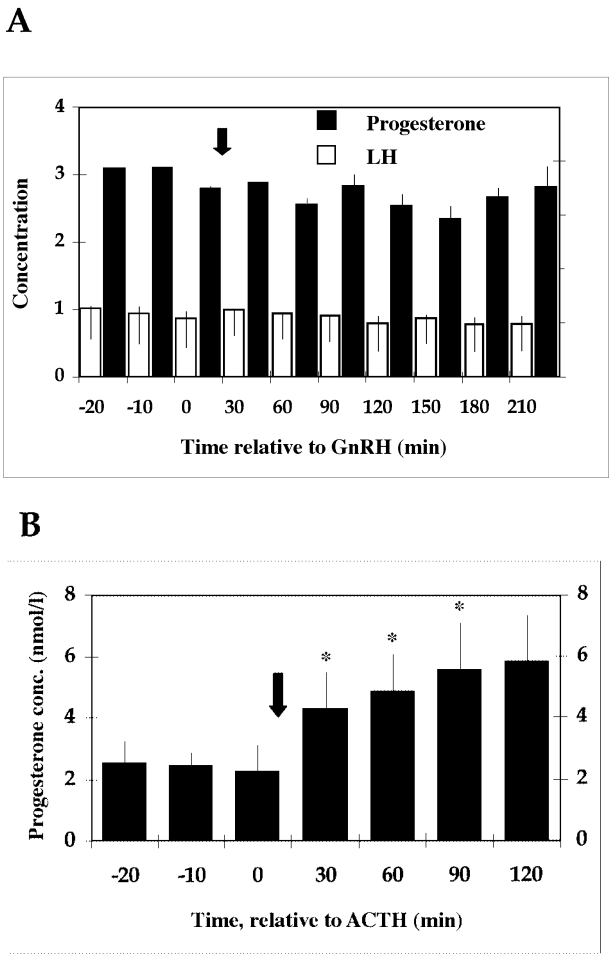
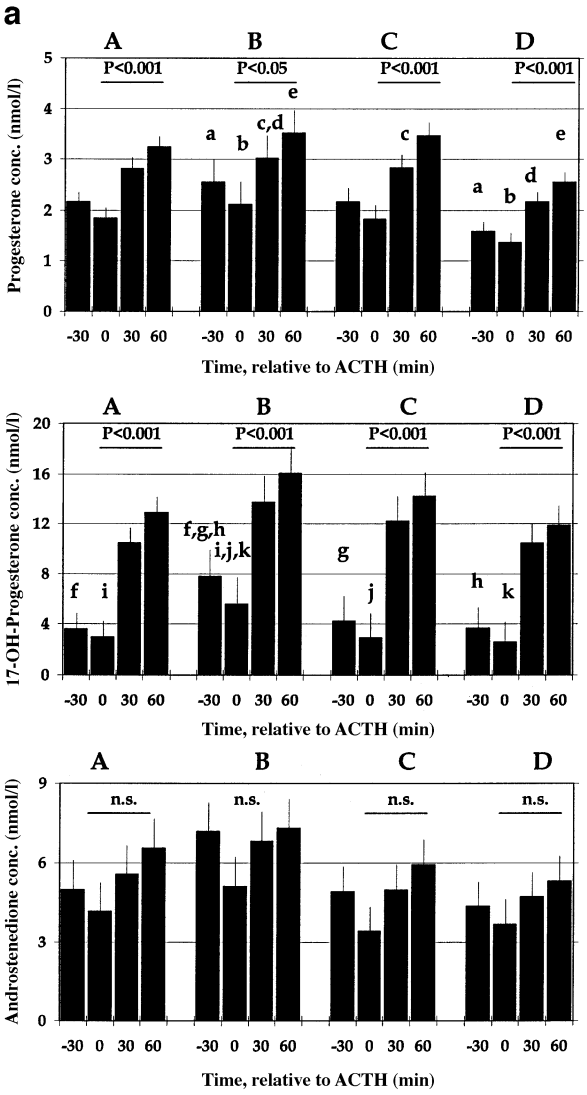


Figure 2. Serum concentrations of progesterone in nmol/l (black columns) and LH in IU/l (empty columns) before and after bolus i.v. injection of 100 μg of GnRH (**A**), or 250 μg of ACTH (**B**) during suppression of endogenous gonadotrophin secretion with a long-acting GnRH agonist. Whereas the concentrations of progesterone and LH were not modified by GnRH, a swift rise of the progesterone concentration was observed after administration of ACTH. *$P < 0.01$ versus 0 min.



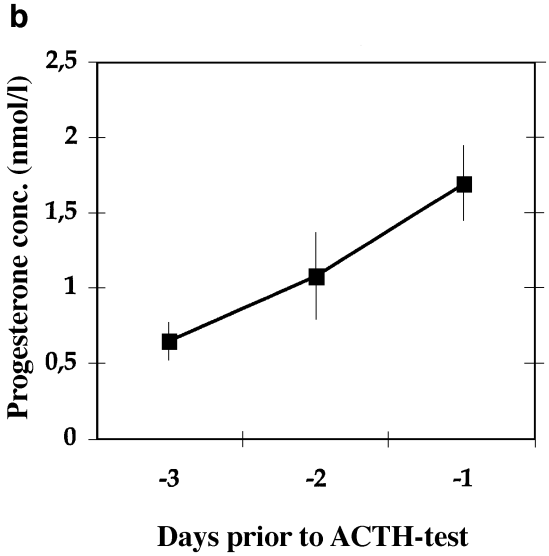


Figure 3. (**a**) Serum concentrations of progesterone (Upper panel), 17α-hydroxyprogesterone (Middle panel) and androstenedione (Lower panel) 30 min before, immediately before, 30 and 60 min after bolus i.v. injection of 250 μg of ACTH. ACTH tests were performed during early follicular phase (A and C), once during the late follicular phase (B) and after 7 days of treatment with 50 μg of ethinyloestradiol (D). Above each ACTH test is indicated whether the endocrine response was statistically significant (Kruskal Wallis) a to e and i, j and k $P < 0.05$ f,g and h $P < 0.01$ (**b**). The ACTH-tests during the late follicular phase (column B in Figure 3a) were performed during the preovulatory rise of progesterone shown here. The latter was determined by daily measurement of early morning progesterone starting on day 12 of an untreated menstrual cycle.

Effect of ACTH on adrenocortical steroid secretion during the natural menstrual cycle and during intake of an oral contraceptive

The higher rise of progesterone levels in patients lacking suppression of adrenocortical steroidogenesis by DXM suggests a significant adrenal contribution during the late follicular phase. Therefore, the origin of preovulatory progesterone was further tested using ACTH to stimulate adrenal steroidogenesis. Nine participants with normal, untreated menstrual cycles were recruited for this study. The results of two participants had to be discarded, because ovulation occurred prior to the second ACTH test. The results of the seven remaining participants were summarized in Figure 3. Whereas the serum concentrations of progesterone and 17α-hydroxyprogesterone rose significantly 30 and 60 min after i.v. administration of ACTH, the changes observed for the androstenedione concentrations before and after administration of ACTH were statistically not significant ($P < 0.05$ and $P < 0.001$ for progesterone, $P < 0.001$ for 17α-hydroxyprogesterone). The basal concentrations of progesterone and 17α-hydroxyprogesterone prior to the administration of ACTH were higher in the late follicular phase as compared with the early follicular phase. However, differences of the basal values of 17α-hydroxyprogesterone and progesterone were significant only in comparison with those after 7 days of treatment with ethinyl estradiol ($P < 0.02$ and < 0.01 respectively). The changes in the concentrations of progesterone and 17α-hydroxyprogesterone provoked by ACTH during the late follicular phase were not different from those during the early follicular phase. The rise of the progesterone concentration induced by ACTH was significantly lower during intake of ethinyl estradiol than in all other tests performed ($P < 0.05$).

Discussion

Despite the overwhelming evidence for the crucial role of progesterone in ovulation, its origin and the regulation of its secretion throughout the follicular phase of the menstrual cycle remains poorly understood. The enzyme 3β-HSD, which is required for the conversion of pregnenolone to progesterone, is strongly expressed throughout the follicular phase in the theca interna as well as in the adrenal cortex but not in the granulosa cells (Bao and Garverick, 1998). In some species,

3β-HSD may be expressed in some granulosa cells of the dominant follicle during the late follicular phase (Richards, 1994). In the guinea pig, 3β-HSD was found to be expressed in a single layer of granulosa cells surrounding the mature oocyte (Dupont *et al.*, 1990), whereas in the granulosa cells of porcine preovulatory follicles no expression of 3β-HSD could be detected (Conley *et al.*, 1994). The ovarian source of late follicular phase progesterone is particularly puzzling in the light of a recent report demonstrating that progesterone biosynthesis by 3β-HSD is inhibited in the presence of high concentrations of estradiol (Gilling-Smith *et al.*, 1997).

The adrenal cortex may also be stipulated as an important source of late follicular phase progesterone (Eldar-Geva *et al.*, 1998). LH receptors have been detected in the human adrenal cortex (Pabon *et al.*, 1996), suggesting an involvement of this organ in the process of ovulation. Conversely, in PCOS patients, elevated levels of progesterone and 17α-hydroxyprogesterone originate in the ovaries rather than in the adrenals (Lachelin *et al.*, 1979; Chetkowski *et al.*, 1984; Azziz *et al.*, 1990). The present communication describes the contribution of the adrenal cortex to circulating progesterone during the follicular phase of women with regular ovulatory menstrual cycles.

The present data clearly establish the adrenals to be the main secretory source of circulating progesterone during the early follicular phase. This is demonstrated by the rapid rise of progesterone after administration of ACTH during suppression of endogenous gonadotrophin secretion with triptoreline acetate. ACTH stimulates the conversion of cholesterol to pregnenolone in the adrenal cortex (Simpson and Waterman, 1988), which is rapidly converted to progesterone, 17α-hydroxyprogesterone and androstenedione by the enzymes 3β-HSD and 17α-hydroxylase/17,20-lyase (cytochrome P450c17) respectively. Although long-term administration of ACTH seems to disrupt ovarian steroidogenesis (Viveiros and Liptrap, 2000), the short-term administration used in this study may not have affected any of the ovarian functions.

During the late follicular phase, the main source of circulating progesterone shifts towards the ovaries, as demonstrated by the lack of suppression by DXM towards the end of follicular development. The activity of the adrenals is not influenced by the cyclic activity of the ovaries, as demonstrated by the similar response of both progesterone and 17α-hydroxyprogesterone secretion induced by ACTH in both the early and late follicular phase of the natural menstrual cycle.

However, an important aspect of the present study revealed a major contribution of the adrenal cortex to circulating progesterone levels during the preovulatory phase. The high progesterone concentration of certain patients rose significantly during the late follicular phase as compared with those patients with consistently low levels of progesterone. There appears to be an individual setpoint, which determines the rate of progesterone secretion both in the theca interna and in the adrenal cortex, in both the early follicular phase and the preovulatory phase. The ovaries mediate the contribution of the adrenal cortex. This is demonstrated by the suppressive effect of ethinyl estradiol on both the basal and the ACTH-stimulated concentrations of progesterone and 17α-hydroxyprogesterone. A similar finding was presented previously by Lobo *et al.* who found a significantly reduced output of adrenal androgens after ACTH stimulation in ovarectomized women as compared with regularly ovulating women (Lobo *et al.*, 1982). Although a direct stimulatory effect of conjugated estrogens on adrenal steroidogenesis was demonstrated in that study, our data suggest rather a suppressive effect of ethinyl estradiol on the adrenal reactivity to ACTH.

Orally administered ethinyl estradiol induces a rise of transcortin, which not only binds cortisol but progesterone as well (Rosner, 1991). The effects of ethinyl estradiol in this study could be explained by the reduced availability of progesterone due to increased binding to transcortin. However, both assay systems measured total serum progesterone: bound and unbound. Therefore, our experimental results suggest a reduced adrenocortical secretion of progesterone in the presence of suppressed ovarian function.

The presence of an endocrine crosstalk between the ovaries and the adrenal cortex in the hormonal regulation of the menstrual cycle has been suggested both experimentally and clinically in the past. Transgenic mice deficient of the inhibin α-subunit all develop gonadal tumours (Matzuk *et al.*, 1994; Kananen *et al.*, 1995). In these animals, adrenocortical tumours will develop after gonadectomy and the shift of tumorigenesis from the gonads to the adrenal cortex is mediated by LH (Rilianawati *et al.*, 1998). Other examples of adrenocortical–ovarian crosstalk is found in PCOS, as ovarian wedge resection results in reduced reactivity of the progesterone and 17α-hydroxyprogesterone concentrations after ACTH administration (Wu *et al.*, 2000), and in ovarectomized women, in whom adrenal androgenesis is suppressed as compared with regularly ovulating controls (Lobo *et al.*,1982).

The present findings clearly demonstrate that the adrenals constitute the main source of circulating progesterone during early follicular development, whereas the ovaries provide most of the circulating progesterone during the late follicular phase. Our data also demonstrate that adrenal steroidogenesis is influenced by ovarian action. The adrenocortical–ovarian crosstalk may be similar to that between the granulosa and the theca interna, in which thecal progesterone synthesis is stimulated by the granulosa (Makris and Ryan, 1977; Kotsuji *et al.*, 1990; Yada *et al.*, 1999). The molecular nature of the endocrine and paracrine mediators between the granulosa, the theca interna and the adrenal cortex remains to be determined.

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