Ovarian Steroidogenesis and Serum Androgen Levels in Patients with Premature Ovarian Failure

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Abbreviations:

A
    Δ^4 -Androstenedione
DHEA-S
    dehydroepiandrosterone sulfate
E2
    estradiol
LHR
    LH receptor
POF
    premature ovarian failure
P450scc
    P450 side-chain cleavage
T
    testosterone

Women with premature ovarian failure (POF) have been reported to have lower serum androgen levels compared with normal women. We reviewed the androgen profiles of 143 POF patients and found androgen levels above normal for post-menopausal women in 16% of these subjects. To determine the source of androgens in those women, we studied the available ovarian biopsy samples of 15 POF patients with increased androgen levels using immunohistochemistry, with a panel of antibodies directed against the main steroidogenic enzymes. Five of the ovarian biopsies exhibited abnormal follicles characterized by hypertrophied theca interna expressing steroidogenic enzymes involved in androgen synthesis. In five other biopsies, the steroidogenic activity was scarce and confined to a small number of ovarian stromal cells, sometimes situated in the proximity of follicular remnants. Finally, in five patients, we found no histological evidence of present or past follicular development beyond the quiescent follicular stage, and no steroidogenic cells were detected by immunohistochemistry. Our findings suggest that ovarian theca-derived cells are a source of androgens in some women with POF, whereas in others, as in most postmenopausal patients, the adrenals or the ovarian hilus cells may synthesize a significant quantity of androgens under LH stimulation. (*J Clin Endocrinol Metab* 90: 2391–2396, 2005)

NATURAL MENOPAUSE IS marked by a decrease in ovarian steroidogenesis, especially of estradiol, but also of androgen levels. A similar pattern of steroid production is to be expected in patients with premature ovarian failure (POF). All available studies on androgen production in these patients show a decrease in androgen synthesis, particularly of testosterone and androstenedione, but not of dehydroepiandrosterone sulfate (DHEA-S), compared with age-matched fertile controls [1] [2] [3] [4] . A sporadic production of ovarian steroids at almost normal levels secondary to the occasional maturation of follicles up to the antral stage has been described in some POF patients [5] . Follicular development in these women is abnormal due to the elevated

LH levels, which cause inappropriate **premature** luteinization of both theca interna and granulosa cells [5] . However, **ovarian** steroidogenesis in POF patients has never been investigated *in situ*. Intraovarian androgen production has been more extensively studied in postmenopausal women, the physiological counterpart of POF patients. When using **ovarian** venous catheterization, an **ovarian** contribution to the androgen pool in postmenopausal subjects has been suggested [6] . A large study comparing testosterone levels before and after menopause in intact and ovariectomized women detects a higher level of androgens in the group of postmenopausal women with intact ovaries [7] . However, some *in situ* studies of the steroidogenic properties of the postmenopausal ovary have failed to show a contribution of interstitial cells to the circulating androgen pool [8] [9] . Indeed, one of those studies demonstrated a prevalent adrenal androgen production after menopause [9] .

We have undertaken a retrospective analysis of androgen levels in women presenting with POF and a normal karyotype. Interestingly, despite **ovarian failure**, in some of our patients we found androgen levels above those considered normal for postmenopausal women. To identify the source of androgens in these women, we decided to study the available **ovarian** biopsy samples of POF patients with elevated androgen levels using immunohistochemistry with a panel of antibodies directed against the main steroidogenic enzymes. As a control group we used **ovarian** samples obtained from POF patients without elevation of androgen levels.

Patients and Methods

Patients

The androgen profiles of 143 consecutive POF patients without history of chemotherapy and/or pelvic radiotherapy and with normal karyotype, referred to our center between 1995 and 2004, were reviewed. In these patients, spontaneous POF was diagnosed before age 40 yr and was defined by at least 4 months of amenorrhea and two FSH levels above 40 mIU/ml, at least 1 month apart. Among them were three patients who had an FSH receptor gene mutation. They have been previously described (patients 2, 3, and 13) [10] [11] [12] . Thirteen patients had thyroid autoimmune disease. None had **ovarian** autoimmune disease. Among these 143 consecutive patients, 23 patients (16.1%) had androgen levels above those in postmenopausal women, defined by plasma testosterone (T) levels greater than 1.4 n M/liter, Δ^4-androstenedione (A) levels greater than 7 n M/liter, and/or DHEA-S levels

greater than 6800 n M/liter [13] . **Ovarian** biopsies were available from 15 of those patients. POF duration was established starting from the first determination of elevated FSH levels to the time of **ovarian** biopsy. Blood samples were taken the day before the **ovarian** biopsy, except for two patients, who did not have elevated androgen levels. Informed consent was obtained from all patients for serum samples, cytogenetic studies,

and surgical procedures. Fifteen patients matched for age and mean POF duration, with normal androgen levels and available **ovarian** biopsies, served as controls.

Hormone measurements

Plasma levels of FSH and LH were measured by conventional RIA (Immunotech Beckman Coulter, Marseille, France). Estradiol (E2), T, and A were determined after previous plasma extraction [DiaSorin (Milan, Italy), Schering CisBio International (Gif-sur-Yvette, France), and Immunotech Beckman Coulter, respectively]. Plasma levels of DHEA-S were measured by conventional RIA (Immunotech Beckman Coulter). The normal range was based on hormone measurements obtained from healthy women during a normal menstrual cycle and provided by the laboratory of hormonal investigations at Necker Hospital. The intraassay coefficient varied from 2.6–8.1%, whereas the interassay coefficient varied from 3.7–11.9%. The inhibin B concentration was measured in duplicate in serum samples using a solid phase sandwich ELISA. The inhibin B assay used a capture monoclonal antibody raised against a sequence near the C terminal of the human β-subunit, immobilized on a hydrazide plate, as described by Groome *et al.* [14] and a second monoclonal antibody specific for the β-subunit of inhibin coupled to alkaline phosphatase. The assay detection limit was 5 pg/ml.

Histological and immunocytochemical studies of the ovaries

For each patient at least two bilateral **ovarian** biopsies of 3–5 mm were available (mean, 2.50; range, 1–5). After formol fixation and paraffin embedding, the biopsies were serially sectioned at 5 μm, then stained with hematoxylin/eosin and Masson's Trichrome stains. Histological examination was performed with a conventional optical microscope (Provis, Olympus, Japan).

Immunohistochemistry was performed as follows. Briefly, serial tissue sections were deparaffinized by successive baths in xylene and alcohol, and antigen retrieval was performed before immunohistochemistry in a commercial microwave oven at full power in pH 6 citrate buffer for 15 min. The sections were then incubated overnight with the following primary antibodies: polyclonal antibodies anti-P450 side-chain cleavage (P450scc; dilution, 1:3000) [15] , anti-3β-HSD (dilution, 1:4000) [16] , anti-P450c17α (dilution, 1:5000) [17] , and antiaromatase (Hauptman-Woodward Medical Research Institute, Inc., Buffalo, NY; dilution, 1:3000) at 4 C in a humid chamber. After primary antibody incubation, endogenous peroxidases were quenched with 3% H_2O_2 in PBS (pH 7.4) for 5 min, and the bound antibodies were revealed with a secondary biotinylated antibody and peroxidase-labeled streptavidin (LSAB2 immunostaining kit, DakoCytomation, Carpinteria, CA) according to the manufacturer's instructions. Aminoethylcarbazol (Sigma-Aldrich Corp., Lyon, France) was used as a chromogen. The sections were counterstained with Mayer's hematoxylin. Sections of a biopsy of a normal ovary from a 26-yr-old woman were used as controls. Each experiment was performed at least twice; immunostaining for P450c17α was performed two additional times on nonconsecutive sections.

Statistical analysis

Analyses were processed with StatView version 5 (Abacus Concepts, Inc., Berkeley, CA). Descriptive statistics were performed for each variable; quantitative results are

presented as the median (range), and qualitative results are presented as a distribution of a number of patients. Hormonal and **ovarian** parameters were compared using the Mann-Whitney test. Proportions for the two groups were compared using the χ^2 test. $P < 0.05$ was accepted as significant.

Results

Clinical and hormonal evaluation

Among the 30 patients, most presented with secondary amenorrhea (n = 24; 80%). Three of the patients with androgen levels above the norm for postmenopausal women also had clinical hyperandrogenism. Of these three patients, one had acnea, and two had mild hirsutism with Ferriman-Gallwey scores of 12 and 13, respectively. The median age (range) at diagnosis in the POF patients with elevated androgen levels was 22 yr (16–39 yr) and was not significantly different from that in control POF patients. There was no significant difference between the groups in the median POF duration [16 months (range, 3–132 months) in patients with elevated androgen levels and 21 months (range, 3–138 months) in control POF patients]. The median body mass index was 24.3 (range, 21.4–37.5) in control patients and 21.2 (range, 17.3–30.1) in POF patients with elevated androgen levels.

In POF patients with elevated androgen levels, median T, A, and DHEA-S levels were significantly higher than in control POF patients [T: 1.60 n M/liter (range, 0.42–2.54 n M/liter) *vs*. 0.69 n M/liter (range, 0.17–0.94 n M/liter); $P < 0.001$; A: 7.68 n M/liter (range, 3.84–15.00 n M/liter) *vs*. 3.84 n M/liter (range, 1.47–6.63 n M/liter); $P < 0.001$; DHEA-S: 6316 n M/liter (range, 1316–9746 n M/liter) *vs*. 3751 n M/liter (range 1314–6408 n M/liter); $P < 0.05$, respectively; Tables 1 and 2]. No significant difference in median FSH and LH levels was observed between the two groups [67 U/liter (range, 20–129 U/liter) and 31 U/liter (range, 6–51 U/liter), respectively, in the POF patients with elevated androgen levels *vs*. 64 U/liter (range, 34–138 U/liter) and 25 U/liter (range, 10–51 U/liter), respectively, in the control POF patients]. However, median E2 levels were significantly higher in patients with elevated androgen levels compared with the control POF patients [75 p M/liter (range, 18–551 p M/liter) *vs*. 18 p M/liter (range, 18–95 n M/liter); $P < 0.05$; Tables 1 and 2]. Inhibin B levels were also more elevated in POF patients with elevated androgen levels, ranging from 5–150 ng/ml, and were undetectable in all control POF patients (Tables 1 and 2).

Immunohistochemical detection of steroidogenic enzymes

The **ovarian** biopsy of all patients was studied by immunohistochemistry for expression of the steroidogenic enzymes P450scc, 3β-HSD, P450c17α, and P450 aromatase. Three different patterns of immunostaining could be identified in the POF patients with elevated androgen levels (Tables 1 and 2).

The first pattern was characterized by an intense expression of steroidogenic enzymes in a relevant number of cells. This pattern was found in five biopsies (patients 1–5); however, there were some differences in both the arrangement of steroidogenic cells and the types of expressed enzymes. Large bands of luteinized thecal cells arranged

around the irregular antrum of atretic follicles were evident in four biopsies. The cells expressed the enzymes P450scc, P450c17α, and 3β-HSD with similar intensities (Fig. 1A , P450scc; Fig. 1B , 3β-HSD; Fig. 1C , P450c17α). In only one of the four cases were a few residual granulosa cells expressing aromatase observed in the antrum of an atretic follicle (Fig. 1D , P450 aromatase). A recent corpus luteum was found in the fifth case. It was characterized by the expression of P450c17α in peripheral theca-derived lutein cells, P450 aromatase immunopositivity of the more central granulosa-derived lutein

2393

	T (nM/liter)	A (nM/liter)	DHEA-S (nM/liter)	E2 (pM/liter)	Inhibin B (ng/ml)	Follicular histology	Interstitial & thecal steroidogenic cells	P450scc	P450C17	3βHSD	Aromatase
1	1.04	7.68	2497	264	15	Primary follicles, CL	+	+	+	+	+
2[a]	1.70	10.82	1316	62	NA	Antral atretic follicles	+	+	+	+	−
3[a]	2.08	4.19	2829	77	73	Antral atretic follicles	+	+	+	+	−
4	2.01	10.47	7507	88	<10	Antral atretic follicles	+	+	+	+	−
5	0.42	7.33	3759	<20	<10	Antral atretic follicles	+	+	+	+	−
6	1.60	5.58	3590	217	105	Antral atretic follicles, CA	+/−	+/−	+/−	+/−	−
7	1.70	7.68	5217	77	18	Antral atretic follicles	+/−	+/−	+/−	−	−
8	1.60	10.12	8030	<20	<10	Primary follicles	+/−	+/−	+/−	−	−
9	1.42	8.03	NA	NA	NA	Absent follicles	+/−	+/−	+/−	−	−

TABLE 1 -- POF patients with elevated androgen levels

	T (nм/liter)	A (nм/liter)	DHEA-S (nм/liter)	E2 (pм/liter)	Inhibin B (ng/ml)	Follicular histology	Interstitial & thecal steroidogenic cells	P450scc	P450C17	3βHSD	Aromatase
							TABLE 1 -- POF patients with elevated androgen levels				
10	1.94	7.68	5130	117	14	Absent follicles	+/−	+/−	+/−	−	−
11	2.53	15.01	7415	551	<10	Antral atretic follicles	−	−	−	−	−
12	0.66	3.84	8089	<20	<10	Primary follicles	−	−	−	−	−
13 [a]	1.25	9.77	9746	<20	<10	Primary follicles	−	−	−	−	−
14	0.90	7.68	8280	73	13	Absent follicles	−	−	−	−	−
15	0.87	5.93	7695	<20	<10	Absent follicles	−	−	−	−	−

The patients are listed in order of abundance of steroidogenic cells. CA, Corpus albicans; CL, corpus luteum; 3βHSD, 3β-hydroxysteroid dehydrogenase; NA, not available.

[a] Those patients with inactivating FSH receptor mutations have been described previously [10] [11] [12] .

cells, and an ubiquitous expression of P450scc and, very intensely, of 3β-HSD on both theca- and granulosa-derived lutein cells (Fig. 1E , P450scc; Fig. 1F , 3β-HSD; Fig. 1G , P450c17α; Fig. 1H , P450 aromatase).

The second pattern was characterized by a variable, but always small, number of immunopositive interstitial cells, isolated or in small groups, which expressed mainly the enzymes P450scc and P450c17α, whereas 3β-HSD expression was observed with less frequency and in a smaller number of cells, and aromatase was not expressed. This pattern characterized five biopsies (patients 6–10), which also exhibited different histologies. Two were totally devoid of normal or atretic follicular structures and of corpora albicantia (Fig. 2A , P450c17α). One showed an isolated primary follicle of normal appearance, one was characterized by the presence of an antral atretic follicle (Fig. 2B , P450c17α), and one exhibited a corpus albicans (Fig. 2C , P450c17α).

The third pattern was characterized by the absence of expression of all four steroidogenic enzymes and was found in the **ovarian** biopsies of patients 11–15. The individual biopsies, however, had a different histological aspect. Two

TABLE 2 -- POF patients with elevated androgen levels

	T (nм/liter)	A (nм/liter)	DHEA-S (nм/liter)	E2 (pм/liter)	Inhibin B (ng/ml)	Follicular histology	Interstitial & thecal steroidogenic cells	P450scc	P450C17	3βHSD	Aromatase
16	0.45	2.16	1831	<20	<10	Antral atretic follicles	+	+	+	+	+
17	0.80	1.67	NA	37	NA	Antral atretic follicles	+	+	+	+	−
18	0.90	5.58	3797	<20	<10	Antral atretic follicles; CA	+/−	+/−	+/−	+/−	−
19	0.66	4.54	NA	<20	<10	Absent follicles, CA	+/−	+/−	+/−	+/−	−
20	0.17	2.34	6272	<20	<10	Antral atretic follicles	+/−	+/−	+/−	−	−
21	0.35	5.93	4466	<20	<10	Absent follicles	+/−	+/−	+/−	+/−	−
22	0.45	4.89	3705	<20	<10	Absent follicles	+/−	+/−	+/−	+/−	−
23	0.69	2.55	NA	<20	<10	Absent follicles	+/−	+/−	+/−	−	−
24	0.66	3.84	2263	<20	<10	Absent follicles, CA	−	−	−	−	−
25	0.38	1.46	1314	95	<10	Absent follicles; CA	−	−	−	−	−
26	0.83	6.28	6408	55	<10	Primary follicles	−	−	−	−	−
27	0.94	6.63	4254	<20	<10	Absent follicles	−	−	−	−	−
28	0.76	2.76	1787	<20	<10	Absent follicles	−	−	−	−	−
29	0.73	3.49	1627	<20	NA	Absent follicles	−	−	−	−	−
30	0.76	4.89	4409	<20	<10	Absent follicles	−	−	−	−	−

The patients are listed in order of abundance of steroidogenic cells. CA, Corpus albicans; 3βHSD, 3β-

						Interstitial & thecal steroidogenic cells				
T (n м/liter)	A (n м/liter)	DHEA-S (n м/liter)	E2 (p м/liter)	Inhibin B (ng/ml)	Follicular histology		P450scc	P450C17	3βHSD	Aromatase

TABLE 2 -- POF patients with elevated androgen levels

hydroxysteroid dehydrogenase; NA, not available.

patients' ovaries did not show evidence of follicular structures, normal or atretic, or of corpora albicantia (Fig. 2D , P450c17α); two exhibited a variable number of small follicles up to the primary stage (Fig. 2E , P450c17α). One biopsy showed the presence of remnants of an antral atretic follicle (Fig. 2F , P450c17α).

Analogous distribution patterns were also observed in the control POF patients, but with different proportions. The biopsies of two controls POF patients (no. 16 and 17), showed significant steroidogenic activity of the hypertrophic theca interna cells of antral atretic follicles. Aromatase was expressed in a few residual follicular granulosa cells in only one of the two. Six other patients of this group (no. 18–23) exhibited a small number of steroidogenic cells in their biopsies, associated with remnants of antral atretic follicles or corpora albicantia in only half the cases. Finally, seven patients were devoid of steroidogenic cells (patients 24–30). Four of those patients lacked follicular structures or remnants in their ovary specimens, one of them exhibited a primary follicle, and corpora albicantia were found in the two others.

Figure 1. Ovaries with abundant expression of steroidogenic enzymes. A–C, A thick band of hypertrophied thecal cells surrounds an irregular antral cavity (a), those cells express P450scc (A), 3β HSD (B), and P450c17α (C) with similar intensity. D, Expression of aromatase in rare residual granulosa cells (*asterisks*) of an atretic follicle with a reduced antral cavity (a) and hypertrophic theca interna (ti). E–H, Steroidogenic enzymes expression in a very recent corpus luteum. E and F, Ubiquitous expression of P450scc (E) and 3β-HSD (F) in thecaderived (tlc) and granulosa-derived (glc) luteinized cells. G and H, P450c17 is expressed only by external, theca-derived cells (tlc; G), whereas aromatase expression is confined to internal, granulosa-derived cells (glc; H). *Bar*: A–D, 100 μm; E–H, 200 μm.

Discussion

We reviewed the androgen profiles of the 143 consecutive POF patients and found, surprisingly, in 23 (16%) of them androgen levels above the normal range for postmenopausal women. We then studied the **ovarian** biopsies available for a subgroup of these POF patients using four different highly specific antibodies directed against the main steroidogenic enzymes to identify the source of androgen production.

Figure 2. Ovaries with absent or scarce steroidogenic enzyme expression; immunolabeling with

anti-P450c17α antibody. A, Some rare steroidogenic cells are dispersed in the interstitial space with no apparent rapport with any follicular structure. B and C, Presence of groups of steroidogenic cells (*arrows*) in the proximity of an atretic follicle (B) and of a corpus albicans (C). D, Cortical **ovarian** tissue devoid of follicles and without interstitial steroidogenic cells. E, Presence of an abundant population of small (mainly primordial and intermediary) follicles devoid of a steroidogenic theca interna. F, Remnant of an antral atretic follicle without evidence of steroidogenesis. *Bar*: A and C, 100 μm; B, D, E, and F, 200 μm.

We found a heterogeneous pattern of expression ranging from the presence of antral atretic follicles with a hypertrophic, functional, androgen-producing theca interna to the total absence of steroidogenic cells. Between these extremes there were several biopsies exhibiting small numbers of interstitial androgen-producing cells. This heterogeneous expression was detected in both groups of patients and is not correlated with the age at diagnosis (median duration of POF), because the two groups of patients with or without elevated androgen levels were comparable in this respect. In particular, the patients presenting with intense expression of steroidogenic enzymes in a relevant number of cells in their **ovarian** biopsies were not the patients with the minimal duration of POF. However, detectable E2 and inhibin B levels were found in some POF patients with elevated androgen levels, suggesting residual follicular activity in these patients.

Abnormal, but functional, follicular structures, characterized by a highly steroidogenic hypertrophied theca interna and apoptotic or absent granulosa cells, were detected in five POF patients with elevated androgen levels. Our observations

suggest that the ovaries of some patients with POF may be capable of advanced follicular development. The abnormal follicular structures are functionally active and capable of steroidogenesis, but orientated mainly toward androgen production. The hypertrophic and hyperplastic androgen-producing thecal cells observed are secondary to the action of persistently elevated LH levels on developing thecae and have been previously described in the ovaries of POF subjects [5] . The absence of aromatase-positive granulosa cells in those anomalous follicles could be secondary to the elevated thecal androgen synthesis, because androgen excess has been implicated in the induction of follicular atresia [18] . The hypertrophied thecae could well be the source of androgens in POF patients with high androgen levels, especially in the presence of an elevated number of those structures in the **ovarian** cortex. Taken together, our results suggest that patients with POF may develop follicles at an advanced stage of development and that those structures are capable of steroidogenesis. However, the finding of very similar (both qualitatively and quantitatively) androgen-producing, abnormal follicles in the ovaries of POF patients either with or without hyperandrogenism suggests that the androgen production of those abnormal follicles is not always sufficient to elevate serum androgen levels in POF patients.

Scarce **ovarian** steroidogenic cells were found in the biopsies of five POF patients with elevated androgen levels and six control POF patients. These ovaries had an aspect similar to that of some normal (nonpathological) postmenopausal ovaries [9] . The few androgen-synthesizing cells observed in these ovaries are probably the thecal remnants of past episodes of advanced follicular maturation. The finding of groups of interstitial

steroidogenic cells in POF patients, therefore, suggests the existence of reserve follicles capable of being recruited. This limited follicular reserve might already be totally spent, as in truly postmenopausal ovaries [19] , or conversely, some follicles might still persist and be susceptible to additional development. This small group of interstitial androgen-producing cells, however, is insufficient to explain the elevated androgen levels found in some POF patients with this pattern of expression of steroidogenic enzymes. Moreover, an identical immunohistochemical aspect is found in a similar number of control POF patients (and in normal postmenopausal women).

Finally, the **ovarian** biopsies of five POF patients with elevated androgen levels and seven control POF patients had no evidence of steroidogenic activity by immunohistochemistry. Some of these biopsies from patients 14 and 15 and from patients 27–30 did not contain reserve follicles and showed no traces (fibrous tissue scars, glassy membranes, or corpora albicantia) of past antral follicular development, suggesting complete exhaustion of the follicular reserve and, possibly, absence of follicular development beyond the secondary stage.

The source of the elevated androgen levels found in some POF patients might also be extrafollicular. Interestingly, the **ovarian failure** in one of the patients with elevated androgen levels devoid of steroidogenic cells was secondary to a totally inactivating mutation of the FSH receptor gene [10] . This woman, who exhibited only primordial and primary follicles at the **ovarian** biopsy, is incapable of follicular development beyond the primary stage. In this particular case, the existence in the ovaries of thecal or interstitial (theca derived) steroid-producing cells capable of androgen production is very unlikely. Conversely, two patients with elevated androgen levels with only partial inactivation of the FSH receptor, and therefore capable of more advanced follicular development [11] [12] , exhibited hypertrophied thecae.

A possible intraovarian source of steroids independent of thecal cells could be the **ovarian** hilus cells, which are similar to testicular Leydig cells. Hilus cells are capable of androgen production [20] and might be sensible to elevated LH levels, as are their testicular homologs [21] . There is one report of a POF patient with hyperandrogenemia associated with **ovarian** hilus cells hyperplasia [22] . However, an unavoidable limit of the **ovarian** biopsy is the lack of sampling of structures in the deep portions of the cortex and the medullary; therefore, it was not possible to detect an abnormal proliferation of hilus cells in our patients. In contrast, the examination of postmenopausal ovaries, removed whole, allows assessment of the respective contributions to steroidogenesis of hilus and cortical cells [9] .

Another possible source of androgens in POF patients with elevated androgen levels could be the adrenal gland. In fact, seven POF patients had elevated DHEA-S levels, suggesting an adrenal contribution to androgen production, and five of them were devoid of **ovarian** steroid-producing cells. Some researchers have reported the presence of the LH receptor (LHR) gene in the fasciculata and reticularis regions of the human adrenal gland [23] , and we have detected the presence of LHR in the human adrenal cortex (Meduri, G., unpublished observations). Participation of the corticosurrenal gland in the circulating androgen pool in postmenopausal women has previously been described [9] , and the presence of functional LHR has recently been demonstrated in adrenocortical cell lines [24] . Additionally, adrenal expression of LHR has been shown to increase with chronically elevated serum LH levels in female mice [25] , and

gonadotropins are chronically elevated in POF patients. Persistently elevated LH levels, however, were common to all patients: the existence of variable individual sensitivity to LH stimulation of hilus or adrenocortical androgen-producing cells, secondary to different levels of expression of LHR or of factors synergic to LH, might explain the difference.

Our findings on the steroidogenic activity of the ovaries in POF patients are limited by the possibility of incomplete sampling. The diagnostic efficacy of the **ovarian** biopsy, especially in assessing **ovarian** reserve, has been discussed and is quite controversial due to the uneven distribution of small follicles in the **ovarian** cortex [26] [27] [28] . However, some researchers have reported the usefulness of **ovarian** biopsies in the diagnosis of POF [26] [29] [30] . Moreover, multiple and bilateral **ovarian** samples more than 3 mm in diameter were available for most of our patients (78%).

In conclusion, we investigated *in situ*, for the first time, the steroidogenic production by ovaries of patients with POF. We found that although the steroidogenic aspect of the ovaries of some POF patients is not dissimilar to that of the ovaries of normal postmenopausal women, a substantial portion of POF patients is still capable of follicular steroid hormone production. The analysis of the particular enzymes

2396

involved indicates that theca interna- and interstitial theca-derived cells are the main source of steroids in these patients and that they synthesize mainly androgens. Our results also suggest that the androgen levels of a portion of POF patients might not always be correlated to the presence of androgen-producing cells in the **ovarian** biopsy or to age and **ovarian failure** duration. The possibility of a contribution of the **ovarian** hilus cells or of the adrenal gland to the elevated androgen levels of some POF patients during LH stimulation was discussed and requires additional investigation.

Acknowledgments

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