PHYSIOLOGY

The Use of a Combined Regimen of GnRH Agonist Plus a Low-Dose Oral Contraceptive Improves the Spontaneous Pulsatile LH Secretory Characteristics in Patients with Polycycstic Ovary Disease After Discontinuation of Treatment

ALESSANDRO D. GENAZZANI,[1,4] CESARE BATTAGLIA,[1] OMBRETTA GAMBA, FELICE PETRAGLIA,[2] BARBARA MALAVASI,[1] and ANDREA R. GENAZZANI[3]

Submitted: November 5, 1999
Accepted: January 29, 2000

Purpose: *The fertility rate in women with polycystic ovary disease (PCOD) is influenced by the type of treatment received. The present study evaluated the possible correlation between treatment and pulsatile release of gonadotropins.*
Methods: *Spontaneous episodic secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and hormonal parameters were monitored before and after 1, 3, and 6 months after treatments suspension. Twenty-four PCOD patients were randomnly divided into two groups of 12 subjects. Group A was treated with gonadotropin-releasing hormone (GnRH)-analogue plus oral contraceptive (OC). Group B was treated only with OC. Both groups were treated for 6 months and followed up for 6 months.*
Results: *In all subjects the therapeutic regimens reduced the androgenic milieau and the gonadotropin plasma levels. Spontaneous pulsatile secretion of LH and FSH was significantly modified in both groups, but patients who received the combined regimen showed a significantly greater reduction of LH plasma levels and a significantly greater decrease of LH pulse amplitude throughout the 6 months after treatment suspension. Ferriman–Gallway score and ovarian volumes were significantly reduced in patients who received the combined treatment than in the OC-treated patients.*
Conclusions: *These data support the evidence of a higher efficacy of the combination of GnRH-a + OC than OC alone in restoring a normal and adequate spontaneous episodic gonadotropin discharge and in decreasing Ferriman–Gallway score and ovarian volumes in patients with PCOD.*

KEY WORDS: GnRH-agonist; polycystic ovary disease; hyperandrogenism; anovulation; episodic gonadotropin release.

INTRODUCTION

Polycystic ovary disease (PCOD) typically is characterized by a variety of signs and symptoms such as oligo- or amenorrhea, overweight/obesity, hirsutism, acne and biochemical disturbances such as high plasma levels of luteinizing hormone (LH), androgens, and insulin (1). As a consequence of these, anovulation and reduced fertility have always been the most frequent complaint of PCOD patients. In fact, PCOD patients often show anovulatory cycles and higher LH secretion than normal (2), probably in relation to an abnormal spontaneous pulsatile release of LH. The evidence of such inappropriate gonadotropin secretion has been proposed to be dependent on the increased LH pulse amplitude (3–7), in part related to a higher pituitary

[1] Department of Obstetrics and Gynecology, University of Modena, Italy.
[2] Department of Obstetrics and Gynecology, University of Pisa, Italy.
[3] Department of Obstetrics and Gynecology, University of Udine, Italy.
[4] To whom correspondence should be addressed at Department of Obstetrics and Gynecology, University of Modena, Via del pozzo 71, 41100 Modena, Italy.

1058-0468/00/0500-0269$18.00/0

sensitivity to GnRH stimulation (4,8,9) and to an abnormal neuromodulation of hypothalamic gonadotropin-releasing hormone (GnRH) secreting neurons (8,10). As a consequence of these, the altered gonadotropin release induces a reduced follicle development and an abnormal ovarian steroidogenesis (2–10). Several reports have demonstrated that clinical symptoms of PCOD are dramatically reduced by the long-term administration of oral contraceptives (OC) alone or in association with a variety of drugs that act on metabolism, i.e., metformin (11) or troglitazone (12), or that are antiandrogenic, i.e., flutamide (13) or finasteride (14,15), but no dramatic changes in gonadotropins secretion have been demonstrated (13). Conversely, recently several reports described the significant efficacy on gonadotropin secretion of the use of GnRH agonist (GnRH-a) alone or in combination with OCs (16–18); since this last combined regimen was clinically effective in the restoration of a normal hypothalamus–pituitary control of ovarian function (18), a specific effect on gonadotropin spontaneous pulsatile release from pituitary has been supposed. On these bases, we aimed to evaluate the secretory characteristics, i.e., the spontaneous pulsatile release, of both gonadotropins in patients suffering for PCOD undergoing a combined treatment of GnRH-a plus an OC in comparison to those undergoing a treatment with an OC alone.

MATERIALS AND METHODS

Subjects

A group of 24 PCOD patients [mean age, 24.5 ± 2.5 years (mean ± SEM)] were enrolled after informed consent. They were referred to our department for menstrual irregularities and/or hirsutism and acne, and were selected on the basis of the following criteria: (a) presence of micropolycystic ovaries at ultrasound (US); (b) oligo- or amenorrhea for at least 6 months prior the study and/or chronic anovulation; (c) plasma androstenedione levels above the normal range for our laboratory (3.0 ng/100ml; conversion factor to pmole/liter: 34.92) and a LH/follicle-stimulating hormone (FSH) > 2.5, (d) hirsutism and or acne (from grade mild to severe); (e) absence of any adrenal and/or other endocrine disease; and (f) no hormonal treatment in the 6 months prior the study. All patients were in a range of +7% to +15% of their ideal body weight and the mean body mass index (BMI) was 23.5 ± 1.2. They were randomnly subdivided in two groups of 12 subjects: (a) group A: patients were treated with leuprolide acetate intramuscularly (im) (Enantone, 11.25 mg every 12 weeks; Takeda, Rome, Italy) plus an OC (Mercilon; Organon, Rome, Italy); and (b) group B: patients were treated with only the OC. Both groups were treated for 6 months and then monitored for the following 6 months. The choice of this OC was made in order to follow previous study protocol (18).

All patients underwent a 6-hr pulsatility study on day 7 of the menstrual cycle if eu- or oligomenorrhoic before any treatment, and on day 7 of the first, third, and sixth cycle after the suspension of the therapy, sampling every 10 min, for LH and FSH determinations. On the same days of the pulsatilities plasma estradiol (E_2), androstenedione (A), 17-hydroxyprogesterone (17-OHP), and testosterone (T) levels were determined. Vaginal US and the Ferriman–Gallway score were performed before, at the third and sixth month of therapy, at the third and sixth month after treatment suspension. Before and after 6 months of treatment lumbar bone density analysis was performed using a dual-energy photon absorptiometry with a Lunar DPX (Lunar Radiation, Madison, WI).

The study protocol was approved by the Human Investigation Committee of the University of Pisa.

Assay

All samples from each subject were assayed in duplicate in the same assay. Plasma LH and FSH concentrations were determined using an immunofluorometric assay (IFMA) previously described (19,20). All samples from the same subjects were analyzed in duplicate in tha same assay. The sensitivity of the assay expressed as the minimal detectable dose was 0.1 IU/ml. The cross-reactivities with free α- and β-subunits of LH, FSH, and thyroid-stimulating hormone (TSH) were less than 2% (19). Intra-assay and interassay coefficients of variation were 4.9 and 7.4%, respectively.

Plasma E_2, 17-OHP, A, and T were determined by radioimmunoassay (Radim, Pomezia, Rome, Italy) as previously described (21). Based on two quality control samples the average within- and between-assay coefficients of variation were 4.0% and 9.7%

Pulse Detection and Statistical Analysis

All LH and FSH time series were first evaluated separately to estimate the random measurement error on the duplicates using the program PREDETEC.WK1 (22,23). Then LH and FSH secretory episodes were

identified using the program DETECT (23), with a *P* value equal to 0.01 (1%) for the nominal false-positive rate. At the nominal *P* level of 0.01 (1%) for false-positive errors, the false-positive rate observed on the data of the plasma pool of each subject assayed together with the time series was not statistically different from 1%.

The presence of significant difference between groups was tested, after analysis of variance (one-way ANOVA), using Student's *t*-test for paired and unpaired data, as appropriate. Linear regression analysis was used to test correlation between individual hormonal values.

RESULTS

All results are showed as mean ± SEM. Hormonal parameters of both groups of PCOD patients resulted similar in baseline conditions and have been reported in Table I, which shows plasma LH and FSH levels as means of the first three samples of the pulsatility. Mean BMI was perfectly similar in both groups (group A: 23.3 ± 1.0; group B: 23.6 ± 1.3). Before any treatment all subjects showed similar high LH and androgen mean plasma levels (Table I) and gonadotropin pulsatile characteristics were perfectly superimposable (Table II). Also, integrated LH and FSH means of the pulsatility profiles were similar (Table II). When treatment was supended, both groups showed the significant reduction of both androgens and LH plasma levels. Indeed, on the first and third month after treatment suspension, patients of group A showed significantly lower LH plasma levels, LH pulse frequency, and LH and FSH pulse amplitudes than in baseline conditions and lower than patients of group B (Table II). Six months after treatment suspension, patients of group B showed all pulsatile parameters perfectly similar to baseline conditions, while patients of group A maintained an LH pulse frequency and amplitude significantly lower than in baseline conditions and lower than group B (Fig. 1). Interestingly, during the 6 months of follow-up, FSH episodic discharge showed a significant and constant reduction of pulse amplitude only in group A, while no changes were observed for patients of group B (Table II).

Menstrual cyclicity was 28 ± 0.8 days for group A and 34 ± 2.5 days ($P < 0.05$) for group B (these values were means over the 6 months of follow-up). Interestingly, it was more stable in group A than in group B for the entire follow-up interval (Table III), as demonstration of a more proper activity of the reproductive axis. In addition, among the patients enrolled for the combined treatment regimen, three of ten remained pregnant (33.3%) in the first month after the follow-up, while no pregnancy was monitored in the other group.

Ultrasound evaluation showed the significant reduction of the ovarian volume in both groups after 6 months of treatment. Results were similar for the Ferriman–Gallway score, which was reduced in both groups after 6 months of treatment. Interestingly, both ovarain volume and the score increased again more rapidly during the follow-up in patients of group B than group A (Fig. 2).

No changes were observed in either group in terms of lumbar bone density after treatment suspension.

DISCUSSION

The present study reported the restoration of the spontaneous episodic secretion of LH and FSH during

Table I. Hormonal Characteristics of the Patients Under Study[a]

	Baseline		After treatment					
			1st month		3rd month		6th month	
	A	B	A	B	A	B	A	B
LH (mIU/ml)	9.8 ± 1.3	10.0 ± 1.6	1.6 ± 0.4**	5.0 ± 0.7**	5.3 ± 1.3**	7.1 ± 1.1*	7.2 ± 1.1**	10.2 ± 1.2
FSH (mIU/ml)	4.2 ± 0.4	4.5 ± 0.4	3.9 ± 0.3	4.4 ± 0.6	4.6 ± 0.3	5.3 ± 1.1	4.3 ± 0.3	4.4 ± 0.4
E_2 (pg/ml)	48.1 ± 9.2	46.2 ± 7.7	22.8 ± 4.8	23.5 ± 3.2	39.8 ± 11.0	36.0 ± 9.4	50.0 ± 9.1	51.2 ± 11.3
17-OHP (ng/ml)	1.2 ± 0.1	1.3 ± 0.3	0.9 ± 0.1**	0.9 ± 0.1**	1.0 ± 0.1**	1.0 ± 0.1**	1.2 ± 0.1	1.5 ± 0.3
Δ4 A (ng/100ml)	4.1 ± 0.3	3.9.2 ± 0.3	2.7 ± 0.2**	2.6 ± 0.2**	3.1 ± 0.3*	3.3 ± 0.2*	3.5 ± 0.2*	3.7 ± 0.2
T (ng/100ml)	72.5 ± 6.3	68.2 ± 5.5	36.1 ± 7.0	41.5 ± 5.2**	45 ± 7.6*	47.5 ± 7.4*	51.1 ± 6.1	55.2 ± 4.2

[a] Mean ± SEM; Group A: patients treated with GnRH-a + OC; Group B: patients treated with OC.
Conversion factors to SI units: LH and FSH IU/liter: 1.00; E_2 pmole/liter: 3.671;17-OHP nmole/liter: 0.0303; A pmole/liter: 34.9; T nmole/liter: 0.0347. *$P < 0.05$ vs. baseline; ** $P < 0.01$ vs. baseline.

Table II. LH and FSH Pulsatile Characteristics[a]

	Baseline			1st Month after suspension			3rd Month after suspension			6th Month after suspension		
	mIU/ml	Pulses No./6 hr	Amplitude mIU/ml	mIU/ml	Pulses No./6 hr	Amplitude mIU/ml	mIU/ml	Pulses No./6 hr	Amplitude mIU/ml	mIU/ml	Pulses No./6 hr	Amplitude mIU/ml
LH												
Group A												
(GnRH-a + OC)	8.6 ± 1.7	6.6 ± 0.4	3.4 ± 0.7	2.1 ± 0.7[b,e]	4.4 ± 0.5[e]	0.8 ± 0.3[c,e]	4.5 ± 0.7[b,e]	5.2 ± 0.6[d]	1.6 ± 0.3[b,e]	7.0 ± 1.4	5.3 ± 0.4[d]	2.4 ± 0.5[b]
Group B (OC)	8.4 ± 1.7	6.1 ± 0.5	3.2 ± 0.7	4.0 ± 0.9[e]	4.5 ± 0.9	2.5 ± 0.5	6.6 ± 1.0	4.1 ± 0.8[d]	2.6 ± 0.3	6.6 ± 1.5	5.8 ± 0.7	4.4 ± 1.5
FSH												
Group A												
(GnRH-a + OC)	4.2 ± 0.4	3.9 ± 0.5	1.0 ± 0.1	3.7 ± 0.5	3.0 ± 0.4	0.6 ± 0.1[b]	3.7 ± 0.2[d]	2.6 ± 0.3[d]	0.6 ± 0.1[d]	4.1 ± 0.5	3.5 ± 0.5	0.6 ± 0.1[d]
Group B (OC)	4.8 ± 0.5	3.8 ± 0.7	1.1 ± 0.3	4.5 ± 1.1	3.2 ± 0.7	1.1 ± 0.2	4.2 ± 0.4	3.6 ± 0.4	0.9 ± 0.3	4.0 ± 0.5	3.9 ± 0.4	0.9 ± 0.1

[a] Mean ± SEM.
[b] $P < 0.05$ vs. group B.
[c] $P < 0.01$ vs. group B.
[d] $P < 0.05$ vs. baseline.
[e] $P < 0.01$ vs. baseline.

a 6-month follow-up in patients with PCOD treated with a combined regimen of GnRH-a plus OC in comparison to OC alone. Our study supports the specific importance of the recovery of the spontaneous gonadotropin discharge to permit an optimal ovarian function in terms of ovulation.

PCOD has been demonstrated to have an inappropriate gonadotropin secretion characterized by altered LH pulse frequency, amplitude, and LH plasma levels (3–7), and several therapeutic strategies have been suggested to treat the physiopathological situation and eliminate the anovulatory condition that is typical in PCOD patients. A variety of drugs have been used to eliminate the hyperandrogenic condition and restore ovulation (11–15), but only recently a greater consideration has been given to the use of GnRH-a. In fact, several reports documented the efficacy of this combined regimen to induce a significant improvement of gonadotropin secretion, ovarian volume and function, and ovulatory cycles (18) as well as to reduce hirsutism (16,24–27). Such a positive therapeutic effect is mainly related to the pseudomenopausal condition induced by the blockade of the ovarian function and its androgen production (16). To avoid the menopausal disturbances and the possible bone resorption an estroprogestin preparation has been associated with GnRH-a in this study as well as in others (16–18,28).

Our study for the first time documented the positive effect of the administration of GnRH-agonist, in its 3-monthly preparation, on the LH pulsatile secretion from pituitary in terms of episodic secretion and indirectly on the hypothalamic function. In fact, even if other authors described the clinical efficacy of the combined regimen we used, none described the pulsatile characteristics of LH secretion immediately after treatment suspension and for a 6-month follow-up. Indeed, our data show that other than the specific positive effect of the clinical symptoms that were observed during the 6 months of treatment, the specific efficacy of the combined regimen of treatment was demonstrated by the fact that during posttreatment observation interval the LH pulsatile release that resulted was similar to normal subjects (29) and significantly different from starting conditions as well as OC-treated subjects. Patients treated with the combined regimen (group A) showed the reduction of both LH pulse frequency and amplitude, and such changes in LH spontaneous release remained significantly lower for the entire period of posttreatment observation. Conversely, patients treated with OC alone showed only a slight reduction of LH pulse frequency within the third month of follow-up, and after 6 months all parameters were already similar to baseline conditions.

These data give support to what previously was reported (18) and showed that the combined regimen of the 3-monthly preparation of GnRH-a plus OC gave a stronger blockade on the hypothalamus–pituitary unit than the OC alone. This combined treatment permits a better recovery of this functional unit to a normal activity, inducing the reduction of all LH pulsatile parameters, with special regard to pulse amplitude. Indeed, this regimen also resolved the problem of the ovarian androgen hyperproduction, since through the gonadotropin blockade it indirectly acted on the local factors participating in the genesis of the cystic ovarian disease. Our data showed that after treatment suspension the reproductive axis, namely the hypophysis, secreted lower amounts of LH with reduced pulse fre-

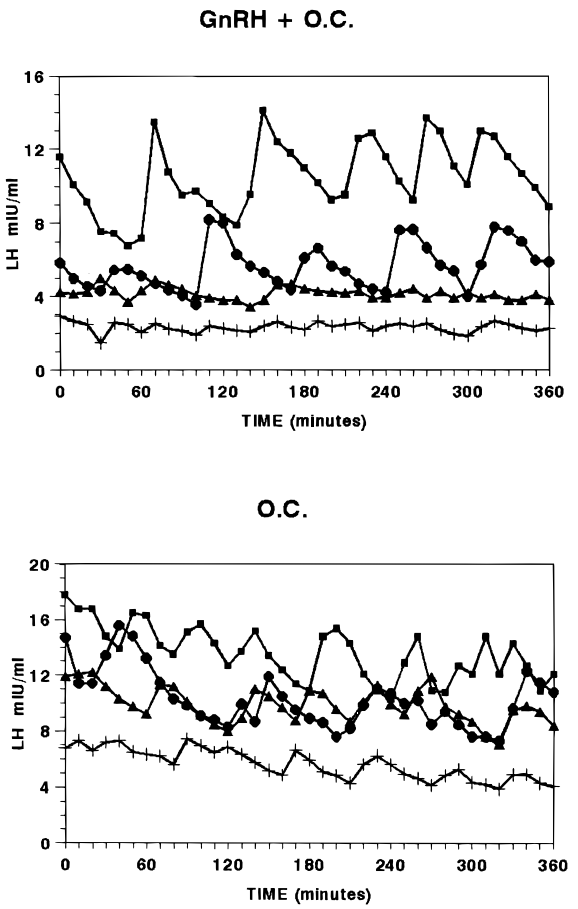

Fig. 1. Spontaneous episodic secretion of LH and FSH for two subjects undergoing GnRH-a + OC (upper panel), and to OC alone (lower panel) on day 7 of the menstrual cycle. Patient treated with the combined regimen showed a more distinct and stable recovery of the normal episodic discharge of LH for the entire period of follow-up, with a significant reduction of LH pulse amplitude and frequency. ■ baseline; +. 1st cycle, ▲ 3rd cycle, ● 6th cycle after treatment suspension.

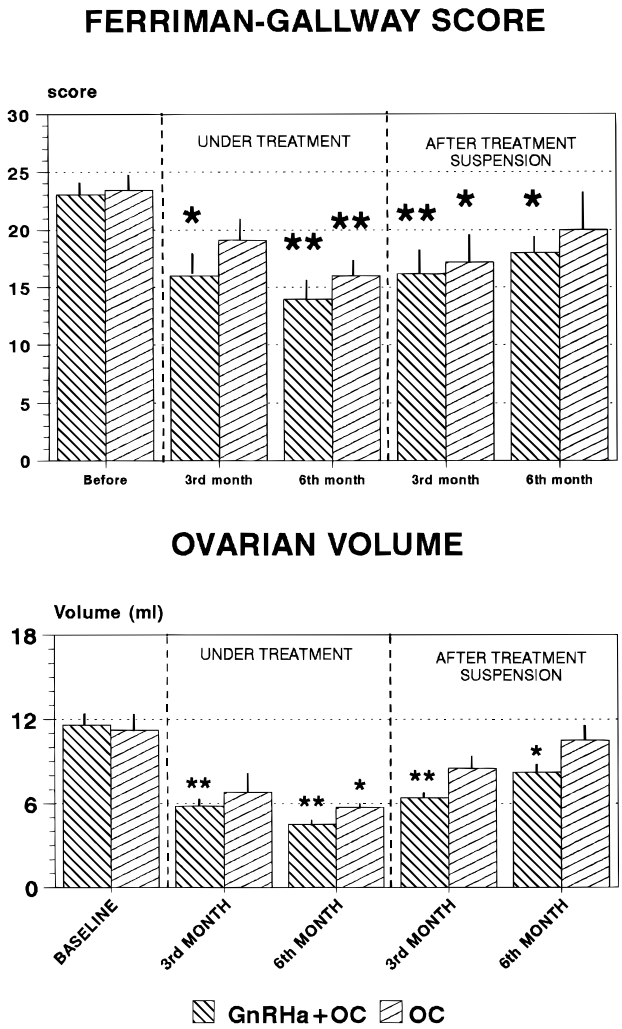

Fig. 2. Mean Ferriman–Gallway scores (upper panel) and mean ovarian volumes (lower panel) for both groups of PCOD patients. Those treated with the combined regimen GnRH-a + OC demonstrated a more stable clinical effect of the treatment during the 6 months of follow-up.

quency and amplitude only in patients treated with GnRH-a plus OC, demonstrating a greater efficacy of this treament in comparison to OC alone. Since patients in group A showed a slower increase of the ovarian volumes as well as of the Ferriman–Gallway score, our data let us infer that the restoration of a normal gonadotropin episodic discharge is essential but not enough to avoid the intrinsic propensity of these patients to go back to their original clinical condition. This supports the hypothesis of the presence of an abnormal (constitutional) peripheral (i.e., ovarian and/

Table III. Mean Intermenstrual Interval Duration (days)[a]

	1st month	2nd month	3rd month	4th month	5th nmonth	6th month
Group A	31 ± 1.5	30 ± 1.2	29 ± 1.0	29 ± 0.9	28 ± 0.9	28 ± 0.8
Group B	29 ± 1.8	30 ± 2.1	32 ± 2.2	32 ± 2.4[b]	33 ± 2.2[b]	34 ± 2.5[b]

[a] Mean ± SEM.
[b] $P < 0.05$ vs. group A.

or metabolic) more than central (i.e., hypothalamic–pituitary) causal factor.

This study confirms previous reports (16–18,24) that demonstrated the restoration of a more regular menstrual cyclicity and ovulation in patients undergoing this combined regimen and supports the fact that GnRH-a blockade of hypothalamus–pituitary function determined a positive recovery of a normal gonadotropin secretion when treatment was suspended. In addition, since the results we observed were in agreement with others (16–18,24), our study shows that the 3-monthly preparation of GnRH-a is effective as is the monthly preparation. Our data suggest that the specific targets of action of therapeutic strategies are all the reproductive axis components. Moreover, our data confirm that OC alone has a relatively limited effect on the overall tonic release of both gonadotropins (30), and hence, on the gonadotropin-induced production of androgens from the thecainterstitial cells of the ovary (16). This last aspect is clearly supported by the fact that OC-treated patients returned to pretreatment conditions at the end of the posttreatment observation interval. The present study also reveals an interesting aspect of FSH secretion. In fact, while LH showed significant changes after both kinds of treatments, FSH showed only the modifications of pulse amplitude in GnRH-a-treated patients and a slight (but not significant) reduction of its plasma levels. These data seem to confirrm that FSH-secreting cells might have a different regulatory system and/or sensitivity as recently proposed (31,32). Obviously the possibility also has to be considered that a different postreceptorial activatory pathway(s) for FSH secretion might be responsible of such different FSH behavior.

From a clinical point of view, in addition to the lower ovarian volumes and Ferriman–Gallway score, patients treated with the combined regimen showed a more stable intermenstrual interval in comparison to OC-treated subjects. This is an important aspect, since it has been demonstrated that the reproductive axis activity gained a higher efficacy in all its components, thus permitting an optimal endocrine control of the ovarian function, that is, an optimal control of the ovulation. Even using the 3-monthly GnRH-a preparation, our data are in agreement with a previous report (32) that showed that the combined regimen facilitated spontaneous ovulation as demonstrated by the optimal secretion of both gonadal steroids, estradiol, and progesterone. In the present study we did not measure gondal steroids plasma levels during the follow-up interval, but the mean intermenstrual interval observed suggested that patients in group A recovered a normal endocrine control of the ovarian function; this observation was supported by not only the reduced ovarian volume which remained lower than baseline in patients of group A for a longer period than group B but also by the spontaneous ability to conceive that three of ten patients demonstrated as soon as they exited from our follow-up interval.

In conclusion, the present study demonstrated that in PCOD patients the use of a combined regimen of GnRH-a plus OC significantly modified the hypothalamus–pituitary–gonadal axis activity [so that to determine a stable recovery of the reproductive axis function than using the oral contraceptive alone.] In fact, the combined regimen improved LH spontaneous release throughout the posttreatment observation interval, reducing the frequency and amplitude of the spontaneous episodic discharge. Moreover, the use of the 3-monthly preparation of the GnRH-a was successfully similar to the monthly preparation, thus suggesting that the main advantage is the shift from a monthly to a 3-monthly puncture. Our study let us infer also that patients affected by PCOD waiting for assisted reproductive programs might try to conceive spontaneously, undergoing to a therapeutic protocol similar to the one we used.

ACKNOWLEDGMENTS

This study was supported in part by CNR grant "Altri Interventi" (No. AI98.00136.04) given to Dr. A. D. Genazzani. We are grateful to Peter Munson, PhD, Department of Computer Research and Technology, DCRT, National Institutes of Health, Bethesda, MD, for his helpfull suggestions and criticism.

REFERENCES

1. Balen AH, Conway GS, Kaltsas G, Techatraisak K, Manning PJ, West C, Jacobs HS: Polycystic ovary syndrome: the spectrum of the disorder in 1741 patients. Hum Reprod 1995;10:2107–2111
2. Yen SSC, Vela P, Rankin J: Inappropriate secretion of follicle-stimulating hormone and luteinizing hormone in polycystic ovarain disease. J Clin Endocrinol Metab 1970;30:435–442
3. Venturoli S, Porcu E, Fabbri R, Magrini O, Gammi L, Paradisi R, Forcacci M, Bolzani R, Flamigni C: Episodic pulsatile secretion of FSH, LH, prolactin, oestradiol, oestrone, and LH circadian variations in polycystic ovary syndrome. Clin Endocrinol 1988;28:93–107
4. Waldstreicher J, Santoro NF, Hall JE, Filicori M, Crowley Jr WF: Hyperfunction of the hypothalamic–pituitary axis in women with polycystic ovarian disease: Indirect evidence for

partial gonadotroph desensitization. J Clin Endocrinol Metab 1988;66:165–172
5. Berga SL, Guzick DS, Winters SJ: Increased luteinizing hormone and alpha-subunit secretion in women with hyperandrogenic anovulation. J Clin Endocrinol Metab 1993;77:895–901
6. Graf MA, Bielfeld P, Distter W, Weiers C, Kuhn-Velten W: Pulsatile luteinizing hormone secretion pattern in hyperandrogenic women. Fertil Steril 1993;59:761–767
7. Apter D, Butzow T, Laughlin GA, Yen SSC: Accelerated 24-h LH pulsatile activity in adolescent girls with ovarian hyperandrogenism: Relevance to the developmental phase of polycystic ovarian syndrome. J Clin Endocrinol Metab 1994;79:119–125
8. Rebar R, Judd HL, Yen SSC, Rakoff J, VandenBerg G, Naftolin F: Characterization of the inappropriate gonadotropin secretion in polycystic ovary syndrome. J Clin Invest 1976;57:1320–1329
9. Hayes FJ, Taylor AE, Martin KA, Hall JE: Use of gonadotropin-releasing hormone antagonist as a physiologic probe in polycystic ovary syndrome: assessment of neuroendocrine and androgen dynamics. J Clin Endocrinol Metab 1998;83:2343–2349
10. Yen SSC: Polycystic ovary syndrome. Clin Endocrinol 1980;12:177–208
11. Nestler JE, Jakubowicz DJ: Decreases in ovarian cytochrome *p*450c17alfa activity and serum free testosterone after reduction of insulin secretion in polycystic ovary syndrome. N Engl J Med 1996;335:617–623
12. Dunaif A, Scott D, Finegood D, Quintana B, Whitcomb R: The insulin-sensitizing agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. J Clin Endocrinol Metab 1996;81:3299–3306
13. Pucci E, Genazzani AD, Monzani F, Lippi F, Angelini F, Gargani M, Barletta D, Luisi M, Genazzani AR: Prolonged treatment of hirsutism with flutamide alone in patients affected by polycystic ovary syndrome. Gynecol Endocrinol 1995;9:221–228
14. Ciotta L, Cianci A, Calogero AE, Palumbo MA, Marletta E, Sciuto A, Palumbo G: Clinical and endocrine effects of finasteride, a 5α-reductase inhibitor, in women with idiopathic hirsutism. Fertil Steril 1995;64:299–306
15. Tolino A, Petrone A, Sarnacchiaro F, Cirillo D, Ronsini S, Lombardi G, Nappi C: Finasteride in the treatment of hirsutism: New therapeutic perspectives. Fertil Steril 1996;66:61–65
16. Ciotta L, Cianci A, Giuffrida G, Marletta E, Agliano A, Palumbo G: Clinical and hormonal effects of gonadotropin-releasing hormone agonist plus an oral contraceptive in severely hirsute patients with polycystic ovary disease. Fertil Steril 1996;65:61–67
17. Heiner JS, Greendale GA, Kawakami AK, Lapolt PS, Fisher M, Young D, Judd HL: Comparison of a gonadotropin-releasing hormone agonist and a low dose oral contraceptive given alone or together in the treatment of hirsutism. J Clin Endocrinol Metab 1995;80:3412–3418
18. Genazzani AD, Petraglia F, Battaglia C, Gamba O, Volpe A, Genazzani AR: A long-term treatment with gonadotropin-releasing hormone agonist plus a low-dose oral contraceptive improves the recovery of the ovulatory function in patients with polycyctic ovary syndrome. Fertil Steril 1998;67:463–468
19. Genazzani AD, Petraglia F, Fabbri G, Monzani A, Montanini V, Genazzani AR: Evidence of luteinizing hormone secretion in hypothalamic amenorrhea associated with weight loss. Fertil Steril 1990;54:222–226
20. Genazzani AD, Petraglia F, Benatti R, Montanini V, Algeri I, Volpe A, Genazzani AR: Luteinizing hormone (LH) secretory burst duration is indipendent from LH, prolactin or gonadal steroid plasma levels in amenorrheic women. J Clin Endocrinol Metab 1991;72:1220–1225
21. Genazzani AD, Petraglia F, Pianazzi F, Volpogni C, Genazzani AR: The concomitant release of androstenedione with cortisol and luteinizing hormone pulsatilee releases distinguishes adrenal from ovarian hyperandrogenism. Gynecol Endocrinol 1993;7:33–41
22. Oerter KE, Guardabasso V, Rodbard D: Detection and characterization of peaks and estimation of instantaneous secretory rate for episodic pulsatile hormone secretion. Computers Biomed Res 1986;19:170–191
23. Genazzani AD, Rodbard D: Evaluation of methods for detection of pulsatile hormone secretion: sensitivity vs. specificity. Acta Endocrinol 1991;124:295–306
24. Falsetti L, Pasinetti E: Treatment of moderate and severe hirsutism by gonadotropin-releasing hormone agonists in women with polycystic ovary syndrome and idiopatic hirsutism. Fertil Steril 1994;61:817–822
25. Adashi EY: Potential utility of gonadotropin-releasing hormone agonists in the menagement of ovarian hyperandrogenism. Fertil Steril 1990;53:765–779
26. Lemay A, Surrey ES, Friedman AJ: Extending the use of gonadotropin-releasing hormone agonists: The emerging role of steroidal and nonsteroidal agents. Fertil Steril 1994;61:21–34
27. Azziz R, Ochoa TM, Bradley EL, Dowing Potter H, Boots LR: Leuprolide and estrogens versus oral contraceptive pills for the treatment of hirsutism: A prospective randomized study. J Clin Endocrinol Metab 1995;80:3406–3411
28. Carr BR, Breslau NA, Givens C, Byrd W, Barnett-Hamm C, Marshburn PB: Oral contraceptive pills, gonadotropin-releasing hormone agonists, or use in combination for treatment of hirsutism: A clinical reasearch center study. J Clin Endocrinol Metab 1995;80:1169–1178
29. Genazzani AD, Rodbard D, Forti G, Petraglia F, Baraghini GF, Genazzani AR: Estimation of instantaneous secretory rate of luteinizing hormone in women during the menstrual cycle and in men. Clin Endocrinol 1990;32:573–581
30. Speroff L, Glass RH, Kase NG. Steroid contraception. *In* L Speroff, RH Glass, NG Kase (eds), Clinical Gynecologic Endocrinology and Infertility. Baltimore, Williams & Wilkins, 1989, pp 461–498
31. Genazzani AD, Petraglia F, Gastaldi M, Volpogni C, Gamba O, Massolo F, Genazzani AR: Evidence suggesting an additional control mechanism regulating episodic secretion of luteinizing hormone and follicle stimulating hormone in prepubertal children and post-menopausal women. Hum Reprod 1994;9:1807–1812
32. Genazzani AD, Massolo F, Ferrari E, Gandolfi A, Petraglia F, Genazzani AR: Long-term GnRH agonist administration revealed a GnRH independent mechanism stimulating FSH discharge in humans. Eur J Endocrinol 1996;134:77–83