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Modulatory Effects of Ethinyl Estradiol Plus Drospirenone Contraceptive Pill on Spontaneous and GnRH-Induced LH Secretion

Alessandro D. Genazzani [1,*], Alessandra Sponzilli [1], Marcello Mantovani [1], Emma Fusilli [1], Francesco Ricciardiello [1], Elisa Semprini [1], Tommaso Simoncini [2] and Christian Battipaglia [1]

[1] Gynecological Endocrinology Center, Department of Obstetrics and Gynecology, University of Modena and Reggio Emilia, Via l Pozzo 71, 41100 Modena, Italy; alessandra.sponzilli@gmail.com (A.S.); 252403@studenti.unimore.it (M.M.); emmafusillimex@gmail.com (E.F.); francesco.ricciardiello96@gmail.com (F.R.); elisasemprini93@gmail.com (E.S.); christianbattipaglia@gmail.com (C.B.)

[2] Department of Obstetrics and Gynecology, University of Pisa, 56126 Pisa, Italy; tommaso.simoncini@med.unipi.it

* Correspondence: algen@unimo.it

Abstract: Background: Combined oral contraceptives (COCs) work mostly by preventing the pre-ovulatory gonadotropin surge, but the action of COCs on spontaneous episodic and GnRH (gonadotropin-releasing hormone)-induced LH (luteinizing hormone) release has been poorly evaluated. Oral contraceptives are known to act on the spontaneous hypothalamic–pituitary functions reducing both GnRH and gonadotropin release and blocking ovulation. Aim: To evaluate spontaneous and GnRH-induced LH release during both phases of the menstrual cycle or under the use of the contraceptive pill. Methods: A group of 12 women, subdivided into two groups, volunteered for the study. Group A (n = 6, controls) received no treatments, while Group B (n = 6) received a 21 + 7 combination of ethinyl-estradiol (EE) 30 µg + drospirenone (DRSP) 3 mg. Both groups were evaluated twice: Group A during follicular and luteal phases, Group B during pill assumption and during the suspension interval, performing a pulsatility test, GnRH stimulation test, and hormonal parameters evaluation. Spontaneous and GnRH-induced secretory pulses were evaluated, as well as the instantaneous secretory rate (ISR). Results: COC treatment lowered LH and FSH (follicle stimulating hormone) levels significantly if compared to the follicular phase of spontaneous cycles. During the suspension interval, hormone levels rapidly rose and became comparable to those of the follicular phase of the control group. The LH pulse frequency under COC administration during the suspension interval was similar to that observed during the follicular phase (2.6 ± 0.3 pulses/180 min and 2.3 ± 0.2 pulses/180 min, respectively). The GnRH-induced LH peaks were greater in amplitude and duration than those observed after ISR computation in both groups. The GnRH-induced LH release during the luteal phase of the control subjects was higher than in the follicular phase (51.2 ± 12.3 mIU/mL and 14.9 ± 1.8 mIU/mL, respectively). Conversely, subjects under COC showed a GnRH-induced LH response similar during COC and during the suspension interval. Conclusions: Our data support that the EE + DRSP preparation acts on both spontaneous pulsatile release and GnRH-induced LH release during the withdrawal period of the treatment, and that after 5–7 days from the treatment suspension, steroidal secretion from the ovary is resumed, such as that of androgens. This suggests that in hyperandrogenic patients, a suspension interval as short as 4 days might be clinically better.

Keywords: hormonal contraception; drospirenone; follicular phase; luteal phase; GnRH test; LH; pulsatile secretion

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1. Introduction

The hypothalamus–pituitary axis is the main driver of female reproduction and its activity is greatly modulated and partly affected by peripheral signals. Endocrine, as well as metabolic and environmental, signals are able to induce specific functional adaptations both directly and indirectly on hypothalamic GnRH-secreting neurons, thus determining changes in the spontaneous episodic pulsatile release [1–3]. Gonadotrope cells inside the pituitary obey GnRH stimulation, releasing both LH and FSH in a pulsatile manner so as to stimulate the recruitment and evolution of the ovarian follicles, thus driving the ovarian cycle [1]. Specific modulations are determined by the activin–inhibin–follistatin system [4] that reflect the combination between the actions caused by the positive and negative feed-back signals on the hypothalamic neurons. These effects are exerted by the gonadal steroids, such as estradiol (E2), androstenedione (A), and progesterone [5,6]. Such ovarian signals also modulate GnRH-neurons' activity through the action of kisspeptin, a neuropeptide produced in the hypothalamus in the rostral periventricular region of the third ventricle (RP3V) and arcuate nucleus (ARC) [7,8]. Gonadal sex steroids stimulate kisspeptin neurons in the RP3V but inhibit kisspeptin neurons in the ARC, which is the underlying mechanism for the positive and negative feedback of estrogens, respectively [7,8]. In addition, progesterone causes specific modulations during the luteal phase, since it inhibits GnRH-induced LH release, reducing LH pulse frequency during the luteal phase [9], probably acting together with the opioid's modulation [10].

In addition to these events, a complex cascade of neuroendocrine peptides [11–13] refines the driving of GnRH-induced gonadotropin release so as to have the ovulation of one dominant follicle [14,15].

Hormonal contraception, i.e., estro-progestins, induce the control of the reproductive function acting on all such biological mechanisms, decreasing gonadotropin secretion [16,17]. The neuro-endocrine control of gonadotropin secretion relies on the combined effects of both estrogens, i.e., EE, and progestins on the spontaneous secretion of GnRH. In fact, it has been clearly evidenced that both estrogens and progestins are able to modulate the endogenous GnRH-induced gonadotropin secretion, progestins' effect being greater than that observed for EE [18]. Moreover, the progestins' effect is essential not only centrally but also peripherally, since it is the main actor for both the prevention of the pre-ovulatory gonadotropin surge and for the changes in endometrial receptivity to embryo implantation [19,20].

On such a basis, the present study aimed to investigate in a group of healthy subjects the intrinsic mechanisms of gonadotropin secretion under a EE + DRSP contraceptive pill, using DETECT: an already validated algorithm for pulse analysis and for the computation of instantaneous secretory rates (ISR) [21].

2. Materials and Methods

A total of 12 women (28 $\pm$ 1.5 years; mean $\pm$ SEM) were enrolled among the nurses and doctors of our unit and volunteered for this study. They were subdivided into two groups of 6 subjects each. The inclusion criteria were: no hormonal therapies for at least 6 months, absence of endocrine pathologies, regular menstrual cycles in the last 6 months (27–34 days), regular body weight (21 < BMI < 24), no strenuous physical activity, and varied regular diet. Group A (n = 6) received no treatment and subjects were considered controls, while Group B (n = 6) was composed by subjects requesting hormonal contraception and therefore receiving a combined oral contraceptive containing ethinylestradiol (0.03 mg) and drospirenone (3 mg) in a 21 + 7 formulation for 3 months. Such a combination was chosen for the positive effects of DRSP on the central nervous system, in particular at the hypothalamic level, as previously reported [22,23].

Basal hormone levels were measured to assess the endocrine profile in both groups, including LH, FSH, prolactin (PRL), estradiol (E2), progesterone (P), androstenedione (A), testosterone (T), and 17-hydroxyprogesterone (17OHP). On the same day, all subjects underwent a pulsatility test: sampling every 10 min for 180 min was repeated after a

gonadotropin-releasing hormone (GnRH) stimulation test using a 10 µg bolus of Leuprolide acetate. Such endocrine evaluations were performed twice for each group: for Group A (controls), the evaluations were conducted between the 4th and 6th days and between the 17th and 21st days of spontaneous menstrual cycles; for Group B (under treatment), the first evaluation was performed between the 17th and 21st day of the estro-progestin treatment, and the second evaluation between the 5th and 7th day of the withdrawal period. For this latter group, the days of hormonal evaluation were chosen so as to have a picture of the effects of at least 17 days of treatment with the contraceptive pill and another after the treatment suspension, very close to the re-start of the treatment.

Informed consent was obtained from all subjects.

2.1. Assay

LH and FSH plasma concentrations were determined using an immunofluorimetric method (IFMA) with a sensitivity of 0.1 IU/mL. The cross-reactivity between the α- and β-subunits of LH, FSH, and TSH was below 2.2%. The coefficient of variation within the same assay and between assays was 4.6% and 7.2%, respectively.

PRL, P, E2, A, T, and 17OHP plasma concentrations were assayed as routine procedure by the Modena Hospital Central Laboratory.

2.2. Pulse Analysis

The presence of the LH secretory pattern was studied on both raw plasma concentrations and on the instantaneous secretory rate (ISR), calculated using the validated DETECT program for pulsatility analysis. LH time series were initially evaluated separately to calculate the random measurement error on sample duplicates using the PREDETECT.wk1 program [21]. Secretory peaks for each time in the time series were then evaluated using the DETECT program with a P value of 0.01 (1%) for the calculated false-positive percentage. DETECT's specificity was compatible with a calculated P level of 0.01 (1%) for false positives, as the observed false-positive percentage from the plasma data of each participant, dosed together with time series, did not statistically differ from 1%. The DETECT program was also used for the computation of the ISR in LH time series and in the LH response to the GnRH bolus [16,24–26].

Plasma hormone levels can be assumed to be the difference between the input from the pituitary and the output from all organs and tissues in charge for the clearance. Because the clearance rate constants and half-life for LH are known, the ISR can be computed by the algorithm included in the DETECT program [16]. LH clearance constants have been previously estimated [22]. The constants for the first and second component rate were set at a half-life time of 17.8 and 90 min, with fractional amplitudes of 0.62 and 0.38, respectively. The variance model used for ISR was calculated as follows: $s^2isr = 2 \times s^2x$, where s is the standard deviation (SD) or measurable error, and s^2 is the variance [16,24,25].

2.3. Statistical Analysis

The amplitude of the LH peak in response to the GnRH bolus was calculated from the plasma levels as the difference (Δ) between the maximum height of the LH response and the plasma LH levels observed before stimulation. When performing the ISR calculation, the amplitude of the LH peak after the GnRH bolus was automatically obtained with the DETECT program in the instantaneous secretory rate profile [16].

After an analysis of variance (one-way ANOVA) to assess the presence of a normal distribution of data and similarity of variance between the groups, data were tested for statistically significant differences between the groups by means of Student's *t*-test for paired and unpaired data where appropriate.

3. Results

Tables 1 and 2 summarize the hormonal parameters of the two groups of subjects under study, controls and those under oral contraceptive treatment (EE + DRSP), respectively.

Table 1. Hormonal parameters of the control group (Group A) during the follicular (Day 4–6), and luteal phases (Day 17–21).

Group A (n = 6)	LH mIU/mL	FSH mIU/mL	PRL ng/mL	E2 pg/mL	P ng/mL	A ng/mL	T ng/dL	17OHP ng/mL
Follicular phase (day 4–6)	6.9 ± 1.7	4.4 ± 0.4	10.6 ± 1.5	72 ± 20.7	1 ± 0.1	182.8 ± 35.1	64.2 ± 10.1	1 ± 0.2
Luteal phase (day 17–21)	9.4 ± 2.1	2.9 ± 0.3	15.7 ± 2.8	155.5 ± 23.8	16.8 ± 3.8	310.6 ± 46.3	69.8 ± 7.3	3.6 ± 0.3
p level vs. follicular phase		0.003	0.02	0.00004	0.0006	0.003		0.001

Table 2. Hormonal parameters of subjects of Group B, under contraceptive pill treatment and during the interval of suspension.

Group B (n = 6)	LH mIU/mL	FSH mIU/mL	PRL ng/mL	E2 pg/mL	P ng/mL	A ng/mL	T ng/dL	17OHP ng/mL
Day 17–21 of treatment	1.1 ± 0.3	0.95 ± 0.3	14.4 ± 0.3	15.5 ± 4.2	0.8 ± 0.07	135.1 ± 19.3	43.5 ± 5.0	0.4 ± 0.09
p level vs. follicular phase (Table 1)	0.005	0.00001		0.007				0.01
Day 5–7 of interval	4.2 ± 0.8	5.7 ± 0.7	10.4 ± 1.9	53 ± 9.8	1.1 ± 0.2	197.8 ± 23.8	57 ± 6.3	0.7 ± 0.09
p level vs. follicular phase (Table 1)	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
p level vs. Day 17–21	0.004	0.0007		0.007		0.04	0.03	0.03

As expected during the luteal phase of the spontaneous cycle, FSH plasma levels decreased significantly, while PRL, E2, P, A, and 17OHP levels increased significantly in comparison to the follicular phase (Table 1).

Patients undergoing EE + DRSP treatment (Table 2) showed significantly lower levels than controls during the treatment (Day 17–21) for LH, FSH, E2, and 17OHP plasma levels.

It is worth noting that subjects under contraceptive treatment did not show hormonal levels completely suppressed or undetectable, and that these levels were lower than the ones during the follicular phase of control subjects (Tables 1 and 2). On the contrary, during the contraceptive-free interval, the hormonal parameters of these patients increased so that they were not significantly different than those observed during the follicular phase of the control subjects (Tables 1 and 2).

When considering the frequency of spontaneous episodic LH secretion in the control group (Group A) during the follicular phase, no differences were observed in terms of amplitude and number of peaks/180 min between the raw data and after ISR calculations (Table 3). As expected, in the luteal phase, the amplitude of LH peaks was higher than in the follicular phase, both in raw data and after ISR calculations (Table 3).

In addition, the amplitude and duration of LH peaks observed in plasma concentrations were significantly longer than those observed after ISR calculations, in both the follicular and luteal phases of the cycle (Table 3). The GnRH test induced a higher response of LH during the luteal phase than during the follicular phase, while the pulse duration was similar. After ISR computation, the resulting amplitude and duration were lower in both phases, the amplitude of the luteal phase being higher than that observed in the

follicular phase, while the resulting pulse duration was similar in both phases and shorter than that observed in the raw data (Table 3).

Table 3. Group A (controls). Characteristics of spontaneous LH pulsatile secretion and after GnRH stimulation tests on raw data and after ISR computation.

Group A (n = 6)	LH Spontaneous Secretion			LH ISR on Spontaneous Secretion		GnRH Test—Raw Data		GnRH Test—ISR	
	Integrated Mean mIU/mL	N Pulse/180 min	Amplitude mIU/mL	N Pulse/180 min	Amplitude mIU/mL	Amplitude mIU/mL	Duration min	Amplitude mIU/mL	Duration min
Follicular phase (Day 4–6)	5.82 ± 1.2	2.6 ± 0.3	1.8 ± 0.3	2.0 ± 0.3	1.6 ± 0.3	14.9 ± 1.8	120 ± 5	7.4 ± 1.4	50.6 ± 6.3
p. vs. ISR on GnRH						0.005	0.0000003		
Luteal phase (Day 17–21)	6.5 ± 1.7	1.7 ± 0.3	4.9 ± 1.2	1.3 ± 0.2	2.3 ± 0.2	51.2 ± 12.3	120 ± 5	26.9 ± 6.5	51.6 ± 6.6
p. vs. follicular phase			0.01	0.05	0.05	0.02		0.01	
p. vs. ISR on GnRH						0.01	0.00009		

In patients under contraceptive pill treatment, the pulsatile release of LH was evaluated only during the drug-free interval (Table 4), because under estrogen–progestin treatment, the secretory peaks were absent or too low to be identified by the DETECT program, even though the LH concentrations were measurable. During the interval of suspension, the LH plasma levels were similar to the follicular phase but lower than the luteal phase of subjects of the control group (Table 4). The resulting LH pulse frequency and amplitude in the raw data and after ISR computation were similar to the follicular phase of the control subjects (Table 4).

Table 4. Group B (under contraceptive pill treatment). Characteristics of spontaneous LH pulsatile secretion and after GnRH stimulation tests. Both as raw data and after ISR computation.

Group B (n = 6)	LH Spontaneous Secretion			LH ISR on Spontaneous Secretion		GnRH Test—Raw Data		GnRH Test—ISR	
	Integrated Mean mIU/mL	N Pulse/180 min	Amplitude mIU/mL	N Pulse/180 min	Amplitude mIU/mL	Amplitude mIU/mL	Duration min	Amplitude mIU/mL	Duration min
Day 17–21 of treatment	0.6 ± 0.1	-	-	-	-	9.1 ± 2.2	85 ± 3.7	5.4 ± 1.3	40 ± 4.9
p. vs. ISR on GnRH						0.007	0.0004		
p. vs. Fol phase						0.05	0.000001		
Day 5–7 of interval	3.4 ± 0.5	2.3 ± 0.2	1.4 ± 0.5	1.5 ± 0.2	1.1 ± 0.2	7.5 ± 2.9	80.5 ± 7.3	6.0 ± 1.6	53.3 ± 3.1
p. vs. Day 17–21	0.0003								
p. vs. ISR on GnRH						0.03	0.00001		
p. vs. Fol phase (Table 3)						0.05	0.00001		
p. vs. Luteal phase (Table 3)	0.05								

Considering GnRH stimulation during contraceptive pill treatment and during the interval of suspension, the pulse and amplitude of the LH peaks observed in the raw data

were lower than those observed in control subjects (Table 4). After ISR computation, the LH pulse amplitude and duration were still lower than those observed in the raw data and similar to those observed after ISR in the control subjects (Table 4).

The mean curves of the LH response to GnRH stimulation for the control subjects during the follicular and luteal phase (Figure 1, left panel) and for patients using the contraceptive pill (Figure 1, right panel) show a different pattern of response, higher during the luteal phase.

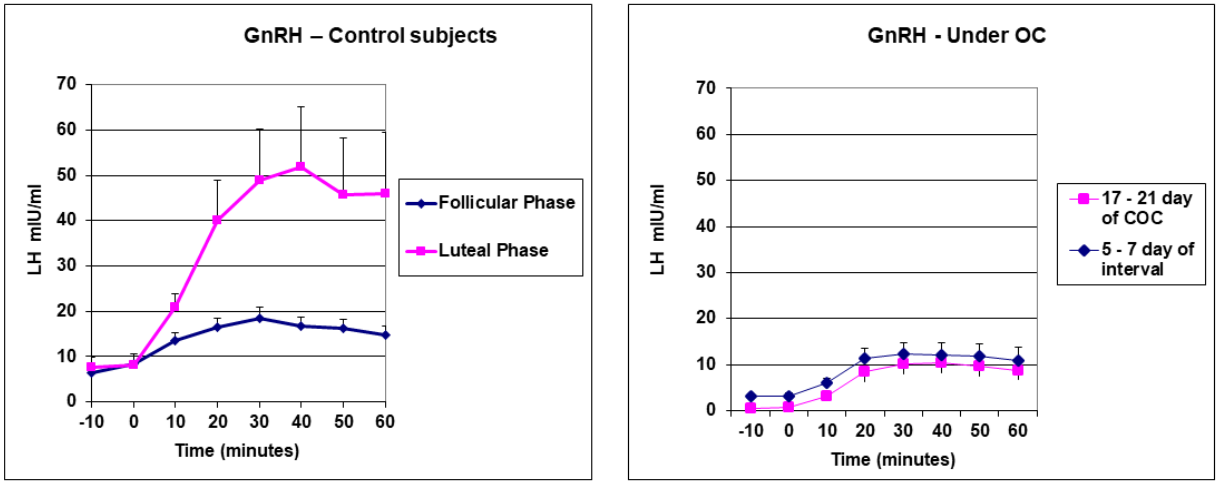

Figure 1. GnRH-induced release during follicular and luteal phase in control subjects (**left**) and in subjects under the contraceptive pill (**right**).

Interestingly, the LH responses to the GnRH test in the contraceptive-treated group did not differ in the amplitude and duration of the LH peak between the treatment and contraceptive-free interval (Figure 1, right panel) and in both cases resulted in similar outcomes to the follicular phase of the control group.

Figure 2 shows the LH response after ISR computation in control subjects (Figure 2, left panel) and in patients under treatment (Figure 2, right panel). As for raw data, the LH response during the luteal phase was higher than during the follicular phase. After ISR computation, patients undergoing contraceptive treatment showed a response to GnRH stimulation under COC similar to the suspension interval, and similar to the follicular phase of the control subjects.

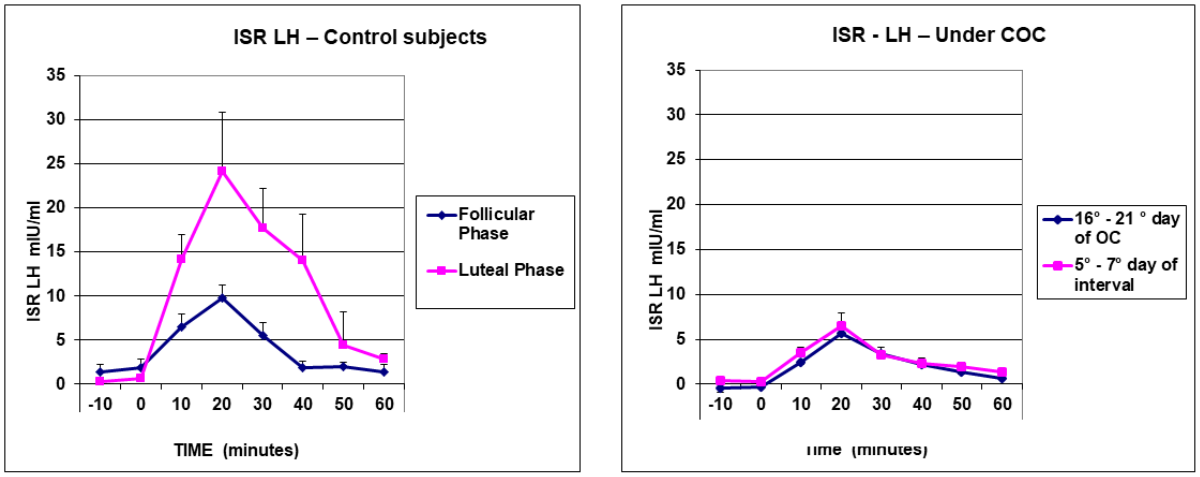

Figure 2. GnRH-induced LH pulse, after ISR computation, in control subjects (**left**) and in subjects under the contraceptive pill (**right**).

4. Discussion

The present study evaluated the effects of a contraceptive pill containing EE + DRSP (EE 0.03 mg + DRSP 3 mg) on spontaneous and GnRH-induced LH secretion and reported specific changes in hypothalamus and pituitary functions during the estro-progestin interval of suspension.

It is well known that any oral contraceptive with an estro-progestin combination is able to block ovulation thanks to the combined effects of ethinylestradiol with that of the progestin on the hypothalamic–pituitary glands. The present study confirmed that the LH and FSH plasma concentrations, although reduced, were detectable during the use of the contraceptive pill. The hypogonadotropic effect of the estro-progestin preparation completely blocks ovulation and permits only a minimal production of gonadal steroids [26]. In fact, our present data support such evidence since under the contraceptive pill treatment, the resulting progesterone, androstenedione, and T levels were similar to those observed during the follicular phase of control subjects. Such androgen levels are probably mainly due to the adrenal function and to a minimal ovarian function which is reduced by the treatment with estro-progestin. It is interesting to note that the hormonal plasma levels observed during the suspension interval of the pill are higher than during the treatment interval, especially 17OHP and androstenedione, and similar to those observed during the follicular phase of the control group. This demonstrates that the ovarian function resumes quite quickly as soon as the pill is suspended and that the theca cell activity again started producing androstenedione, which through the aromatase action is transformed into estradiol.

Such an observation has relevance from a clinical point of view. In fact, it has to be considered when prescribing the use of the contraceptive pill as a treatment to counteract a hyperandrogenic state as in PCOS patients whose elevated androgenic milieu is higher due to an excess in ovarian androgens [27]. Clinically speaking, treatment suspension for a time as long as 7 days might expose these patients to an excessive recovery of androgen secretion, thus being the cause of the recurrence of some dermatologic signs such as acne frequently observed [27]. This fact suggests that a longer duration of treatment (24 instead of 21 days) and a shorter suspension interval (4 instead of 7 days) might be consistently better for these patients, as previously stated [28].

While control subjects showed a distinct different pattern of LH episodic secretion during the follicular and the luteal phases, in the other group, in agreement with previous studies [29], the administration of the combined contraceptive pill completely blunted the typical spontaneous pulsatile release of LH. In fact, no pulses were observed, although a residual LH secretion was observed, still confirming the ability of hormonal oral contraceptives to negatively act on the neuroendocrine control of gonadotropin release [18]. Conversely, during the interval of suspension a distinct spontaneous pulsatile secretion of LH was detected that was superimposable to that observed during the follicular phase of the control subjects. Such data of LH episodic secretion agree with those previously reported [24,25], and the fact that LH secretion is restored similar to the follicular phase of the control group clearly demonstrates that the neuroendocrine control of gonadotropin secretion is rapidly restored within a few days from pill suspension.

It is of interest to note that the patients under COC treatment showed an LH response to GnRH infusion perfectly identical during both the COC treatment and during the suspension interval. Such a reduced response of LH is also evident after ISR computation, which discloses the gonadotrope secretory ability under GnRH stimulation [21]. This observation gives a clear idea in regard to the strength of the DRSP effect on the pituitary cells. In fact, it is well known that progesterone and progestins modulate pituitary functions, thus modulating gonadotropin release [9,27]. The fact that LH shows the same response in both stimulations lets us infer that DRSP induces a specific long-lasting effect at the gonadotrope level which is not countered by the increase in estradiol of ovarian origin during pill suspension. In fact, during the luteal phase of the menstrual cycle of control subjects, the LH response to GnRH stimulation was higher than during the follicular phase,

clearly supporting the amplification determined by progesterone modulation. On the contrary, in patients under oral contraceptive treatment, the LH response to the GnRH stimulus was reduced both in raw data and after ISR computation. These data clearly support those previously reported by Goldzieher et al. [18] that stated the greater negative effect of progestins than EE on endogenous GnRH-induced LH and FSH release. In regard to the DRSP effects, this is probably due to the strong specific activity of this compound at the hypothalamic level to modulate GnRH discharge, since it increases beta endorphin (βEP) contents as previously demonstrated [22], thus participating in the reduced LH response, substantially the opposite to the permissive activity known for progesterone [9,25]. In addition, this specific contraceptive preparation has been demonstrated to be clinically effective in the neuroendocrine control of specific areas of the central nervous system (CMNS), since it has been proposed for the treatment of premenstrual syndrome (PMS) or premenstrual dysphoric disorder (PMDD) [30]. Moreover, Yoshino et al. [31] reported a great efficacy of the EE + DRSP combination on pain perception in sufferers of endometriosis and/or dysmenorrhea. Additionally, confirming the central modulatory role of DRSP, De Berardis et al. [30] sustained that EE + drospirenone should be taken into consideration, not as a first-line treatment but reserved mainly for SSRI-resistant subjects so as to have a better action centrally.

Finally, it is of interest to underline that the use of ISR computation permitted us to assess that the time spent by the gonadotrope cells to release LH under GnRH stimulation was identical in the control group during both phases of the menstrual cycle, as well as during both the contraceptive pill treatment and the suspension interval. Such an observation in these latter groups sustains the hypothesis that the estro-progestin, mainly through DRSP, modulates the amplitude of the GnRH-induced LH release.

In conclusion, our data support the ability of the combination of EE + DRSP to efficiently suppress the reproductive axis and disclose the fact that the inhibitory action of DRSP on GnRH-induced LH release is also maintained during the suspension interval, since it is no different from that observed during the assumption of the contraceptive. Additionally, our data underlie that within 5–7 days after the suspension of the contraceptive pill, the ovarian function is resumed and a gonadal steroid pattern similar to that observed during the follicular phase of the control subjects is shown. This suggests that a suspension interval as long as 7 days should be avoided in hyperandrogenic patients and a suspension interval as short as 4 days might be preferable. This choice should help to better counteract the androgen-induced discomfort.

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