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Effects of oral contraceptive, synthetic progestogen or natural estrogen pre-treatments on the hormonal profile and the antral follicle cohort before GnRH antagonist protocol

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BACKGROUND: Steroid pre-treatments may be useful to program GnRH antagonist IVF/ICSI cycles. This prospective study assessed hormonal and ultrasound data collected during the free period after the discontinuation of three different pre-treatments to provide information on the optimal time interval required before starting stimulation. METHODS: Women were randomized to receive oral contraceptive pill (OCP) [ethinyl estradiol (E_2) 30 µg + desogestrel 150 µg] (*n* = 21) or norethisterone 10 mg/day (*n* = 23) or 17-βE_2 4 mg/day (*n* = 25) or no pre-treatment (*n* = 24) for one cycle before IVF. Assessments were performed on post-treatment day (PD) 1, 3 and 5, or on spontaneous cycle day (CD) 1 and 3. RESULTS: After OCP and progestogen administration, FSH and LH concentrations shifted from strongly suppressed PD1 levels to PD5 values similar to those observed on CD1. Meanwhile, follicle sizes remained small up to PD5. In contrast, estrogen pre-treatment poorly reduced FSH levels on PD1 compared with OCP or progestogen. Consequently, follicle size was more heterogeneous. FSH rebound was maximal on PD3, whereas LH levels were slightly increased up to PD5. CONCLUSIONS: A 5-day free interval after OCP or progestogen offers the advantages of gonadotrophin recovery and homogeneous follicular cohort, whereas early FSH rebound occurring after estrogen pre-treatment argues for a short free period.

Key words: GnRH antagonist/natural estrogen/oral contraceptive pill/pre-treatment/progestogen

Introduction

Compared with GnRH agonist long protocols, the introduction of GnRH antagonist protocols for controlled ovarian hyperstimulation has offered the great opportunity to reduce the duration of treatment and the consumption of gonadotrophins and to lower the physical and psychological burden for patients submitted to pituitary desensitization. However, GnRH antagonist protocols do not allow for programming of IVF or ICSI cycles, which is quite convenient for regulating activity of assisted reproduction treatment (ART) centres and for domestic or work organization of patients. Moreover, some other concerns came out with the use of GnRH antagonists. Indeed, the slight reduction in the number of retrieved oocytes and in the pregnancy rate reported in a meta-analysis (Al-Inany and Aboulghar, 2002) has been partly attributed to the absence of synchronization of the follicular cohort before ovarian stimulation. In that respect, GnRH antagonist protocols largely differ from the so-called GnRH antagonist long protocols. Therefore, it has been speculated that this could explain the discrepancies in the cycle outcome between both the protocols.

For that reason, more attention has been paid to the potential interest of steroid pre-treatments to program cycles, to modify the hormonal environment in relation to the negative feedback exerted by steroids on endogenous gonadotrophin secretion and therefore to synchronize the follicular cohort before stimulation. Indeed, both oral contraceptive pill (OCP) and synthetic progestogens have been largely used for many years to program cycles (Frydman *et al*., 1986; Wardle *et al*., 1986; Gerli *et al*., 1989; Biljan *et al*., 1998). More recently, the use of natural estrogens has also been advocated (de Ziegler *et al*., 1998). Estrogens are believed to primarily inhibit FSH secretion (Tsai and Yen, 1971; le Nestour *et al*., 1993), whereas progestogens are supposed to be mainly involved in the control of LH secretion (Anderson *et al*., 1990). Therefore, consequences of these pre-treatments should be considered separately because their respective impact on the subsequent cycle is more likely to be different.

When considering the effects of steroid pre-treatments, two different issues should be addressed. The first one is related to

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the negative control exerted by steroid administration on the endogenous gonadotrophin secretion. It may be presumed that the stronger the negative hypophyseal feedback exerted by steroid, the more effective the synchronization of the cohort expected. The second issue is related to the timing and intensity of the gonadotrophin secretory rebound that usually follows pre-treatment discontinuation. It should govern the optimal timing for starting FSH stimulation, which has not been established so far. It is more likely, but not proved, that these two consecutive effects of steroids are closely linked and the most suppressive compound will presumably induce the strongest rebound of gonadotrophin secretion.

OCP pre-treatment in GnRH antagonist protocol proved to be effective for scheduling oocyte retrieval during the 5-day working week (Barmat *et al*., 2005). Two large studies recently reported the effects of OCP pre-treatment on follicular growth, hormonal profiles and cycle outcomes (Kolibianakis *et al*., 2006; Rombauts *et al*., 2006). They share the same conclusion that OCP pre-treatment does not actually improve oocyte yield and may even exert a deleterious effect on cycle outcome. This latter conclusion contrasts with previous reports of the studies without GnRH antagonist, taking advantage of the use of OCP before ovarian stimulation (Biljan *et al*., 1998; Fukuda *et al*., 2000). Discrepancies between studies stress the need for a careful evaluation of the endocrine profile during the wash-out period, defined as the interval between the end of pre-treatment and the beginning of FSH stimulation. Serum FSH and LH levels were strongly suppressed after a 2-day wash-out interval (Rombauts *et al*., 2006), whereas normal baseline FSH levels were observed at the end of a 5-day interval (Kolibianakis *et al*., 2006). This is consistent with the previous observation that a plasma FSH rise is usually observed up to 7 days after OCP discontinuation (Fauser and van Heusden, 1997). However, these studies did not actually assess gonadotrophin secretion patterns or consequences on follicle cohort homogenization during the wash-out period.

Synthetic progestogen preparations, mainly norethisterone, have been also previously used as a pre-treatment for programming oocyte retrieval on working days (Zorn *et al*., 1987) and for preventing the occurrence of a spontaneous LH surge during ovarian stimulation in patients whose endogenous gonadotrophins were not suppressed by GnRH agonists (Thatcher *et al*., 1988). Indeed, norethisterone acts through a highly potent suppressive effect on basal pituitary LH secretion (Anderson *et al*., 1990) and on GnRH agonist-induced LH flare-up (Cédrin-Durnerin *et al*., 1996). More recently, the predictive value of LH levels before FSH stimulation on cycle outcome has been addressed in patients treated with GnRH antagonist protocols (Kolibianakis *et al*., 2003). This study suggested that low basal LH values are critical to ensure adequate pregnancy rate. However, the impact of progestogen pre-treatment on GnRH antagonist cycle outcome has not been evaluated so far.

Finally, the potential benefit of natural estrogen pre-treatment has been recently assessed in GnRH antagonist cycles. This approach seemed to be promising because endogenous estrogen secretion is actually the main factor involved in the negative regulation of FSH secretion during the luteal-follicular transition (le Nestour *et al*., 1993; Lahlou *et al*., 1999). Indeed, previous reports have shown that estrogen pre-treatment allows improvement of follicle synchronization within the cohort (Fanchin *et al*., 2003a) and enhances the recovery of mature oocytes (Fanchin *et al*., 2003b). Nevertheless, the optimal interval between the discontinuation of estrogen intake and the starting of FSH stimulation still remains to be determined.

Overall, these data suggest that there is a scope to revisit the concept of scheduling GnRH antagonist cycles by steroid pre-treatment. Indeed, no information still exists neither on the comparative effectiveness of different steroid preparations nor on the optimal time for starting FSH stimulation after pre-treatment discontinuation. The purpose of this prospective randomized study was to assess the effects of three different steroid pre-treatments on follicular growth and on hormonal profiles during the 5-day wash-out period. Data were compared with those of a control group without pre-treatment.

Materials and methods

Subjects

Ninety-three women undergoing an IVF/ICSI cycle were enrolled in this prospective randomized study conducted in six IVF centres from March to December 2004. The inclusion criteria were regular normo-ovulatory cycles (28–35 days), age <38 years and BMI between 18 and 30. The exclusion criteria were high levels of baseline serum FSH or E_2 values, <5 follicles at the antral follicular count performed on day 3 of a spontaneous cycle or a history of high (>20 oocytes) or low (<5 oocytes) ovarian response in a previous IVF attempt. Selected women were randomly assigned to receive one of three pre-treatments or no pre-treatment. Random allocation sequence was generated from a table of random numbers and was concealed to each physician who enrolled and randomized patients. Randomization was stratified by centre and was performed with sealed envelopes. This study was not blind. After study ethical approval, patients provided written informed consent.

Protocols

Pre-treatments were administered daily during the cycle preceding the IVF cycle: 21 patients received a combination of ethinyl [estradiol (E_2)] 30 µg + desogestrel 150 µg (Varnoline®, Organon, Puteaux, France) from cycle day (CD) 2 to 3 for 15–21 days; 23 patients were treated with norethisterone (Primolut-nor®, Schering, Lys-les-Lannoy, France) 10 mg/day from CD15 for 10–15 days and 24 of 25 eligible women actually received micronized 17-βE_2 (Provames®, Aventis, Paris, France) 2 mg twice a day, started 10 days before the presumed menses. Finally, the control group was composed of 24 women without any pre-treatment.

The last administration of pre-treatment was constantly given on Sunday evening. Hormonal and ultrasound assessments were performed on alternate days during the 5 days after steroid discontinuation (Figure 1): on Monday post-treatment day 1 (PD1), Wednesday (PD3) and Friday (PD5). Similar assessments were performed on CD1 and CD3 after spontaneous menses in the control group.

At the last evaluation point (PD5 or CD3), stimulation was started by daily injection of 150–300 IU of recombinant follitropin beta (Puregon®, Organon). The starting dose was chosen according to age, BMI and previous responses to stimulation. This dose was maintained constant for 5 days and then adjusted according to ovarian response. When the leading follicle reached 14 mm in diameter, daily injections of ganirelix 0.25 mg (Orgalutran®, Organon) were performed up to

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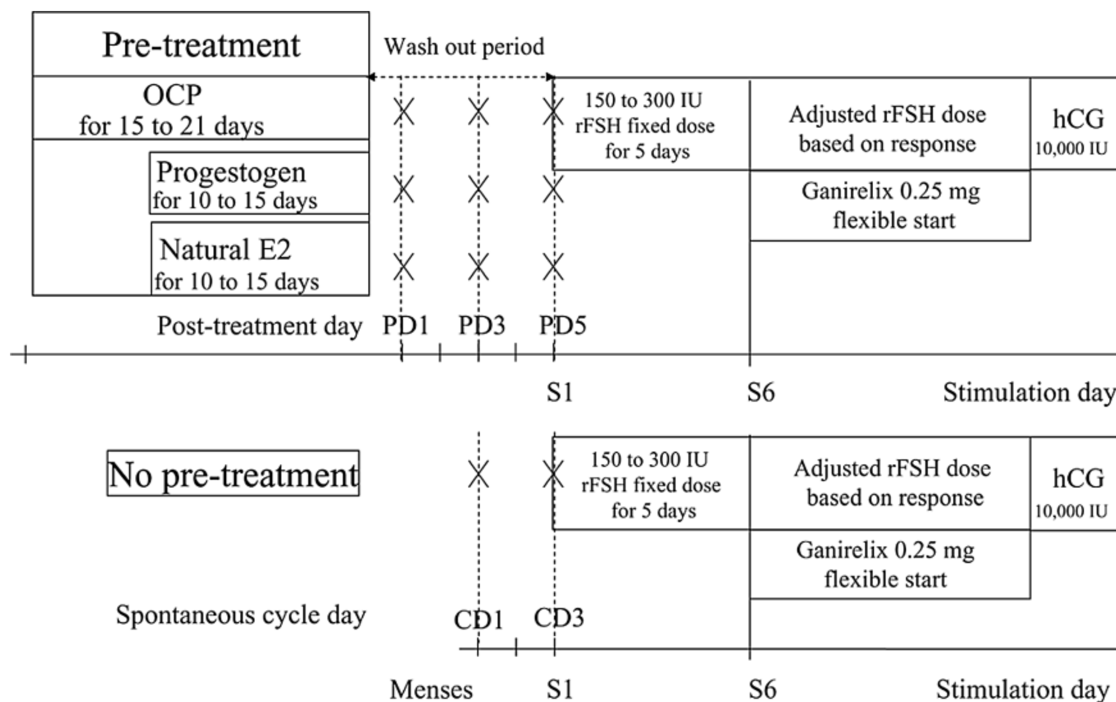

Figure 1. Protocol design. OCP, oral contraceptive pill; rFSH, recombinant FSH.

the time of HCG. Injection of 10 000 IU HCG (Gonadotrophines Chorioniques Endo®, Organon) was given when at least three mature (≥17 mm) follicles were obtained and oocyte retrieval was performed 36 h later. Luteal phase was supported by vaginal administration of micronized progesterone 400 mg/day (Utrogestan®, Besins International, Montrouge, France) from the day of ovarian puncture to the day of pregnancy test. If a pregnancy occurred, progesterone administration was extended up to the evidence of fetal heart activity at ultrasound.

Hormonal measurements

Serum samples were collected for local measurement of E_2 and progesterone concentrations and were also frozen until subsequent centralized analysis of FSH, LH and inhibin B concentrations at each evaluation point. Samples for anti-Müllerian hormone (AMH), testosterone, sex hormone-binding globulin (SHBG), insulin growth factor 1 (IGF1) and insulin growth factor binding protein (IGF BP3) determination were collected at the last point.

Local hormonal measurements were carried out using commercially available chemiluminescence immunoassays with automated Elecsys immunoanalyser (ECLIA, Roche diagnostic, Meylan, France). The sensitivity of the assay was 5 pg/ml and 0.03 ng/ml for E_2 and progesterone, respectively. Intra-assay and inter-assay coefficients of variation were 5 and 10% for E_2 and 3 and 5% for progesterone, respectively.

Central hormonal measurements were carried out from frozen serum samples at the end of the study. FSH and LH were measured by chemiluminescence immunoassays (Advia Centaur, Bayer Diagnostics, Puteaux, France). The sensitivity of the assay was 0.3 IU/l for FSH and 0.07 IU/l for LH. The intra- and inter-assay coefficients of variation were 1.4 and 2.4% for FSH and 2.3 and 2.6% for LH, respectively. Enzyme-linked immunosorbent assay (ELISA) was used to measure inhibin B (IBB, DSL, Webster, USA) and AMH (EIA, Immunotech Beckman Coulter, Marseille, France). The sensitivity was 7 pg/ml for inhibin B and 0.1 ng/ml for AMH (1 ng/ml = 7.14 pmol/l). The intra- and inter-assay coefficients of variation were 4.5 and 4.2% for inhibin B and 12.3 and 14.2% for AMH, respectively. Testosterone and SHBG were measured by radioimmunoassay (RIACT, Cis Bio International, Gif sur Yvette, France). The sensitivity of the assay was 0.03 ng/ml for testosterone and 0.5 nmol/l for SHBG. The intra- and inter-assay coefficients of variation were 5.3 and 5.4% for testosterone and 4.3 and 4.8% for SHBG, respectively. The technique for IGF1 and IGF BP3 measurements was a sandwich radioimmunoassay (IRMA, Immunotech Beckman Coulter). The sensitivity was 30 ng/ml for IGF1 and 140 ng/ml for IGF BP3. The intra- and inter-assay coefficients of variation were 6.3 and 6.8% for IGF1 and 6 and 9.5% for IGF BP3, respectively.

Vaginal ultrasonography

Ultrasound assessments were performed with a 6 MHz vaginal transducer. The same clinician in each centre performed ultrasound examinations. The ovarian surface (length by width by 0.8), the number of follicles sized 2–5 mm and 6–9 mm in both ovaries and the endometrium thickness were recorded. Follicle diameter was calculated as the mean diameter measured in 2Ds.

Sample size estimate

This study was a prospective observational study. As very few data were available so far regarding the effects of steroid pre-treatment, and no data still existed on the respective effects of different steroid preparations on hormonal environment and follicle cohort, no calculation could be performed. However, a sample size of about 25 subjects per group seemed sufficient to display differences in hormonal profile or antral follicle cohort between treatments.

Statistical analysis

Results are expressed as mean ± SD in tables and mean ± 95% confidence interval in figures. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, Chicago, USA) for hormonal data and Statview (Abacus Concepts, Berkeley, USA) for other variables. Nominal or continuous variables were analysed with chi-square or Student's *t*-test or analysis of variance (ANOVA) for

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repeated measures as required. Analyses were adjusted for centre if necessary. For variables with deviation from normality, a non-parametric test of Kruskal–Wallis was used. For comparisons between the three pre-treated groups and the control group, PD1 and PD3 were compared with CD1, and PD5 was compared with CD3. A *P* value <0.05 was considered as statistically significant.

Results

Baseline characteristics

Three patients were excluded from analysis (Figure 2) in the natural estrogen group for reasons independent from treatment (one did not start any treatment, one ovarian cyst and one major protocol violation). Baseline characteristics of patients were similar in all groups (Table I). After the discontinuation of OCP or progestogen, menses occurred on PD4 with a narrow range in both groups, whereas 70% of patients menstruated (median of 2.5 days) before the end of natural estrogen pre-treatment.

Hormonal data

For graphical presentation, data were lined up on the day of stimulation start (CD3 or PD5). As shown in Figure 3, OCP and synthetic progestogen similarly exerted a significant suppressive effect on LH and FSH secretion on PD1 (*P* for Kruskal–Wallis <0.001). No significant correlation was observed within groups between serum FSH and LH values on PD1 and the duration of pre-treatment. Serum FSH values returned to baseline values observed in the control group by PD5, and serum LH values were no longer significantly different from control on PD3. Natural estrogen administration did not significantly suppress serum FSH and LH values compared with the control group. After the discontinuation of estrogen treatment, serum FSH rebound was maximal on PD3 and a slight increase in serum LH levels persisted until PD5. As expected, serum E_2 levels were highly increased in the natural estrogen group on PD1 and were still significantly higher on PD5 compared with the other three groups (*P* for Kruskal–Wallis <0.001 on PD1 and PD5). Serum progesterone levels were not significantly different between groups, although some patients could have persistent secretion from the corpus luteum before the onset of menses. Serum inhibin B levels increased significantly during the wash-out period (*P* for time effect of ANOVA for repeated measures <0.001), but without any significant effect between different pre-treatments and control.

Regarding hormones assessed before the start of ovarian stimulation (Table II), the only significant difference was observed for SHBG levels, which were significantly higher in groups pre-treated with synthetic or natural estrogen intake

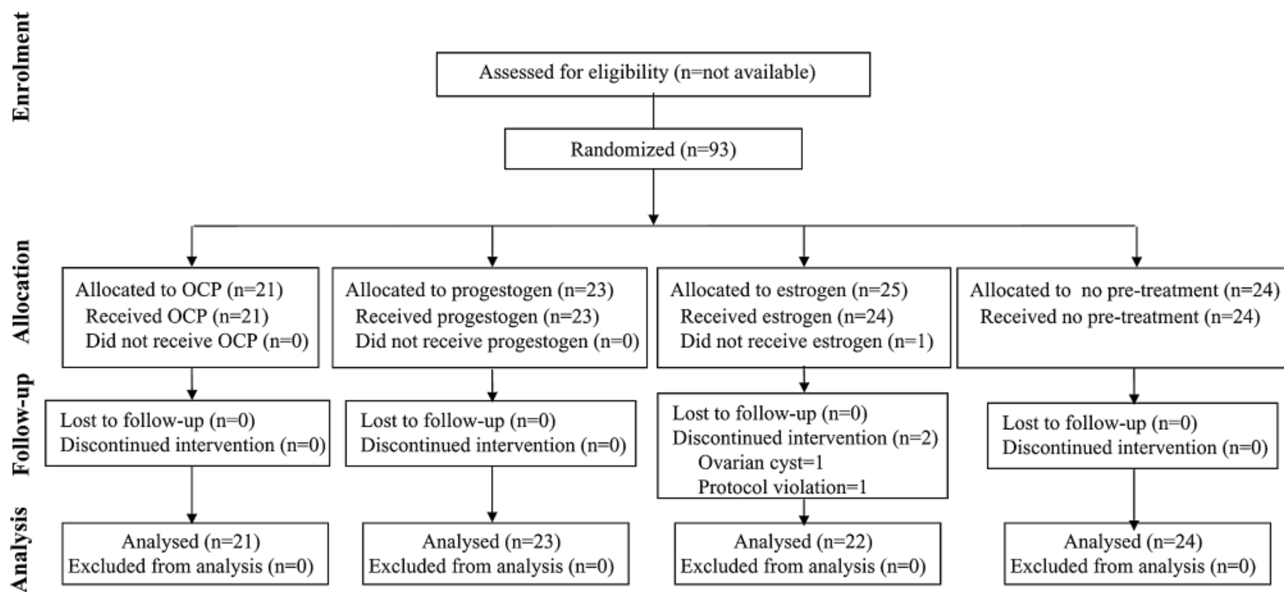

Figure 2. CONSORT statement flow diagram.

Table I. Characteristics of patients in each pre-treatment group and in the control group without pre-treatment

	OCP (*n* = 21)	Progestogen (*n* = 23)	Estrogen (*n* = 22)	Control (*n* = 24)	*P*
Age (years)	30.8 ± 4.6	32.9 ± 2.5	31.8 ± 3.2	31.2 ± 4.3	NS
BMI (kg/m^2)	22.2 ± 2.9	22.4 ± 3.3	22.4 ± 2.7	22.3 ± 3.4	NS
Pre-treatment duration (days)	18.7 ± 3.0	13.3 ± 2.7	10.6 ± 3.6	NA	0.001
Menses onset					
Mean day	+4.1 ± 1.1	+3.7 ± 0.7	–1.3 ± 4.1	NA	0.001
Median	+4	+4	–2.5		
Range	+2 to +7	+3 to +5	–8 to +6		

NB, the onset of menses is expressed with regard to the day of the last pre-treatment intake; OCP, oral contraceptive pill. Results are expressed as mean ± SD.

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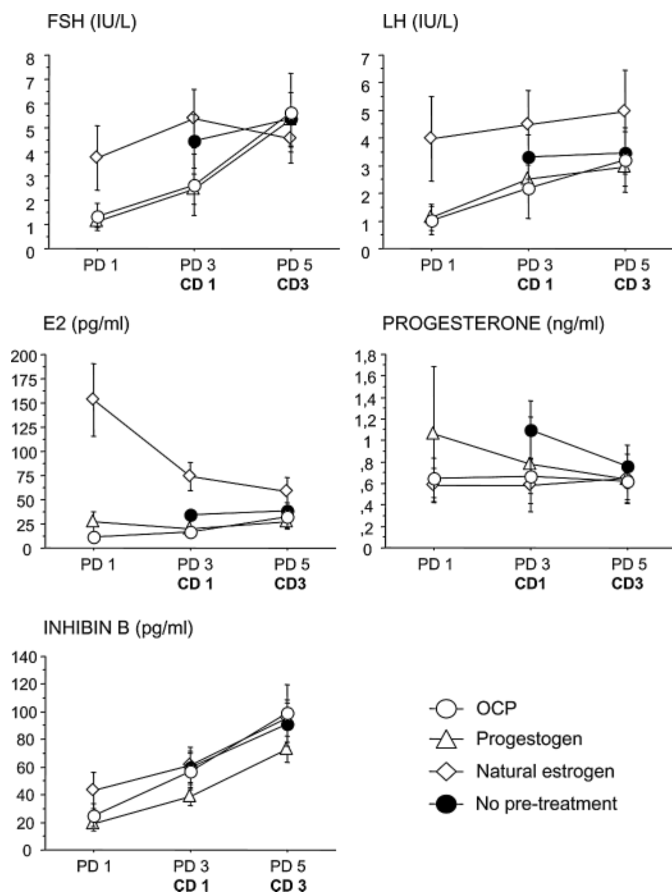

Figure 3. Serum hormone levels [mean ± 95% confidence interval (CI)] of FSH, LH, estradiol (E_2), progesterone and inhibin B on day 1, 3 and 5 post-treatment (PD) after oral contraceptive pill (OCP), progestogen or natural estrogen administration or on spontaneous cycle day (CD) 1 or 3 without pre-treatment. Comparison between the three steroid pre-treatments: treatment effect of analysis of variance (ANOVA) for repeated measures, $P = 0.006$ for FSH, non-significant (NS) ($P = 0.16$) for LH, $P < 0.001$ for E_2, and NS for progesterone and inhibin B. Comparison with the control group: treatment effect of analysis of variance (ANOVA) for repeated measures, $P = 0.02$ (PD1/CD1, PD5/CD3) or $P = 0.04$ (PD3/CD1, PD5/CD3) for FSH, NS for LH, $P < 0.001$ for E_2, NS for progesterone and inhibin B.

(*P* for ANOVA <0.001). It should be noted that serum AMH levels were not significantly different between groups.

Ultrasound data

As shown in Figure 4, the antral follicle count was similar in all groups (13–15 follicles for both ovaries) and did not vary with time in accordance with serum AMH levels. However, the ovarian surface was significantly lower in the two groups with strongly suppressed gonadotrophin secretion (OCP and progestogen groups) compared with natural estrogen or control groups ($P < 0.001$ both for ANOVA for repeated measures and Kruskal–Wallis tests). The number of small follicles from 2 to 5 mm in diameter was not different between groups. In contrast, the number of follicles between 6 and 9 mm in diameter was significantly larger in the two groups that displayed the highest ovarian surface (natural estrogen or control group) ($P < 0.001$ both for ANOVA for repeated measures and Kruskal–Wallis tests). Therefore, in those groups, the follicular cohort was more heterogeneous as attested by a mean of four or five follicles larger than the others. The endometrial thickness was significantly higher in the progestogen group (*P* for Kruskal–Wallis <0.001 on PD1 and PD3) but decreased with the onset of menses and was no longer different at the time to start stimulation.

Ovarian stimulation and cycle outcome

In the two groups with a heterogeneous follicular cohort (natural estrogen or no pre-treatment), the ovarian response (Table III) was advanced on day 6 of ovarian stimulation as attested by higher E_2 levels ($P < 0.001$) and a larger size of the leading follicle ($P < 0.001$). Therefore, the flexible GnRH antagonist administration was started earlier in those two groups ($P = 0.01$). Two cycles were cancelled in the natural estrogen group on day 6 because of premature LH surge, but none for that reason in the other groups. The other cycles were cancelled for the risk of ovarian hyperstimulation syndrome (OHSS) (one in estrogen group, one in OCP group and one in progestogen group) or for progesterone increase after antagonist introduction (one in progestogen group). Moreover, one cycle in progestogen group was converted into intrauterine insemination (IUI) for poor response. Because of the advanced ovarian response, stimulation duration was significantly shorter in natural estrogen or no pre-treatment groups ($P = 0.04$) with a significant reduction in the consumption of gonadotrophin ($P = 0.04$). However, the number of retrieved oocytes and total embryos were not significantly different from those of the two other groups.

Discussion

Our data show that steroid pre-treatments differently affect the hormonal environment and antral follicle size before ovarian stimulation. Indeed, during the wash-out period after both OCP and progestogen pre-treatments, the endocrine profile shifted

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Table II. Hormonal measurements before the start of ovarian stimulation

	OCP (*n* = 21)	Progestogen (*n* = 23)	Estrogen (*n* = 22)	Control (*n* = 24)	*P*
AMH (ng/ml)	3.84 ± 2.4	2.6 ± 1.96	3.7 ± 2.31	2.82 ± 1.78	NS
Testosterone (ng/ml)	0.68 ± 0.14	0.57 ± 0.12	0.63 ± 0.16	0.62 ± 0.16	NS
SHBG (nmol/l)	181 ± 71	50 ± 27	120 ± 44	62 ± 28	<0.001
IGF1 (ng/ml)	237 ± 67	289 ± 77	233 ± 58	250 ± 99	NS
IGF BP3 (ng/ml)	3108 ± 476	3382 ± 691	3080 ± 563	2887 ± 526	NS

AMH, anti-Müllerian hormone; IGF BP3, insulin growth factor binding protein 3; IGF1, insulin growth factor 1; SHBG, sex hormone-binding globulin.
Results are expressed as mean ± SD.

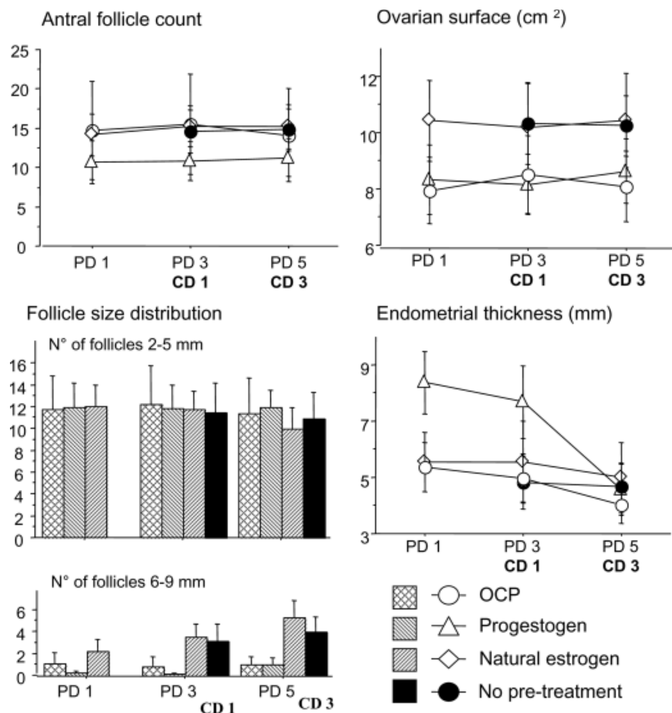

Figure 4. Ultrasound data (mean ± 95% CI) of antral follicle count, ovarian surface, follicle size distribution and endometrial thickness on post-treatment day (PD) 1, 3 and 5 after oral contraceptive pill (OCP), progestogen or natural estrogen administration or on spontaneous cycle day (CD) 1 or 3 without pre-treatment. Comparison between the three steroid pre-treatments: treatment effect of analysis of variance for repeated measures, non-significant (NS) for antral follicle count, $P = 0.001$ for ovarian surface, NS for 2–5 mm follicles, $P < 0.001$ for 6–9 mm follicles and $P = 0.001$ for endometrial thickness. Comparison to the control group: treatment effect of analysis of variance (ANOVA) for repeated measures, NS for antral follicle count, $P = 0.008$ (PD1/CD1, PD5/CD3) or $P = 0.001$ (PD3/CD1, PD5/CD3) for ovarian surface, NS for 2–5 mm follicles, $P < 0.001$ for 6–9 mm follicles and $P = 0.003$ (PD1/CD1, PD5/CD3) or $P = 0.04$ (PD3/CD1, PD5/CD3) for endometrial thickness.

from strongly suppressed FSH and LH values to values similar to those observed in a spontaneous cycle. Meanwhile, the follicle size inside the cohort remained homogeneous. Therefore, these results suggest that a 5-day wash-out period is optimal for patients pre-treated with progestogen and OCP. In contrast, natural estrogen pre-treatment did not significantly reduce serum FSH levels, and follicle sizes within the cohort appeared as heterogeneous as observed on spontaneous CD3. Moreover, the abrupt FSH rebound after stopping estrogen intake with a concomitant increase in follicle sizes argues for a short wash-out interval of 1 or 2 days.

At variance with GnRH agonists extensively used in the long protocol, steroids may interfere with gonadotrophin secretion not only by the suppressive effect induced by steroid intake but also by the rebound of gonadotrophin secretion after the cessation of administration. It remains unclear which steroid is the most suppressive and whether suppressive and rebound periods are closely linked. Considering the negative control exerted by steroid administration on the endogenous FSH secretion, we observed that OCP and progestogen pre-treatments were more suppressive than natural estrogen pre-treatment. This suggests that synthetic progestogens are a major determining factor to deeply suppress endogenous FSH. However, we cannot exclude the possibility that a more pronounced suppression of circulating FSH levels could be obtained by giving higher oral doses of natural estrogens or by using a vaginal route of administration, proven to be more effective for achieving high serum E_2 concentrations (Tourgeman *et al*., 2001). As far as FSH secretory rebound was concerned, we did not observe any difference in maximal FSH concentrations reached after the discontinuation of the different steroid pre-treatments, nor correlation within groups between suppressed levels and maximum rebound. Similarly, Kolibianakis *et al*. (2006) observed serum FSH levels after a 5–day wash-out interval after OCP identical to those of early follicular phase. Moreover, in OCP users, maximum FSH levels reached

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Table III. Ovarian stimulation and cycle outcome

	OCP (*n* = 21)	Progestogen (*n* = 23)	Estrogen (*n* = 22)	Control (*n* = 24)	*P*
Starting FSH dose (IU)	212 ± 43	214 ± 56	195 ± 44	188 ± 26	NS
At S6					
E_2 (pg/ml)	679 ± 388	500 ± 238	1030 ± 477	720 ± 352	<0.001
Follicle size (mm)	11.3 ± 2.7	11.5 ±1.5	14.4 ± 2.2	14 ± 1.9	<0.001
Number of follicles >10 mm	6.6 ± 5.3	6.5 ± 7.1	8.1 ± 4.9	5.6 ± 3.3	NS
Cancelled cycle (*n*)	1	2	3	0	
Antagonist starting day	7.5 ± 1.5	7.3 ± 1.4	6.3 ± 0.7	6.7 ± 1.2	0.01
Antagonist duration	3.6 ± 1	3.2 ± 1.3	4 ± 1	3.3 ± 2.1	NS
HCG day	11 ± 1.7	10.6 ± 0.9	10.1 ± 0.9	10.1 ± 1.9	0.04
FSH dose (IU)	2174 ± 723	2010 ± 670	1700 ± 524	1734 ± 551	0.04
Retrieval (*n*)	20	20	19	24	
Number of oocytes	14 ± 8.3	12.6 ± 7.3	13.1 ± 7	9.9 ± 5.4	NS
Number of embryos	8.1 ± 4.7	6.4 ± 5.4	6.9 ± 3.5	6 ± 3.6	NS
Transfer (*n*)	18	18	15	24	
Transferred embryos	2.1 ± 0.5	2 ± 0.5	2.2 ± 0.4	2 ± 0.6	NS
Positive pregnancy test (*n*)	5	7	4	12	
PR per oocyte retrieval	25%	35%	21%	50%	NS
LB (*n*)	3	5	3	6	
LB per oocyte retrieval	15%	25%	15.8%	29.2%	NS
Live babies (*n*)	5	6	3	8	

E_2, estradiol; LB, live birth; PR, pregnancy rate; S6, day 6 of ovarian stimulation.
Results are expressed as mean ± SD.

at the end of the pill-free period are not correlated with FSH levels on the first day after the discontinuation of OCP (van Heusden *et al*., 2002). Altogether, these data suggest that the amplitude of FSH rebound is not dependent on the intensity of FSH suppression but that the FSH rebound is advanced when FSH suppression is weak.

The effects of steroid pre-treatments on the antral follicular cohort have been assessed by measuring the number and the size of follicles and the ovarian surface, which reflects both follicular and stromal compartments of the ovary. Suppressed FSH levels had no impact on the number of follicles as assessed by measurement of antral follicle count at ultrasound or serum AMH levels, but they may actually homogenize follicle sizes within the cohort. Indeed, although standard ultrasound examination by several observers is less precise to measure sizes of small antral follicles than the previously used tissue harmonic imaging system by a single observer (Fanchin *et al*., 2003a), we did detect differences in follicular sizes between day 3 of a spontaneous cycle or day 1 post-estrogen pre-treatment and day 5 post-pre-treatment by OCP or progestogen. Furthermore, strongly suppressed gonadotrophin secretion by OCP or progestogen led to decreased ovarian surface. Therefore, these data suggest that the stronger the negative FSH feedback exerted by steroid, the more effective the homogenization of the cohort.

Homogenization of the follicular cohort is more likely to play a critical role in ensuring a synchronized follicular growth during ovarian stimulation and, as a consequence, for optimizing the number of retrieved oocytes. However, two studies with many included patients failed to demonstrate a beneficial effect of OCP pre-treatment compared with no pre-treatment on the number of retrieved oocytes (Rombauts *et al*., 2006), even if fixed FSH doses were used during the whole stimulation (Kolibianakis *et al*., 2006). Moreover, OCP pre-treatment increased the duration of ovarian stimulation to a similar extent to that observed with a GnRH agonist long protocol and allowed the retrieval of a similar number of oocytes compared with long protocol (Huirne *et al*., 2006; Rombauts *et al*., 2006). In contrast, Fanchin *et al*. (2003b) showed that the synchronization of the follicular cohort with estrogen pre-treatment, followed by a short wash-out period before starting stimulation, was associated with an increase in the number of follicles and oocytes retrieved. Whether strong homogenization of the follicular cohort by OCP or a more subtle homogenization by estrogen pre-treatment has differential effects on oocyte yield remains to be determined in an adequately powered prospective study.

However, another important difference between steroid pre-treatments concerned LH levels, which were reduced by OCP and progestogen and increased by natural estrogen pre-treatment. This could be clinically relevant because pregnancy rate seems optimal within a certain window of LH secretion in GnRH antagonist cycles (Huirne *et al*., 2005). In our study, after the discontinuation of OCP or progestogen, LH levels returned to baseline levels similar to those of controls. In contrast, Kolibianakis *et al*. (2006) noticed persistently decreased LH levels at the initiation of stimulation in OCP group, and these levels remained lower until HCG administration. Interestingly, although those authors previously suggested the lower the LH levels, the higher the probability of pregnancy (Kolibianakis *et al*., 2004), their recent report showed that OCP pre-treatment was not actually associated with improved pregnancy rate, but conversely with increased miscarriage rate. Similarly, Rombauts *et al*. (2006) observed decreased implantation rate after OCP pre-treatment with a short wash-out interval of 2 days leading to a profound pituitary suppression. Therefore, the ability of serum LH levels to predict cycle outcome in GnRH antagonist cycles remains questionable. Conversely, after natural estrogen pre-treatment, we observed slightly increased LH levels at the initiation of ovarian stimulation and an increased incidence of premature LH surge before the start of GnRH antagonist treatment. This effect was not reported in Fanchin's study (2003b), and this discrepancy might be explained by the different wash-out intervals between the two studies (1 day in the study by Fanchin versus 5 days in this study). Nevertheless, in cycles without pre-treatment, exposure to high LH levels is associated with a reduced chance of pregnancy (Kolibianakis *et al*., 2003). Whether this slight increase in LH levels at the beginning of stimulation could have potential positive or negative effects remains to be determined in a large series of patients.

Finally, steroid pre-treatments also differently affect proliferation and differentiation of the endometrium before and during ovarian stimulation. OCP markedly decreased endometrial thickness during the whole administration, and remnant effects could be observed until mid-ovarian stimulation phase as reported by Kolibianakis *et al*. (2006). Accordingly, OCP pre-treatment could be beneficial because advanced endometrial maturation has been advocated to explain the decreased implantation rate reported with GnRH antagonist protocol (Kolibianakis *et al*., 2002). However, the negative impact of GnRH antagonist protocols on endometrial receptivity has been recently challenged by an extensive assessment of endometrial biopsy (Simon *et al*., 2005). The administration of progestogen pre-treatment during the luteal phase of the preceding cycle was obviously associated with a thick endometrium before the occurrence of menses. However, all patients had menstruated at the initiation of ovarian stimulation and then endometrial thickness was no longer different from that of controls. Similarly, endometrial thickness was not significantly different from that of controls after oral administration of natural estrogen pre-treatment. The weak effect of estrogen pre-treatment on endometrium was more likely related to the short duration of treatment after the onset of menses. Until now, it is not clear whether steroid interference in endometrium cycle before ovarian stimulation may impact on cycle outcome.

In conclusion, these data show that steroid pre-treatments may differently affect the hormonal environment before GnRH antagonist protocol. As previous studies assumed that the endocrine profile before starting FSH stimulation might greatly affect the cycle outcome, the consequences of the higher suppressive effects of contraceptive pill and progestogen on gonadotrophin and ovarian secretion as compared with estrogen need to be further assessed. More specifically, it remains to be determined whether the potential negative effect of increased LH serum levels before starting FSH administration would affect the beneficial effect of oestrogen pre-treatment on the

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follicular cohort synchronization. Moreover, this study allows to set up the endocrine basis to determine the optimal wash-out interval between the cessation of steroid pre-treatment and the starting day of FSH stimulation. According to our results, a 5-day wash-out period seems to be optimal for patients pre-treated with progestogens and OCP, whereas FSH stimulation should be started early, 1 or 2 days after stopping natural estrogen pre-treatment. However, this needs to be confirmed by a direct comparison of various durations of wash-out period.

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References

Al-Inany H and Aboulghar M (2002) GnRH antagonist in assisted reproduction: a Cochrane review. Hum Reprod 17,874–885.

Anderson RE, Stein A, Paulson RJ, Stanczyk FZ, Vijod AG and Lobo RA (1990) Effects of norethindrone on gonadotropin and ovarian steroid secretion when used for cycle programming during *in vitro* fertilization. Fertil Steril 54,96–101.

Barmat LI, Chantilis SJ, Hurst BS and Dickey RP (2005) A randomised prospective trial comparing gonadotropin-releasing hormone (GnRH) antagonist/recombinant follicle-stimulating hormone (rFSH) versus GnRH-antagonist/rFSH in women pretreated with oral contraceptives before in vitro fertilization. Fertil Steril 83,321–330.

Biljan MM, Mahutte NG, Dean N, Hemmings R, Bissonnette F and Tan SL (1998) Effects of pre-treatment with oral contraceptive on the time required to achieve pituitary suppression with gonadotropin-releasing hormone analogues and on subsequent implantation and pregnancy rates. Fertil Steril 70,1063–1069.

Cédrin-Durnerin I, Bulwa S, Hervé F, Martin-Pont B, Uzan M and Hugues JN (1996) The hormonal flare-up following GnRH agonist administration is influenced by a progestogen pretreatment. Hum Reprod 11,1859–1863.

Fanchin R, Cunha-Filho JS, Schonauer LM, Kadoch IJ, Cohen-Bacri P and Frydman R (2003a) Coordination of early antral follicles by luteal estradiol administration provides a basis for alternative controlled ovarian hyperstimulation regimens. Fertil Steril 79,316–321.

Fanchin R, Salomon L, Castelo-Branco A, Olivennes F, Frydman N and Frydman R (2003b) Luteal estradiol pre-treatment coordinates follicular growth during controlled ovarian hyperstimulation with GnRH antagonists. Hum Reprod 18,2698–2703.

Fauser BCJM and van Heusden AM (1997) Manipulation of human ovarian function: physiological concepts and clinical consequences. Endocr Rev 18,71–106.

Frydman R, Forman R, Rainhorn JD, Belaisch-Allart J, Hazout A and Testart J (1986) A new approach to follicular stimulation for in vitro fertilization: programmed oocyte retrieval. Fertil Steril 46,657–662.

Fukuda M, Fukuda K, Andersen CY and Byskov AG (2000) Does anovulation induced by oral contraceptives favor pregnancy during the following two menstrual cycles? Fertil Steril 73,742–747.

Gerli S, Remohi J, Partrizio P, Borrero C, Balmaceda JP, Silber SJ and Asch RH (1989) Programming of ovarian stimulation with norethindrone acetate in IVF/GIFT cycles. Hum Reprod 4,746–748.

van Heusden AM, Coelingh Bennink HJT and Fauser BCJM (2002) FSH and ovarian response: spontaneous recovery of pituitary ovarian activity during the pill-free period vs. exogenous recombinant FSH during high-dose combined oral contraceptives. Clin Endocrinol 56,509–517.

Huime JAF, van Loenen ACD, Schats R, McDonnell J, Hompes PGA, Schoemaker J, Homburg R and Lambalk CB (2005) Dose-finding study of daily GnRH antagonist for the prevention of premature LH surges in IVF/ICSI patients: optimal changes in LH and progesterone for clinical pregnancy. Hum Reprod 20,359–367.

Huirne JA, Hugues JN, Pirard C, Fischl F, Sage JC, Pouly JL, Obruca A, Braat DM, van Loenen AC and Lambalk CB (2006) Cetrorelix in an oral contraceptive-pretreated stimulation cycle compared with buserelin in IVF/ICSI patients treated with r-hFSH: a randomized, multicentre, phase IIIb study. Hum Reprod 21,1408–1415.

Kolibianakis EM, Bourgain C, Albano C, Osmanagaoglu K, Smitz J, Van Steirteghem AC and Devroey P (2002) Effects of ovarian stimulation with recombinant follicle-stimulating hormone, gonadotropin releasing hormone antagonists, and human chorionic gonadotropin on endometrial maturation on the day of oocyte pick-up. Fertil Steril 78,1025–1029.

Kolibianakis EM, Albano C, Kahn J, Camus M, Tournaye H, Van Steirteghem AC and Devroey P (2003) Exposure to high levels of luteinizing hormone and estradiol in the early follicular phase of gonadotropin-releasing hormone antagonist cycles is associated with a reduced chance of pregnancy. Fertil Steril 79,873–880.

Kolibianakis EM, Zikopoulous K, Schiettecatte J, Smitz J, Tournaye H, Camus M, Van Steirteghem AC and Devroey P (2004) Profound LH suppression after GnRH antagonist administration is associated with a significantly higher ongoing pregnancy rate in IVF. Hum Reprod 19,2490–2496.

Kolibianakis EM, Papanikolaou EG, Camus M, Tournaye H, Van Steirteghem AC and Devroey P (2006) Oral contraceptive pill pretreament on ongoing pregnancy rates in patients stimulated with GnRH antagonists and recombinant FSH for IVF. A randomized controlled trial. Hum Reprod 21,352–357.

Lahlou N, Chabbert-Buffet N, Christin-Maitre S, Le Nestour E, Roger M and Bouchard P (1999) Main inhibitor of follicle stimulating hormone in the luteal-follicular transition: inhibin A, oestradiol, or inhibin B? Hum Reprod 14,1190–1193.

le Nestour E, Marraoui J, Lahlou N, Roger M, de Ziegler D and Bouchard P (1993) Role of estradiol in the rise in follicle-stimulating hormone levels during the luteal-follicular transition. J Clin Endocrinol Metab 77,439–442.

Rombauts L, Healy D and Norman RJ. On behalf of the Orgalutran Scheduling Study Group. (2006) A comparative randomized trial to assess the impact of oral contraceptive pretreatment on follicular growth and hormone profiles in GnRH antagonist-treated patients. Hum Reprod 21,95–103.

Simon C, Oberyé J, Bellver J, Vidal C, Bosch E, Horcajadas JA, Murphy C, Adams S, Riesewijk A, Mannaerts B *et al*. (2005) Similar endometrial development in oocyte donors treated with either high-or standard-dose GnRH antagonist compared to treatment with a GnRH agonist or in natural cycles. Hum Reprod 20,3318–3327.

Thatcher SS, Boyle HP, Glasier AF, Hillier SG and Baird DT (1988) A comparison of dosages of norethisterone for synchronisation of cycles in a fixed regimen of follicular augmentation and in vitro fertilization. Fertil Steril 49,848–853.

Tourgeman DE, Slater CC, Stanczyk FZ and Paulson RJ (2001) Endocrine and clinical effects of micronized estradiol administered vaginally or orally. Fertil Steril 75,200–202.

Tsai CC and Yen SCC (1971) The effect of ethinyl-estradiol administration during early follicular phase on the gonadotrophin levels and ovarian function. J Clin Endocrinol Metab 33,917–923.

Wardle PG, Foster PA, Mitchell JD, McLaughlin EA, Williams JAC, Corrigan E, Ray BD, McDermont AM and Hull MGR (1986) Norethisterone treatment to control timing of IVF cycle. Hum Reprod 1,455–457.

de Ziegler D, Jääskelaïnen AS, Brioschi PA, Fanchin R and Bulletti C (1998) Synchronisation of endogenous and exogenous FSH stimuli in controlled ovarian hyperstimulation (COH). Hum Reprod 13,561–564.

Zorn JR, Boyer P and Guichard A (1987) Never on a Sunday: programming for IVF-ET and GIFT. Lancet 1(8529),385–386.

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