

Original Article

Oral continuous combined 0.5 mg estradiol valerate and 5 mg dydrogesterone as daily add-back therapy during post-operative GnRH agonist treatment for endometriosis in Chinese women

Shien Zou¹, Qiqi Long¹, Shaofen Zhang¹, Yi Han¹, Wei Zhang²

¹Department of Gynecology, Obstetrics and Gynecology Hospital of Fudan University, Shanghai 200011, China;

²Department of Biostatistics, School of Public Health, Fudan University, Shanghai 200032, China

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Abstract: Objective: To evaluate the lowest effective dose of combined estrogen and progestogen (E₂+P) add-back therapy during post-operative gonadotropin-releasing hormone agonist (GnRHa) treatment for endometriosis in Chinese women. Study design: The study enrolled 81 patients aged 18 to 50 years with stage III or IV endometriosis, as diagnosed by surgery. All patients were given GnRHa 3-6 mg by subcutaneous injection once every 28 days for a total of three times. Patients were divided into three groups: the first (n = 35; GnRHa only group) received GnRHa only without add-back therapy, the second (n = 35; 0.5 mg E₂+P add-back group) received GnRHa plus 0.5 mg estradiol valerate and 5 mg dydrogesterone orally every day, and the third (n = 11; 1 mg E₂+P add-back group) received GnRHa plus 1 mg estradiol valerate and 10 mg dydrogesterone orally every day for the duration of treatment. All patients were required to follow up at our hospital at 4, 8 and 12 weeks after treatment initiation to assess efficacy and levels of serum reproductive hormones. Results: Compared with baseline levels, serum levels of the four reproductive hormones assessed (E₂, LH, P₄ and FSH) were significantly decreased in both the GnRHa only and the 0.5 mg E₂+P add-back groups at 4, 8, and 12 weeks after treatment; and levels reached a stable state at 4 weeks of treatment. In the 1 mg E₂+P add-back group, LH and FSH serum levels were significantly decreased, while those of E₂ and P were not significantly different at any of the time points assessed. In the 0.5 mg E₂+P add-back group, E₂ serum levels decreased drastically at first, then gradually over the course of the study. In contrast, pre- and post-treatment E₂ serum levels in the 1 mg E₂+P add-back group were not significantly different, and these levels were over 45 pg/mL for the entire study duration. Comparison among groups showed that E₂ levels in both add-back groups were significantly higher than in the GnRHa only group at 12 weeks after treatment. Furthermore, E₂ serum levels in the two add-back groups at 8 and 12 weeks after treatment were significantly different. Conclusion: Oral continuous combined 0.5 mg/d estradiol valerate and 5 mg/d dydrogesterone as immediate add-back therapy during post-operative GnRH agonist treatment for severe endometriosis may be the most suitable regimen for Chinese women.

Keywords: Endometriosis, GnRH agonist, ultra-low dose estrogen, immediate add-back therapy

Introduction

Endometriosis is a common gynecological disease, with a prevalence of approximately 6% to 10% during childbearing years [1, 2]. Surgical therapy, typically laparoscopic, is commonly used to diagnose or treat endometriosis. In addition, gonadotropin-releasing hormone agonists (GnRHa) can be effective in preventing recurrence and controlling pain [3-7]. However, long-term GnRHa treatment (usually 3 to 6 months) can induce a hypoestrogenic state,

thereby causing such side effects as vasomotor defects, vaginal dryness, and loss of bone mineral density (BMD) [7]. To prevent or minimize these deleterious side effects, GnRHa treatment can be combined with low doses of estrogen and progestogen in a process known as "add-back therapy" [7-10].

The typical add-back therapy regimen for Caucasian is 1 mg/d estradiol valerate or 0.65 mg/d conjugated equine estrogen combined with several kinds of progestones. Because of ethnic

differences, clinical data are still lacking to determine whether Chinese women should receive a lower dose of estrogen add-back therapy in order to reduce endometriosis recurrence. As such, we wanted to determine the lowest possible dose of continuous combined estrogen and progestogen add-back therapy for Chinese women with endometriosis being treated with GnRHa. The overall goal was to control serum estradiol (E_2) levels in patients to achieve therapeutic efficacy while minimizing adverse effects as much as possible, in order to improve compliance and prolong the potential duration of therapy.

We therefore designed a clinical trial to assess the therapeutic effects, including the effect on serum E_2 and FSH levels, of 0.5 mg/d or 1 mg/d estradiol valerate combined with progestogen as add-back therapy during post-operative GnRHa treatment for patients with endometriosis.

Materials and methods

Patients

Eighty-one women with endometriosis, aged 18 to 50 years, being treated at the Obstetrics and Gynecology Hospital of Fudan University from June 2007 to October 2008 were enrolled in the study. A diagnosis of stage III or IV endometriosis according to the revised American Fertility Society (rAFS) classification was confirmed by laparoscopy or laparotomy for all patients. None of the patients had received additional hormone treatment for at least 3 months prior to enrollment. In addition, none of the patients had hepatic, renal or immune system disorders.

The design and protocol of this clinical trial were approved by the hospital ethics committee, and all patients provided written informed consent before the study.

Study design

All subjects were randomly divided into three treatment groups using a random digits table: GnRHa only, 0.5 mg E_2 +P add-back, and 1 mg E_2 +P add-back. GnRHa (Zoladex 3.6 mg, Astra-Zeneca, UK) was administered subcutaneously to all patients once every 28 days for a total of three times, beginning at 3 to 5 days after the operation or 1 to 2 days into the menstrual cycle. Thirty-five patients were assigned to the

GnRHa only group and did not receive add-back therapy. Another 35 patients were assigned to the 0.5 mg E_2 +P add-back group and received 0.5 mg estradiol valerate (Progynova, Bayer Healthcare Co. Ltd. Guangzhou Branch) and 5 mg dydrogesterone (Solvay Pharmaceuticals B.V., now belongs to Abbott, USA) via oral administration every day in addition to GnRHa treatment. The remaining 11 patients were assigned to the 1 mg E_2 +P add-back group, and were treated with a combination of GnRHa as well as 1 mg estradiol valerate and 10 mg dydrogesterone daily. Based on the ethics committee's approval, participation was terminated when serum E_2 levels were found to exceed 45 pg/mL.

Patients were required to make follow-up visits just after surgery; at 4, 8 and 12 weeks after initiating treatment; and after the onset of menses. A flow chart of the trial is provided in **Table 1**, which indicates all of the parameters that were measured at each visit.

Assessment of reproductive hormone levels

Fasting cubital venous blood (2 mL) was collected from all patients before surgery and at 4, 8, and 12 weeks after treatment. Serum samples were separated within 2 hours to assay hormone levels. The serum levels of four reproductive hormones: E_2 , Progesterone (P_4), follicular stimulating hormone (FSH), and luteinizing hormone (LH), were measured using an Access immune luminescence analyzer (Beckman DXI800, USA). The system was operated in accordance with the manufacturer's instructions, and the intra- and inter-assay error rates were both less than 10%.

Statistical analysis

All data are presented as mean \pm standard deviation (SD). Data were compared using two-way ANOVA, followed by Bonferroni's correction where appropriate. SAS 9.2 software was used for statistical analyses, with a p value of 0.05.

Results

Patient demographics

Thirty-five patients were assigned to the GnRHa only group, 35 to the 0.5 mg E_2 +P add-back group, and 11 to the 1 mg E_2 +P add-back group. Overall baseline demographics were

Table 1. Trial flow chart

	Screening	Baseline	Treatment		
Visit number	1	2	3	4	5
Week	-2 to -1	0	4	8	12
Allowed time deviation (days)	-	-	±5	±7	±10
Informed consent	x				
Inclusion/exclusion criteria	x	x			
Randomization		x			
Demographics	x				
Medical history	x				
Gynecological history	x				
History of ERT/HRT	x				
Vital signs	x	x	x	x	x
Height	x				
Physical examination	x				x
Gynecological examination	x				x
Cervical smear	x				
Transvaginal/transanal ultrasound	x				x
Serum E ₂ /P/FSH/LH levels		x	x	x	x
Issue/review/collect diary cards	x	x	x	x	x
Concomitant illness	x				
Concomitant medication	x	x	x	x	x
Adverse events	x	x	x	x	x
Drug dispensation		x	x	x	
End of trial					x

Table 2. Baseline demographics of trial population. Values represent mean ± SD or n

	GnRHaonly	1 mg E ₂ +P add-back	0.5 mg E ₂ +P add-back
n	35	11	35
Age (years)	30.7 ± 6.7	32.2 ± 6.9	32.2 ± 7.0
Body mass index (kg/m ²)	21.7 ± 2.4	20.1 ± 1.8	20.3 ± 3.3
rAFS	35.9 ± 17.2	28.8 ± 8.2	42.3 ± 23.9

comparable among all three groups, with no significant differences observed in age, height, body mass index or rAFS score (**Table 2**).

Three patients each from the GnRHa only and 0.5 mg E₂+P add-back groups failed to complete the follow-up schedule. Among them, one patient in the GnRHa only group withdrew due to thyroid disease, one in the 0.5 mg E₂+P add-back group because they required orally-administered doses, and another four patients did not return for treatment after the first injection. No patients dropped out or withdrew due to adverse reactions. Overall, 32 patients in

both the GnRHa only group and the 0.5 mg E₂+P add-back group, and 11 patients in the 1 mg E₂+P add-back group completed the trial. One patient in the GnRHa only group became pregnant after therapy without first menstruating.

Serum reproductive hormone levels

Serum levels of the four reproductive hormones measured were comparable among all groups at baseline, and decreased significantly in both the GnRHa only and 0.5 mg E₂+P add-back groups at 4, 8 and 12 weeks after treatment compared to baseline. After 4 weeks of treat-

ment, hormones levels in all three groups were mostly stable. In the 1 mg E₂+P add-back group, serum levels of both LH and FSH were significantly decreased at 4, 8 and 12 weeks after treatment compared to baseline, while levels of E₂ and P were not significantly different (Figure 1).

In the GnRHa only group, E₂ serum levels decreased significantly, from 64.39 ± 52.52 pg/mL at baseline to 22.06 ± 32.53 pg/mL at 4 weeks, 18.50 ± 24.00 pg/mL at 8 weeks, and 14.68 ± 14.19 pg/mL at 12 weeks after treatment. Serum levels of FSH were 11.80 ± 8.53 mIU/mL at baseline and were significantly lower at all measured time points after treatment.

In the 0.5 mg E₂+P add-back group, E₂ serum levels were 82.84 ± 82.22 pg/mL at baseline and decreased drastically to 31.32 ± 21.33 pg/mL at 4 weeks after treatment. Levels continued to decrease very gradually to 29.10 ± 17.28 pg/mL at 8 weeks and 25.61 ± 19.36 pg/mL at 12 weeks after treatment. Baseline FSH serum levels were 15.54 ± 15.70 mIU/mL, and were lower than 3 mIU/mL at all measured time points after treatment.

In contrast, no significant differences were observed between pre- and post-treatment serum levels of E₂ and P₄ in the 1 mg E₂+P add-back group. E₂ serum levels were over 45 pg/mL at all measured time points: 58.40 ± 34.86, 46.14 ± 23.50, 52.10 ± 37.91 and 47.70 ± 20.24 pg/mL at baseline and at 4, 8 and 12 weeks after treatment, respectively.

Comparison among groups showed that serum levels of E₂ in both add-back groups were significantly higher than in the GnRHa only group at 12 weeks after treatment. Furthermore, there was a significant difference in E₂ serum levels at 8 and 12 weeks after treatment between the two add-back groups, with the 1 mg E₂+P add-back group showing a more pronounced increase. Serum levels of LH and P₄, however, were not significantly different between the three treatment groups at any of the measured time points, except for LH levels at 8 weeks after treatment between the two add-back groups (Figure 2).

Discussion

GnRHa therapy is commonly used in the post-operative treatment of endometriosis, and can

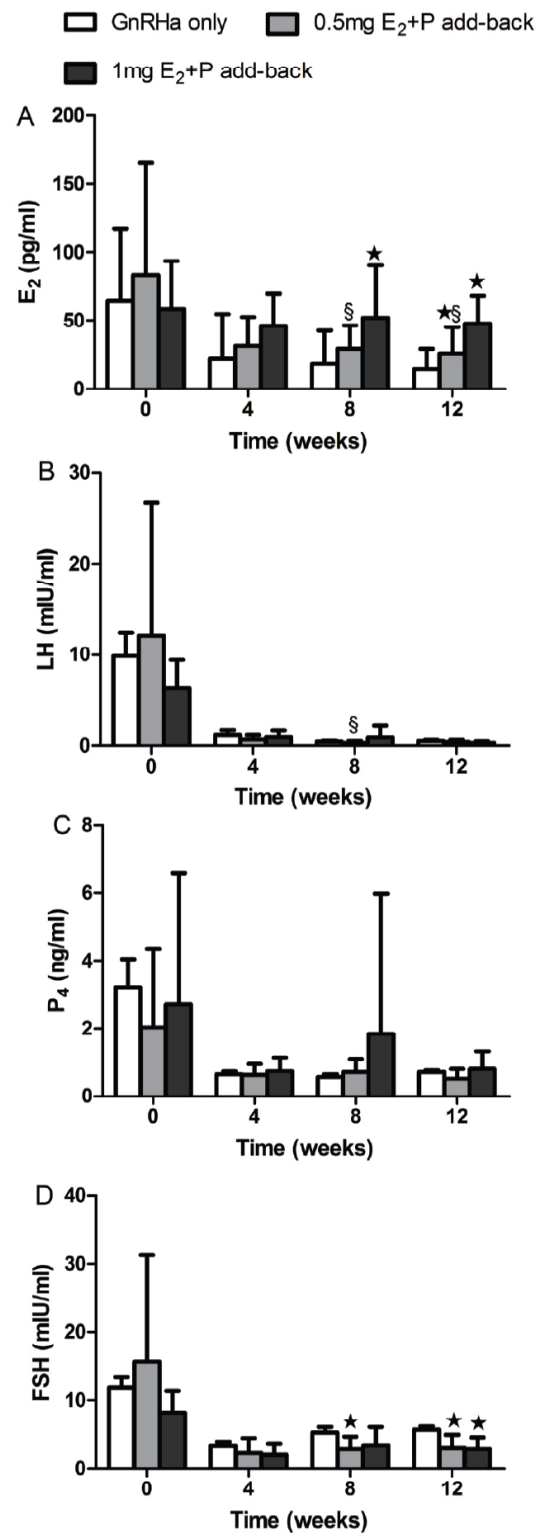


Figure 1. Serum reproductive hormones levels compared among three groups. Values represent mean±SD; ★, p<0.05 vs GnRHa only; §, p<0.05 vs 1mg E₂+P add-back.

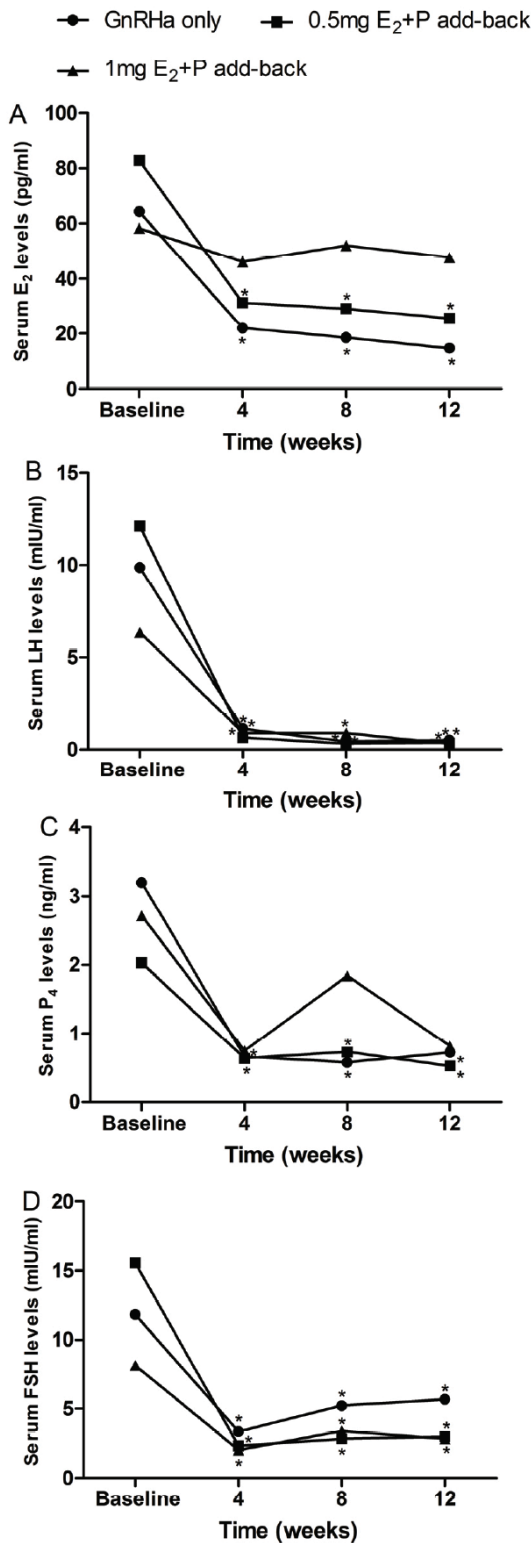


Figure 2. Changes in the levels of the indicated serum reproductive hormones after GnRHa therapy. Values represent mean±SD; *, p<0.01 vs baseline.

both ameliorate disease-related symptoms and inhibit relapse [3-7]. Owing to the hypoestrogenic state caused by long-term use of GnRHa, add-back therapy is often required in order to minimize adverse effects. Typical add-back regimens include the following: low-dose estrogen and progestin combination, estrogen alone, Tibolone, selective estrogen receptor modulators (Raloxifene), as well as others [11]. Importantly, estrogen and progesterone combination add-back therapy can protect bones and prevent climacteric symptoms such as hot flashes and genitourinary tract atrophy [7-10].

It is generally recommended to limit GnRHa therapy to 6 months of at a time, because although the initial dose of GnRHa can cause a transient increase in FSH and LH secretions (the so-called “flare-up effect”), FSH and LH levels decline sharply shortly thereafter. Ovarian hormones levels also decrease, to levels approximating those of menopause. Indeed, estrogen levels in premenopausal women undergoing GnRHa treatment are similar to levels seen in postmenopausal women. Studies have confirmed that add-back therapy with estrogen alone (such as 0.5 mg/d or 1 mg/d estradiol valerate, 0.3 mg/d or 0.45 mg/d Premarin, or 0.3 mg/d esterified estrogen), or with estrogen combined with progestogen can effectively alleviate menopausal symptoms, enhance patient compliance, and prolong GnRHa use [3, 7, 12-16]. On the other hand, add-back therapy with a relatively high dose of estrogen (1.25 mg/d Premarin) combined with progestogen can accelerate dysmenorrheal recovery after drug withdrawal [8].

In our trial, continuous oral add-back therapy with 0.5 or 1 mg/d estradiol valerate combined with 5 mg/d dydrogesterone was initiated concurrently with GnRHa treatment in post-operative Chinese patients with endometriosis. Throughout the 12 weeks of treatment, E₂ serum levels in the 1 mg/d E₂+P group were continuously greater than 45 pg/mL, beyond the threshold range of 30-45 pg/mL (110-165 pmol/L) proposed by Barbieri [17]. E₂ serum levels in the 0.5 mg/d E₂+P group, however, ranged from 25 to 32 pg/mL between 4 and 12 weeks after initiating treatment. According to Barbieri’s estrogen threshold hypothesis [17], E₂ levels ranging between 30 and 45 pg/mL represent the ideal concentrations to promote the atrophy of endometriotic lesions while also

alleviating the side effects due to GnRHa-induced hypoestrogenism, namely the loss of BMD and the occurrence of vasomotor defects. In the GnRHa only group, levels of serum E₂ at 4 to 12 weeks after treatment ranged from 10 to 20 pg/mL; these levels would thus be associated with deleterious hypoestrogenic adverse effects. In the 0.5 mg/d E₂+P add-back group, however, even though the observed E₂ serum levels were slightly lower than the prescribed therapeutic window, they were nevertheless too low to stimulate growth of endometriosis lesions, but sufficient to help relieve pain, decrease the loss of BMD, alleviate menopause-associated symptoms and improve overall quality of life, data showed in the literature [18]. Furthermore, Hornstein *et al.* also reported that endometriotic lesions were effectively inhibited and that pain was alleviated when E₂ levels were maintained under 30 pg/mL [19].

The observed effectiveness of our minimal E₂ dose add-back regimen is probably due at least in part to the fact that add-back therapy was initiated concurrently with GnRHa treatment. If add-back therapy had been delayed until 3 months after GnRHa treatment, a different estrogen dose may have been found to be most effective. In addition, ethnic differences may be another reason as to why the lower dose was most effective.

Baseline serum levels of FSH and LH were not significantly different among the three groups, and all reached a steady state at 4 weeks. Although FSH serum levels were slightly higher in the GnRHa only group than in the two add-back groups, FSH levels in all three groups were still equivalent to levels typical of the follicular phase. We thus speculated that add-back therapy was having an inhibitory action on FSH serum levels. After treatment initiation, LH levels decreased significantly in all three groups and were not significant different among groups for the duration of the study, suggesting that add-back therapy does not affect LH production. However, the specific mechanisms underlying these effects remain incompletely understood and will require further study.

Of note, the estradiol valerate formulation used in the present trial can metabolize into one of the body's natural estrogens (17 β estradiol) after oral administration and is currently widely used in hormone replacement therapy. Dydro-

gesterone is the 6-dehydro reversal isomeride of progesterone and as such its molecular structure is extremely similar to that of natural progesterone [20]. Moreover, in contrast to synthetic depogeston, dydrogesterone has not been shown to increase the risk of breast cancer and is therefore an ideal choice for add-back therapy [21].

On the whole, our trial showed that oral continuous combined 0.5 mg estradiol valerate and 5 mg dydrogesterone as daily, immediate add-back therapy during post-operative GnRH agonist treatment of severe endometriosis may be the most suitable regimen for Chinese women. This combination therapy can not only reliably relieve pain symptoms, reduce bone mass loss, alleviate menopausal symptoms and improve quality of life, but can also minimize overall adverse effects, improve patient compliance and prolong GnRHa treatment duration.

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Address correspondence to: Dr. Shaofen Zhang, The Obs/Gyn Hospital of Fudan University, 128 Shenyang Rd. Shanghai 200090 China. Tel: 0086 13501831796; Fax: 0086 21 63455090; E-mail: zhangshaofen@163.com

References

- [1] Hummelshoj L, Prentice A and Groothuis P. Update on endometriosis. *Womens Health (Lond Engl)* 2006; 2: 53-56.
- [2] Giudice LC and Kao LC. Endometriosis. *Lancet* 2004; 364: 1789-1799.
- [3] Kim NY, Ryoo U, Lee DY, Kim MJ, Yoon BK and Choi DS. The efficacy and tolerability of short-term low-dose estrogen-only add-back therapy during post-operative GnRH agonist treatment for endometriosis. *Eur J Obstet Gynecol Reprod Biol* 2011; 154: 85-89.
- [4] Olive DL. Optimizing gonadotropin-releasing hormone agonist therapy in women with endometriosis. *Treat Endocrinol* 2004; 3: 83-89.
- [5] Batzer FR. GnRH analogs: options for endometriosis-associated pain treatment. *J Minim Invasive Gynecol* 2006; 13: 539-545.
- [6] Olive DL. Gonadotropin-releasing hormone agonists for endometriosis. *N Engl J Med* 2008; 359: 1136-1142.

- [7] Surrey ES. Gonadotropin-releasing hormone agonist and add-back therapy: what do the data show? *Curr Opin Obstet Gynecol* 2010; 22: 283-288.
- [8] Surrey ES and Hornstein MD. Prolonged GnRH agonist and add-back therapy for symptomatic endometriosis: long-term follow-up. *Obstet Gynecol* 2002; 99: 709-719.
- [9] Fernandez H, Lucas C, Hédon B, Meyer JL, Mayenga JM and Roux C. One year comparison between two add-back therapies in patients treated with a GnRH agonist for symptomatic endometriosis: a randomized double-blind trial. *Hum Reprod* 2004; 19: 1465-1471.
- [10] Bedaiwy MA and Casper RF. Treatment with leuprolide acetate and hormonal add-back for up to 10 years in stage IV endometriosis patients with chronic pelvic pain. *Fertil Steril* 2006; 86: 220-222.
- [11] Magon N. Gonadotropin releasing hormone agonists: Expanding vistas. *Indian J Endocrinol Metab* 2011; 15: 261-267.
- [12] Gambacciani M, Cappagli B, Ciaponi M, Pepe A, Vacca F and Genazzani AR. Ultra low-dose hormone replacement therapy and bone protection in postmenopausal women. *Maturitas* 2008; 59: 2-6.
- [13] Langer RD. Efficacy, safety, and tolerability of low-dose hormone therapy in managing menopausal symptoms. *J Am Board Fam Med* 2009; 22: 563-573.
- [14] Stevenson JC, Durand G, Kahler E and Pertyński T. Oral ultra-low dose continuous combined hormone replacement therapy with 0.5 mg 17 β -oestradiol and 2.5 mg dydrogesterone for the treatment of vasomotor symptoms: results from a double-blind, controlled study. *Maturitas* 2010; 67: 227-232.
- [15] Liu J, Allgood A, Derogatis LR, Swanson S, O'Mahony M, Nedoss B, Soper H, Zbella E, Prokofieva SV, Zipfel L and Guo CY. Safety and efficacy of low-dose esterified estrogens and methyltestosterone, alone or combined, for the treatment of hot flashes in menopausal women: a randomized, double-blind, placebo-controlled study. *Fertil Steril* 2011; 95: 366-368.
- [16] Olive DL. The role of add-back therapy in the United States. *Drugs Today (Barc)* 2005; 41: 23-26.
- [17] Barbieri RL. Hormone treatment of endometriosis: the estrogen threshold hypothesis. *Am J Obstet Gynecol* 1992; 166: 740-745.
- [18] Long QQ, Zhang SF, Han Y, Chen H, Li XL, Hua KQ and Hu WG. Clinical efficacy and safety of gonadotropin releasing hormone agonist combined with estrogen-dydrogesterone in treatment of endometriosis. *Zhonghua Fu Chan Ke Za Zhi* 2010; 45: 247-251. [Article in Chinese]
- [19] Hornstein MD, Yuzpe AA, Burry KA, Heinrichs LR, Buttram VL Jr and Orwoll ES. Prospective randomized double-blind trial of 3 versus 6 months of nafarelin therapy for endometriosis associated pelvic pain. *Fertil Steril* 1995; 63: 955-962.
- [20] de Kraker AT, Kenemans P, Smolders RG, Kroeks MV and van der Mooren MJ. The effects of 17 beta-oestradiol plus dydrogesterone compared with conjugated equine oestrogens plus medroxyprogesterone acetate on lipids, apolipoproteins and lipoprotein(a). *Maturitas* 2004; 49: 253-263.
- [21] Gadducci A, Biglia N, Cosio S, Sismondi P and Genazzani AR. Progestagen component in combined hormone replacement therapy in postmenopausal women and breast cancer risk: a debated clinical issue. *Gynecol Endocrinol* 2009; 25: 807-815.