参考資料1

日健栄協第３号
平成１８年1月１２日

食品安全委員会
委員長　寺　田　雅　昭　先生

財団法人　日本健康・栄養食品協会
理事長　細　谷　憲　政

大豆イソフラボンの安全性に関する情報提供の件

現在貴委員会で検討されている大豆イソフラボンのホルモン作用に関しまして、当協会「大豆イソフラボン加工食品作業部会」の有志による意見書及び情報提供をさせて頂いております。

この度、第 30 回貴委員会新開発食品専門調査会で出されました『大豆イソフラボンを含む特定保健用食品の安全性評価の基本的な考え方』（第 30 回会合修正案）に関し、当協会「大豆イソフラボン加工食品作業部会」の有志から新たな情報提供と意見が別添のとおり当協会宛提出されました。

つきましては、「大豆イソフラボン加工食品作業部会」からの願い書及び参考文献等を、下記の通り情報提供として提出いたしますので、ご査収の上よろしくお取り計らい賜りますようお願い申し上げます。

記

大豆イソフラボンを含む特定保健用食品等の安全性評価にあたってのお願い
：財団法人日本健康・栄養食品協会　大豆イソフラボン食品作業部会、
平成 18 年 1 月 12 日付け

以　上

平成18年1月12日

財団法人　日本健康・栄養食品協会
理事長　細　谷　憲　政　殿

財団法人　日本健康・栄養食品協会
大豆イソフラボン加工食品作業部会

大豆イソフラボンを含む特定保健用食品等の安全性評価にあたってのお願い

拝啓　時下，ますますご清祥のこととお慶び申しあげます。

現在，内閣府食品安全委員会新開発食品専門調査会において検討されております大豆イソフラボンの安全性評価について，貴協会を通じ当作業部会よりこれまでに平成17年7月5日付，9月12日付で意見書を提出いたしております。

さて，度重なるお願いとなりますが，去る12月12日に行われました第30回会合の資料「大豆イソフラボンを含む特定保健用食品の安全性評価の基本的な考え方（第30回会合修正案)」を拝読し，国際的な動向も踏まえ是非とも下記の件についてご検討いただきたいと存じ，本意見書を提出いたします。

どうかご高配賜りますよう，よろしくお願い申しあげます。

記

Ⅰ．大豆イソフラボンの安全な一日摂取目安量の上限値について

健康被害発現量であることが予想されたUnferらの大豆イソフラボン150 mg/日の5年間の摂取試験（文献96）の結果に関しては，現在，多くの議論が行われております。

1．Fothらは，Unferらの論文に対して，試験開始時における子宮内膜増殖症者をどのように除外したのかについて疑問を呈しています[1)]。

2．Ariciらは，異型のない子宮内膜増殖症はサンプリングの時期が評価に影響する場合があること，また，Unferら論文には投与による異常出血などの報告がなく，ほとんどが単純増殖症であったことから，それらはイソフラボンの弱いエストロゲン様の作用を示すものであり，癌につながるリスクは低いと思われることを述べています[2)]。

3．米国Medscapeメディカルニュースでは，Unferらの論文を精査し，5年間にわたるイソフラボン150 mg/日の摂取は，子宮内膜増殖症のリスクをわずかに増加させるかもしれないが，子宮内膜悪性腫瘍の発症と関係しないと結論付けています[3)]。

4．文献96の著者であるUnferらは，その後の研究でイソフラボン1500 mg/日の大量投与が不妊症治療に有効で妊娠率が有意に高まり，問題となる事象はなかったことを報告しています[4,5]。

5．産婦人科専門医[注]の見解でも，「（1）なぜ，一日摂取量の仮の上限値を70～75 mgと設定する必要があるのか。（2）なぜ、子宮内膜癌の診断もなく子宮内膜の増殖が多少あるだけで，あたかもそれが危険であるごとく，健康危害発現量を定めているのか疑問である。不正出血など何らかの臨床症状があるのか。子宮内膜の増殖は，不妊症などではポジティブな場合もあり，一概にこの増殖が悪いとは限らない」ということです。

Ⅱ．特定保健用食品としての大豆イソフラボンの一日上乗せ摂取量について

国民栄養調査に基づく大豆イソフラボンの摂取量の分布からも明らかなように，50パーセントタイル値や最頻出値は平均値と比較して低いものになっています。この摂取量の分布やばらつきは日常的に多量に摂取している人，または，全く摂取していない人の割合を示すものではないと解説されていますが，平成8年の国民栄養調査では週にどのくらいの頻度で大豆・大豆製品を摂取しているかが調べられており，それによると，総数では，ほとんど食べない人が10.5%，週に2～3回が43.3%，週に4～5回が23.7%，ほとんど毎日が22.4%となっています。これは，個人の摂取量のバラツキが大きく，日常的に摂取量が平均値以下の人が多数存在することが示されていると考えられます。

従って，特定保健用食品としての大豆イソフラボンの一日上乗せ摂取量を一律に30mgと規定してしまうことは，多数存在する大豆食品からの摂取量が少ない人の日常食生活の摂取量の応じた適正なサプリメント活用を阻害し，健康の維持・増進において好ましくない影響を与える可能性があると考えられます。

Ⅲ．公表に際してのお願い

一般の大豆食品と大豆イソフラボンの抽出物を添加した食品を明確に区別した評価であることは十分理解していますが，適切な情報発信がなされないと国民に大きな誤解を与えることは必至です。特に一日摂取目安量の上限値である70～75 mgは，具体的には納豆（50 g/パック）ではおよそ2パック，豆乳（200 g/パック）ではおよそ1.5パックに相当する量であり，前回の食品安全委員会新開発食品専門調査会の報道でも消費者の誤解を招いたこともあり，どうしても大豆食品の安全性に危惧の念を抱かせます。

そのために，食品安全委員会ならびに厚生労働省におかれましては，国民に誤解を与えることのないよう適切な情報発信をしていただくと共にマスコミに対する指導をお願いいたします。

また，大豆食品は日本食の中心的存在であり，国民の健康に大きく貢献していることから食育の観点からも重要な位置を占めています。日本が世界に誇るべき大豆食文化を自ら破壊することがないよう十分な議論とその適切な公表をお願いします。

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以上

注）見解をいただいた産婦人科専門医の先生

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参考1)

We also searched for the presence of *C. trachomatis* DNA by polymerase chain reaction using DNA extracted from the paraffin-embedded tissue from the hydrosalpinges and endometrium. All of the hydrosalpinges were positive for chlamydia DNA. Chlamydia DNA was not found in the endometrium (data not shown). These findings suggest that downstream fluid might not cause *C. trachomatis* infection in endometrium. This is a very important conclusion of this study. Additional antibiotic therapy might not resolve lymphocyte infiltration in the endometrium without salpingectomy, because it might not be caused by endometritis. Salpingectomy is a prerequisite to eliminate the lymphocyte infiltration in the endometrium, despite the appearance of hydrosalpinges after chronic *C. trachomatis* infection.

The persistent, residual cloistering of lymphocytes in the endometrium in the absence of microorganisms seemed to be overlooked. Immunostaining for CD56, CD8, and CD3 has demonstrated pathologic infiltration in all the tested cases. Clustered infiltration and positive immunostaining was absent in all control cases (data not shown). We could not demonstrate the mechanism of implantation failure from studies of these lymphocytes. However, immunohistopathologic study of the endometrium is a good monitoring method to study implantation failure. The major problem of hydrosalpinges is tubal infertility, but implantation failure during IVF-ET is frequently encountered. It is difficult but important to know whether hydrosalpinges caused by mechanisms other than chronic *C. trachomatis* infection evoke implantation failure.

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Effect of phytoestrogens on the endometrium?

To the Editor:

We read with interest the article by Unfer et al. (1) and would like to further comment on it. The authors present the longest randomized clinical trial to determine the endometrial effects of 150 mg of isoflavones per day. They concluded that long-term treatment with soy isoflavones was associated with an increased occurrence of endometrial hyperplasia in postmenopausal women. This result is contradictory to further studies.

Epidemiologic investigations indicate a relatively low endometrial cancer risk in populations that consume high amounts of soy. In long-term studies, 129 mg isoflavones per day did not stimulate uterine proliferation in postmenopausal monkeys. The cynomolgus monkey (*Macaca fascicularis*) model offers a unique opportunity for the study of hormonal and dietary effects on the endometrium (2, 3).

In previous placebo-controlled, randomized clinical trials of shorter duration, no increase of endometrial thickness and no histologic changes of the endometrium were found under isoflavone supplementation.

The time around menopause is characterized by a high incidence of endometrial bleeding disorders and hyperplasia. In the study by Unfer et al. (1), only postmenopausal women with intact uterus, absence of menses for $\geq$12 months, and FSH $\geq$30 IU/L were included. The mean age of menopause is nearly 51 years in European women. The age at start of treatment and mean age at menopause of participants were nearly 50 years, but at baseline patients were 5.7 $\pm$ 4.4 years beyond menopause.

On the other hand, in the study by Unfer et al. (1), approximately 25% of endometrial tissue at baseline and after 30 months of treatment was classified as unassessable.

Judging from patient numbers, it seems that after 5 years of treatment all patients continued to participate in the study. Our question is, how was endometrial hyperplasia at baseline excluded in these patients? Endometrial biopsy with a Pipelle is superior to other techniques in the detection of postmenopausal endometrial carcinoma and atypical hyperplasia (4). However, the number of successful biopsies varies in the literature and from clinical experience between 70% and 100%. Additional information on the endometrium, such as endometrial thickness or bleeding patterns, is not given. A baseline sample that is classified as insufficient does not exclude uterine pathology.

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Reply of the Authors:

We appreciate the comments from Drs. Foth and Nawroth regarding our study (1). We agree that some epidemiologic studies indicate a relatively low endometrial cancer risk in populations that consume high amounts of soy. On the other hand, there is no general consensus concerning the real daily quantity of soy intake in those populations. Recent studies have questioned the previously calculated values attributed to soy consumption in the Asian population and discovered that it seems to be lower. Furthermore, daily intake should comprise between 3 mg and 28 mg per day (2, 3). Consequently, we should re-evaluate the importance of other factors that might play a role in protecting those populations from endometrial cancer. It is well known that phytoestrogens can act as estrogenic agonists or antagonists. The characteristics of this action rely on many factors, including dosage, duration of use, protein binding affinity, individual metabolism, and intrinsic estrogenic state, as well as the distribution of the estrogen receptor subtypes at the tissue level. This could be the reason that the results concerning the effects of phytoestrogens on the uterus are contradictory. Cline et al. (4) recently demonstrated a dramatic estrogenic effect of soy isoflavone aglycones in both male and female mice. Effects in females included endometritis and effects typical of estrogenic stimulation (i.e., uterine enlargement, keratinization of vaginal epithelium, increased height of endometrial surface epithelial cells, and uterine squamous metaplasia).

In our work, we classified the following as unassessable: no tissue identified; tissue insufficient for diagnosis; no endometrium identified; and endometrium tissue insufficient for diagnosis. Our results correspond to the majority of studies that used Pipelle for biopsies, considering that our patients were in menopause for 12 months. However, all patients underwent vaginal sonography both at the beginning and periodically during the study (data not shown). The results of these examinations were compatible with those obtained by vaginal biopsies. Although we did not find a statistically significant difference in the percentage of unassessable biopsies, a tendency to lower values was found in group A after 5 years. These findings could be in accordance with the hypothesis of an estrogenic effect exerted by phytoestrogens. In conclusion, we would like to emphasize again the necessity to perform more studies to better investigate the in vivo effects and the pharmacology of phytoestrogens (5).

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Erratum

An error occurred in the report, *"Revised guidelines for human embryology and andrology laboratories,"* by the Practice Committee of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology (Fertil Steril 2004;82:1736–53). On page 1752, first column, under "J." the first sentence was incomplete and should read:

"There must be sufficient space available for working."

doi:10.1016/j.fertnstert.2004.09.005

参考2)

Phyto-oestrogens and the endometrium

Vittorio Unfer and colleagues[1] recently reported a 5-year randomised double-blind study on the endometrial effects of the use of soy phyto-oestrogen in postmenopausal women. Of 154 patients receiving 150 mg isoflavones a day for 5 years, six (4%) developed endometrial hyperplasia (five simple, one complex; no endometrial hyperplasia with atypia), compared with none of 165 patients on placebo. The same group[2] also reported a randomised trial of high-dose phyto-oestrogens added to clomiphene citrate, to reverse the antioestrogenic effects of clomiphene on the endometrium during intrauterine insemination. The endometrium was thicker, miscarriage rates lower, and ongoing pregnancy rate higher in patients on phyto-oestrogen plus clomiphene than in those on clomiphene alone.

These findings are neither surprising nor alarming, confirming what we know about phyto-oestrogens acting as a selective oestrogen-receptor modulator (SERM).[3] Soy phyto-oestrogens (isoflavones genistein, daidzin, and glycitin) induce endometrial stromal-cell proliferation in vitro.[4] The proliferative effect occurred at high concentrations and was 8–15% lower than that induced by oestradiol. In the presence of oestradiol, however, the isoflavones antagonised the proliferative effect of oestradiol by 10–20%, which indicates that isoflavones are weak oestrogens and their antioestrogenic effects are only seen in high concentrations when combined with physiological concentrations of oestradiol. Animal models also show that high doses of isoflavones, particularly genistein, stimulate uterine growth and expression of several genes regulated by uterine oestrogen, and isoflavones have weak oestrogenic activity in mammary gland and hypothalamic/pituitary cells.

Any clinical application of these findings might result in different outcomes, possibly due to various doses and preparations used for isoflavones and individual differences in metabolism. A 4-week study of soy-supplemented diet in postmenopausal women did not have oestrogenic effects in liver and pituitary-gland function.[5] In a small randomised trial, 25 mg soy-protein isolate daily with 120 mg isoflavones added to oestradiol for 6 months did not protect endometrium from oestradiol-induced hyperplasia.[6]

The natural history of endometrial hyperplasia is not fully understood and some simple and complex hyperplasias will regress without treatment.[7] However, the time over which regression occurs is unknown, as is the time from a normal endometrium progressing to hyperplasia. False-positive diagnoses of endometrial hyperplasia might be due to the sampling during natural regression, and false-negative diagnoses are possible when sampling is done during progression of benign or atrophic endometrium to hyperplasia.[7] Untreated simple hyperplasia without atypia sometimes progress to carcinoma, and this risk might be greater with complex hyperplasia.[8] Untreated hyperplasia with atypia is more likely to progress to endometrial cancer.[8,9] Hyperplasia without atypia tends to spontaneously regress, whereas atypical hyperplasias are more likely to progress.[10] Concomitant endometrial carcinoma is also more frequent in the presence of atypia.

Unopposed moderate or high-dose oestrogen therapy in women with an intact uterus is associated with a significant increase in rates of endometrial hyperplasia, with increasing rates at longer duration of treatment and follow-up.[11] In the 3-year PEPI trial,[12] in 119 women randomised to 0·625 mg conjugated equine oestrogen daily, 74 (62%) developed some type of hyperplasia. In 119 women on placebo,

however, three women (3%) developed endometrial hyperplasia and carcinoma. 66% of women on unopposed oestrogen had unscheduled endometrial biopsies because of abnormal uterine bleeding. Unfer and colleagues[1] make no mention of findings with unopposed oestrogen. Possibly there was no abnormal uterine bleeding in patients on isoflavones because they do not describe any unscheduled biopsies. Endometrial hyperplasia was first detected after 5 years and most of the cases had simple hyperplasia, which confirms the weak oestrogenic effect of isoflavones on the endometrium. The risk for postmenopausal women to develop an endometrial cancer when on chronic soy supplementation seems to be low. Nevertheless, there is one case of endometrial cancer in a woman who was taking excessively high amounts of isoflavones.[13]

As may be true for all weak agonists, the effects of isoflavones seem to be dependent on dose and the duration of treatment. In a small 12-week randomised study, women on 40 and 160 mg isoflavones did not show any difference in frequency of climacteric symptoms, vaginal pH, and blood tests compared those on with placebo.[14] Another 12-week study used 90 mg isoflavones daily and found no benefit in hot flashes.[15] A 4-month study of 150·9 mg soy protein and 100 mg isoflavones showed some relief of menopausal symptoms and decreased total cholesterol and low-density lipoprotein levels.[16] Isoflavones 150 mg daily for 4 weeks did not give symptomatic benefit.[17] A 6-month study found that 72 mg soy-derived isoflavones daily was no more beneficial than placebo in reducing hot flashes; endometrial thickness was also not affected by isoflavones.[18] Unfer and colleagues' study[1] should be further analysed for any long-term effects of isoflavones on climacteric complaints, bone-mineral density, and blood tests, including lipid profile.

Discrepancies between studies might also be due to differences in the type and quantity of isoflavones used. Because botanicals are defined as dietary supplements and are not regulated as drugs, they can vary greatly in production and amounts of active ingredients.

Research into prevention of menopausal effects has been directed to finding ideal SERMs with activities in the brain to prevent climacteric complaints, in the bone to prevent osteoporosis, and in the vagina to prevent atrophy-related complaints, but without the stimulating effects of oestrogens on the breast and endometrium. Apart from aggressive commercial advertisements for botanicals, the scientific interest in phyto-oestrogens mostly originated from the hope for an ideal SERM. Some developments are already on their way. For example, pharmaceutical-quality ipriflavone (synthetic genistein) is approved in Europe and Japan for the treatment of osteoporosis.[19] Before making a recommendation, the long-term risks and benefits of phyto-oestrogens need to be known.

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We declare we have no conflict of interest.

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Medscape Medical News

Soy Supplements Linked to Endometrial Hyperplasia in Some Women CME

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Complete author affiliations and disclosures, and other CME information, are available at the end of this activity.

Release Date: July 9, 2004; Valid for credit through July 9, 2005

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July 9, 2004 — Soy (phytoestrogen) supplements induce endometrial hyperplasia in a statistically significant but small group, according to the results of a five-year randomized trial published in the July issue of *Fertility & Sterility*.

"Some clinical studies on the effects of phytoestrogens on the endometrium in postmenopausal women have been carried out during the past few years, but their results are discordant," write Vittorio Unfer, MD, from the Obstetrics and Gynecology Centre in Rome, Italy, and colleagues.

In this double-blind study, 376 healthy postmenopausal women, all with intact uterus, were randomized to receive soy tablets containing 150 mg of isoflavones per day or placebo tablets for five years. The main outcome measure was endometrial histology from biopsies obtained at baseline, 30 months, and five years after beginning treatment.

Of the 376 women, 298 women completed the five-year study, and none had malignancy detected on biopsy. Endometrium was classified as atrophic or nonassessable in 70% of women receiving soy and in 81% of women receiving placebo. Endometrial hyperplasia occurred in 3.37% of women receiving soy and in none of the women receiving placebo ($P < .05$).

Although the Italian Ministry of Health recommends that isoflavone supplements not be given at dosages greater than 80 mg/day, the dosage in this study was 150 mg/day.

"Long-term treatment (up to 5 years) with soy phytoestrogens was associated with an increased occurrence of endometrial hyperplasia. These findings call into question the long-term safety of phytoestrogens with regard to the endometrium," the authors write. "We need more information on the effects of these extracts on the endometrium. However, phytoestrogenic supplements should be reconsidered, particularly in women at high risk for endometrial cancer."

Fertil Steril. 2004;82:145-148

Learning Objectives for This Educational Activity

Upon completion of this activity, participants will be able to:

- Compare the incidence of endometrial hyperplasia in women receiving phytoestrogens compared with those taking placebo for five years.
- Describe the potential effects of long-term phytoestrogens on the endometrium.

Clinical Context

Phytoestrogens are nonsteroidal estrogen compounds found in plants and offered in dietary supplements. They are purported to provide protection against estrogen-dependent cancers, such as breast cancer, and are used for a variety of conditions including menopausal symptoms. Results of studies conducted on postmenopausal women are conflicting with regard to phytoestrogens effects on the endometrium. Both agonistic and antagonistic effects on the estrogen receptor have been described. In a study published in the October 1999 issue of the *Journal of Clinical Endocrinology and Metabolism*, Duncan and colleagues demonstrated the absence of clinical effects on the endometrium of women taking a soy isoflavone-enhanced diet for 90 days. Hale and colleagues showed in a study reported in the September 2001 issue of *Menopause* that 30 months of treatment using 50 mg of red clover isoflavones a day did not increase the incidence of endometrial hyperplasia or increase endometrial thickness. Most isoflavones are given at a dosage of 80 mg/day or less.

This is the longest randomized, double-blinded controlled trial examining the effects of five years of isoflavone supplementation at a dose of 150 mg daily on the endometrial histology of healthy volunteer postmenopausal women who are not receiving estrogen replacement therapy.

Study Highlights

- 376 healthy postmenopausal women not receiving estrogen were randomized to receive orally either 150 mg isoflavones (consisting of 40% to 45% genistein, 40% to 45% diadzein, and 10% to 20% glycitein) daily (n = 179) or identical-appearing placebo for 5 years (n = 197).
- Inclusion criteria were intact uterus, no menses for at least 12 months, follicle-stimulating hormone level of 30 IU/L or more and body weight no more than 20% of "normal" weight.
- Exclusion criteria were use of estrogen, progestin, or androgens within 8 weeks and presence of endometrial hyperplasia.
- All women had a baseline history, physical examination, and endometrial biopsy by Pipelle endometrial suction. Samples were all read by a single pathologist blinded to assignment who classified them by standard criteria.
- Primary outcome was incidence of endometrial hyperplasia.
- Baseline menopausal symptoms (if any), dietary habits, and intake of isoflavones was not reported. Medication adherence was not documented. Use of other dietary supplements or hormonal medications during the 5-year study was not reported.
- The power of the study and the projected sample size required to meet the hypothesis were not given.
- 298 (79%) of 376 women completed the 5-year study. Reasons for dropout were not given.
- Endometrial biopsies were available for 376 women at baseline, 369 at 30 months, and 319 after 5 years of treatment. The disposition of the remaining 57 women was not given.
- 70% of women in the isoflavone group had biopsies that were atrophic or nonassessible compared with 81% in the placebo group.
- At baseline, the two groups were similar for age (50 years), weight (164 kg), mean age at menopause (50 years), and parity (2.7). Presence or absence of menopausal symptoms was not described.
- At 30 months of therapy, there were no cases of hyperplasia or malignancy in either group.
- At 5 years, there were 6 cases (3.37%) of hyperplasia (five simple and one complex) in the isoflavone group and none in the placebo group. This difference was statistically significant at $P < .05$.
- There were no cases of endometrial carcinoma.

Pearls for Practice

- Long-term oral isoflavones at 150 mg daily compared with placebo may slightly increase the risk of endometrial hyperplasia in women not receiving hormone replacement therapy.
- Use of isoflavones at 150 mg daily for five years is not associated with endometrial malignancy.

Instructions for Participation and Credit

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This article is intended for primary care physicians, gynecologists, gerontologists, and other specialists who care for postmenopausal women.

Goal

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IN VITRO FERTILIZATION

Phytoestrogens may improve the pregnancy rate in in vitro fertilization–embryo transfer cycles: a prospective, controlled, randomized trial

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Objective: To compare the effectiveness of IM P and IM P plus oral phytoestrogens for luteal phase support in patients undergoing IVF-ET cycles.

Design: Prospective, controlled, randomized trial.

Setting: University Hospital, Perugia, Italy.

Patient(s): Two hundred thirteen infertile patients undergoing IVF-ET were included in the study. The inclusion criteria were use of a GnRH agonist for pituitary down-regulation and age <40 years. The total number of cycles performed was 274.

Intervention(s): Patients were assigned to receive either IM P (50 mg daily) plus placebo or P (50 mg daily) plus phytoestrogen supplementation (1,500 mg daily) for luteal phase support starting from the evening of oocyte retrieval until either a serum pregnancy test result was negative or embryonic heartbeat was sonographically confirmed.

Main Outcome Measure(s): The outcomes of IVF-ET were evaluated in both study groups in terms of implantation rate, biochemical pregnancy rate (PR), clinical PR, spontaneous abortion rate, and ongoing pregnancy/delivered rate.

Result(s): Statistically significant higher values for implantation rate, clinical PR, and ongoing pregnancy/delivered rate were recorded in the patients who received P plus phytoestrogens for luteal phase support in comparison with patients receiving P and placebo.

Conclusion(s): Although the results of this study encourage the use of phytoestrogens for luteal phase support in patients undergoing IVF-ET program, more studies are necessary to support the hypothesis that phytoestrogens have a beneficial effect in IVF cycles. (Fertil Steril® 2004;82:1509–13. ©2004 by American Society for Reproductive Medicine.)

Key Words: Phytoestrogens, progesterone, IVF-ET cycles, luteal phase support

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The major limiting step in the establishment of a successful pregnancy is implantation (1–4). It requires complex transformations of the endometrium, which begin in the proliferative phase and go on through the luteal phase (5). In IVF cycles, the E_2 of ovarian origin, acting on endometrial tissues, determines the stage of endometrial development that is reached in the follicular phase (6). This action becomes clinically evident with the increase of endometrial thickness and echogenicity, which are considered markers of endometrial receptivity.

Serum levels of E_2 and P begin to decline from the midluteal phase in IVF-ET cycles in which pituitary suppression is used to obtain controlled ovarian hyperstimulation (COH). Presently, luteal phase support with P is used routinely in IVF-ET cycles (7–13). This therapeutic approach is based on the observation that P supplementation increases the implantation rates in IVF cycles in which a pituitary down-regulation protocol is used. Usually, P supplementation is given as a daily IM injection of P in oil (50–100 mg). By

contrast, the importance of E_2 supplementation is still controversial.

Few studies have been conducted to evaluate the efficacy of E_2 in improving the implantation rate in IVF cycles when GnRH agonist (GnRH-a) is used for pituitary down-regulation (14, 15). Some consideration can be given to support the important role E_2 plays during the luteal phase in the preparation of the endometrium for implantation. During the natural cycles in fertile women, midluteal phase serum E_2 levels are significantly higher in conception cycles compared with nonconception cycles (16–18). The depletion of E_2 during the luteal phase has a negative effect on implantation in women undergoing oocyte donation (19). An association between elevated and steadily increasing serum E_2 levels in the luteal phase of IVF-ET cycles and higher pregnancy rates has also been reported (20–23). All this evidence demonstrates the positive correlation existing between elevated E_2 levels in the luteal phase and conception.

Many plants produce isoflavones that possess estrogenic activity in animals and are thus called phytoestrogens (PEs). Phytoestrogens are nonsteroidal compounds present in a variety of dietary products (24). Some epidemiological studies have also demonstrated that the ingestion of food that is rich in PEs may provide a protection against certain estrogen-dependent cancers, such as breast and prostate cancers (25, 26). Phytoestrogens continue to be of increasing interest because of their possible influence on the physiology of the reproductive tract (27). A brief summary of the studies that have investigated the estrogenic effects of PEs as well as their ability to bind the estrogen receptors (ERs) has already been presented in previous work by our group (28).

Despite the evidence that PEs have an estrogenic-like action on experimental animal models and on in vitro models, contradicting results emerged from recent clinical studies of the effects of PEs on endometrium in postmenopausal women (29–31). We have hypothesized that the dosage of PEs administered in these studies could be too low to determine any estrogen-like effect on the endometrium. In two previous studies in which a higher dosage of PEs was used, we demonstrated an evident estrogenic-like effect of PEs on endometrium. This was evident both in long-term treatment in postmenopausal women (32) and in women undergoing IUI (for whom PEs reversed the anti-estrogenic effects of clomiphene citrate) (28).

The aim of this prospective, controlled, randomized study was to compare the outcome of IVF-ET cycles in which either IM P alone or IM P combined with high dosages of PEs were used for luteal phase support when a GnRH-a was used.

MATERIALS AND METHODS

Patients

All patients treated in our IVF units between January 2000 and September 2002 were asked to participate in the study. The inclusion criteria were the use of GnRH analogue for pituitary down-regulation and age <40 years. Patients received either IM P plus placebo (P + placebo) or P plus PE supplementation (P + PE) according to a randomization table. The Institutional Review Board approved the protocol, and all patients gave written informed consent before entering the study.

Patients were prescribed either P in oil (50 mg IM daily) plus placebo tablets or P in oil (50 mg IM daily) plus PE in tablets (1,500 mg daily) starting on the evening of oocyte retrieval. Phytoestrogens were in tablet form containing 1,500 mg of soy isoflavones per tablet. The composition in isoflavones was 40%–45% by weight of genestein, 40%–45% diadzein, and 10%–20% glycitein.

Controlled Ovarian Hyperstimulation

All patients underwent pituitary desensitization by SC administration of a GnRH-a (400 μg twice daily) from day +20 of the previous menstrual cycle until the IM administration of hCG (10,000 IU). Then COH was performed in all patients by administration of urinary FSH (uFSH). Patients were monitored by measuring the plasma concentration of 17β-E_2 and the size of follicles on days +5, +7, and +12 of the stimulation. The amount of gonadotropin administered was adjusted according to the individual response. Human chorionic gonadotropin (10,000 IU) was injected IM in all patients when serum 17β-E_2 exceeded 200 pg per follicle and there were at least three follicles with a minimum diameter of 18 mm.

In Vitro Procedure

Oocytes were retrieved 34–36 hours after hCG administration by transvaginal echo-guided aspiration. In vitro fertilization medium (Medi-Cult A/S, Innogenetics, Denmark) was used as the culture medium. Spermatozoa were prepared using the swim-up technique. All cases underwent conventional IVF techniques with gametes and embryos cultured under oil. The ET was performed at the 2- to 4-cell stage 40–44 hours after insemination. No more than three embryos were transferred.

Luteal Phase

On the evening of oocyte retrieval, all patients were randomly allocated to two groups:

Group A (P + PE) (n = 115): IM administration of P in oil (50 mg daily) plus PE (1,500 mg daily).

Group B (P + placebo) (n = 98): IM administration of P in oil (50 mg daily) plus placebo tablets.

Both treatments were continued until either a serum pregnancy test result was negative or embryonic heartbeat was sonographically confirmed.

TABLE 1

Characteristics of patients who received P + phytoestrogens (group A) or P + placebo (group B).

Variable	Group A	Group B	*P*
No. of patients	115	98	—
No. of cycles	155	129	—
Mean (±SD) age (y)	31 ± 5.1	29 ± 4.9	NS
Mean (±SD) duration of infertility (mo)	46.1 ± 18.5	37.7 ± 9.6	NS
Body mass index	26.7 ± 7.5	26.3 ± 6.8	NS
Causes of infertility			
Ovulatory factor[a] (%)	11 (9.6)	9 (9.2)	NS
Endometriosis (%)	3 (2.6)	3 (3.1)	NS
Male factor (%)	47 (40.9)	41 (41.8)	NS
Tubal factor (%)	36 (31.3)	34 (34.7)	NS
Unexplained (%)	18 (15.7)	11 (11.2)	NS

Note: No statistical differences were found between groups, thus *P* values ($P<.05$) are not shown. NS = not significant.

[a] Polycystic ovaries, clomiphene = resistant, anovulatory/normogonadotropic.

Unfer. PE and pregnancy rate in IVF-ET cycles. Fertil Steril 2004.

Concentrations of β-hCG, P, and 17β-E_2 were measured by commercially available methods (RSL-E_2 and RSL-P4 radioimmunoassays, ICN Biomedical, Costa Mesa, CA; Tandem hCG, Hybritech, San Diego, CA). Interassay and intra-assay coefficients of variation never exceeded 5% and 8%, respectively.

Determination of Pregnancy States

A biochemical pregnancy was defined as a small and transitory increase in β-hCG levels. A clinical pregnancy was determined by the visualization of an embryo with cardiac activity at 6–7 weeks of pregnancy. Spontaneous abortion was classified as the loss of the pregnancy between the 5th and 12th week of gestation. Ongoing pregnancies were those reaching 20 weeks of gestation.

Statistical Analysis

A commercial statistical software package (SPSS KIT SigmaStat for Windows, version 2.03S; SPSS, Chicago, IL) was used for data analysis. Clinical characteristics were analyzed using the unpaired Student's *t*-test or the Mann-Whitney rank sum test. All other analyses were performed using χ^2 analysis of Fisher's exact test. $P<.05$ was considered statistically significant.

RESULTS

During the study period, 213 patients conforming to the inclusion criteria were randomized into two groups as previously described. Group A (P + PE) consisted of 155 cycles (n = 115) and group B (P + placebo) consisted of 129 cycles (n = 98). No differences were found between the two groups in mean age, body mass index, and duration of infertility (Table 1). In addition, the causes of infertility did not differ after randomization in the two groups. Progesterone and 17β-E_2 plasma levels were measured throughout the luteal phase. Estradiol and P levels were similar in both groups (data not shown).

No differences were found in the mean number of oocytes retrieved, the mean number of oocytes fertilized, or the mean number and quality of embryos transferred (data not shown).

The outcome of IVF in both study groups was evaluated for implantation rate, biochemical PR, clinical PR, spontaneous abortion, and ongoing/delivered pregnancies (Table 2). Statistically significant differences were found in implantation rate, clinical PR, and ongoing pregnancy/delivered rates, with all three parameters being higher in the P + PE group. The administered dosage of PEs was well tolerated by all patients, and no adverse effects were recorded.

DISCUSSION

The high incidence of luteal defects in patients undergoing a down-regulation protocol for an IVF program may possibly be related to the heterogeneous population of follicles during the time of ovulation induction; under these circumstances the P synthesized by the smaller follicles is probably less than that synthesized by follicles ≥18 mm in diameter.

TABLE 2

Pregnancy outcome of patients who received phytoestrogens + P and P + placebo (percentages are in parentheses).

Variable	Group A	Group B	*P*
Implantation rate (%)	115/452[a] (25.4)	79/390[a] (20.2)	<.05
Biochemical PR (%)	3/155[b] (1.9)	3/129[b] (2.3)	NS
Clinical PR (%)	61/155[b] (39.3)	27/129[b] (20.9)	<.05
Spontaneous abortion rate (%)	4/61 (6.5)	2/27 (7.4)	NS
Ongoing pregnancies/delivered PR (%)	47/155[b] (30.3)	21/129[b] (16.2)	<.05

Note: NS = not significant; PR = pregnancy rate.

[a] Total no. of embryos transferred.

[b] No. of cycles.

Unfer. PE and pregnancy rate in IVF-ET cycles. Fertil Steril 2004.

Smaller follicles that still have not reached full maturity at the time of the hCG injection may become luteinized at an immature stage, which may cause an early and unavoidable demise of the corpus luteum itself. This demise can lead to a relative decline in P levels and the P/E_2 ratio between implantation and the luteo-placental shift and can cause premature resumption of uterine activity. These events may possibly cause the loss of the pregnancy.

According to the hypothesis stated above, P supplementation of the luteal phase is routinely prescribed to women undergoing IVF. The therapeutic need to support the luteal phase with E_2 remains debatable, even though the endometrial development in IVF cycles (in which a down-regulation protocol with GnRH-a is used) depends on ovarian E_2 production (see the introduction). A few studies on the improvement of PRs and implantation rates were carried out to evaluate the effectiveness of E_2 supplementation in IVF cycles. The results were discrepant (14, 15).

In previous work of our group, we showed that PEs could have an estrogenic-like effect on endometrium, in spite of the results reported by other investigators, due probably to the higher dosage and longer treatment protocol of PEs used (1,500 mg/day) (32). Moreover, in clomiphene citrate–treated patients undergoing IUI, that is, in a patient in whom high circulating levels of estrogens are present (<800 pg/mL), we found an improvement in endometrial thickness that was correlated with higher pregnancy rates when high doses of PEs were administered (28). The present study showed that PEs, when administered at high dosages, can have a positive effect on IVF-ET cycle outcome when a down-regulation protocol is used, that is, even when exceptionally high circulating levels of estrogens are present (>2,000 pg/mL). Results show that supplementation with PEs seems to have a beneficial effect on the implantation rate, on the clinical PR, and on ongoing pregnancy/delivered rates. All three rates were statistically significantly higher in the group treated with P plus PE (group A) in comparison with those of the group treated with P at the same dosage and placebo (group B). Considering that the quality of the embryo transferred was similar in both groups, the beneficial effect of PEs must be attributed to a positive effect on endometrial receptivity. Given the estrogenic-like effects demonstrated by PEs in in vitro and in vivo studies on animal models, as well as their effects when administered at a high dosage (1,500 mg/day) on postmenopausal endometrium (32) and in women undergoing IUI (28), we could conclude that the estrogenic-like effects of PEs on endometrium can improve the outcome of IVF-ET treatment.

Another mechanism that may be postulated to explain the positive clinical effect shown by PEs on implantation in IVF-ET cycles could be the "antiestrogenic-like" properties of PEs. Binding characteristics and effects at the receptor level of many PEs have been extensively studied (28, 33, 34). Phytoestrogens bind to the estrogenic receptors (ERs) ERα and ERβ with a higher affinity compared with E_2 but show a lower estrogenic activity. In the high estrogenic milieu of an IVF cycle, high circulating levels of PEs could compete with E_2 at the receptor level. When administered at high concentrations, and having a higher affinity in comparison with E_2 to the ERs, they could displace endogenous estrogen. At the same time, because they have a lower activity compared with E_2, they could finally exert an antiestrogenic-like effect at a clinical level, that is, modulate the action of the high levels of E_2 in IVF-ET cycles toward lower levels. This could influence and ameliorate endometrial receptivity.

According to this proposed mechanism, the improvement of implantation in PE-supplemented IVF cycles could result from the ability of PEs to antagonize the effect of high E_2 exposure on the uterine lining, exerting a weaker estrogenic activity. As far as we know, this is the first study performed in women undergoing IVF-ET cycles in which high doses of PEs have been administered when high levels of endogenous estrogens are present.

Although more studies should be carried out to confirm this evidence and to study the mechanism of action of PEs on endometrium in IVF-ET cycles, these findings may suggest not only that the importance of estrogenic action should be better investigated for its influence on implantation but also that more attention should be given to the effects on endometrium of PEs. However, some further consideration should be given to PE pharmacology.

It is well known that PEs demonstrate estrogenic-like effects in in vivo and in vitro models and that they bind ERs. We also know that the two identified ERs, ERα and ERβ, have a different distribution in reproductive organs and that they activate different metabolic pathways (35). Phytoestrogens bind both of these ERs with typically different affinity (33, 34) and may act both as an agonist or an antagonist on the receptor they bind. Therefore, the pharmacology of PEs seems to be more complex than that of E_2 itself (36, 37). As a consequence, there is the possibility of more complex and diversified patterns of interaction at the tissue level and of different therapeutic implications. Furthermore, this could explain the more evident beneficial effect on the suitability of the endometrium for implantation.

Studies have been carried out to delineate the action of PEs on many critical molecular targets of implantation. For example genistein, biochanin A, daidzein, formononetin, and equol are known to induce the synthesis of the leukemia inhibitory factor, a glycoprotein essential for implantation (38). We still do not know if PEs can exert an action on other factors that are important for successful implantation different from the action of endogenous estrogens.

This is the first study on IVF-ET outcome in which PEs were used at high dosages in the perimplantation period. Our findings seem to emphasize a positive action of PEs

at a high dosage on the outcome of IVF-ET when a down-regulation protocol was used. Notwithstanding these results, before suggesting the implementation of PE administration in IVF-ET cycles, we recommend that a larger randomized, placebo-controlled study of the efficacy and safety of these compounds be carried out. Nevertheless, our findings suggest new avenues for future fertility research and treatment with PEs and strengthen the importance of investigating the features of estrogenic action on endometrium before implantation.

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参考5)

High Dose of Phytoestrogens Can Reverse the Antiestrogenic Effects of Clomiphene Citrate on the Endometrium in Patients Undergoing Intrauterine Insemination: A Randomized Trial

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OBJECTIVE: *To compare the effectiveness of clomiphene citrate (CC) alone or combined with phytoestrogens (PE) in ovulation induction in patients who had intrauterine insemination in a randomized, double-blind study.*

METHODS: *A total of 134 women aged 25–35 years, who were infertile for at least 2 years and who had oligomenorrhea or amenorrhea associated with a positive menstrual response to the intramuscular progesterone-challenge test were enrolled. They were randomly treated with CC (100 mg daily for 5 days) and CC (100 mg daily for 5 days) in combination with PE (1500 mg daily for 10 days). We estimated the difference in uterine artery pulsatily index, number of preovulatory follicles, endometrial thickness, and pregnancy rate.*

RESULTS: *Both treatments increased follicle-stimulating hormone, luteinizing hormone, and 17β-estradiol plasma concentrations, but the differences were not statistically significant. However, the differences in endometrial thickness of the two groups were statistically significant. No significant differences in the pulsatility index values and in the number of preovulatory follicles were noted.*

CONCLUSION: *A high dose of phytoestrogens can reverse the deleterious effects of clomiphene citrate on endometrial thickness and could contribute to higher pregnancy rates. (J Soc Gynecol Investig 2004;11: 323–8)*

KEY WORDS: Clomiphene citrate, phytoestrogens, isoflavone, intrauterine insemination.

Clomiphene citrate (CC), a nonsteroidal estrogen agonist and antagonist, was synthesized in 1956. It was reported to be effective in ovulation induction by Greenblatt et al[1] in 1961 and is now the most commonly used drug to treat infertility.[2] Clomiphene is most effective in inducing ovulation in women in the World Health Organization group II, which consists of women with anovulation or oligo-ovulation, a wide variety of menstrual disorders, relatively normal (or elevated) gonadotropin levels, and evidence of significant endogenous estrogen production.[3] The Food and Drug Administration–approved dosages for CC are 50 or 100 mg/day for a maximum of 5 days per cycle.[4] After spontaneous menses or the induction of menses by progesterone, CC is started on cycle day 3, 4, or 5 at 50 mg daily for 5 days. Obese women tend to require higher doses of CC to achieve ovulation.[5,6] CC is not stored in adipose tissue, and the increased dose used in obese women is more likely the result of a more intensive anovulatory state with higher androgen levels producing a more resistant hypothalamic-pituitary-ovarian axis.[7] The higher dosage of CC will eventually help to achieve the same therapeutic success in overweight women as in the lean ones.[8,9]

Over the years, evidence has accumulated that CC is successful in inducing ovulation in 50–75% of the cases,[10,11] but the number of pregnancies achieved after ovulation induction is much lower than expected.[12] This discrepancy has been attributed to a negative effect of CC on the endometrium, ie, to its prolonged antiestrogenic effects on endometrial receptivity[13] and cervical mucus.[14] Moreover, Hsu and colleagues[15] demonstrated that CC affects uterine blood flow, which was lower in the early luteal phase and in the perimplantation phase, compared with that of untreated women.

Many plants produce isoflavones that possess estrogenic activity in animals and are, thus, called phytoestrogens (PE). PE are nonsteroidal compounds present in a variety of dietary products.[16] Among the foods consumed by humans, soybeans contain the highest concentration of isoflavones, such as daidzin, genistin, and glycitin. Some of their metabolites, eg, daidzein, genistein, and glycitein, also show estrogenic activity.[17] These PE are of increasing interest for their possible

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influence on the physiology of the reproductive tract.[18] Epidemiologic studies have shown that the ingestion of food rich in PE might provide protection against certain estrogen-dependent cancers, such as breast and prostate cancer.[19,20] Several reports have suggested that some of the effects of PE are mediated by their ability to bind the estrogen receptor (ER).[21–24] This has been confirmed recently by numerous studies in which it was clearly shown that different PE such as genistein, coumestrol, diadzein, zearalenone, glycitein, and many others bind both the ERs (ERα and ERβ) and modulate a variety of estrogen-dependent processes.[17,25–28] PE exert an estrogenic-like effect on the uterine and vaginal morphology and uterine growth in ovariectomized rats and can stimulate the growth of ER-positive cells. Moreover, uterine response to estrogens also involves the activation of a large pattern of estrogen-sensitive genes. The analysis of this cluster of estrogen-sensitive endometrial genes is of help in identifying estrogenic substances, assessing their potency, and elucidating their mechanism of action.[29] The effects of many PE on estrogen-sensitive genes, as well as their uterotropic activity and their selective affinity to estrogenic-receptor subtypes, have been investigated and characterized in numerous studies.

The effects of genistein was investigated in normal and malignant experimental uterine models in vivo by Diel et al.[30] The effects of the 3-day oral administration of genistein (25, 50, or 100 mg/kg of body weight per day) on uterine and vaginal morphology, uterine growth, and uterine gene expression in the uterus and vagina of ovariectomized DA/Han rats were compared with those of ethinyl estradiol (0.1 mg/kg of body weight per day). A dose-dependent increase of the uterine wet weight and uterine and vaginal epithelial height, a dose-dependent up-regulation of complement C3, down-regulation of clusterin mRNA expression, and stimulation of the vaginal cornification were observed after administration of genistein. Uterine gene expression and vaginal epithelium responded to genistein at doses where no significant effects on uterine wet weight were detectable. In conclusion, four independent uterine and vaginal parameters indicated that genistein is a weak estrogen receptor agonist in the uterus and vagina of female DA/Han rats, and evidence was provided for a selective estrogen receptor modulator-like action of genistein in normal and malignant uterine tissues.

In another study by Diel et al,[29] daidzein also provoked a significant stimulation of the uterine wet weight and was able to modulate the expression of a large pattern of estrogen-sensitive genes. Although daidzein was a very weak stimulator of uterine growth in comparison to ethynyl estradiol, it was able to strongly alter the expression of the androgen receptor, ERs, and complement C3.[18]

Other studies have also confirmed that glycitein, diadzein, and genistein show clear estrogenic activity in in vitro and in vivo models and have high binding affinity for ERα and ERβ.[27,31–37]

Some clinical studies of the effects of PE on the endometrium have also been done recently, but their results are discordant.[38–41] Despite the evidence that PE act as an estrogen regarding uterotropic activity in animal models and the modulation of the expression of estrogen-sensitive genes, PE seem to exert an antagonistic effect clinically opposing the effect of estrogens on the endometrium. We have hypothesized that the dosage of PE administered in these studies may have been too low to cause any estrogen-like effects on the endometrium.

The aim of the present study was to compare pregnancy rates after intrauterine insemination (IUI) in two groups of women receiving CC, with or without high doses of phytoestrogens. We also investigated plasma hormonal levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E2); follicular recruitment; and the differences in endometrial thickness and uterine artery blood flow, which are sensitive indicators of uterine receptivity.[42]

MATERIALS AND METHODS

Patients

A total of 134 women were enrolled in this study. Inclusion criteria were age 25–35 years, infertility for at least 2 years, and oligomenorrhea or amenorrhea associated with a positive menstrual response to progesterone challenge test (performed with 100 mg progesterone in oil). All patients had normal serum concentrations of thyroid-stimulating hormone, prolactin, and total testosterone. It was their first cycle of ovulation induction using CC, and no patient had received fertility medications in the past.

We excluded couples with male factor infertility (semen analysis according to World Health Organization criteria[43]), uterine or tubal abnormalities (hysterosalpingogram), and overweight women (body mass index >25).

Treatment Protocol

The protocol was approved by the Institutional Board of Research, and all patients gave written informed consent before being enrolled in the study. The day in which the menses started was designated day 1 of the treatment cycle. All patients were randomly distributed in a double-blind manner into the following two groups: in group A (65 patients), stimulation began on day 3 with the administration of 100 mg of CC daily for 5 days. From day 3 and for 10 days they received phytoestrogens (1500 mg daily). In group B (69 patients), stimulation began on day 3 with the administration of 100 mg of CC daily for 5 days. From day 3 and for 10 days placebo tablets identical to those containing phytoestrogens were administered to all patients of this group.

The soy product was formulated in tablets each containing 500 mg of soy isoflavones. The components of soy isoflavones were 40% to 45% genistein, 40% to 45% daidzein, and 10% to 20% glycitein.

Plasma 17β-estradiol concentrations and ultrasonographic analysis of follicular size and number were assessed on days 5, 7, and 12 of the stimulated cycles.

All patients were given 10,000 IU of intramuscular human chorionic gonadotropin (hCG) when serum 17β-estradiol

concentration exceeded 200 pg per mature follicle and when there was at least one follicle with a minimum diameter of 18 mm.

Intrauterine Insemination

A single intrauterine insemination was performed 24 to 36 hours after the administration of hCG.

Laboratory Determinations and Ultrasound Analysis

Plasma concentrations of FSH, LH, and 17β-estradiol were determined by radioimmunoassay on blood samples on days 1, 5, 9, and 12 of the menstrual cycle. Ultrasound scans were performed daily starting from day 1 (to rule out ovarian cysts) until the mean follicular diameter reached a length of 18 mm. The endometrial thickness was estimated on the day of hCG administration. Pulsatility index (PI) was recorded for both uterine arteries. A gynecologist experienced in transvaginal sonography performed all examinations with a 5-MHz broadband probe.

The examiner was blinded to the patient's group assignment. Color Doppler sonography was used for imaging the uterine arteries on cycle days 2, 8, and 12. The PI was calculated by subtracting the peak end-diastolic-shifted frequency from systolic-shifted frequency and dividing the result by the mean Doppler shift over the cardiac cycle. The intraobserver coefficient of variation for measurement of PI was 5.3%. All examinations were performed between 9:00 and 11:00 AM to reduce the effects of the circadian variation of PI.[42]

Determination of Pregnancy States

A biochemical pregnancy was defined as a small transitory increase in β-hCG levels followed by a decrease in β-hCG levels within 1 week. Clinical pregnancies were defined by visualization of a gestational sac at the first planned ultrasound examination obtained at 6–7 weeks of pregnancy or a serum β-hCG level over 1400 mIU in the absence of a scan. Ongoing pregnancies were defined as gestations that reached 20 weeks' gestation.

Statistical Analysis

Statistics were performed with the SPSS statistical package (SPSS Inc., Chicago, IL). Chi-square and Fisher exact test were used. Statistical significance was defined as a P value $<$ .05.

RESULTS

On day 1, before the beginning of treatment, there were no statistically significant differences in the plasma levels of FSH, LH, and 17β-estradiol. Both treatments increased FSH, LH, and 17β-estradiol plasma concentrations, but the differences between the two groups were not statistically significant. However, the mean of FSH value in the group treated with CC in combination with PE was lower than that of the group treated with CC alone. There were no statistically significant differences in the plasma levels of FSH, LH, and 17β-estradiol between the groups during the entire treatment period (data not shown).

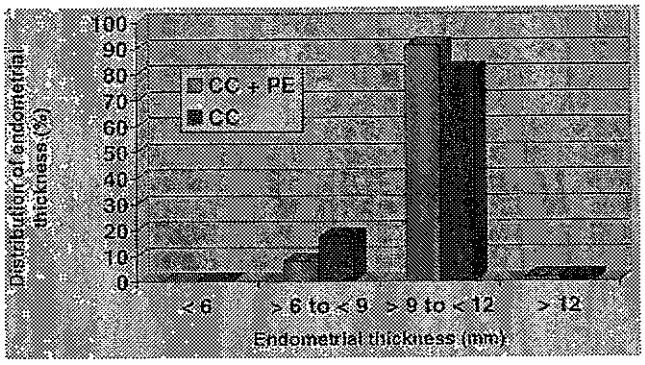

Figure 1. Percentage distribution of endometrial thickness on the day of hCG administration.

There were no statistically significant differences between the two groups in follicular development and in the number of preovulatory follicles (data not shown).

Endometrial Thickness and Uterine Blood Flow

The endometrial thickness was estimated on the day of hCG administration. In all cases it was more than 6 mm. It was between 6 and 9 mm in five cases in group A (7.7%) compared with 12 cases in group B (17.4%); between 9 and 12 mm in 59 cases (90.8%) in group A compared with 56 cases (81.2%) in group B; and greater than 15 mm in one case (1.5%) in group A compared with one case (1.5%) in group B (Figure 1). No significant differences in PI values were noted (Figure 2).

Pregnancy Rate

The miscarriage rate was 3.1% in group A compared with 8.7% in group B. The difference was statistically significant (Table 1). At the same time, the percentage of the ongoing pregnancies was higher in the group treated with CC in combination with PE (group A, 20.0%) than in the group treated with CC alone (group B, 4.4%; $P < .05$) (Table 1).

DISCUSSION

Clomiphene, which was introduced in 1967, is considered to increase the incidence of spontaneous abortion.[8,12] The in-

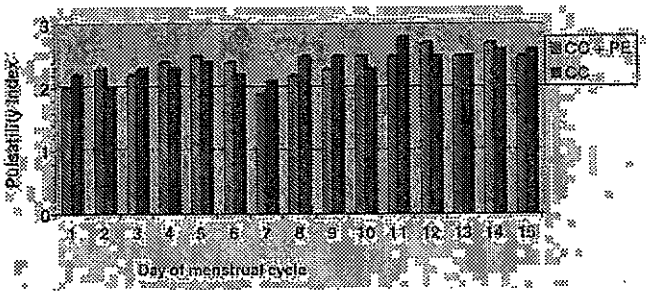

Figure 2. Pulsatility index (expressed as mean ± standard error) in the patients who had ovarian stimulation with clomiphene citrate alone (CC) or combined with phytoestrogens (CC + PE).

Table 1. Characteristics of Patients Who Received Clomiphene Citrate Plus Phytoestrogens or Clomiphene Citrate Alone

Characteristic	CC + PE Group A	CC Group B	P
No. of patients	65	69	—
Mean (± SD) age (y)	28 ± 5.6	26 ± 4.2	NS
Mean (+SD) duration of infertility (mo)	48.1 ± 18.5	36.7 ± 9.6	NS
No. (%) of pregnancies per cycle			
Biochemical	3 (4.6)	4 (5.8)	NS
Miscarriages	2 (3.1)	6 (8.7)	<.05
Ongoing pregnancies	13 (20.0)	3 (4.4)	<.05

CC = clomiphene citrate; PE = phytoestrogens; SD = standard deviation; NS = not significant.

crease in spontaneous abortion in clomiphene-treated pregnancies has been attributed to several factors, including impaired endometrial development.[9,44,45]

Goldstein et al[46] reported that estradiol concentrations that were either too low or too high and associated with normal progesterone concentrations caused desynchronized endometrial development. An antagonistic effect of CC on the endometrium has long been recognized in histologic studies.[44,47–49] Furthermore, numerous ultrasound studies have shown that CC may reduce endometrial thickness.[50–55]

It has been shown that the adverse effects of CC on the endometrium can be prevented by adding estrogens with clomiphene or after clomiphene.[56] However, in 1990, Bateman et al[57] demonstrated that exogenous estrogens did not improve the quality or quantity of cervical mucus in CC-treated women. Similarly, the use of CC for in vitro fertilization does not seem to reduce the implantation rate,[58] possibly because the contemporary use of human menopausal gonadotropin (HMG) with its more sustained estradiol production could have a positive effect on the endometrium.

Our study shows that adding PE to CC increased endometrial thickness and decreased the risk of abortion. A better pregnancy rate may have resulted from the improved endometrial characteristics caused by the administration of high-dosage PE (possibly having an estrogen-like action that balances the antiestrogenic effect of clomiphene), which facilitates embryo implantation. Results supporting this hypothesis were found in studies on IUI cycles.[56,58] These studies estimated that ovulation induction with sequential clomiphene-HMG resulted in fecundity that is double that of CC alone, which reaffirmed a possible positive role of estrogens on the endometrium. Furthermore, Dickey et al[58] noticed that the increased pregnancy rate achieved when HMG was administered after CC was related to the increased number of preovulatory follicles, and a significant doubling of the implantation rate per follicle was also calculated. In that study, the estradiol level per follicle nearly doubled for clomiphene-HMG treated women compared with women treated only with CC.

We determined the endometrial effects of high doses of PE. In vitro studies demonstrated that PE have estrogenic-like effects and promote the transcription of estrogen-sensitive genes. The in vivo studies on ovariectomized rats also confirmed the estrogenic effects of PE on the endometrium. Studies on the effects of PE on the endometrium in hormone replacement therapy has not only not confirmed the efficacy of these PE in hormone replacement therapy but also has often shown an antiestrogen-like effect of PE on the endometrium.[38–41] We hypothesized that this could be attributable to the insufficient length of the treatments, the insufficiency of the daily dosage administered, or both.

Phytoestrogens bind both estrogen receptors, ERα and ERβ, as shown in many studies,[17,25–28] with different affinity and efficacy, acting sometimes as a partial or a full agonist, depending of the ER.[26,27] For example, genistein acts as a partial agonist of ERβ, although it binds ERβ with an approximately 30-fold higher affinity than ERα in humans, and genistein binds ERα acting as a slight superagonist (range, 107–130% of the efficacy shown by the endogenous agonist estradiol).[27] Moreover, tissue distribution of ERs varies during the menstrual cycle and menopause[59–61] and is modulated by the same estrogens.[59] Finally, it is important to remember that isoflavones reduce the serum concentration of estradiol by feedback regulation.[17] The modulation of ERs is much more complex than thought before the discovery of ERβ. Thus, depending on tissue distribution and specific prevalence of ERα and ERβ on the target organ, PE could act in different ways resulting in different clinical effects. For example, a higher affinity combined with a lower efficacy in one of the ERs can result in an antiestrogen-like effect on the target organ, while the dosage could be insufficient to permit the displacement of the endogenous ligand on the other ER, where the PE could show a superagonist effect. The administration of a higher dosage of PE can help to displace the endogenous estrogen and bind to the receptor, or longer therapy could affect the tissular expression of ER subtypes. However, in two in vitro studies examining the effects of phytoestrogens on human endometrial cells, phytoestrogens were added to the cells at concentrations up to 10^{-5} M and, in the presence of estradiol, clear antiestrogenic effects were demonstrated.[62,63] An alternative hypothesis could be that phytoestrogens compete with the antiestrogenic isomer of clomiphene for estrogen receptors and have a less potent antiestrogenic effect than the cis-isomer of clomiphene citrate.

A previous study performed on postmenopausal women by our group[64] found that a longer therapy (up to 5 years) with PE had an estrogen-like effect on the endometrium, ie, led to an increased occurrence of endometrial hyperplasia in the PE-treated subjects compared with a placebo-treated group. This new study confirms our hypothesis.

In conclusion, in accordance with other studies, we noted that inadequate endometrial development might have a negative influence on the outcome of implantation. In fact, preovulatory endometrial thickness is predictive of a high risk of miscarriage. The addition of high doses of phytoestrogens to the treatment protocol of women treated with CC provoked a positive response of the endometrium. Based on our data, we conclude that a combined regimen of CC combined with high

doses of phytoestrogens could reverse the deleterious effect of CC on the endometrial development.

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