参考資料2

（参考論文その1）

FERTILITY AND STERILITY®
VOL. 82, NO. 1, JULY 2004

Published by Elsevier Inc.
Printed on acid-free paper in U.S.A.

Endometrial effects of long-term treatment with phytoestrogens: a randomized, double-blind, placebo-controlled study

Vittorio Unfer, M.D.,[a] Maria Luisa Casini, Ph.D.,[b] Loredana Costabile, M.D.,[a] Marcella Mignosa, M.D.,[c] Sandro Gerli, M.D.,[c] and Gian Carlo Di Renzo, M.D., Ph.D.[c]

University of Perugia, Perugia, Italy

Objective: To determine the effects of 5 years of treatment with soy phytoestrogens on histological characteristics of endometrium in postmenopausal women.

Design: Randomized, double-blind, placebo-controlled study.

Setting: Centre of Perinatal and Reproductive Medicine, Department of Gynecological, Obstetrical, and Pediatric Sciences, University of Perugia, Italy.

Patient(s): Three hundred seventy-six postmenopausal healthy women, all with intact uterus.

Intervention(s): Women were distributed in two different groups using randomized criteria: group A (n = 179) patients received soy tablets (150 mg of isoflavones per day) for 5 years; group B (n = 197) patients received identical appearing placebo tablets for 5 years.

Main Outcome Measure(s): Results of endometrial histology from biopsies obtained at baseline, 30 months, and 5 years after the beginning of the treatment.

Result(s): Two hundred ninety-eight women completed the 5-year treatment. No cases of malignancy were detected during biopsy. Seventy percent of women undergoing treatment with soy phytoestrogens had an endometrium classified as atrophic or nonassessable versus 81% receiving placebo. The occurrence of endometrial hyperplasia was significantly higher in group A (3.37% vs. 0%).

Conclusion(s): 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. (Fertil Steril® 2004;82:145–8. ©2004 by American Society for Reproductive Medicine.)

Key Words: Phytoestrogens, endometrial effects, postmenopausal therapy

Received July 14, 2003; revised and accepted November 12, 2003.

Reprint requests: Prof. Vittorio Unfer, A.G.UN.CO. Obstetrics and Gynecology Centre, Via G. Cassiani, 15-00155 Rome, Italy (FAX: 39-06-3241284; E-mail: vittorio.unfer@lycos.com).

[a] A.G.UN.CO., Obstetrics and Gynecology Centre, Rome, Italy.

[b] Department of Human Physiology and Pharmacology "Vittorio Erspamer," University "La Sapienza," Rome, Italy.

[c] Centre of Perinatal and Reproductive Medicine, Department of Gynecological, Obstetrical, and Pediatric Sciences, University of Perugia.

0015-0282/04/$30.00
doi:10.1016/j.fertnstert.2003.11.041

Estrogens are the treatment of choice for women with menopausal symptoms. However, estrogens have been postulated by some investigators to promote breast cancer and thus have often been contraindicated (1). Many women reject hormone therapy because of this possible long-term effect. Phytoestrogens (PEs) are nonsteroidal compounds present in a variety of dietary products (2). Epidemiological studies have demonstrated that the ingestion of food that is rich in PEs may provide protection against certain estrogen-dependent cancers, such as breast and prostate cancers (3, 4).

Several reports have suggested that some effects of PEs are mediated through their ability to bind and antagonize the activity of estrogen receptors (ERs) (5–8). This is supported by the observation that certain PEs such as genestein, coumestrol, or zearalenone are able to bind the ERs and modulate a variety of estrogen-dependent processes. Phytoestrogens are able to stimulate the growth of ER-positive cells, increase uterine wet weight, modulate the estrus cycle, compete with E_2 for binding ER, and stimulate the activity of reporter genes in an ER-dependent manner (9–11). Some clinical studies on the effects of PEs on the endometrium in postmenopausal women have been carried out during the past few years, but their results are discordant (12–16). The aim of this study was to investigate the effects of PEs on the endometrium when they are administered for a long period.

MATERIALS AND METHODS

Patients

This randomized, double-blind, placebo-controlled study was approved by the Institutional Review Board, and all patients gave written informed consent before enrollment.

A total of 376 healthy voluntary postmenopausal women were enrolled in this study, which started in November 1996 and ended in December 2002. All women had initial screenings that included a complete medical history and a complete physical examination. Inclusion criteria were intact uterus, absence of menses for at least 12 months, FSH ≥ 30 IU/L, and body weight range within 20% of their normal weight. Exclusion criteria were use of medication containing estrogens, progestins, or androgens within 8 weeks of the beginning of the study or the presence of endometrial hyperplasia.

Treatment Protocol

The 376 enrolled patients were randomly assigned to one of the following two groups: group A (n = 179) patients received three soy tablets (150 mg of isoflavones per day) for 5 years; and group B (n = 197) patients received identical appearing placebo tablets for 5 years.

The soy PEs were formulated in 600-mg tablets containing 50 mg of soy isoflavones in each tablet. The formulated percentages of isoflavones were 40%–45% genistein, 40%–45% diadzein, and 10%–20% glycitein.

Endometrial Biopsies

Endometrial biopsies were performed at the beginning of the study (baseline values), after 30 months, and at the end of the study. Biopsies were performed with Pipelle endometrial suction (Pipelle de Cornier samplers) (17). Specimens were then fixed in 4% unbuffered formalin, and 4-μm sections were stained with hematoxylin and eosin. The same pathologist, who was blinded to the patient's protocol regimen, interpreted biopsies and classified them according to standard criteria (18).

TABLE 1

Characteristics of participants at baseline.

Characteristic	Group A (n = 179)	Group B (n = 197)
Age at start of treatment (years)	49 ± 4.3	50 ± 3.9
Height (cm)	165.5 ± 5.3	163.4 ± 5.6
Weight (kg)	67 ± 10.2	65.8 ± 10.7
Mean age at menopause (y)	50.2 ± 6.5	49.8 ± 6.3
Years of menopause	5.6 ± 4.3	5.8 ± 4.5
Parity (n)	2.8 ± 1.8	2.7 ± 1.7

Note: Unless otherwise specified, values are mean ± SD. The *P* value for all data was not significant.

Unfer. Phytoestrogen long-term treatment. Fertil Steril 2004.

Statistical Analysis

The data were expressed as means ± SD and percentages. The between-group differences were measured by means of one-way analysis of variance or Student's *t*-test, as appropriate. Fisher's exact test was used post hoc to determine significant differences. $P<.05$ was considered statistically significant.

RESULTS

Table 1 shows the demographic characteristics of the two groups of patients. There were no statistical differences in terms of age, weight, height, or years of menopause between the groups. Weight was monitored throughout the study, and there were no statistically significant differences in these values within each group between trial entry and exit (data not shown).

Table 2 shows the endometrial effects after 30 months

TABLE 2

Number (%) of women with different histological classifications of endometrial tissue at baseline (T0), after 30 months (T1), and after 5 years (T2) of treatment (group A) or placebo administration (group B).

Classification	Group A (n = 179) T0	Group B (n = 197) T0	Group A (n = 176) T1	Group B (n = 193) T1	Group A (n = 154) T2	Group B (n = 165) T2
Unassessable	48 (26.8)	49 (24.8)	45 (25.5)	48 (24.9)	30 (19.5)	41 (24.8)
Inactive	67 (37.4)	73 (37.0)	71 (40.3)	69 (35.7)	70 (45.4)	60 (36.3)
Atrophic	62 (34.6)	71 (36.0)	60 (34.1)	76 (39.4)	43 (27.9)	64 (38.8)
Proliferative	2 (1.1)	3 (1.5)	0	0	5 (3.2)[a]	0
Secretory	0	0	0	0	0	0
Pseudo-decidual change	0	0	0	0	0	0
Simple hyperplasia	0	0	0	0	5 (3.2)[a]	0
Complex hyperplasia	0	0	0	0	1 (0.6)	0
Atypical hyperplasia	0	0	0	0	0	0

[a] Statistically significant ($P<.05$).

Unfer. Phytoestrogen long-term treatment. Fertil Steril 2004.

and 5 years of treatment. Data about endometrial biopsies were available for 376 patients at baseline, for 369 after 30 months, and for 319 after 5 years of treatment. No cases of endometrial hyperplasia or malignancy were detected in women whose biopsy specimens were assessable after 30 months of therapy. After 5 years of treatment, six cases (3.8%) of endometrial hyperplasia (five of simple and one of complex hyperplasia) were detected in group A. No cases of endometrial hyperplasia were detected in group B. The difference between the two groups was statistically significant ($P<.05$). No cases of endometrial carcinoma occurred during the 5 years of the study.

DISCUSSION

Phytoestrogens are estrogenic compounds found in plants. The classical definition of PEs refers to compounds that exert estrogenic effects on the central nervous system, induce estrus, and stimulate the growth of the genital tract of female animals (9), but antiestrogenic effects have also been observed (5–8). Given that the endometrium is a target tissue for estrogens and that an excess of estrogen stimulation is linked to type I endometrial cancer, the aim of our study was to assess whether PEs exert an estrogenic or an antiestrogenic effect on endometrium.

Although many PEs have been characterized by their in vitro affinity for ERs, only a few of them have been studied concerning their specific activity on the endometrium. Phytoestrogens increase the production of complement C3 protein, primarily by increasing its steady state mRNA level (19), and enhance alkaline phosphatase activity (20); both are known to exert estrogenic stimulatory effects in rat endometrium. These studies suggest that PEs may act as agonists at the endometrial level. Duncan et al. (21) demonstrate the absence of significant effects in endometrial biopsy samples taken from women on a soy isoflavone-enhanced diet for 90 days. It is still unclear whether there is an absence of endometrial effects in longer exposure to PEs.

This is the longest randomized clinical trial performed to determine the endometrial effects of PE administration. Previous studies involved a small number of patients or had a shorter duration or both, and their results were discordant (12–16). Thirty months of treatment with 150 mg of isoflavones per day did not result in an increased incidence of endometrial hyperplasia, which is in agreement with previous studies (22, 23). In contrast, our results indicated that a longer treatment resulted in an increased incidence of endometrial effects. Furthermore, most isoflavone supplements are given at a dosage of no more than 80 mg/day. In Italy, as of July 2002, the Ministry of Health recommends that isoflavone supplements do not exceed this dosage. During our study, we administered a higher dosage (150 mg/day).

Our hypothesis is that PEs may act as a partial agonist on the ERs, exerting an antagonist-like action at the endometrial level. This means that they bind the ERs and act as an agonist. Because their action is weaker than that of endogenous estrogens, the final effect on endometrium seems to be antagonistic in the sense that it seems to counteract the effects of endogenous estrogens. This mechanism of action could explain the antiestrogenic effects observed in previous studies (5–8). Studies on the modification of the ERα/ERβ three-dimensional structure after the interaction with different isoflavones show that PEs modify the receptors in different ways depending on their particular molecular structure (24, 25). This can lead to different patterns of activation of the two ERs, and, consequently, PEs show an agonist, partial agonist, or antagonist character depending on the ER subtype considered. This allows us to hypothesize that the estrogenic-like effects demonstrated at the endometrial level in the patients treated with PEs could be ascribed to a different sensibility to PEs because of a different quantitative and qualitative distribution of ER subtypes on endometrial tissue (26).

When the treatment is prolonged, the agonist action could become more evident, and in this case it is possible to see an estrogenic-like effect at the endometrial level in predisposed patients. If this estrogenic effect is not properly counterbalanced with the administration of a progestogen, it may increase the risk of endometrial hyperplasia. It is evident that these findings require further study before PEs can be safely and properly used. 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.

Acknowledgments: The authors thank Dr. Suzette Paolella for her support in (English) translation and language assistance.

References

1. Loprinzi CL, Michalak JC, Quella SK, O'Fallon JR, Hatfield AK, Nelimark RA, et al. Megestrol acetate for the prevention of hot flashes. N Engl J Med 1994;331:347–52.
2. Xu X, Wang HJ, Murphy PA, Hendrich S. Neither background diet nor type of soy food affects short-term isoflavone bioavailability in women. J Nutr 2000;130:798–801.
3. Adlercreutz H. Phyto-oestrogens and cancer. Lancet Oncol 2002;3: 364–73.
4. Barnes S, Peterson TG, Coward L. Rationale for the use of genistein-containing soy matrices in chemoprevention trials for breast and prostate cancer. J Cell Biochem Suppl 1995;22:181–7.
5. Mueller SO, Kling M, Arifin Firzani P, Mecky A, Duranti E, Shields-Botella J, et al. Activation of estrogen receptor alpha and ERbeta by 4-methylbenzylidene-camphor in human and rat cells: comparison with phyto- and xenoestrogens. Toxicol Lett 2003;142:89–101.
6. Rickard DJ, Monroe DG, Ruesink TJ, Khosla S, Riggs BL, Spelsberg TC. Phytoestrogen genistein acts as an estrogen agonist on human osteoblastic cells through estrogen receptors alpha and beta. J Cell Biochem 2003;89:633–46.
7. Lee Y, Jin Y, Lim W, Ji S, Choi S, Jang S, et al. A ginsenoside-Rh1, a component of ginseng saponin, activates estrogen receptor in human breast carcinoma MCF-7 cells. J Steroid Biochem Mol Biol 2003;84: 463–8.

8. Pearce V, Nawaz Z, Xiao W, Wiedenfeld D, Boyle N, Smith D. 4-Ethoxymethylphenol: a novel phytoestrogen that acts as an agonist for human estrogen receptors. J Steroid Biochem Mol Biol 2003;84: 431–9.
9. Jefferson WN, Padilla-Banks E, Clark G, Newbold RR. Assessing estrogenic activity of phytochemicals using transcriptional activation and immature mouse uterotrophic responses. J Chromatogr B Analyt Technol Biomed Life Sci 2002;777:179–89.
10. Diel P, Smolnikar K, Schulz T, Laudenbach-Leschowski U, Michna H, Vollmer G. Phytoestrogens and carcinogenesis-differential effects of genistein in experimental models of normal and malignant rat endometrium. Hum Reprod 2001;16:997–1006.
11. Markaverich BM, Webb B, Densmore CL, Gregory RR. Effects of coumestrol on estrogen receptor function and uterine growth in ovariectomized rats. Environ Health Persp 1995;103:574–81.
12. Balk JL, Whiteside DA, Naus G, DeFerrari E, Roberts JM. A pilot study of the effects of phytoestrogen supplementation on postmenopausal endometrium. J Soc Gynecol Invest 2002;9:238–42.
13. Penotti M, Fabio E, Modena AB, Rinaldi M, Omodei U, Vigano P. Effect of soy-derived isoflavones on hot flushes, endometrial thickness, and the pulsatility index of the uterine and cerebral arteries. Fertil Steril 2003;79:1112–7.
14. Upmalis DH, Lobo R, Bradley L, Warren M, Cone FL, Lamia CA. Vasomotor symptom relief by soy isoflavone extract tablets in postmenopausal women: a multicenter, double-blind, randomized, placebo-controlled study. Menopause 2000;7:422.
15. Johnson EB, Muto MG, Yanushpolsky EH, Mutter GL. Phytoestrogen supplementation and endometrial cancer. Obstet Gynecol 2001;98:947–50.
16. Sammartino A, Di Carlo C, Mandato VD, Bifulco G, Di Stefano M, Nappi C. Effects of genistein on the endometrium: ultrasonographic evaluation. Gynecol Endocrinol 2003;17:45–9.
17. Cornier E. The Pipelle: a disposable device for endometrial biopsy. Am J Obstet Gynecol 1984;148:109–10.
18. Hofmeister FJ, Vondrak B, Barbo DM. The value of the endometrial biopsy. A study of 14,655 office endometrial biopsies. Am J Obstet Gynecol 1966;95:91–8.
19. Heikaus S, Winterhager E, Traub O, Grummer R. Responsiveness of endometrial genes Connexin26, Connexin43, C3 and clusterin to primary estrogen, selective estrogen receptor modulators, phyto- and xenoestrogens. J Mol Endocrinol 2002;29:239–49.
20. Wober J, Weisswange I, Vollmer G. Stimulation of alkaline phosphatase activity in Ishikawa cells induced by various phytoestrogens and synthetic estrogens. J Steroid Biochem Mol Biol 2002;83:227–33.
21. Duncan AM, Underhill KE, Xu X, Lavalleur J, Phipps WR, Kurzer MS. Modest hormonal effects of soy isoflavones in postmenopausal women. J Clin Endocrinol Metab 1999;84:3479–84.
22. Murray MJ, Meyer WR, Lessey BA, Oi RH, DeWire RE, Fritz MA. Soy protein isolate with isoflavones does not prevent estradiol-induced endometrial hyperplasia in postmenopausal women: a pilot trial. Menopause 2003;10:456–64.
23. Hale GE, Hughes CL, Robboy SJ, Agarwal SK, Bievre M. A double-blind randomized study on the effects of red clover isoflavones on the endometrium. Menopause 2001;8:338–46.
24. Morito K, Hirose T, Kinjo J, Hirakawa T, Okawa M, Nohara T, et al. Interaction of phytoestrogens with estrogen receptors alpha and beta. Biol Pharm Bull 2001;24:351–6.
25. Pike AC, Brzozowski AM, Hubbard RE, Bonn T, Thorsell AG, Engstrom O, et al. Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist. EMBO J 19991;18:4608–18.
26. Lecce G, Meduri G, Ancelin M, Bergeron C, Perrot-Applanat M. Presence of estrogen receptor beta in the human endometrium through the cycle: expression in glandular, stromal, and vascular cells. J Clin Endocrinol Metab 2001;86:1379–86.

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.

Masafumi Ito, M.D., Ph.D.
Department of Pathology
Nagoya University Hospital
Nagoya, Japan

Chikako Ito, M.D., Ph.D.
Narita Hospital
Nagoya, Japan

September 26, 2004

doi:10.1016/j.fertnstert.2004.10.020

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.

Dolores Foth, M.D., Ph.D.
Department of Obstetrics and Gynaecology
University of Cologne
Cologne, Germany

Frank Nawroth, M.D., Ph.D.
Centre of Reproductive Medicine and Gynecologic Endocrinology
Endokrinologikum Hamburg
Hamburg, Germany

September 14, 2004

REFERENCES

1. Unfer V, Casini MA, Costabile L, Mignosa M, Gerli S, Di Renzo GC. Endometrial effects of long-term treatment with phytoestrogens: a randomized, double-blind, placebo-controlled study. Fertil Steril 2004;82: 145–8.
2. Wood CE, Register TC, Anthony MS, Kock ND, Cline JM. Breast and uterine effects of soy isoflavones and conjugated equine estrogens in

postmenopausal female monkeys. J Clin Endocrinol Metab 2004;89:3462–8.
3. Foth D, Cline JM. Effects of mammalian and plant estrogens on mammary glands and uteri of macaques. Am J Clin Nutr 1998;68:1413S–7S.
4. Dijkhuizen FP, Mol BW, Brolmann HA, Heintz AP. The accuracy of endometrial sampling in the diagnosis of patients with endometrial carcinoma and hyperplasia: a meta-analysis. Cancer 2000;89:1765–72.

doi:10.1016/j.fertnstert.2004.10.021

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).

Vittorio Unfer, M.D.
A.G.UN.CO. Obstetrics and Gynecology Centre
Rome, Italy

Maria Luisa Casini, Ph.D.
Department of Human Physiology and Pharmacology
"Vittorio Erspamer" University "La Sapienza"
Rome, Italy

Gian Carlo Di Renzo, M.D., Ph.D.
Centre of Perinatal and Reproductive Medicine
Department of Gynecological, Obstetrical and
Paediatric Sciences
University of Perugia
Perugia, Italy

September 28, 2004

REFERENCES

1. Unfer V, Casini ML, Costabile L, Mignosa M, Gerli S, Di Renzo GC. Endometrial effects of long-term treatment with phytoestrogens: a randomized, double-blind, placebo-controlled study. Fertil Steril 2004;82:145–8.
2. Chen J, Campbell TC, Li J, Peto R. Diet, lifestyle and mortality in China. A study of the characteristics of 65 counties [monograph]. Joint publication of Oxford University Press, Cornell University Press, China People's Medical Publishing House, 1990.
3. Nakamura Y, Tsuji S, Tonogai Y. Determination of the levels of isoflavonoids in soybeans and soy-derived foods and estimation of isoflavonoids in the Japanese daily intake. J AOAC Int 2000;83:635–50.
4. Cline JM, Franke AA, Register TC, Golden DL, Adams MR. Effects of dietary isoflavone aglycones on the reproductive tract of male and female mice. Toxicol Pathol 2004;32:91–9.
5. Unfer V, Casini ML, Costabile L, Mignosa M, Gerli S, Di Renzo GC. High dose of phytoestrogens can reverse the antiestrogenic effects of clomiphene citrate on the endometrium in patients undergoing intrauterine insemination: a randomized trial. J Soc Gynecol Investig 2004;11:323–8.

doi:10.1016/j.fertnstert.2004.10.022

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