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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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