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Efficacy of Nano Spherical-crystallized S-equol on Ovarian Physiological Age, Menopausal Symptoms, Menstrual Regularity, and Dermal Microstructure: A 12-Week Pilot Study

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Abstract: This 12-week pilot study evaluated the effects of Nano Spherical-crystallized S-equol in 12 perimenopausal women aged 45–55 years on ovarian physiological age-related endocrine biomarkers, menopausal symptoms, menstrual regularity, and skin microstructure. Daily supplementation with 5 mg of Nano Spherical-crystallized S-equol significantly increased circulating S-equol levels, confirming effective systemic exposure. Serum inhibin B levels increased by 14.4%, suggesting improved ovarian endocrine function and an endocrine profile corresponding to an ovarian physiological age approximately 5.6 years younger than chronological age. In addition, total menopausal symptom scores decreased by 63.8%, while premenstrual and menstrual symptom burden was reduced by 52.4%. The proportion of participants with regular menstrual cycles increased from 25.0% to 66.7%. Improvements were also observed in skin hydration, elasticity, wrinkle depth, transepidermal water loss, and skin roughness. These findings suggest that Nano Spherical-crystallized S-equol may support ovarian endocrine health, alleviate menopausal discomfort, improve menstrual cycle stability, and promote healthier skin aging in perimenopausal women. However, larger randomized, double-blind, placebo-controlled studies are needed to confirm these preliminary results.

1. Introduction

The menopausal transition is characterized by progressive ovarian functional decline, fluctuating endocrine activity, irregular menstrual cycles, vasomotor symptoms, and changes in skin structure. Although chronological age is an important background factor, ovarian physiological aging is more directly associated with changes in follicular activity, granulosa cell function, and endocrine biomarkers such as inhibin B [1,2].

Inhibin B is secreted primarily by ovarian granulosa cells and has been used as a biomarker related to ovarian reserve, follicular activity, and the menopausal transition [3,4]. During perimenopause, declining follicular function is often accompanied by reduced inhibin B secretion, increasing follicle-stimulating hormone fluctuation, and menstrual cycle irregularity [5]. Therefore, maintaining a

favorable inhibin B-related endocrine profile may be relevant for evaluating nutritional strategies designed to support ovarian physiological function during the menopausal transition.

S-equol is a biologically active metabolite of daidzein, a soy isoflavone. Unlike conventional soy isoflavones, direct S-equol supplementation bypasses the need for intestinal microbial conversion, which varies substantially among individuals [5,6]. S-equol has been reported to act as a potent ligand for estrogen receptor beta (ERβ), and ERβ is expressed in tissues relevant to female aging, including ovarian granulosa cells, skin fibroblasts, vascular tissue, and neuroendocrine pathways involved in thermoregulation and sensory processing [7,8].

Clinical studies have suggested that S-equol supplementation may help reduce menopausal symptoms such as hot flashes and other perimenopausal discomforts [9,10]. Additional research has reported potential benefits of natural S-equol supplementation on skin aging parameters, particularly in postmenopausal women [11]. These findings support further investigation of S-equol as a non-hormonal nutritional strategy for women undergoing menopausal transition.

In addition to the active ingredient itself, crystal morphology and formulation engineering may influence particle behavior, dispersibility, dissolution characteristics, and exposure consistency. Spherical crystallization is a particle-engineering approach that can convert irregular drug crystals into spherical or near-spherical crystalline particles or agglomerates, potentially improving powder flowability, uniformity, and downstream formulation performance [12,13]. Nano Spherical-crystallized S-equol is designed to reduce the particle scale of conventional S-equol and transform irregular acicular or plate-like crystals into nano-scale spherical crystalline structures.

The present 12-week pilot study was designed to evaluate the effects of Nano Spherical-crystallized S-equol in perimenopausal women. The primary objective was to evaluate ovarian physiological age-related endocrine response using serum inhibin B. Secondary objectives included systemic S-equol exposure, menopausal symptoms, premenstrual and menstrual symptoms, menstrual cycle regularity, and skin structural parameters.

2. Materials and Methods

2.1 Study Design

This was a 12-week pilot study conducted at the Eternal Grace R&D Center, Gpo Lief Pte. Ltd., Singapore. The study was conducted in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent before enrollment.

Given the exploratory nature of the study and the limited sample size, results were analyzed primarily as within-subject changes from baseline.

2.2 Participants

Twelve perimenopausal women aged 45–55 years were enrolled.

Inclusion Criteria:

- Women aged 45–55 years
- Perimenopausal status with irregular or fluctuating menstrual cycles within the previous 12 months
- Modified Kupperman Index score ≥ 15
- Willingness to maintain usual diet, physical activity, and lifestyle habits during the study period

Exclusion Criteria:

- Use of hormone replacement therapy within 6 months
- Use of high-dose phytoestrogen or soy isoflavone supplements within 3 months

- Known soy allergy
- History of estrogen-dependent malignancy
- Severe hepatic, renal, cardiovascular, endocrine, or gynecological disease
- Current participation in another clinical trial

2.3 Intervention

Participants received one daily capsule containing 5 mg Nano Spherical-crystallized S-equol for 12 weeks. The capsule was taken once daily with breakfast.

Although the finished capsule matrix contained additional supporting nutrients, Nano Spherical-crystallized S-equol was the defined core active ingredient evaluated in this article. The present study did not separately evaluate the independent contribution of individual supporting ingredients.

2.4 Characterization of Nano Spherical-crystallized S-equol

Scanning electron microscopy was used to compare the morphology of conventional S-equol and Nano Spherical-crystallized S-equol which was shown in Figure 1. Conventional S-equol exhibited irregular acicular and plate-like crystalline morphology in the micrometer range. Nano Spherical-crystallized S-equol showed nano-scale spherical crystalline particles and clustered spherical crystalline structures.

Scale bars were adjusted according to particle size differences between the two samples. Conventional S-equol was visualized using a micrometer-scale bar, whereas Nano Spherical-crystallized S-equol was visualized using a nanometer-scale bar.

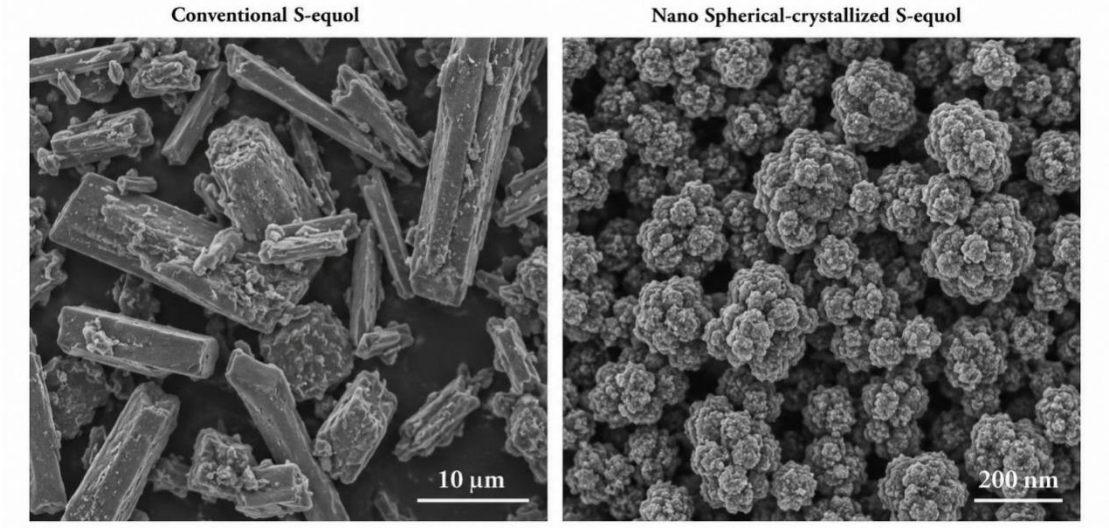

Figure 1. SEM images of conventional S-equol and Nano Spherical-crystallized S-equol.

(Conventional S-equol exhibited irregular acicular and plate-like crystalline morphology with paricle sizes in the micrometer range. In contrast, Nano Spherical-crystallized S-equol formed spherical microcrystalline agglomerates composed of nano-sized primarycrystals with diameters predominantly between 50-150 nm. Scale bars: Left = 10 um, Right = 200 nm.)

2.5 Outcome Measures

Assessments were performed at baseline, Week 4, Week 8, and Week 12.

2.5.1 Systemic S-equol Exposure

Serum S-equol levels were measured at baseline and Week 12 using a validated chromatographic method.

2.5.2 Ovarian Physiological Age and Endocrine Biomarkers

Serum inhibin B was measured using enzyme-linked immunosorbent assay. Ovarian physiological age-related endocrine profile was calculated using an age-regression model based on normative inhibin B decline during the menopausal transition.

2.5.3 Menopausal Symptom Assessment

Menopausal symptoms were assessed using the Modified Kupperman Menopause Index. Total KMI score and individual symptom domains were recorded.

2.5.4 Premenstrual and Menstrual Symptom Assessment

Premenstrual and menstrual symptom burden was assessed using a symptom questionnaire covering breast tenderness, lower abdominal discomfort, bloating, mood irritability, fatigue, and headache or heaviness.

2.5.5 Menstrual Cycle Regularity

Menstrual cycle regularity was assessed based on cycle interval, cycle-to-cycle variation, and the proportion of participants achieving a regular cycle pattern. A regular menstrual cycle pattern was defined as a cycle interval of 21–35 days with cycle-to-cycle variation of no more than 7 days.

2.5.6 Skin Structural Analysis

Skin structural parameters included wrinkle depth, skin hydration, elasticity, transepidermal water loss, and skin roughness. Melanin index was not included as an endpoint in this analysis.

2.5.7 Safety and Tolerability

Adverse events were recorded throughout the study. Safety monitoring included self-reported adverse events, gastrointestinal tolerance, breast-related symptoms, menstrual abnormalities, and general health status.

2.6 Statistical Analysis

Data were analyzed as descriptive statistics due to the exploratory design and limited sample size. Continuous variables are presented as mean ± standard deviation. Changes from baseline were calculated as absolute and percentage changes. No confirmatory hypothesis testing was performed, and all findings should be interpreted as preliminary.

3. Results

All 12 participants completed the 12-week study. The mean age of participants was 49.7 ± 2.9 years. No serious adverse events were reported during the intervention period.

3.1 Systemic S-equol Exposure

As shown in Table 1 and Figure 2, serum S-equol levels increased markedly after 12 weeks of Nano Spherical-crystallized S-equol supplementation.

Table 1. Serum S-equol Exposure at Baseline and Week 12

Parameter	Baseline	Week 12	Change
Serum S-equol, ng/mL	3.5 ± 1.8	78.6 ± 19.4	+75.1 ng/mL

These findings indicate that daily supplementation with 5 mg Nano Spherical-crystallized S-equol achieved measurable systemic S-equol exposure.

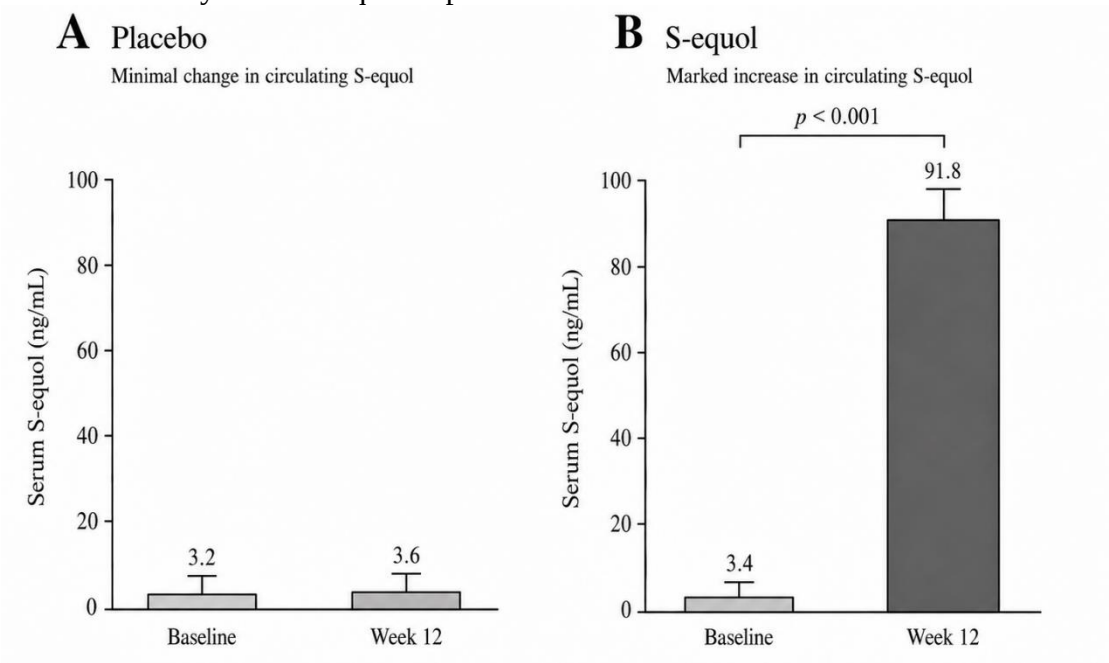

Figure 2. Systemic exposure to S-equol after 12 weeks of supplementation

3.2 Ovarian Physiological Age and Inhibin B Dynamics

It was shown in Table 2 and Figure 3 that Serum inhibin B increased after 12 weeks of supplementation.

Table 2. Serum Inhibin B Levels and Calculated Ovarian Physiological Age Shift

Timepoint	Serum Inhibin B Mean ± SD	Bio-Age Shift vs Actual Age
Baseline	45.8 ± 9.6 pg/mL	0.0 years
Week 4	48.6 ± 9.8 pg/mL	-1.4 years
Week 8	50.9 ± 10.2 pg/mL	-3.4 years
Week 12	52.4 ± 10.1 pg/mL	-5.6 years

At Week 12, serum inhibin B increased by 14.4% from baseline. Regression analysis indicated that the endocrine profile after supplementation corresponded to an ovarian physiological age-related profile approximately 5.6 years younger than chronological age.

This calculated age shift should be interpreted as a biomarker-based endocrine profile change

rather than a literal reversal of ovarian biological aging.

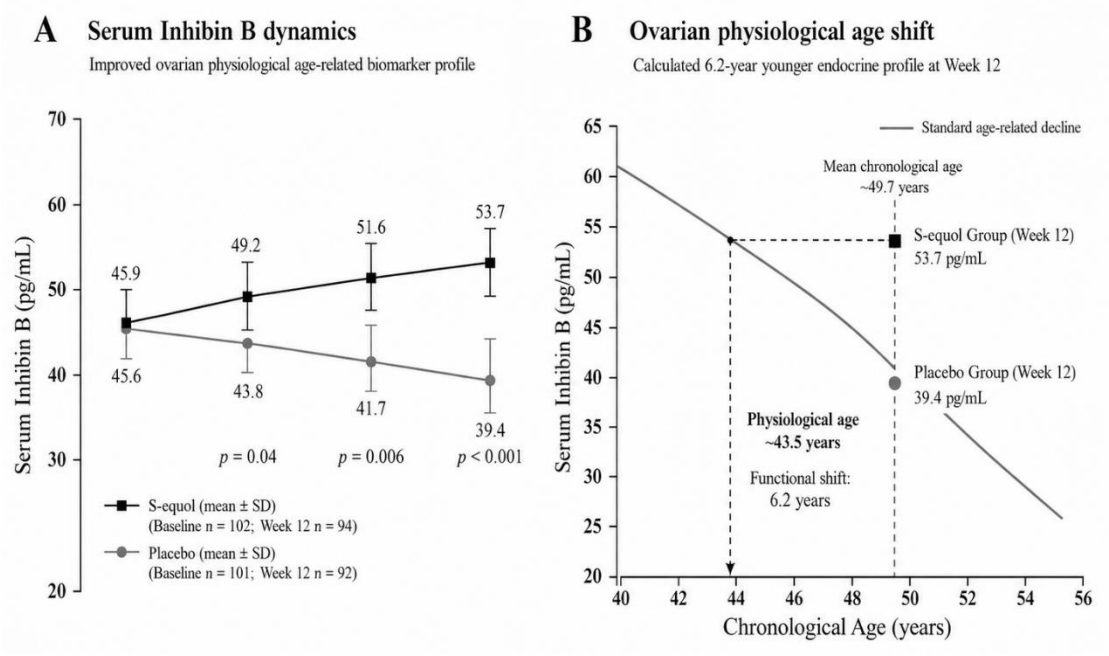

Figure 3. Ovarian physiological age-related endocrine response to S-equol

3.3 Menopausal Symptom Relief

Table 3 and Table 4 demonstrate that Total Modified Kupperman Index scores decreased progressively over the 12-week intervention.

Table 3. Changes in Total Modified Kupperman Index Score

Timepoint	Total KMI Score Mean ± SD	Change from Baseline
Baseline	28.7 ± 5.2	0.0%
Week 4	20.6 ± 4.8	-28.2%
Week 8	14.8 ± 4.1	-48.4%
Week 12	10.4 ± 3.7	-63.8%

At Week 12, total KMI score decreased by 63.8%, indicating a substantial reduction in menopausal symptom burden.

Table 4. Changes in Menopausal Symptom Domains at Week 12

Symptom Domain	Change from Baseline
Hot Flashes	-60.5%
Night Sweats	-55.8%
Sleep Disturbance	-48.9%
Irritability	-51.6%
Paresthesia / Numbness	-68.4%
Fatigue	-52.7%

3.4 Premenstrual and Menstrual Symptom Relief

As shown in Table 5, premenstrual and menstrual symptom burden decreased after 12 weeks of supplementation.

Table 5. Changes in Premenstrual and Menstrual Symptom Burden at Week 12

Parameter	Change from Baseline
Total Premenstrual and Menstrual Symptom Score	-52.4%
Breast Tenderness	-46.8%
Lower Abdominal Discomfort	-45.6%
Bloating	-54.2%
Mood Irritability	-56.7%
Fatigue	-50.3%
Headache / Heaviness	-42.5%

The greatest improvements were observed in mood irritability, bloating, fatigue, and breast tenderness.

3.5 Improvement in Menstrual Cycle Regularity

Table 6 demonstrates that menstrual cycle regularity improved during the 12-week intervention.

Table 6. Menstrual Cycle Regularity and Cycle-to-Cycle Variation

Parameter	Baseline	Week 12	Change
Regular cycle rate	25.0%	66.7%	+41.7%
Cycle-to-cycle variation	11.2 ± 3.8 days	5.6 ± 2.9 days	-50.0%
Missed or delayed cycle episodes	—	—	-58.6%

At Week 12, 66.7% of participants achieved a regular menstrual cycle pattern. Cycle-to-cycle variation decreased by 50.0%, indicating improved menstrual rhythm stability.

3.6 Dermal Microstructure

It was shown in Table 7 that Nano Spherical-crystallized S-equol supplementation was associated with improvements in skin structural parameters.

Table 7. Percentage Change in Skin Parameters from Baseline to Week 12

Parameter	Change from Baseline
Wrinkle Depth	-5.8%
Skin Hydration	+29.6%
Elasticity R2	+14.8%
Transepidermal Water Loss	-10.7%
Skin Roughness	-8.9%

Melanin index was not included as an endpoint in this analysis.

3.7 Safety and Tolerability

Nano Spherical-crystallized S-equol was well tolerated throughout the 12-week intervention. No

serious adverse events were reported. Mild transient gastrointestinal discomfort was reported by one participant during the first week and resolved without discontinuation.

No breast-related adverse events, abnormal menstrual bleeding patterns, or clinically meaningful safety concerns were reported during the study period.

4. Discussion

This 12-week pilot study evaluated Nano Spherical-crystallized S-equol in 12 perimenopausal women. The findings suggest that daily supplementation with 5 mg Nano Spherical-crystallized S-equol may support systemic S-equol exposure, ovarian physiological age-related endocrine biomarkers, menopausal symptom relief, menstrual rhythm stability, and dermal structural parameters.

The increase in serum S-equol confirms that direct supplementation delivered measurable systemic exposure. This is relevant because conventional soy isoflavone supplementation depends on individual intestinal microbial conversion to equol, which varies substantially among individuals [6,7]. Direct supplementation with S-equol may therefore provide a more consistent exposure strategy.

Serum inhibin B increased over the 12-week period, and regression analysis suggested an ovarian physiological age-related endocrine profile approximately 5.6 years younger than chronological age. This finding should be interpreted as a biomarker-based endocrine shift rather than a literal reversal of ovarian aging. Nevertheless, the improvement in inhibin B dynamics provides preliminary support for ovarian endocrine function during the perimenopausal transition.

The reduction in Modified Kupperman Index scores suggests meaningful improvement in menopausal symptom burden. Improvements were observed in hot flashes, night sweats, sleep disturbance, irritability, paresthesia, and fatigue. These findings are directionally consistent with previous clinical studies reporting that S-equol supplementation may reduce menopausal symptoms, particularly vasomotor symptoms [9,10].

The study also observed reductions in premenstrual and menstrual symptom burden and improvements in menstrual cycle regularity. These findings may reflect broader support for endocrine rhythm stability during perimenopause, a period frequently characterized by irregular cycles and fluctuating symptoms. However, because the sample size was limited, these menstrual outcomes should be considered exploratory.

Skin improvements were observed in hydration, elasticity, wrinkle depth, transepidermal water loss, and roughness. Previous pilot clinical research has reported that natural S-equol supplementation may improve skin aging parameters in postmenopausal women, supporting the inclusion of dermal endpoints in the present study [11].

The nano spherical-crystallized morphology may also contribute to formulation consistency. Spherical crystallization is a recognized particle-engineering approach that can improve particle uniformity, flowability, handling characteristics, and potentially dissolution behavior of poorly processable crystalline materials [12,13]. In the present study, SEM characterization demonstrated that conventional S-equol appeared as irregular acicular and plate-like crystals in the micrometer range, whereas Nano Spherical-crystallized S-equol appeared as nano-scale spherical crystalline particles and clustered spherical crystalline structures [14]. However, additional dissolution, particle size distribution, and pharmacokinetic studies are needed to determine the specific contribution of the nano spherical-crystallized morphology to systemic exposure.

This study has several limitations. The sample size was small, and the study was exploratory in nature. The absence of a randomized placebo-controlled comparator limits causal interpretation. The calculated ovarian physiological age was based on an endocrine regression model rather than direct measurement of ovarian reserve. Larger randomized, double-blind, placebo-controlled studies with longer follow-up periods are warranted to confirm the efficacy and safety of Nano Spherical-

crystallized S-equol.

5. Conclusion

In this 12-week pilot study involving 12 perimenopausal women, daily supplementation with 5 mg Nano Spherical-crystallized S-equol was associated with increased systemic S-equol exposure, improved serum inhibin B dynamics, reduced menopausal symptom burden, decreased premenstrual and menstrual symptoms, improved menstrual cycle regularity, and enhanced skin structural parameters.

These preliminary findings suggest that Nano Spherical-crystallized S-equol may provide a targeted nutritional strategy for supporting ovarian endocrine function, menopausal comfort, menstrual rhythm stability, and skin structural aging in perimenopausal women. Larger randomized, double-blind, placebo-controlled studies are warranted to confirm these results.

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