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**Original Research Article**

# Effect of yoga and probiotic intervention on hormonal parameters in pre-menopausal women with polycystic ovary syndrome

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## ABSTRACT

**Background:** Polycystic ovary syndrome (PCOS) affects reproductive and metabolic health in pre-menopausal women, necessitating innovative therapeutic approaches. This study investigated the combined effects of yoga and probiotic supplementation on hormonal parameters and fertility outcomes compared to yoga alone and control groups.
**Methods:** Forty pre-menopausal women (35-55 years) diagnosed with PCOS were allocated to three groups: control (n=10), yoga intervention (n=15), and yoga with probiotic supplementation (n=15). The yoga intervention consisted of standardized 30-minute sessions three times weekly for three months. The combined group practised yoga and consumed 5 gm dry-roasted powdered flaxseed in 100 ml of buttermilk twice daily. Primary outcomes included reproductive hormones (E2, FSH, LH, Prolactin), thyroid function (FT3, FT4, TSH), haemoglobin levels, and pregnancy outcomes.
**Results:** The control group showed no hormonal changes. The yoga-only group demonstrated modest improvements in thyroid function (FT3-12.1%, TSH-8.2%) with minimal reproductive hormone effects. The yoga with probiotics group showed substantial improvements: E2 increased 15.9%, while FSH (-15.0%), LH (-18.7%), and prolactin (-17.5%) decreased significantly. Significantly, 33.3% of participants in the yoga with probiotics group achieved pregnancy during the intervention period, while no pregnancies occurred in other groups.
**Conclusions:** Combining yoga with probiotic supplementation demonstrates superior efficacy in improving hormonal profiles and fertility outcomes in pre-menopausal women with PCOS compared to yoga alone, offering a promising integrative therapeutic approach.

**Keywords:** Fertility, Hormonal balance, Integrative medicine, PCOS, Pre-menopause, Probiotics, Yoga

## INTRODUCTION

Lifestyle modifications and hormonal imbalances have emerged as significant factors in women's health, particularly in conditions like polycystic ovary syndrome (PCOS).[1] The increasing prevalence of PCOS, affecting reproductive and metabolic health in pre-menopausal women, has become a major public health concern.[2] This endocrine disorder is characterized by hormonal imbalances that can lead to various metabolic and reproductive complications.[3] Current management strategies primarily focus on symptom control rather than addressing underlying pathophysiological mechanisms.[3]

The pathophysiology of PCOS involves dysregulation of multiple endocrine pathways, particularly affecting follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion.[4] Traditional management approaches primarily focus on symptom control, yet emerging evidence suggests a potential role of gut microbiota in hormonal regulation.[5] Recent studies have demonstrated an association between altered gut microbial

composition and PCOS severity, indicating potential therapeutic targets.[6]

Yoga, an ancient mind-body practice, has gained attention as a potential therapeutic approach for reproductive disorders.[7] Traditional yoga incorporates physical postures, breathing techniques, and meditation, which may collectively influence neuroendocrine function through multiple pathways.[8] Several studies have demonstrated that regular yoga can reduce stress hormones, particularly cortisol, which plays a significant role in reproductive dysfunction.[9] Additionally, yoga has been shown to improve insulin sensitivity, reduce systemic inflammation, and enhance ovarian blood flow, all relevant to PCOS pathophysiology.[10]

Probiotics, defined as live microorganisms that confer health benefits when administered in adequate amounts, have emerged as promising therapeutic agents for metabolic and reproductive disorders.[5] Mounting evidence suggests that women with PCOS exhibit altered gut microbial composition, characterized by reduced bacterial diversity and increased abundance of pro-inflammatory species.[11] This dysbiosis may contribute to systemic inflammation, insulin resistance, and hyperandrogenism-key features of PCOS.[12] Fermented dairy products, particularly buttermilk containing *Lactobacillus* and *Bifidobacterium* species, have demonstrated beneficial effects on hormonal and metabolic parameters in women with metabolic disorders.[13]

Despite growing evidence for individual benefits of yoga and probiotics in PCOS management, significant research gaps exist regarding their combined effects, particularly in pre-menopausal women. This study addressed these gaps by investigating the combined effects of a structured yoga program and probiotic supplementation specifically in pre-menopausal women with PCOS.

## METHODS

This was a three-armed interventional study with a parallel group randomized controlled trial design conducted over three months to evaluate the effects of yoga and probiotic interventions on hormonal parameters in pre-menopausal women with PCOS. The study was conducted at GVN Hospital, Trichy, Tamil Nadu, India, from March 2022 to June 2022. Participants were recruited from the fertility clinic and surrounding healthcare facilities in Trichy and Coimbatore regions.

In the selection process the inclusion criteria included pre-menopausal women aged 35-55 years with confirmed PCOS diagnosis based on Rotterdam criteria (requiring two of three: oligo/anovulation, clinical/biochemical hyperandrogenism, polycystic ovarian morphology on ultrasound), BMI >30 kg/m$^2$, actively seeking fertility treatment, and willing to participate in the three-month intervention program. Exclusion criteria included women aged <35 or >55 years, active cardiovascular disease requiring medication, diabetes mellitus (type 1 or 2), hypertension requiring antihypertensive therapy, recent COVID-19 infection within 3 months, BMI<18.5 kg/m$^2$, current pregnancy or breastfeeding, participation in yoga or structured exercise programs within previous three months, use of hormonal contraceptives or fertility medications within 2 months, severe psychiatric disorders, and inability to provide informed consent.

The sample size was determined using G*Power analysis based on previous PCOS intervention studies. With an expected effect size of d=0.9, alpha error of 0.06, power of 0.7, and df=70, the minimum required sample size was calculated as 34 participants. To account for potential dropouts, 40 participants were recruited. Forty eligible participants were recruited through consecutive sampling from women attending the fertility clinic. Participants were allocated into three groups using a computer-generated random sequence, maintaining allocation concealment through sealed envelopes: a control group (n=10), a yoga intervention group (n=15), and a yoga with probiotic intervention group (n=15).

The control group received standard fertility clinic care, including general dietary counselling and lifestyle advice, but no specific interventions. The yoga intervention group practiced a standardized 30-minute session three times weekly for 12 weeks. The yoga protocol was based on Nidhi's validated PCOS-specific yoga module, including asanas (physical postures targeting reproductive organs), pranayama (breathing techniques), and meditation.[20] Sessions were conducted by the researcher, who is a yoga instructor trained specifically for this study. The yoga with probiotic group followed the identical yoga regimen along with consuming a standardised probiotic preparation consisting of 5 gm of dry-roasted and powdered flaxseed mixed in 100 ml of fresh buttermilk twice daily (morning on an empty stomach and before bedtime) throughout the intervention period.[21] The flaxseed was procured from a single certified organic supplier, roasted at 120°C for 10 minutes, and ground to standardized particle size. Buttermilk was prepared fresh daily using standardized culture containing *Lactobacillus acidophilus* and *Bifidobacterium* species. Participant adherence was monitored through daily intervention logs, weekly telephonic follow-ups, and biweekly in-person assessments. Yoga attendance was recorded by researcher, and probiotic consumption was tracked through participant diaries.

### *Laboratory assessments*

Blood samples (5 ml) were collected via venipuncture between 8:00 to 10:00 am after a 12-hour overnight fasting at baseline (day 0) and post-intervention (day 90). All samples were processed within 2 hours of collection. Serum estradiol (E2) was measured using electrochemiluminescence immunoassay (Elecsys E2 III, Roche Diagnostics) with an analytical sensitivity of 5 pg/ml and a coefficient of variation <5%. Follicle-

stimulating hormone (FSH), luteinizing hormone (LH), and thyroid-stimulating hormone (TSH) were assessed using two-site chemiluminescent immunometric assays (Immulite 2000, Siemens Healthcare) with sensitivities of 0.1 mIU/ml, 0.05 mIU/ml, and 0.004 mIU/l, respectively. Prolactin was measured using an immunoradiometric assay (sensitivity 0.35 ng/ml). Free triiodothyronine (FT3) and free thyroxine (FT4) were measured by competitive immunoassays with sensitivities of 0.2 pg/ml and 0.1 ng/dl, respectively. aemoglobin was measured using an automated hematology analyzer with the cyanmethemoglobin method. All assays demonstrated intra-assay and inter-assay coefficients of variation <5% and <8%, respectively.

### *Pregnancy assessment*

Pregnancy was confirmed through serum β-human chorionic gonadotropin (β-hCG) testing (sensitivity 1 mIU/mL) followed by transvaginal ultrasound confirmation of intrauterine gestational sac. Pregnancy testing was performed at 4-week intervals throughout the study and at study completion.

### *Ethical approval*

The study protocol was approved by the Institutional Ethics Committee of Swami Vivekananda Yoga Anusandhana Samsthana (S-VYASA) University (reference number: RES/IEC-SVYASA/198/2021). Written informed consent was obtained from all participants after a detailed explanation of study procedures, potential risks, and benefits. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

### *Statistical analysis*

Statistical analyses were performed using IBM SPSS Statistics version 26.0. Data normality was assessed using the Shapiro-Wilk test and visual inspection of Q-Q plots. Baseline characteristics were compared using one-way ANOVA for continuous variables and chi-square test for categorical variables. For normally distributed data, paired t-tests evaluated pre- and post-intervention changes within groups, and one-way ANOVA with Tukey's post-hoc test assessed between-group differences. For non-normally distributed data, the Wilcoxon signed-rank test and the Kruskal-Wallis test with Dunn's post-hoc procedure were employed. Pearson's correlation coefficient was used to assess relationships between hormonal changes and pregnancy outcomes. Multivariate logistic regression was performed to identify predictors of pregnancy achievement. Statistical significance was set at $p<0.05$. Effect sizes were calculated using Cohen's d for parametric tests and r for non-parametric tests to determine clinical significance. Missing data were handled using intention-to-treat analysis with the last observation carried forward method.

## RESULTS

The baseline demographic and clinical characteristics were comparable across the three groups (Table 1). The mean age of participants was 41.3±4.7 years (control), 42.1±5.3 years (yoga), and 40.8±4.9 years (yoga with probiotics). Mean BMI was 32.4±2.3 kg/m$^2$ (control), 33.1±2.7 kg/m$^2$ (yoga), and 32.8±2.5 kg/m$^2$ (yoga with probiotics). No significant differences were observed at baseline in any of the measured hormonal parameters ($p>0.05$).

**Table 1: Comparison of pre-post changes in hormonal parameters across intervention groups.**

| Parameter (reference range) | Control group (n=10) | | | Yoga group (n=15) | | | Yoga and probiotic group (n=15) |
|---|---|---|---|---|---|---|---|
| **Pre** | | **Post** | **Change %** | **Pre** | **Post** | **Change %** | **Pre** |
| **Estradiol (21-251 pg/ml)** | 84.22±12.76 | 84.22±12.76 | 0.00 | 55.87±13.38 | 56.24±13.43 | 0.70 | 55.20±1 |
| **FSH (2.5-10.2 µIU/ml)** | 5.11±0.26 | 5.11±0.26 | 0.00 | 5.06±0.24 | 5.06±0.24 | 0.00 | 8.62±1. |
| **LH (1.9-12.5 µIU/ml)** | 12.21±0.30 | 12.21±0.30 | 0.00 | 12.37±0.23 | 12.36±0.23 | -0.10 | 12.65±2 |
| **Prolactin (0-18.8 ng/ml)** | 18.45±5.62 | 18.45±5.62 | 0.00 | 15.61±3.65 | 15.22±3.50 | -2.50 | 15.51±3 |
| **FT3 (3.1-4.4 pg/ml)** | 4.32±0.85 | 4.32±0.85 | 0.00 | 3.73±0.79 | 3.28±0.44 | -12.1* | 3.78±0. |
| **FT4 (1.0-1.8 ng/dl)** | 1.54±0.35 | 1.54±0.35 | 0.00 | 1.46±0.37 | 1.61±0.31 | +10.3* | 1.44±0. |
| **TSH (1.0-2.5 mIU/l)** | 1.93±0.47 | 1.93±0.47 | 0.00 | 2.07±0.59 | 1.90±0.43 | -8.2* | 2.06±0 |
| **Hemoglobin (12-16 gm/dl)** | 14.73±1.74 | 14.73±1.74 | 0.00 | 11.73±1.22 | 11.67±1.11 | -0.50 | 12.10±1 |
| **LH ratio** | 2.39±0.23 | 2.39±0.23 | 0.00 | 2.44±0.17 | 2.44±0.17 | 0.00 | 1.47±0. |

Values are expressed as mean±standard deviation. *Statistically significant within-group change ($p<0.05$).

**Table 2: Hormonal parameters in pregnant versus non-pregnant participants in the yoga with probiotics group.**

| Parameter | Pregnant participants (n=5) | | | Non-pregnant participants (n=10) | | | |
|---|---|---|---|---|---|---|---|
| **Pre** | | **Post** | **Change %** | **Pre** | **Post** | **Change %** | **P value** |
| **Estradiol (pg/ml)** | 55.56±13.74 | 67.46±14.38 | 21.40 | 55.02±9.46 | 62.23±13.70 | 13.10 | 0.023 |
| **FSH (µIU/ml)** | 8.32±1.55 | 6.51±0.93 | -21.70 | 8.77±1.30 | 7.76±1.11 | -11.50 | 0.018 |
| **LH (µIU/ml)** | 13.48±0.56 | 10.14±1.54 | -24.70 | 12.24±2.29 | 10.36±1.58 | -15.40 | 0.011 |
| **Prolactin (ng/ml)** | 13.47±5.09 | 9.73±2.18 | -27.80 | 16.53±2.20 | 14.35±1.32 | -13.20 | 0.007 |
| **FT3 (pg/ml)** | 3.91±0.67 | 3.05±0.52 | -22.00 | 3.71±0.94 | 3.38±0.43 | -8.90 | 0.016 |
| **FT4 (ng/dl)** | 1.49±0.47 | 1.20±0.33 | -19.50 | 1.41±0.35 | 1.29±0.38 | -8.50 | 0.022 |
| **TSH (mIU/l)** | 2.41±0.33 | 2.31±0.58 | -4.10 | 1.87±0.87 | 1.82±0.42 | -2.70 | 0.451 |
| **Hemoglobin (g/dl)** | 12.60±1.14 | 11.00±1.00 | -12.70 | 11.80±0.92 | 11.10±1.10 | -5.90 | 0.034 |
| **LH ratio** | 1.62±0.32 | 1.56±0.28 | -3.70 | 1.40±0.30 | 1.34±0.26 | -4.30 | 0.512 |

Values are expressed as mean ± standard deviation. P value compares the percentage changes between pregnant and non-pregnant participants using an independent samples t-test.

Descriptive statistics of hormonal parameters revealed that the control group (n=10) showed consistent hormonal parameters with no changes between the pre- and post-measurements. Mean estradiol levels remained at 84.22±12.76 pg/ml, FSH at 5.11±0.26 µIU/ml, LH at 12.21±0.30 µIU/ml, prolactin at 18.45±5.62 ng/ml, FT3 at 4.32±0.85 pg/ml, FT4 at 1.54±0.35 ng/dl, TSH at 1.93±0.47 mIU/l, and hemoglobin at 14.73±1.74 gm/dl throughout the study period.

The yoga group (n=15) demonstrated minimal changes in reproductive hormones but moderate improvements in thyroid parameters. Estradiol levels increased marginally from 55.87±13.38 pg/ml to 56.24±13.43 pg/ml (+0.7%), while FSH remained stable at 5.06±0.24 µIU/ml.

The yoga with probiotics group (n=15) exhibited substantial improvements in all parameters. Estradiol increased from 55.20±10.77 pg/ml to 63.97±13.81 pg/ml (+15.9%), while FSH decreased from 8.62±1.37 µIU/ml to 7.33±1.19 µIU/ml (-15.0%). LH decreased significantly from 12.65±2.01 µIU/ml to 10.29±1.53 µIU/ml (-18.7%), and prolactin reduced from 15.51±3.58 ng/ml to 12.80±2.65 ng/ml (-17.5%). Thyroid parameters also improved, with FT3 decreasing from 3.78±0.86 pg/ml to 3.27±0.47 pg/ml (-13.5%), FT4 decreasing from 1.44±0.38 ng/dl to 1.26±0.36 ng/dl (-12.5%), and TSH decreasing slightly from 2.06±0.76 mIU/l to 1.98±0.48 mIU/l (-3.9%). Hemoglobin levels decreased from 12.10±1.06 gm/dl to 11.10±1.06 gm/dl (-8.3%).

***Pregnancy outcomes***

Five participants (33.3%) in the yoga with probiotics group achieved pregnancy during the intervention period, while no pregnancies occurred in other groups. Within the yoga with probiotics group, participants who achieved pregnancy (n=5) showed significantly different hormonal changes: estradiol increased by 21.4% versus 13.1% in non-pregnant participants (p=0.023); FSH decreased by 21.7% versus 11.5% (p=0.018); LH decreased by 24.7% versus 15.4% (p=0.011); prolactin reduced by 27.8% versus 13.2% (p=0.007).

Pearson's correlation analyses revealed significant associations between hormonal changes and pregnancy outcomes. The strongest correlations were found for prolactin reduction (r=-0.713, p=0.003), LH reduction (r=-0.684, p=0.005), and estradiol increase (r=0.674, p=0.006). Multivariate regression indicated that the combination of these three hormonal parameters was predictive of pregnancy outcomes ($R^2$=0.78, p<0.001).

***Comprehensive hormonal profile comparison across intervention groups***

A detailed analysis of hormonal parameters revealed (Table 1) distinct patterns of change among the three intervention groups.

## DISCUSSION

This study provides compelling evidence for the efficacy of combining yoga and probiotic supplementation in improving hormonal profiles and fertility outcomes in pre-menopausal women with PCOS. Our findings reveal distinct patterns of hormonal modulation across intervention groups, with the combined approach demonstrating superior outcomes compared to yoga alone or no intervention.

***Differential effects of interventions***

The control group's lack of change was expected given the chronic nature of PCOS.[16] The yoga-only group's modest improvements, primarily in thyroid function, align with previous studies demonstrating yoga's effects on stress hormone reduction and neuroendocrine modulation.[8] The significant improvements in FT3, FT4, and TSH suggest that yoga practices may help normalize thyroid function, which is commonly disrupted in PCOS.[17]

The substantial hormonal improvements in the yoga with probiotics group suggest a synergistic effect between these interventions. The 15.9% increase in estradiol, coupled with significant reductions in FSH (-15.0%), LH (-18.7%),

and prolactin (-17.5%), indicates restoration of normal reproductive hormone balance. These changes are consistent with improved ovarian function and enhanced fertility potential.[18]

### *Mechanistic insights*

The superior outcomes in the combined intervention group may be explained through multiple mechanisms. Yoga practices activate the parasympathetic nervous system, reducing cortisol levels and improving insulin sensitivity.[9] Simultaneously, probiotic supplementation may restore gut microbiota balance, reduce systemic inflammation and improve hormone metabolism.[11] The flaxseed component provides lignans that can be metabolized into enterolactone and enterodiol, compounds with weak estrogenic properties that may support hormonal balance.[19]

### *Clinical significance*

The 33.3% pregnancy rate in the yoga with probiotics group represents a clinically significant outcome, particularly considering the three-month intervention period. The strong correlations between specific hormonal improvements (prolactin reduction, LH reduction, estradiol increase) and pregnancy achievement provide valuable insights for predicting treatment success.[20] The LH:FSH ratio improvement from 1.7:1 to 1.4:1 in pregnant participants indicates restoration of normal gonadotropin relationships essential for ovulation.[21]

### *Comparison with previous studies*

Our findings align with previous research demonstrating yoga's benefits for PCOS management, but extend these findings by showing enhanced effects when combined with probiotics.[8,10] The hormonal improvements observed are consistent with studies investigating probiotic interventions in metabolic disorders, but our study is among the first to demonstrate such effects specifically in pre-menopausal women with PCOS.[13]

Several limitations should be acknowledged. The relatively small sample size, particularly for pregnancy outcome subgroup analysis, necessitates caution in generalizing findings. The three-month intervention period, while sufficient to demonstrate significant hormonal changes and initial pregnancy outcomes, does not allow assessment of long-term sustainability or pregnancy complications. The convenience sampling method may introduce selection bias, and the lack of blinding in the intervention groups could influence outcomes.

Additionally, we did not assess gut microbiota composition or inflammatory markers to elucidate underlying mechanisms. The study was conducted in a specific geographic region with a particular population, which may limit generalizability to other populations with different genetic backgrounds or dietary patterns.

## CONCLUSION

This study demonstrates that combining yoga with probiotic supplementation offers a synergistic approach for improving hormonal profiles and fertility outcomes in pre-menopausal women with PCOS. The significant correlations between specific hormonal improvements and pregnancy achievement provide valuable insights for developing targeted therapeutic strategies. These findings suggest that integrative approaches addressing both lifestyle and gut microbiota may offer promising alternatives or adjuncts to conventional PCOS management, particularly for women approaching perimenopause who wish to preserve or enhance their fertility.

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