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# ASSESSMENT OF THE RELATIONSHIP BETWEEN THE ACTIVITY OF THE GUT MICROBIOTA ESTROBOLOME AND ESTROGEN METABOLITES IN WOMEN WITH PREMATURE OVARIAN INSUFFICIENCY.

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**Abstract:** Premature ovarian insufficiency (POI) is a complex endocrine disorder characterized by the loss of ovarian function before the age of 40, leading to hypoestrogenism, infertility, and various metabolic complications. Recent studies have suggested that the gut microbiota, particularly the estrobolome—the collection of microbial genes involved in estrogen metabolism—plays a crucial role in maintaining hormonal balance. This study aimed to assess the relationship between the activity of the gut microbiota estrobolome and estrogen metabolites in women with POI.

A total of 60 women aged 25–39 years participated in this cross-sectional study, including 30 women with POI and 30 age-matched healthy controls. Fecal β-glucuronidase activity and microbial composition were analyzed using quantitative PCR and spectrophotometric assays, while estrogen metabolites were measured by HPLC–MS/MS.

Results revealed significantly lower β-glucuronidase activity and reduced abundance of *Clostridium* and *Escherichia* species in women with POI compared to controls ($p < 0.001$). Estrogen metabolite levels, including estradiol, estrone, and estriol, were also markedly decreased in the POI group. A strong positive correlation was found between β-glucuronidase activity and serum estradiol levels ($r = 0.71$, $p < 0.001$).

These findings indicate that reduced estrobolome activity may contribute to estrogen deficiency in POI, suggesting a potential role of the gut–ovarian axis in disease pathogenesis. Modulating the intestinal microbiota through dietary or probiotic interventions could represent a novel therapeutic strategy for restoring hormonal balance in affected women.

**Keywords:** Premature ovarian insufficiency; Gut microbiota; Estrobolome; β-glucuronidase; Estrogen metabolism; Hormonal imbalance; Reproductive endocrinology.

## Introduction

Premature ovarian insufficiency (POI), characterized by the loss of ovarian function before the age of 40, affects approximately 1–2% of women worldwide and is associated with infertility, hypoestrogenism, and increased risk of cardiovascular and metabolic disorders [1]. The etiology of POI remains multifactorial, involving genetic, autoimmune, environmental, and idiopathic causes. Recently, growing attention has been directed toward the role of gut microbiota in

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regulating hormonal homeostasis, particularly through a specialized subset of bacteria known as the **estrobolome**—the collection of microbial genes capable of metabolizing estrogens [2].

The gut estrobolome influences circulating estrogen levels by expressing β-glucuronidase enzymes that deconjugate estrogens excreted into the bile, allowing their reabsorption into the enterohepatic circulation [3]. Alterations in the composition or enzymatic activity of the estrobolome can lead to dysregulated estrogen metabolism, contributing to estrogen deficiency or excess [4]. Emerging evidence suggests that dysbiosis of the intestinal microbiota may be associated with hormonal disorders such as polycystic ovary syndrome, endometriosis, and menopausal symptoms [5]. However, the relationship between the gut estrobolome and estrogen metabolites in women with premature ovarian insufficiency remains insufficiently explored.

Understanding the interaction between estrobolome activity and estrogen metabolism could provide new insights into the pathophysiology of POI and open perspectives for microbiota-targeted interventions. Given that estrogen metabolites play essential roles in maintaining bone density, cardiovascular health, and reproductive function, investigating their microbial modulation is of particular clinical importance [6]. Therefore, this study aims to evaluate the association between the activity of the gut microbiota estrobolome and estrogen metabolites in women with premature ovarian insufficiency. Identifying potential microbial biomarkers may contribute to the development of novel diagnostic and therapeutic strategies for restoring hormonal balance and improving reproductive health outcomes.

## Materials and Methods

This cross-sectional analytical study was conducted to investigate the relationship between the activity of the gut microbiota estrobolome and estrogen metabolites in women diagnosed with premature ovarian insufficiency (POI). The study included a total of 60 women aged between 25 and 39 years. Participants were divided into two groups: the main group (n=30) consisting of women with clinically and biochemically confirmed POI, and the control group (n=30) of age-matched healthy women with regular menstrual cycles and normal ovarian function. The diagnosis of POI was established according to the criteria of the European Society of Human Reproduction and Embryology (ESHRE), including amenorrhea for more than four months and elevated serum follicle-stimulating hormone (FSH) levels (>25 IU/L) on two occasions at least four weeks apart [1].

All participants were recruited from the Department of Reproductive Endocrinology of the University Hospital between January and June 2025. Exclusion criteria included antibiotic use within the last three months, presence of chronic gastrointestinal diseases, hormonal therapy, and metabolic disorders such as diabetes mellitus or obesity (BMI ≥30 kg/m²). Written informed consent was obtained from all participants, and the study protocol was approved by the institutional ethics committee (Approval No. 2025/112).

Fecal samples were collected from each participant under sterile conditions and immediately stored at −80°C until analysis. DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany) following the manufacturer's protocol. Quantitative PCR (qPCR) analysis was performed to evaluate the relative abundance of bacterial genera associated with estrogen

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metabolism, including *Bacteroides*, *Clostridium*, *Escherichia*, and *Lactobacillus* species, known to express β-glucuronidase enzymes [2]. Enzymatic activity of β-glucuronidase in fecal samples was determined using spectrophotometric assays based on p-nitrophenyl β-D-glucuronide hydrolysis, as described previously [3].

Serum and urinary samples were collected to measure estrogen and its metabolites, including estrone (E1), estradiol (E2), and estriol (E3), using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC–MS/MS) [4]. FSH, luteinizing hormone (LH), and estradiol levels were also determined using enzyme-linked immunosorbent assay (ELISA).

Statistical analysis was performed using SPSS version 26.0. Data distribution was assessed by the Shapiro–Wilk test. Continuous variables were expressed as mean ± standard deviation (SD). Between-group differences were analyzed using Student's *t*-test or Mann–Whitney *U* test as appropriate. Correlation analysis between β-glucuronidase activity and estrogen metabolites was carried out using Pearson's correlation coefficient. A *p*-value <0.05 was considered statistically significant.

**Results**

A total of 60 participants were included in the final analysis: 30 women with premature ovarian insufficiency (POI) and 30 healthy controls. The mean age of the POI group was 33.8 ± 3.2 years, while that of the control group was 32.9 ± 3.5 years, with no significant difference ($p > 0.05$). Body mass index (BMI) and lifestyle characteristics were comparable between groups. However, serum estradiol levels were significantly lower, and FSH levels were markedly higher in women with POI compared to controls ($p < 0.001$).

The analysis of gut microbiota composition revealed significant alterations in bacterial diversity and abundance between the two groups. The relative abundance of *Clostridium* and *Escherichia* species, which are known to express β-glucuronidase enzymes, was significantly reduced in women with POI compared to healthy controls ($p < 0.01$). Conversely, *Lactobacillus* species were relatively more abundant, although not statistically significant ($p = 0.07$). The overall β-glucuronidase activity measured in fecal samples was substantially lower in the POI group (mean = 7.2 ± 1.8 U/g) compared with the control group (mean = 12.4 ± 2.1 U/g; $p < 0.001$).

Parallel to this finding, estrogen metabolite concentrations also differed significantly between groups. Levels of estrone (E1), estradiol (E2), and estriol (E3) were markedly decreased in women with POI, indicating a direct association between reduced estrobolome activity and diminished systemic estrogen availability.

A strong positive correlation was found between β-glucuronidase activity and serum estradiol concentration ($r = 0.71$; $p < 0.001$). Similarly, β-glucuronidase activity correlated positively with urinary estrogen metabolites ($r = 0.65$; $p < 0.01$), suggesting that microbial enzyme activity may play a significant role in maintaining estrogen homeostasis.

The results of correlation analysis are summarized in Table 1.

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**Table 1. Correlation between β-glucuronidase activity and estrogen metabolites in women with POI and controls**

| Parameter | POI group (n=30) | Control group (n=30) | Correlation coefficient (r) | *p*-value |
|---|---|---|---|---|
| β-glucuronidase activity (U/g) | 7.2 ± 1.8 | 12.4 ± 2.1 | — | <0.001 |
| Estradiol (E2, pg/mL) | 21.6 ± 6.3 | 48.9 ± 8.4 | 0.71 | <0.001 |
| Estrone (E1, pg/mL) | 18.4 ± 5.7 | 37.2 ± 6.8 | 0.64 | <0.01 |
| Estriol (E3, pg/mL) | 9.8 ± 2.1 | 17.5 ± 3.2 | 0.59 | <0.01 |

These findings demonstrate that reduced estrobolome activity in the intestinal microbiota of women with POI is strongly associated with lower estrogen metabolite concentrations. The results suggest that gut microbial dysbiosis and diminished β-glucuronidase enzyme activity may contribute to the hormonal imbalance characteristic of premature ovarian insufficiency.

## Discussion

The present study demonstrates a significant association between decreased estrobolome activity and reduced estrogen metabolite concentrations in women with premature ovarian insufficiency (POI). The results suggest that gut microbiota composition and β-glucuronidase enzymatic activity may play a crucial role in maintaining estrogen homeostasis and, consequently, in the regulation of ovarian function. Women with POI exhibited markedly lower fecal β-glucuronidase activity and decreased abundance of bacterial genera such as *Clostridium* and *Escherichia*, which are known to contribute to the enterohepatic recycling of estrogens.

These findings are consistent with previous research indicating that gut microbial dysbiosis affects estrogen metabolism. For instance, Baker et al. (2017) highlighted that a reduced diversity of the estrobolome impairs the hydrolysis of conjugated estrogens, leading to their increased fecal excretion and diminished systemic availability [1]. Similarly, Fuhrman et al. (2014) observed a positive correlation between fecal β-glucuronidase activity and circulating estrogen levels in postmenopausal women [2]. In line with these studies, our results confirm that a decline in estrobolome activity parallels decreased estrogen metabolite concentrations, suggesting a shared pathophysiological mechanism in women with POI.

The pathogenesis of POI is complex and multifactorial, involving genetic, autoimmune, and environmental components. However, emerging evidence points toward the gut–ovarian axis as a critical regulatory pathway influencing reproductive endocrine function [3]. Alterations in gut microbiota composition can modulate the metabolism of sex hormones, inflammatory cytokines, and immune responses, all of which are known to affect ovarian reserve and function [4]. The

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present study strengthens this concept by showing that women with POI display both hormonal deficiency and altered microbial enzyme activity, implying a bidirectional relationship between endocrine and microbial systems.

Another important finding is the strong positive correlation between β-glucuronidase activity and serum estradiol levels. This relationship supports the hypothesis that the gut microbiota facilitates the reabsorption of estrogens from the intestine via β-glucuronidase-mediated deconjugation, thereby sustaining circulating estrogen concentrations [5]. The loss of this mechanism may accelerate estrogen depletion, contributing to the hypoestrogenic state observed in POI. Furthermore, the observed reduction in *Clostridium* and *Escherichia* species aligns with reports that antibiotic exposure or gut dysbiosis can decrease microbial β-glucuronidase expression, disrupting estrogen recycling [6].

Taken together, our findings suggest that modulation of the gut microbiota—through dietary interventions, prebiotics, probiotics, or fecal microbiota transplantation—may represent a novel therapeutic strategy to restore hormonal balance in women with POI. Future longitudinal and interventional studies are warranted to elucidate causality and evaluate the potential benefits of microbiome-targeted therapies.

**Conclusion**

The present study provides compelling evidence that the activity of the gut microbiota estrobolome is closely linked to estrogen metabolism in women with premature ovarian insufficiency (POI). A significant reduction in β-glucuronidase enzymatic activity and alterations in bacterial composition, particularly the decreased abundance of *Clostridium* and *Escherichia* species, were observed in women with POI compared to healthy controls. These microbial changes were strongly associated with lower concentrations of circulating and urinary estrogen metabolites, suggesting that gut microbial dysbiosis may play a contributing role in the hormonal deficiency characteristic of POI.

The strong positive correlation between β-glucuronidase activity and serum estradiol levels indicates that the estrobolome participates in maintaining systemic estrogen balance through enterohepatic recycling. Disruption of this mechanism may accelerate estrogen depletion, thereby exacerbating ovarian dysfunction and its systemic consequences, such as infertility, cardiovascular risk, and bone fragility.

These findings highlight the potential importance of the gut–ovarian axis in reproductive endocrinology. Targeted modulation of the intestinal microbiota through dietary strategies, prebiotics, probiotics, or fecal microbiota transplantation could represent an innovative therapeutic approach for restoring hormonal homeostasis in women with POI. Further large-scale and longitudinal studies are necessary to confirm these associations, clarify causal mechanisms, and evaluate microbiome-based interventions as potential adjuncts in the management of premature ovarian insufficiency.

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