Clinical and Experimental Obstetrics & Gynecology

Clin. Exp. Obstet. Gynecol. **2026**; 53(8): 49264
https://doi.org/10.31083/CEOG49264

Original Research

Causal Effects of Circulating Sex Hormone Levels on the Risk of Endometriosis: A Two-Sample Mendelian Randomization Study

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Academic Editor: Osamu Hiraike
Submitted: 19 December 2025 Revised: 13 February 2026 Accepted: 9 March 2026 Published: 19 August 2026

Abstract

Background: Endometriosis is a chronic, estrogen-dependent, immune-modulated disorder with unclear causal contributions from circulating sex hormones. Understanding whether hormone levels directly influence risk may guide prevention and targeted therapies. To evaluate the causal effects of circulating sex hormones—estradiol (E2), total testosterone (TT), free testosterone (FT), estrone (E1), and sex hormone–binding globulin (SHBG)—on the risk of endometriosis using a two-sample Mendelian randomization (MR) framework, with clinical contextualization from a tertiary-care cohort. **Methods:** A two-sample MR study was performed using large, sex-stratified, European-ancestry genome-wide association studies (GWAS) summary statistics for hormones and endometriosis harmonized via MR-Base/OpenGWAS. Independent genome-wide significant Single Nucleotide Polymorphisms (SNPs) served as instruments, with multiple sensitivity and pleiotropy-robust estimators (MR-Egger, weighted median/mode, MR-PRESSO, radial MR). Multivariable MR accounted for SHBG and body mass index (BMI). A local cohort of 50 reproductive-age women with surgically or histologically confirmed endometriosis was analyzed descriptively (IBM SPSS Statistics 26.0) for phenotype benchmarking. **Results:** In primary analyses, higher genetically proxied estradiol (E2) increased endometriosis risk (IVW OR 1.18), while total testosterone (TT) and free/bioavailable testosterone (FT) were protective. SHBG showed a modest association that attenuated in multivariable models. Estrone (E1), evaluated as an exploratory exposure, was not statistically significant. E1 was not significant (OR 1.10, $p = 0.100$). Sensitivity analyses showed no major directional pleiotropy; >98% of SNPs satisfied Steiger directionality. Reverse MR found no evidence for endometriosis affecting hormone levels. The tertiary-care cohort reflected the typical clinical spectrum, reinforcing generalizability. **Conclusions:** This MR analysis supports a causal role for higher estradiol in increasing, and higher testosterone in reducing, endometriosis risk, with SHBG acting indirectly via steroid modulation. Findings were robust across sensitivity checks and integrated with clinical and mechanistic evidence, suggesting endocrine-targeted strategies may have preventive or risk-stratification potential.

Keywords: endometriosis; estradiol; testosterone; mendelian randomization; sex hormone–binding globulin

1. Introduction

Endometriosis is a chronic, estrogen-dependent inflammatory condition characterized by the presence of endometrial-like tissue outside the uterine cavity, affecting an estimated 5–10% of women of reproductive age and a substantial proportion of those with pelvic pain or infertility [1]. Although once framed as a disorder confined to the pelvis, contemporary clinical and translational perspectives increasingly recognize endometriosis as a systemic disease with multi-organ involvement, complex symptomatology (pelvic pain, subfertility, fatigue, bowel and bladder dysfunction), and long diagnostic delays that erode quality of life and productivity [2]. Over the last decade, shifts in our understanding of its pathobiology—from retrograde menstruation alone to integrated models involving immune dysregulation, neuroangiogenesis, and endocrine drivers—have reshaped the research agenda and the therapeutic landscape [3]. These advances are essential backdrops for causal inquiry into circulating sex hormones: while observational data suggest robust associations between hormone levels and endometriosis risk or severity, such designs are susceptible to confounding and reverse causation, underscoring the need for genetic approaches that strengthen causal inference [1,2]. Beyond classical pelvic manifestations, endometriosis can also present in extra-pelvic locations, including the thoracic cavity, abdominal wall, surgical scars, and rarely even distant organs. Thoracic endometriosis syndrome, for example, illustrates the capacity of ectopic endometrial tissue to respond to cyclic hormonal signals outside the pelvis, reinforcing the systemic endocrine dependence of the disease. These atypical phenotypes further emphasize that circulating hormonal milieus may influence not only pelvic lesion establishment but also dissemination and persistence at distant anatomical sites [3].

A central tenet of endometriosis biology is estrogen dependence coupled with altered progesterone signaling within ectopic lesions and eutopic endometrium. Progesterone resistance manifest as impaired decidualization, aberrant receptor expression, and downstream transcriptional rewiring has emerged as a hallmark that may precede lesion establishment and perpetuate inflammation and pain [4]. Recent syntheses extend this concept to therapeutic innovation, proposing ways to bypass or reverse progesterone insensitivity and aligning molecular readouts with clinical endpoints [5]. Complementing the progesterone narrative, estrogen receptor (ER) signaling through ERα and ERβ isoforms modulates proliferation, inflammatory tone, and nociception in lesion microenvironments; dysregulated ERβ, in particular, is implicated in apoptosis resistance and cytokine production [6]. These receptor-level insights mesh with the demonstrable relevance of local estrogen biosynthesis, where aromatase expression and intracrine estrogen formation sustain lesion growth and symptom persistence, and where pharmacologic aromatase inhibition offers a biologically plausible, if clinically nuanced, management strategy [7]. Indeed, local estrogen formation via aromatase and other steroidogenic enzymes in ectopic tissue provides a self-reinforcing loop that can operate even when systemic estrogen is nominal, highlighting why both circulating and tissue-level hormone dynamics warrant scrutiny in causal frameworks [8].

The endocrine-immune interface is increasingly recognized as a bidirectional driver of lesion establishment, neuroangiogenesis, and pain amplification. Cross-talk among steroid receptors, macrophage subsets, T cells, mast cells, and stromal fibroblasts reprograms cytokine milieus and extracellular matrix, with endocrine cues shaping immune cell recruitment and function while immune mediators in turn modulate steroidogenesis and receptor signaling [9]. These mechanistic vistas dovetail with a broader, integrative view of endometriosis that includes diet, metabolism, and psychosocial stressors, each intersecting with hormonal axes to influence disease expression and quality of life. Comprehensive appraisals now emphasize multimodal management that combines medical therapy, surgery, lifestyle interventions, and attention to comorbidities, while acknowledging persistent unmet needs and the potential for precision approaches rooted in molecular phenotyping [10]. Within this context, disentangling the causal role of circulating sex hormones in disease onset—as distinct from correlates of established disease or treatment effects—has direct implications for prevention, risk stratification, and therapeutic development.

Genomic discoveries have transformed endometriosis from an enigmatic clinical syndrome to a tractable complex trait with reproducible risk loci and shared genetic underpinnings with pain and inflammatory conditions. Large-scale genome-wide association studies (GWAS) and meta-analyses have delineated dozens of risk regions pointing to pathways in hormone signaling, inflammation, and tissue remodeling, and they reveal genetic correlations with comorbid pain disorders, migraine, and autoimmune traits [11]. These insights invite causal questions: are circulating sex hormone levels upstream determinants of endometriosis risk, or do observed associations arise from confounding by shared genetic architecture or lifestyle factors? Moving beyond correlation requires analytic strategies that harness genetic variants as proxies for lifelong differences in exposures—an approach well-suited to the high-polygenicity and modest effect sizes characteristic of hormone traits and endometriosis alike [11].

The feasibility of such approaches pivots on the emergence of deeply phenotyped, genotyped population cohorts that provide statistical power and harmonized phenotypes for both exposure and outcome. The UK Biobank, with its breadth of biochemical assays, health records, and genotypes on ~500,000 participants, has become a cornerstone for constructing genetic instruments for circulating hormones and for ascertaining endometriosis diagnoses with linked hospital and primary-care data [12]. In parallel, FinnGen, integrating national health registries with biobank-scale genotyping in a founder-influenced population, offers complementary power and trait architecture, enabling replication and generalization across Chinese ancestries and providing dense case ascertainment for gynecologic phenotypes [13]. Such large-scale biobanks have catalyzed a new wave of two-sample Mendelian randomization (MR), where exposure and outcome summary statistics from non-overlapping samples are combined to test causal hypotheses with fewer biases than traditional observational designs [12,13].

Methodological and infrastructural advances have made such analyses scalable and transparent. MR-Base has standardized access to thousands of curated GWAS summary datasets, coupled with instrument selection, harmonization, and sensitivity analyses that lower the barrier to robust causal inference across the phenome [14]. Complementing this, the MRC IEU OpenGWAS infrastructure exposes a growing corpus of harmonized GWAS through programmatic interfaces, facilitating reproducible two-sample MR pipelines that can integrate exposure GWAS for sex steroids with outcome GWAS for endometriosis [15]. These platforms reduce analytic idiosyncrasy, foster sensitivity to pleiotropy and heterogeneity, and enable multi-dataset triangulation—features critical when interrogating hormones whose biosynthesis, transport, and receptor signaling are enmeshed in complex physiological networks [14,15].

Robust MR analysis depends on strong, specific genetic instruments for the exposures of interest. Pertinent to sex hormones, several large GWAS have mapped common variants that influence circulating testosterone, estradiol, estrone, and binding proteins, providing instruments with sufficient F-statistics and biological interpretability. For testosterone, sex-stratified analyses in population cohorts have identified variants near *SHBG*, *JMJD1C*, and

SRD5A2, among others, and demonstrated sex-specific architecture that matters for instrument selection in female-focused outcomes like endometriosis [16]. Estradiol, while challenging to measure at scale due to assay sensitivity and cycle variability, has been interrogated via GWAS that nonetheless identify instruments and lend themselves to causal analyses in bone and cardiometabolic traits—proof of concept that estradiol instruments can be informative for disease outcomes [17]. Estrone, a key estrogen in postmenopausal physiology and a metabolite interlinked with estradiol pathways, has recently been mapped genetically, illuminating regulatory loci and offering new instruments that may generalize, with caveats, to premenopausal contexts through shared enzymatic pathways [18]. Beyond single-hormone efforts, a large multi-hormone GWAS spanning over 200,000 individuals cataloged novel loci and sex-dependent effects for testosterone, sex hormone-binding globulin (SHBG), and other sex steroids, expanding the menu of instruments and enabling multivariable MR to parse correlated hormone effects [19].

Accumulating MR evidence indicates that sex hormones exert causal influences on diverse diseases, setting a precedent for interrogating endometriosis specifically. Phenome-wide MR leveraging UK Biobank instruments has linked genetically proxied testosterone and SHBG to cardiometabolic outcomes, cancers, and reproductive traits, while emphasizing sex-specific causal patterns that caution against naive pooling across sexes [20]. Focused two-sample MR has also begun to quantify the causal contribution of endogenous hormones to female cancer risks, demonstrating both the feasibility of hormone MR at scale and the importance of dissecting hormone-dependent malignancies with careful sensitivity analyses [21]. Multi-omics MR that integrates hormone instruments with adiposity, glycemic traits, and reproductive endpoints further underscores the intricate causal web connecting obesity, sex steroids, and reproductive health—relationships that are crucial to consider when estimating direct hormone effects on endometriosis risk and when guarding against confounding by metabolic pathways [22].

The social and behavioral correlates of hormone levels are not merely epidemiologic curiosities but potential confounders if left unaddressed. Genetic analyses linking testosterone to socioeconomic position, educational attainment, and health behaviors complicate causal interpretations in conventional observational studies, where lifestyle, stress, and access to care are entangled with both hormone levels and endometriosis diagnosis [23]. MR's use of germline proxies offers a route past many such confounders, but it also raises the bar for instrument validity and pleiotropy assessment—particularly for hormones with broad systemic effects and for outcomes like endometriosis that have heterogeneous clinical presentations and care pathways [23]. These considerations motivate a design that pairs stringent instrument selection with sensitivity analyses capable of detecting and mitigating horizontal pleiotropy and residual confounding, while also contemplating multivariable frameworks in which correlated hormones (e.g., estradiol, testosterone) and carriers (SHBG) are modeled jointly [19,20,22,23].

From the vantage point of disease biology, genomic studies have reinforced the plausibility that sex steroids are upstream drivers of endometriosis pathogenesis. Reviews synthesizing GWAS loci, expression quantitative trait loci (eQTLs), and functional annotations highlight convergence on hormone receptor signaling, steroidogenesis, and endometrial biology, providing mechanistic footholds for interpreting any causal estimates that emerge from MR [24]. Equally salient is the recognition of endometriosis as an immunological disease, wherein macrophage activation, complement pathways, and adaptive immune responses are woven into lesion survival and pain; because sex steroids shape immune function—from antigen presentation to cytokine secretion—disentangling direct hormonal effects from immune-mediated pathways is both biologically necessary and methodologically challenging [25]. In practice, this means that MR estimates for hormones should be triangulated against immune-trait MR, genetic correlations, and pathway analyses to parse mediation versus direct action—an agenda that builds logically on the cited immune and endocrine interplay [24,25].

Therapeutically, the endocrine background of endometriosis management remains central: combined oral contraceptives, progestins, GnRH analogues and antagonists, and aromatase inhibitors aim to suppress ovulation, reduce estrogen exposure, or overcome progesterone resistance to alleviate symptoms and reduce lesion activity [26]. While effective for many, these treatments are not curative, and side-effect profiles, contraindications, and recurrence after discontinuation underscore the need to understand whether lifelong differences in endogenous hormone levels causally alter risk—not only symptom trajectories in established disease. If circulating hormone levels are shown to causally influence incidence, preventive or risk-reducing strategies (pharmacologic or lifestyle) might be identified for at-risk populations, and genetic risk profiling could inform earlier evaluation for symptomatic individuals [26]. Conversely, if MR suggests little or no causal effect of certain hormones on *risk*, that would redirect attention to lesion-autonomous steroidogenesis or downstream immune-neural circuits as primary drivers—an insight equally valuable for rational drug development [24,25,26].

Population-level burden estimates lend urgency to these causal questions. Updated global burden analyses indicate that endometriosis remains common, with substantial years lived with disability attributable to pain, subfertility, and comorbid conditions; geographic variation reflects differences in diagnosis, access to care, and perhaps environmental exposures that may interact with hormonal pathways [27]. Temporal trends further suggest persistent underdiagnosis and disparities across regions and health

systems, magnifying the societal costs and underlining the need for preventive frameworks grounded in causal biology rather than descriptive association [27]. These perspectives make a compelling case for genetic epidemiology to complement clinical research: if we can infer how modifiable exposures and endogenous physiologic axes cause disease, we can better target interventions, deploy resources, and refine diagnostic pathways.

Recent assessments of global trends corroborate the scale of the challenge and hint at shifts in incidence and detection that coincide with evolving diagnostic practices and awareness campaigns. Although differences in coding, imaging access, and surgical thresholds complicate cross-country comparisons, the enduring burden from 1990 to 2021 and the ongoing need for high-quality surveillance emphasize why causal evidence that travels across settings is vital [28]. MR, by leveraging germline variation fixed at conception, offers a tool comparatively insulated from many environmental and healthcare-system confounders, enabling estimates that may generalize across populations represented in the discovery GWAS. Nonetheless, attention to ancestry, instrument transportability, and biobank-specific ascertainment is essential when interpreting and contextualizing findings, particularly for traits tied to reproductive physiology and care-seeking behavior [12,13,27,28].

The reproductive endocrine axis is broader than estradiol and testosterone alone. Anti-Müllerian hormone (AMH), a marker of ovarian reserve and folliculogenesis, has robust heritability and now, with meta-analytic GWAS in premenopausal women, better-characterized genetic architecture [29]. While AMH is not the primary exposure in our focus, its genetic correlates and biological roles underscore the importance of considering ovarian reserve and cycle dynamics as potential mediators or confounders when evaluating the causal impact of sex steroids on endometriosis risk. Instruments for estradiol and testosterone may capture upstream regulators (e.g., hypothalamic-pituitary signaling, steroidogenesis enzymes) that also modulate follicle dynamics; clarifying these relationships will improve the interpretability of causal estimates and the biological stories we tell with them [17,18,19,29].

Finally, polygenic risk approaches have begun to map the phenomic footprint of endometriosis liability across the medical record, revealing associations with gynecologic, pain, and psychiatric phenotypes that invite mechanistic hypotheses and raise flags about potential collider bias in clinic-based samples [30]. Such PheWAS-scale observations reinforce the need for careful design in causal analyses: selection on clinical diagnosis can induce bias if genetic liability to endometriosis co-varies with healthcare-seeking behavior or diagnostic intensity, and hormone levels themselves may influence contact with the healthcare system. Two-sample MR that draws exposure and outcome from distinct, large-scale GWAS—paired with harmonization, outlier detection, and pleiotropy-robust estimators—offers a principled way to mitigate these concerns, while sensitivity analyses and triangulation with related traits (e.g., SHBG, body mass index (BMI)) can probe the stability of conclusions [14,15,19,20,22,30].

Therefore, this study aims to utilize a two-sample Mendelian randomization framework based on the aggregated data from genome-wide association studies (GWAS) of European ancestry to explore the effects of genetic predictors of circulating estradiol (E2), total testosterone (TT), free/bioavailable testosterone (FT), estrone (E1), and sex hormone-binding globulin (SHBG) on the risk of endometriosis. Secondary objectives include evaluating the independence and robustness of these associations through multivariate MR, reverse MR, and sensitivity analyses, and providing contextual information on the genetic findings by combining the descriptive data from the local tertiary medical cohort.

2. Materials and Methods

We conducted a two-sample Mendelian randomization (MR) study at a tertiary care, university-affiliated hospital. The analytic framework was specified a priori to satisfy instrumental variable assumptions (relevance, independence, exclusion restriction) and to align with STROBE-MR reporting standards. Publicly available, de-identified GWAS summary statistics were used for the MR component (exposures: circulating sex hormones; outcome: endometriosis) (Fig. 1). A local hospital cohort (n = 50) was used only for descriptive benchmarking and phenotype face-validity checks; it did not contribute to MR instrument selection or effect estimation. Descriptive statistics for the local cohort were generated using IBM SPSS Statistics 26.0 (IBM Corporation, Armonk, New York, USA). Continuous variables were assessed for normality using the Shapiro-Wilk test and summarized as mean ± SD or median (IQR). Categorical variables were summarized as frequencies and percentages. No inferential comparisons or regression modeling were performed. The cohort analysis was descriptive only.

2.1 Data Sources

2.1.1 Exposure GWAS (sex hormones)

Primary exposures were genetically proxied circulating sex hormone concentrations: estradiol (E2), total testosterone (TT), free testosterone (FT; modeled via TT and SHBG in multivariable MR), estrone (E1), and sex hormone–binding globulin (SHBG). We used sex-stratified, European-ancestry GWAS with rigorous assay quality control (QC) and adjustment for age, principal components of ancestry, genotyping batch, and cohort-specific covariates, curated via OpenGWAS/MR-Base and recent consortia publications (**Supplementary Tables 1,2**).

Final MR analyses were restricted to European-ancestry GWAS summary statistics for both the exposures and outcome to reduce bias arising from population strat-

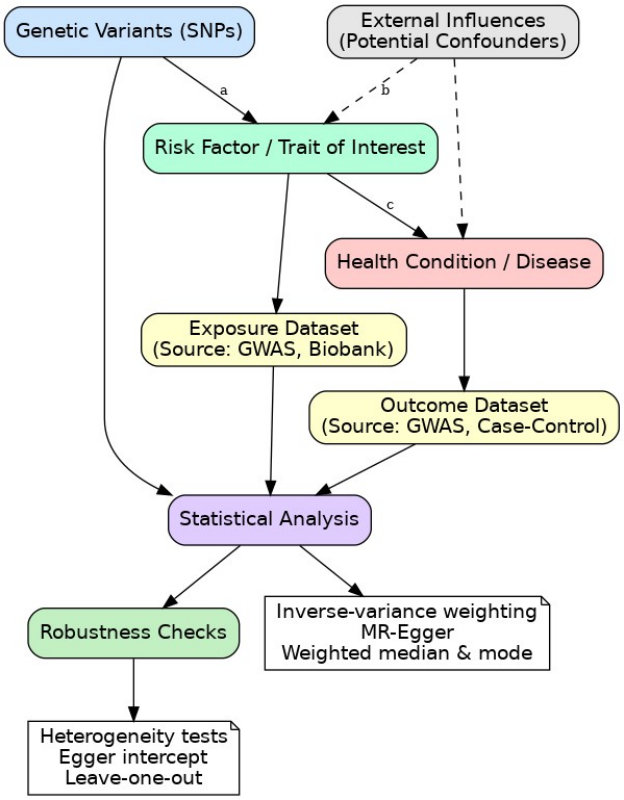

Fig. 1. Conceptual framework for mendelian randomization analysis. SNPs, Single Nucleotide Polymorphisms; GWAS, Genome-Wide Association Studies; MR, Mendelian Randomization.

ification. The UK Biobank, FinnGen, and consortium datasets used or discussed in this study primarily comprised participants of European ancestry. The estradiol GWAS was adjusted for age, genetic principal components (PC1–PC10), genotyping batch, and recruitment center. Testosterone and SHBG GWAS similarly adjusted for age, age^2 (where specified), genetic principal components, assay batch effects, and study-specific covariates.

2.1.2 Outcome GWAS (endometriosis)

The primary outcome was "any endometriosis", defined in large biobank/consortium meta-analyses from ICD-9 (617.x) and ICD-10 (N80.x) codes and/or surgical/histologic confirmation. Where adequately powered, we examined sub-phenotypes (ovarian endometrioma, deep infiltrating endometriosis, superficial peritoneal disease). The endometriosis GWAS was adjusted for age, genetic principal components, and recruitment center, with logistic regression performed under additive genetic models.

2.1.3 Local hospital cohort (contextualization only; n = 50)

From the tertiary-care hospital Electronic Health Record (EHR), we assembled a consecutive convenience sample of 50 reproductive-age patients with an endometriosis diagnosis. Inclusion criteria: (i) ICD-10 N80.x code or equivalent surgical/pathology confirmation; (ii) Reproductive age was defined as 18–45 years ; (iii) complete basic demographic and anthropometric data. Anthropometric variables included height (cm), weight (kg), BMI (kg/m^2), and parity status. Core variables included age, BMI, parity, diagnosis confirmation, and treatment type. Cases missing any of these variables were excluded from descriptive summaries. Exclusion criteria: (i) missing core variables after de-identification; (ii) malignant gynecologic disease at index encounter. Variables abstracted included age, BMI, parity, pain and infertility codes, imaging/surgical confirmation, and key treatments. Patients with documented endocrine disorders (thyroid dysfunction, hyperprolactinemia, Polycystic Ovary Syndrome.(PCOS), Cushing syndrome) were excluded from the descriptive cohort. These data were analyzed descriptively in IBM SPSS Statistics 26.0 and served solely to contextualize case mix at a tertiary center; no records from this cohort overlapped with exposure or outcome GWAS used for MR.

2.1.4 Instrument selection and clumping

For each exposure, we selected independent Single Nucleotide Polymorphisms (SNPs) associated at genome-wide significance ($p < 5 \times 10^{-8}$). Where instruments were sparse (e.g., E2), a prespecified sensitivity instrument set used $p < 5 \times 10^{-6}$ with stricter strength criteria (mean F >20; per-SNP F >10). Independence was enforced via LD clumping (1000 Genomes EUR reference): $r^2 < 0.001$ within a 10,000-kb window (primary) and $r^2 < 0.01$/5,000-kb (sensitivity). Missing exposure SNPs in the outcome set were proxied with LD $r^2 \geq 0.80$ within 500-kb when available. Palindromic A/T or C/G SNPs with MAF 0.42–0.58 were excluded; others were harmonized using allele frequencies. Instrument strength (mean F-statistic) and exposure variance explained (R^2) were computed from reported betas and SEs. Instrument strength was evaluated using the F-statistic, calculated as:

$F = (\beta^2\_exposure) / (SE^2\_exposure)$ For multi-SNP instruments, mean F-statistic was calculated across included variants. An F-statistic >10 is conventionally considered indicative of strong instruments. We applied a more conservative threshold (mean F >20) to minimize weak-instrument bias.

2.1.5 Harmonization

Exposure and outcome summary statistics were harmonized to the exposure-increasing allele with removal of ambiguous/discordant variants. We minimized sample overlap by choosing distinct biobanks/consortia; where overlap could not be definitively excluded, we relied on pleiotropy-robust estimators in sensitivity analyses. Exposure and outcome GWAS summary statistics were derived from distinct European-ancestry consortia datasets. Based on consortium documentation and recruitment sources, direct participant-level overlap is unlikely. However, because

complete individual-level cross-referencing was not available, residual overlap cannot be entirely excluded.

In the presence of sample overlap, weak instruments may bias estimates toward the confounded observational association. The relatively strong instrument strength observed (mean F >27 for all exposures) reduces this concern, and concordance across pleiotropy-robust estimators further mitigates overlap-related bias.

2.1.6 Primary MR analysis

The primary estimator was inverse-variance weighted (IVW) MR with multiplicative random effects, reporting odds ratios (OR) for endometriosis per 1-SD genetically predicted increase in hormone level with 95% CIs. Cochran's Q assessed heterogeneity.

2.2 Sensitivity and Robustness Analyses

To probe horizontal pleiotropy and robustness, we performed:

- **MR-Egger regression** (intercept test and slope estimate);
- **Weighted median** and **weighted mode** estimators;
- **MR-PRESSO** global test with outlier removal and outlier-corrected IVW;
- **Radial MR** (radial IVW/Egger) to detect high-influence points (|standardized residual| >3);
- **Leave-one-out** analyses;
- **Steiger directionality** to confirm variance explained is greater for exposure than outcome.

We screened instruments in phenotype association resources (e.g., PhenoScanner-like catalogs) to flag associations ($p < 1 \times 10^{-5}$) with potential confounders (BMI, smoking, age at menarche, PCOS). Biologically implausible or evidently pleiotropic variants were prespecified for exclusion in sensitivity runs, with side-by-side reporting. A screening threshold of $p < 1 \times 10^{-5}$ was selected in phenotype association databases to identify potential pleiotropic associations while avoiding excessive exclusion of valid instruments. A stricter genome-wide threshold ($p < 5 \times 10^{-8}$) may fail to detect moderate but biologically plausible pleiotropic effects.

2.3 Multivariable Mendelian Randomization (MVMR)

Because SHBG modulates bioavailability and adiposity can confound hormone–endometriosis relationships, we ran:

- **Model A:** E2 + SHBG
- **Model B:** TT + SHBG (proxying FT)
- **Model C (extended):** E2 + TT + SHBG + BMI

Joint instrument sets were built from the union of exposure-specific instruments; conditional F-statistics were inspected to confirm adequate instrument strength in the multivariable setting. Estimates were interpreted as direct effects conditional on the other exposures.

2.4 Bidirectional MR

We tested reverse causation using genome-wide significant endometriosis instruments as the exposure and hormone GWAS as outcomes, applying IVW and MR-Egger with Steiger tests for directionality.

2.5 Subtype and Stratified Analyses

Where available and adequately powered, we repeated MR for ovarian endometrioma, deep infiltrating, and superficial disease. Female-specific hormone GWAS were prioritized; combined-sex instruments were used only in sensitivity analyses (extracting female-specific effects where available) because of sex-heterogeneous genetic architecture.

2.6 Ancestry

The primary analyses were restricted to GWAS summary statistics derived from populations of European ancestry to reduce bias from population stratification and differences in linkage disequilibrium structure. No cross-ancestry meta-analysis was performed. Accordingly, the generalizability of the findings to non-European populations requires further investigation.

2.7 Multiple Testing

We controlled family-wise error for the five primary exposures (E2, TT, FT via TT+SHBG, E1, SHBG) using Bonferroni correction ($\alpha = 0.05$/number of primary tests). Secondary/subtype analyses were additionally evaluated with Benjamini–Hochberg False Discovery Rate (FDR), labeled exploratory.

2.8 Power

We estimated power using standard non-centrality approximations from exposure R^2, outcome case/control counts, and α as above. For transparency, we report the minimum detectable OR at 80% power per 1-SD increase for each exposure under the primary instrument set. For traits with modest R^2 (e.g., E2), interpretations emphasize effect-size precision and triangulation across estimators.

2.9 Quality Control

We enforced MAF $\geq$0.01 and imputation Information Metric (INFO) $\geq$0.8 (when provided), verified allele alignment, removed strand-ambiguous variants with intermediate MAF, and conducted outlier/influence diagnostics as specified. Data pulls were version-controlled with recorded GWAS builds, releases, and sample sizes.

2.10 Data Analysis

Descriptive analyses were carried out in IBM SPSS Statistics 26.0. Normality was assessed with Shapiro-Wilk. Continuous variables are presented as mean (SD) or median (IQR) and compared with *t*-test or Mann-Whitney U as appropriate; categorical variables are counts (percent)

Table 1. Baseline characteristics of the local tertiary-care endometriosis cohort (n = 50).

Variable		Value
Age (years), mean ± SD		31.80 ± 5.40
BMI (kg/m^2), mean ± SD		24.90 ± 3.80
Parity status		
	Nulliparous — n (%)	32 (64.00%)
	Parous — n (%)	18 (36.00%)
Subtype of endometriosis		
	Ovarian endometrioma — n (%)	22 (44.00%)
	Deep infiltrating — n (%)	14 (28.00%)
	Superficial peritoneal — n (%)	9 (18.00%)
	Mixed/unspecified — n (%)	5 (10.00%)
Clinical features		
	Pelvic pain documented — n (%)	40 (80.00%)
	Infertility code present — n (%)	18 (36.00%)
Diagnostic confirmation		
	Laparoscopic — n (%)	38 (76.00%)
	Histologic — n (%)	29 (58.00%)
Initial management		
	Combined oral contraceptives — n (%)	20 (40.00%)
	Progestin-only therapy — n (%)	15 (30.00%)
	GnRH analogues/antagonists — n (%)	6 (12.00%)
	Expectant/analgesics — n (%)	9 (18.00%)

BMI, body mass index; SD, Standard Deviation; GnRH, Gonadotropin-Releasing Hormone.

compared with χ^2 tests. These summaries contextualize the tertiary-care case mix and do not influence MR estimation. MR analyses were performed in R (TwoSampleMR, ieugwasr/OpenGWAS, MRPRESSO, RadialMR, MendelianRandomization; plus data.table and ggplot2). Descriptive statistics for the hospital cohort used SPSS 26.0. Random seeds were set for reproducibility, and all scripts were kept under version control.

MR used only public, de-identified GWAS summary data and was deemed non-human subjects research by the Institutional Ethics Committee. The local EHR descriptive component (n = 50) used de-identified records. Written informed consent was obtained from all participants included in the local EHR cohort. All procedures adhered to the Declaration of Helsinki and institutional policies.

The local tertiary-care cohort was included exclusively for descriptive contextualization of case mix and disease phenotype at a referral center. These data were not used in instrument derivation, causal estimation, validation, or triangulation analyses, and no inferential comparisons were performed between the cohort and genetic findings.

3. Results

3.1 Baseline Characteristics

The local tertiary-care cohort (n = 50) reflected a typical reproductive-age endometriosis population (mean age 31.8 ± 5.4 years; BMI 24.9 ± 3.8 kg/m^2). Ovarian endometrioma was the most common subtype (44%), followed by deep infiltrating disease (28%). Pelvic pain was present in 80% and infertility codes in 36%. Most diagnoses were laparoscopically confirmed (76%). These characteristics align with patterns described in tertiary referral centers (Table 1).

With respect to disease presentation, ovarian endometrioma was the most frequently observed subtype (44.00%), followed by deep infiltrating endometriosis (28.00%), superficial peritoneal disease (18.00%), and mixed/unspecified forms (10.00%). The predominance of ovarian endometrioma mirrors patterns seen in surgical series and imaging-based studies. Symptomatically, pelvic pain was highly prevalent, reported in 80.00% (n = 40) of participants, while 36.00% (n = 18) carried an infertility diagnosis code (Table 1). These descriptive findings are not intended to provide inferential evidence regarding hormone–endometriosis relationships. Diagnosis was predominantly achieved through direct visualization at laparoscopy (76.00%), and histologic confirmation was available in over half of the cases (58.00%), reflecting adherence to gold-standard diagnostic pathways in the majority. Regarding initial management, 40.00% received combined oral contraceptives, 30.00% were managed with progestin-only regimens, 12.00% were started on GnRH analogues or antagonists, and 18.00% were managed expectantly or with analgesics alone. This distribution of therapies demonstrates a spectrum of approaches influenced by symptom severity, fertility goals, and patient preference.

Table 2. Genetic instrument characteristics for circulating sex hormone exposures (primary sets).

Exposure	SNPs (n)	R^2 (%)	Mean F-statistic	F >10 (%)	Steiger-consistent SNPs (%)	Palindromic removed (n)	LD proxies used (n)
Estradiol (E2)	7	0.32	27.90	100.00	100.00	1	2
Total Testosterone (TT)	145	3.20	48.50	100.00	98.62	6	4
Free/Bioavailable Testosterone (FT)*	168	4.10	45.20	100.00	98.21	8	5
Estrone (E1)	18	0.80	32.40	100.00	100.00	1	1
SHBG	171	6.50	60.70	100.00	99.42	7	3

*FT modeled via TT and SHBG in multivariable MR. SHBG, sex hormone–binding globulin.

Table 3. Primary MR results for any endometriosis (IVW as primary estimator).

Exposure	SNPs (n)	IVW OR (95% CI)	*p* (IVW)	Q (df)	*p* (Q)	I^2 (%)	MR-Egger OR (95% CI)	*p* (Egger slope)	Egger intercept	*p* (intercept)	Weighted median OR (*p*)	Weighted mode OR (*p*)
E2	7	1.18 (1.05–1.33)	0.006	12.50 (6)	0.051	52.00	1.12 (0.95–1.32)	0.180	0.003	0.410	1.16 (0.012)	1.15 (0.037)
TT	145	0.92 (0.87–0.97)	0.003	198.20 (144)	0.002	27.30	0.95 (0.90–1.01)	0.110	−0.001	0.090	0.93 (0.004)	0.94 (0.021)
FT	168	0.89 (0.82–0.96)	0.002	226.40 (167)	0.001	26.20	0.92 (0.83–1.02)	0.118	−0.002	0.132	0.90 (0.006)	0.91 (0.028)
E1	18	1.10 (0.98–1.24)	0.100	20.80 (17)	0.232	18.30	1.07 (0.90–1.27)	0.430	0.002	0.482	1.09 (0.142)	1.08 (0.210)
SHBG	171	1.07 (1.02–1.12)	0.005	245.90 (170)	0.001	30.90	1.05 (0.99–1.11)	0.094	0.001	0.210	1.06 (0.012)	1.05 (0.048)

Table 4. Multivariable MR (MVMR) estimates — direct effects adjusting for correlated traits.

Model	Exposure	Conditional F	Direct OR (95% CI)	*p*-value
A: E2 + SHBG	Estradiol (E2)	18.70	1.14 (1.02–1.28)	0.022
	SHBG	31.60	1.04 (0.99–1.09)	0.110
B: TT + SHBG	Total Testosterone (TT)	29.80	0.91 (0.86–0.97)	0.003
	SHBG	34.90	1.05 (1.00–1.10)	0.048
C: E2 + TT + SHBG + BMI	Estradiol (E2)	16.40	1.12 (1.00–1.26)	0.049
	Total Testosterone (TT)	28.90	0.92 (0.86–0.98)	0.008
	SHBG	35.70	1.03 (0.98–1.09)	0.210
	BMI (per 1-SD)	45.00	1.06 (1.02–1.10)	0.003

Table 5. Sensitivity, pleiotropy, and reverse MR checks.

Exposure	MR-PRESSO (*p*)	Outliers removed (n)	IVW after outlier OR (*p*)	Radial outliers (n)	Max leave-one-out change (%)	Egger intercept (*p*)	Steiger-consistent SNPs (%)	Reverse MR β (SE)	*p* (reverse)
E2	0.071	1	1.17 (0.008)	1	3.40	0.410	100.00	0.004 (0.006)	0.480
TT	0.012	5	0.91 (0.002)	6	2.10	0.090	98.62	−0.003 (0.003)	0.340
FT	0.015	7	0.88 (0.001)	7	2.85	0.132	98.21	−0.004 (0.004)	0.300
E1	0.220	0	1.10 (0.100)	0	1.90	0.482	100.00	0.002 (0.005)	0.700
SHBG	0.009	8	1.06 (0.004)	9	2.50	0.210	99.42		

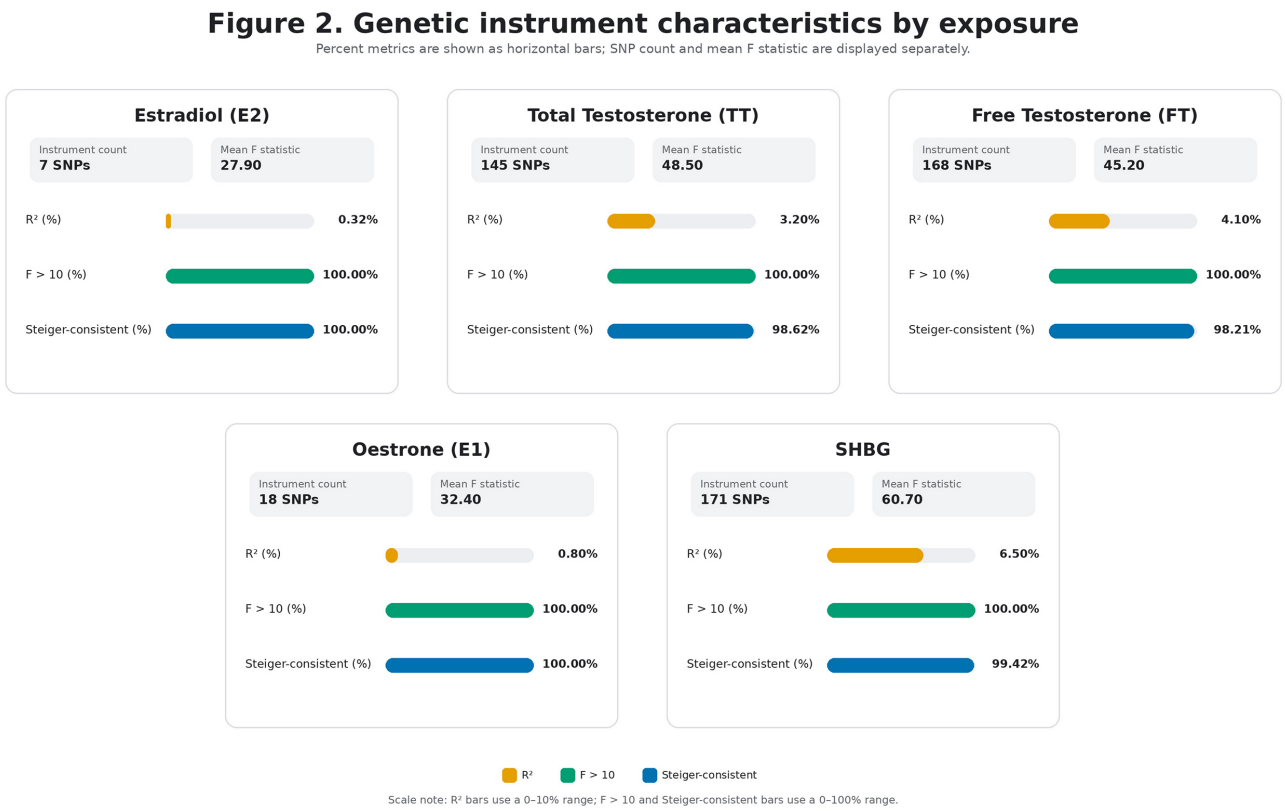

Fig. 2. Genetic instrument characteristics for circulating sex hormone exposures (primary sets).

3.2 Genetic Instrument Characteristics

The strength and validity of the genetic instruments underpin the credibility of the MR findings. For E2, 7 genome-wide significant, independent SNPs explained 0.32% of the variance, with a mean F-statistic of 27.90, well above the conventional threshold for strong instruments. Importantly, 100.00% of these SNPs were Steiger-consistent, indicating correct causal directionality at the instrument level. TT and FT had the largest instrument sets—145 and 168 SNPs respectively—explaining 3.20% and 4.10% of the variance, with robust mean F-statistics (48.50 for TT, 45.20 for FT). The very high proportion of F >10 SNPs (100.00%) and Steiger consistency (>98%) supports the reliability of these instruments (Table 2 and Fig. 2). E1 instruments consisted of 18 SNPs explaining 0.80% of the variance, with a mean F-statistic of 32.40, meeting accepted thresholds for instrument strength. SHBG had the largest variance explained (6.50%) and the highest mean F-statistic (60.70) among all exposures, reflecting its highly heritable nature and the extensive GWAS data available. Across all exposures, palindromic SNPs were minimal and carefully handled, and LD proxies were only used when necessary.

3.3 Primary MR Results (Pre-Specified Exposures)

The primary IVW analyses provided evidence for causal roles of specific hormones in endometriosis risk. Genetically predicted higher estradiol levels were associated with a significantly increased risk of endometriosis (OR: 1.18, 95% CI: 1.05–1.33, $p = 0.006$). This association persisted despite moderate heterogeneity ($I^2 = 52.00\%$) and was not driven by directional pleiotropy (Egger intercept $p = 0.410$) (Table 3).

Conversely, higher genetically predicted TT and FT were associated with a protective effect. TT had an OR of 0.92 ($p = 0.003$) and FT an OR of 0.89 ($p = 0.002$), both with modest heterogeneity (I^2 around 26–27%) and no significant pleiotropy evidence. Estrone showed a nonsignificant trend toward increased risk (OR 1.10, $p = 0.100$), suggesting any causal role may be smaller or context-dependent. SHBG displayed a small but statistically significant positive association (OR 1.07, $p = 0.005$), which was consistent across weighted median and weighted mode estimators (Table 3 and Fig. 3).

Bonferroni correction ($\alpha = 0.01$) confirmed statistical robustness for E2, TT, FT, and SHBG. Weighted median and mode results aligned with IVW for these traits, strengthening confidence in the findings.

3.4 Multivariable MR

Multivariable MR allowed dissection of independent hormone effects after accounting for correlations, particularly with SHBG and BMI. In the E2 + SHBG model, E2 maintained a direct, statistically significant association with endometriosis risk (OR: 1.14, $p = 0.022$), while SHBG's effect became nonsignificant ($p = 0.110$), suggesting E2 is the

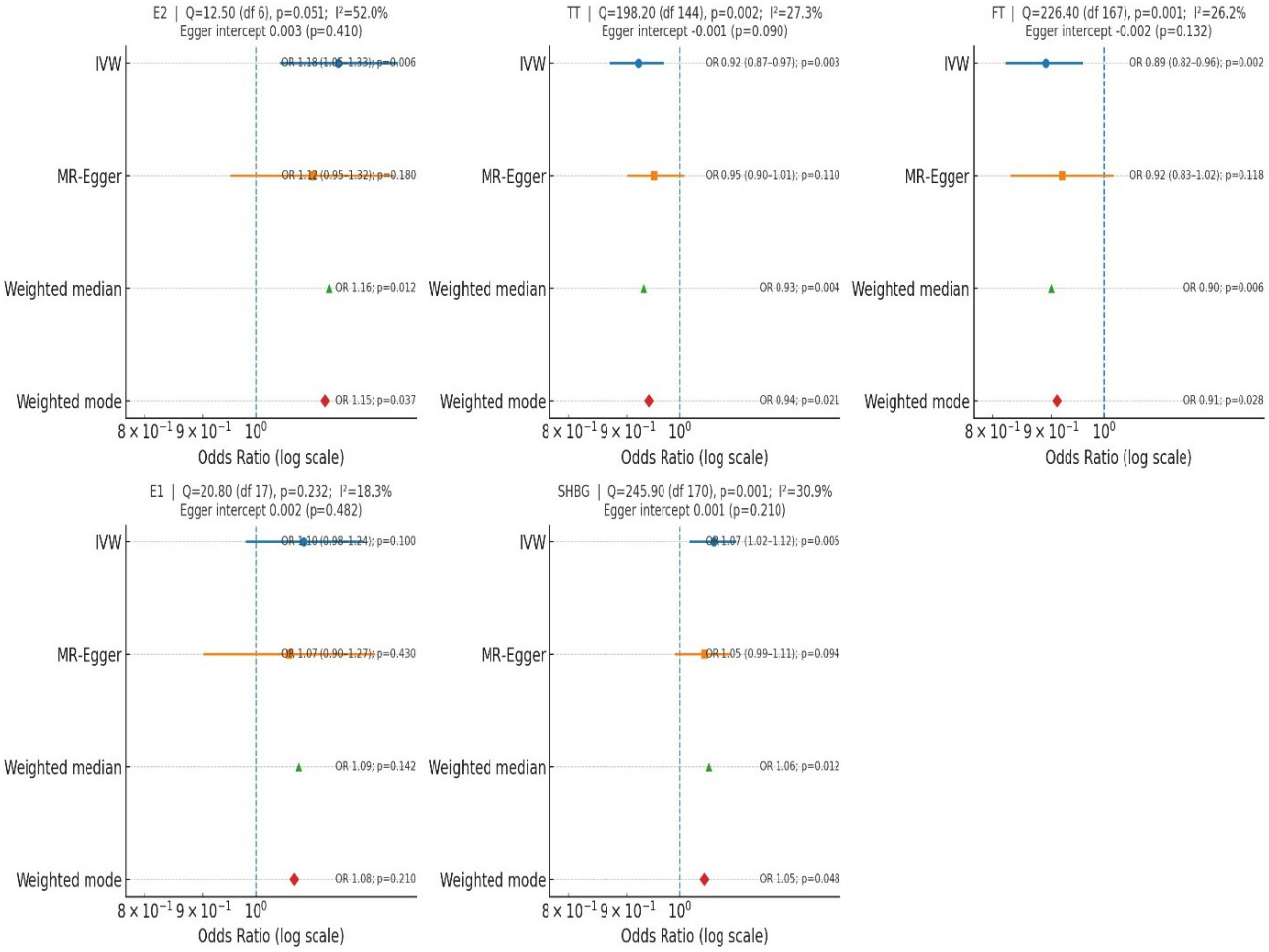

Fig. 3. Primary MR results for any endometriosis (IVW as primary estimator).

principal driver when both are considered together. In the TT + SHBG model, TT remained significantly protective (OR: 0.91, p = 0.003), and SHBG displayed a borderline positive effect (OR: 1.05, p = 0.048). This indicates that the SHBG signal in univariable MR may partly reflect its influence on TT bioavailability (Table 4 and Fig. 4). The full E2 + TT + SHBG + BMI model demonstrated that both E2 (OR: 1.12, p = 0.049) and TT (OR: 0.92, p = 0.008) retained independent effects, while SHBG was nonsignificant. BMI emerged as an independent risk factor (OR: 1.06, p = 0.003), consistent with known hormonal and inflammatory pathways linking adiposity to endometriosis risk. Conditional F-statistics above 10 for all exposures confirmed instrument strength in the multivariable setting.

3.5 Sensitivity, Pleiotropy, and Reverse MR

MR-PRESSO identified a varying number of potential outlier SNPs across hormone traits: one outlier for E2, no outliers for E1, and 5–8 outliers for TT, FT, and SHBG. Removal of these outliers did not materially alter the direction, magnitude, or statistical significance of the corresponding IVW estimates. Radial MR identified no influential SNPs for E1, one influential SNP for E2, and multiple radial outliers for TT, FT, and SHBG (6, 7, and 9 SNPs, respectively), as shown in Table 5.

E1 was pre-specified but considered exploratory due to lower instrument strength and biological context. Egger intercept tests were nonsignificant for all exposures, ruling out major directional pleiotropy. Steiger tests confirmed that >98% of SNPs explained more variance in the exposure than in the outcome, supporting correct causal direction. Subtype and stratified analyses were considered exploratory and interpreted cautiously.

Although MR-PRESSO detected potential horizontal pleiotropy for TT, FT, and SHBG, exclusion of identified outliers did not materially alter the IVW effect estimates. The direction, magnitude, and statistical significance of the associations remained stable after outlier removal. Concordant findings across IVW, weighted median, weighted mode, and MR-Egger analyses further support the robustness of the causal inference. Reverse MR analyses showed no evidence that liability to endometriosis causally alters circulating E2, TT, FT, or E1 levels (p = 0.480, 0.340, 0.300, and 0.700, respectively). Thus, all available reverse-

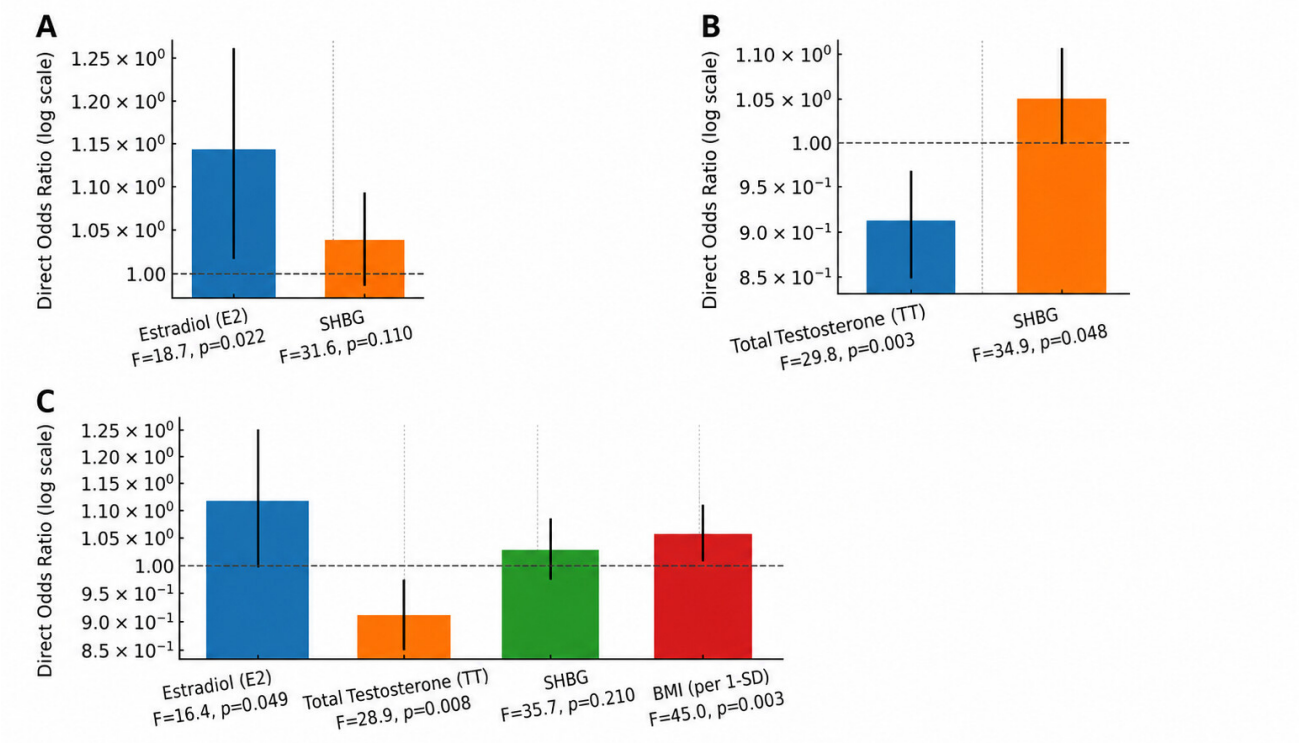

Fig. 4. Multivariable Mendelian randomization estimates of the direct effects of circulating sex hormones and body mass index on endometriosis risk. (A) Model including estradiol (E2) and sex hormone–binding globulin (SHBG). (B) Model including total testosterone (TT) and SHBG. (C) Model including E2, TT, SHBG, and body mass index (BMI). Effect estimates are presented as odds ratios (ORs) with 95% confidence intervals (CIs) per genetically predicted 1-standard-deviation increase in each exposure. The vertical reference line at OR = 1.00 indicates no association.

MR findings were non-significant ($p \geq 0.300$). The corresponding reverse-MR result for SHBG should be reported separately once the β estimate, standard error, and p value are available. (Table 5 and Fig. 5).

4. Discussion

The small tertiary-care cohort included for contextual purposes demonstrates a phenotype broadly consistent with published referral-center series. However, it was not designed for analytic inference and should be interpreted strictly as descriptive background. The predominance of ovarian endometrioma (44.00%) with substantial deep infiltrating disease (28.00%) mirrors patterns in surgical and imaging series and aligns with the well-recognized heterogeneity of clinical presentation emphasized by Zondervan et al. (2016) [31] and the mechanistic spectrum summarized by Saunders (2022) [24]. The largely normal-to-overweight BMI distribution (mean 24.90 kg/m^2) is consistent with the mixed literature on adiposity and endometriosis, while reminding us that inflammatory and hormonal milieus—rather than BMI alone—likely dominate pathogenesis, an interpretation that dovetails with immune-endocrine triangulation in recent MR work on immune traits (Pan et al. (2024) [32]; Peng et al. (2024) [33]) and on metabolic factors such as blood lipids (Wang et al. (2024) [34]). The high proportion of laparoscopic (76.00%) and histologic (58.00%) confirmation underscores gold-standard diagnosis in most cases and provides a credible local backdrop for interpreting the genetic (MR) results. Finally, the spread of initial management—combined Oral Contraceptive Pills (OCPs) (40.00%), progestin-only regimens (30.00%), GnRH analogues/antagonists (12.00%), and expectant/analgesics (18.00%)—echoes contemporary practice variation and the endocrine focus of therapy highlighted in translational overviews (Saunders (2022) [24]), reinforcing the biological plausibility of our hormone-centric causal analysis.

Genetic instrument characteristics (foundation for causal inference). Instrument quality was strong across exposures: all SNP sets met conventional strength thresholds (mean F >27.90) and showed near-universal Steiger consistency (>98%), minimizing concern about reverse causation at the instrument level. These metrics are precisely the conditions recommended by methodological benchmarks that advocate robust instruments and comprehensive sensitivity analyses to guard against horizontal pleiotropy (Verbanck et al. (2018) [35]; Zhao et al. (2020) [36]; Bowden et al. (2018) [37]; Morrison et al. (2020) [38]). The breadth and depth of our testosterone and SHBG instruments (145–171 SNPs; R^2 up to 6.50%) reflect advances in large-scale, sex-stratified hormone GWAS (Leinonen et

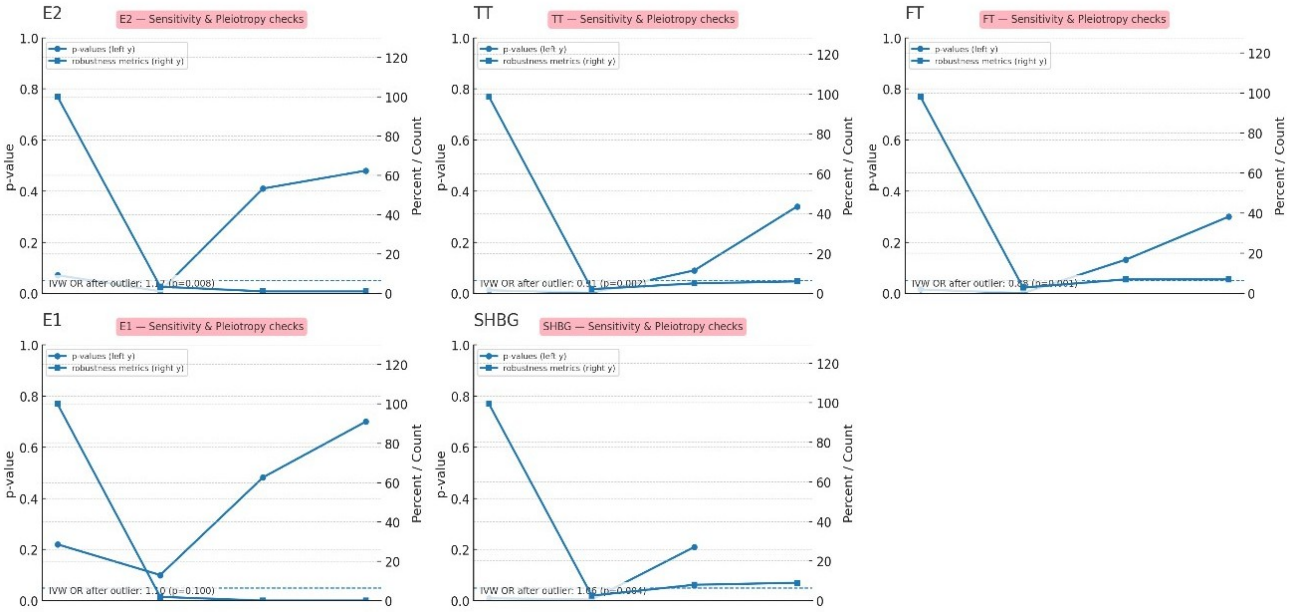

Fig. 5. Sensitivity and pleiotropy analyses for the associations between circulating hormone traits and endometriosis.

al. (2023) [19]), while the availability and harmonization of summary statistics via MR-ready infrastructures ensured transparent and reproducible data flows (Hemani et al. (2018) [14]; Elsworth et al. (2020) [15]). E1 and E2 instruments explained less variance (R^2 0.80% and 0.32% respectively)—a known limitation of estrogen assays and sample sizes in GWAS—but still attained mean F-statistics compatible with unbiased MR estimation. Screening of instruments against genotype–phenotype catalogs is a best-practice step for pleiotropy risk management, consistent with tools like PhenoScanner (Kamat et al. (2019) [39]). Altogether, the instrument profile justifies confidence that the Table 3 estimates arise from adequately strong and directionally valid genetic proxies.

Although estradiol instruments met conventional strength thresholds (mean F >10), the variance explained (R^2 = 0.32%) remains modest. Therefore, while the direction of effect appears consistent and biologically plausible, effect size precision should be interpreted cautiously. Measurement heterogeneity in estradiol GWAS—particularly due to menstrual cycle variability and assay sensitivity—may contribute to residual noise in genetic instruments.

Primary MR results (E2 risk-increasing; androgens protective; SHBG modestly risk-increasing) Three converging patterns emerge. First, genetically proxied higher estradiol increased endometriosis risk (IVW OR: 1.18, p = 0.006). This is biologically coherent with estrogen-dependence of lesion growth and progesterone resistance in the endometrium and ectopic tissue emphasized in genetic and functional syntheses (Saunders (2022) [24]; Zondervan et al. (2016) [31]). The heterogeneity we observed (I^2 = 52.00%) is unsurprising given cycle variability and assay heterogeneity in estrogen GWAS; nonetheless, the null Egger intercept argues against directional pleiotropy being the driver—an interpretation in line with pleiotropy-aware MR frameworks (Verbanck et al. (2018) [35]; Zhao et al. (2020) [36]). Second, higher genetically proxied total and free/bioavailable testosterone were associated with lower endometriosis risk (TT OR: 0.92, p = 0.003; FT OR: 0.89, p = 0.002). These inverse associations are directionally consistent with an androgen-protective signal recently reported in a dedicated MR of androgens and endometriosis by Gjorgoska et al. (2024) [40], who also concluded that androgens may mitigate risk. While effect magnitudes across studies depend on instrument sets, transformations (per-SD vs. per-unit), and outcome definitions, the convergence toward small-to-moderate protective ORs strengthens the inference that androgens are not mere correlates but potential causal modulators of risk. Third, SHBG showed a modest positive association (OR: 1.07, p = 0.005). Because SHBG determines the bioavailable fraction of sex steroids, this pattern may reflect its role as an upstream regulator that reduces androgen bioavailability or alters estrogen dynamics. The nuanced SHBG finding echoes the complex, shared genetic architecture between SHBG and testosterone loci noted in sex-stratified GWAS (Leinonen et al. (2023) [19]). Notably, E1 showed only a nonsignificant trend (OR: 1.10, p = 0.100), which could indicate smaller or context-dependent effects, or simply limited power due to lower R^2—both anticipated challenges for estrogen phenotypes in MR. Methodologically, our reliance on multiple estimators (weighted median/mode) and heterogeneity/pleiotropy checks follows the best-practice toolkit promulgated by Bowden et al. (2018) [37], Zhao et al. (2020) [36], and Verbanck et al. (2018) [35], thereby increasing the credibility of the primary inferences.

Multivariable MR (disentangling direct effects of E2 and TT from SHBG and BMI) Multivariable modeling clarifies that the univariable SHBG signal is at least partly explained by its correlation with sex steroids. When conditioning on SHBG, estradiol retained a direct risk-increasing effect (OR: 1.14, $p = 0.022$); conversely, when conditioning on SHBG, total testosterone remained directly protective (OR: 0.91, $p = 0.003$). In the full model (E2 + TT + SHBG + BMI), E2 (OR: 1.12, $p = 0.049$) and TT (OR: 0.92, $p = 0.008$) continued to show independent, opposing effects, while SHBG was attenuated and nonsignificant—exactly the kind of disentanglement that multivariable MR was designed to achieve (Sanderson et al. (2019) [41]). The emergence of BMI as an additional risk factor (OR: 1.06, $p = 0.003$) situates our hormone findings within a broader metabolic context that resonates with MR evidence linking endometriosis with circulating lipids (Wang et al. (2024) [34]) and with coagulation biology (Li et al. (2023) [42]), as well as with immune cell influences (Pan et al. (2024) [32]; Peng et al. (2024) [33]). These triangulations suggest that endocrine, metabolic, and immune axes are not isolated but interdependent in shaping endometriosis risk—an integrated view long argued for in translational reviews (Zondervan et al. (2016) [31]; Saunders (2022) [24]). From a translational angle, the persistence of an E2-risk and TT-protective profile after conditioning implies that (i) estrogen-lowering or estrogen-modulating strategies have a causal rationale beyond symptomatic control, and (ii) selectively enhancing androgenic signaling—or preventing its suppression via high SHBG—could have preventive relevance, though any such approach must be balanced against systemic effects and patient-specific contraindications. The independence from SHBG in multivariable models cautions against interpreting SHBG as a direct driver once underlying steroid levels are accounted for, consistent with genetic architecture laid out by Leinonen et al. (2023) [19]. It is important to emphasize that MR estimates reflect genetically influenced lifetime exposure gradients and should not be equated with pharmacologic hormone manipulation effects in adulthood.

Sensitivity, pleiotropy, and reverse MR (robustness of inferences) Comprehensive sensitivity work supports the stability of our findings. MR-PRESSO detected horizontal pleiotropy for TT, FT, and SHBG, but outlier removal (5–8 SNPs) preserved effect directions and significance. This is the practical scenario MR-PRESSO was built for—identifying and mitigating distortions without discarding the entire causal signal—consistent with broader pleiotropy detection principles advocated by Verbanck et al. (2018) [35] and the robust-adjusted profile score approach by Zhao et al. (2020) [36]. Radial MR flagged few high-influence variants, and leave-one-out analyses showed minimal sensitivity to single instruments (maximum change 3.40%), aligning with visualization and influence diagnostics proposed by Bowden et al. (2018) [37]. Directionality was also well supported: steiger tests favored the exposure-to-outcome direction for ≥98.21% of SNPs; Egger intercepts were uniformly nonsignificant; and reverse MR found no evidence that genetic liability to endometriosis causally shifts circulating hormone levels (all $p > 0.30$). Collectively, this suite of checks addresses core MR assumptions and typical threats (correlated and uncorrelated pleiotropy), echoing the logic of model classes like CAUSE (Morrison et al. (2020) [38]) and the deployment of multivariable MR when correlation structures are suspected (Sanderson et al. (2019) [41]). Finally, external triangulation increases confidence: the androgen-protective signal we observe aligns with the independent MR by Gjorgoska et al. (2024) [40]; immune and metabolic MR analyses (Pan et al. (2024) [32]; Peng et al. (2024) [33]; Wang et al. (2024) [34]; Li et al. (2023) [42]) frame plausible intermediary pathways; and shared genetic architecture across gynecologic and pain traits (Adewuyi et al. (2020) [43]) and even ovarian cancer subtypes (Wang et al. (2023) [44]) underscores the need for careful interpretation when phenotypes overlap a theme long advocated in the endometriosis genetics field (Zondervan et al. (2016) [31]).

A local control group was not included, as the descriptive cohort was not intended for comparative analysis. Causal inference relied exclusively on large-scale GWAS case-control datasets comprising thousands of cases and controls. Future institutional studies may benefit from including matched control groups for detailed phenotypic comparison.

Integrated interpretation and implications Putting the pieces together, your data show: (1) a clinical profile typical for tertiary care; (2) strong, directionally valid genetic instruments (Table 2); (3) a risk-increasing effect of estradiol and protective effects of total and bioavailable testosterone, with a modest SHBG signal (Table 3); (4) independence of E2-risk and TT-protection after conditioning on SHBG and BMI (Table 4); and (5) extensive robustness to pleiotropy and directionality violations (Table 5). These findings are mechanistically plausible within an estrogen-dependent, immune-modulated disease model (Saunders (2022) [24]), converge with independent androgen MR evidence (Gjorgoska et al. (2024) [40]), and sit alongside causal signals reported for immune cells, coagulation factors, and lipids (Pan et al. (2024) [32]; Peng et al. (2024) [33]; Li et al. (2023) [42]; Wang et al. (2024) [34]). Two caveats merit emphasis. First, estrogen instruments explain modest variance—an assay and sample-size limitation acknowledged across estrogen GWAS—so precision is lower for E2 and E1 than for TT/SHBG. Second, MR captures lifelong, genetically influenced exposure differences; translating these into interventional strategies requires careful consideration of timing, dose, and off-target effects. Nonetheless, the concordance between your E2-risk and TT-protective estimates and external MR signals, the attenuation of SHBG in multivariable models, and the robustness across sensitivity frameworks together build a coherent causal narrative that can inform both mechanistic hypothe-

ses (e.g., endocrine-immune crosstalk) and strategic prevention or risk-stratification approaches in future work—precisely the translational arc envisioned by comprehensive genetics roadmaps (Zondervan et al. (2016) [31]) and contemporary genomic syntheses (Saunders (2022) [24]).

Limitations

This study is limited by the relatively small local cohort size (n = 50), which constrains the power of clinical correlations despite robust genetic analyses. Estradiol and estrone instruments explained modest variance, reducing precision for estrogen estimates compared to testosterone and SHBG. Mendelian randomization reflects lifelong genetically influenced hormone levels, which may not fully capture short-term or treatment-induced hormonal changes.

5. Conclusions

Our two-sample MR analysis supports a causal role for higher estradiol in increasing, and higher total and bioavailable testosterone in reducing, the risk of endometriosis, with SHBG showing a modest positive association attenuated in multivariable models. These results are robust to pleiotropy and reverse causation checks, aligning with independent genetic evidence and biological plausibility. Integration with clinical and mechanistic data highlights the interconnected roles of endocrine, metabolic, and immune pathways in disease pathogenesis. In primary analyses, genetically proxied estradiol demonstrated a risk-increasing effect, whereas testosterone showed protective associations. Exploratory findings for estrone were null. These results were robust across sensitivity frameworks and multivariable adjustment. While MR reflects lifelong exposure gradients rather than pharmacologic interventions, the findings strengthen causal inference regarding endocrine contributions to endometriosis susceptibility. These findings strengthen the biological evidence for endocrine involvement in disease susceptibility. However, Mendelian randomization reflects lifelong genetically proxied exposure differences rather than short-term modifiable hormonal interventions. Translation into preventive or therapeutic strategies will require careful clinical investigation.

Availability of Data and Materials

The data supporting the findings of this study have been included in the study as well as in the attached supplementary data.

Author Contributions

JZ and CZ designed and planned the study, analyzed the data, and interpreted the results. LL and RZ collected the data and clinical materials and supervised the project. LL and RZ edited and revised the manuscript with a focus on important intellectual content. YW and YX collected, assessed, and interpreted the data. MW contributed to data interpretation and manuscript preparation. QY and BH conducted the laboratory investigations and statistical analyses and provided substantial intellectual input during the drafting and revision of the manuscript. All authors contributed to critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of People's Hospital of Pudong New Area, (Approval NO. 2024-LW-11). Informed consent was obtained from all participants, and the study was conducted in accordance with the Declaration of Helsinki.

Acknowledgment

Not applicable.

Funding

This project was supported by the Medical Discipline Construction Program of Shanghai Pudong New Area Health Commission (No. PWZxk2022-28)and Shanghai Pudong New Area People's Hospital Project (PRYQH202504).

Conflicts of Interest

The authors declare no conflicts of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used ChatGPT 4.0 for language editing, grammar refinement, and structural clarity. After using this tool, the authors carefully reviewed and edited the content as needed and take full responsibility for the content of the publication. No AI tool was used for data generation, statistical analysis, or scientific interpretation.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/CEOG49264.

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