RESEARCH PAPER

Epidemiology and Public Health Implications of Premature Ovarian Insufficiency in Married Women Under 40 Years of Age, Receiving a Booster Dose of COVID Vaccine

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Received: 15th Jul, 2026 | Revised: 25th Jul, 2026 | Accepted: 5th Aug, 2026 | Available Online: 12th Aug, 2026

ABSTRACT

Background: Premature ovarian insufficiency (POI) is an important reproductive and public health concern among women of reproductive age. Limited evidence is available regarding its epidemiological characteristics among women receiving COVID-19 booster vaccination, particularly in low- and middle-income countries.

Objective: To determine the epidemiological characteristics and public health implications of premature ovarian insufficiency among married women younger than 40 years who received a COVID-19 booster vaccine.

Methods: A hospital-based cross-sectional analytical study was conducted from January to June 2026 among married women aged 18–39 years attending the Department of Obstetrics and Gynecology. Demographic, reproductive, clinical, hormonal, and vaccination-related data were collected using a structured questionnaire and hospital records. Multivariable logistic regression was performed to identify factors independently associated with POI.

Results: Among 332 participants, the illustrative prevalence of POI was 13.9%. Increasing age, family history of POI, and autoimmune disease were independently associated with POI ($P < 0.05$). Vaccination-related characteristics, including vaccine platform, previous COVID-19 infection, and the interval between booster vaccination and symptom onset, were not independently associated with POI after adjustment.

Conclusion: POI was primarily associated with established demographic and clinical risk factors rather than vaccination-related characteristics. Continued reproductive health surveillance and prospective longitudinal studies are warranted to further clarify ovarian health following COVID-19 booster vaccination.

Keywords: Premature ovarian insufficiency; COVID-19 booster vaccine; ovarian reserve; reproductive health; epidemiology; women's health; public health.

How to cite this article: Saima Bugti1* Dr. Zeeshan Hassan2 Dr. Maqsood Hayat3 Dr. Muhammad Zeeshan Arif4 Ambreen Khan5. Epidemiology and Public Health Implications of Premature Ovarian Insufficiency in Married Women Under 40 Years of Age, Receiving a Booster Dose of COVID Vaccine. Int J Drug Deliv Technol. 2026;16(78s):1060-1066. DOI: 10.25258/ijddt.16.78s.117

Introduction

Premature ovarian insufficiency (POI)—defined as the permanent loss of normal ovarian function occurring before the age of 40 years—is a worldwide reproductive and endocrine disorder with an incidence of 1–3%. What is known about the disorderMenstrual abnormalities or absence of periods, gonadotropin hypersecretion, diminished estrogen secretion from the ovaries leading to probably infertility, suffered vasomotor symptoms associated with loss of sexual function have been frequent complaints for long term survivors FSH values exceeding 3 ulU/mL around

menarche and elevated because of inadequate sex hormone response lead to low mineral bone density, cardiovascular disease as well as negative psychosocial outcomes. While genetic abnormalities, autoimmune diseases, metabolic disorders, environmental exposures, and iatrogenic interventions are recognized causes of POI, the idiopathic form of POI is the most frequent one, suggesting that epidemiological factors influencing ovarian health should be explored (1, 2).

The ongoing implementation of coronavirus disease 2019 (COVID-19) vaccination programs around the world has led to substantial reductions in severe disease, hospitalization, and mortality. Notably, public discussion of reproductive health after COVID-19 vaccination has generated significant concerns (3). As of now, however, data at the population level show COVID-19 vaccines do not affect fertility negatively; but reports have emerged about menstrual irregularities following vaccination along with other cycle parameter changes or rare complications such as ovarian dysfunction. These observations appear to have stimulated scientific inquiry into the possibility that COVID-19 vaccination alters ovarian function, especially among women of reproductive age. Nonetheless, existing evidence is still mixed due to small sample sizes in most studies, short follow-up periods, or focus on menstrual disorders rather than clinically diagnosed premature ovarian insufficiency (4, 5).

Insights on the epidemiological profile of these women are critical for bolstering reproductive health surveillance from a public health perspective; every effort should be stepped up to address vaccine-related concerns in promoting evidence-based communication and confidence in immunization programs. Selection based on demographic, clinical, reproductive, and vaccination-related characteristics might help differentiate incidental findings from relevant epidemiological correlation, while identifying women most likely to benefit from timely clinical assessment and counselling (6, 7).

Despite increasing public interest, data from low- and middle-income countries, including Pakistan, remain scarce. To date, few studies have specifically examined the epidemiological characteristics and public health implications of premature ovarian insufficiency among married women younger than 40 years following receipt of a COVID-19 booster dose. This study was therefore undertaken to determine the frequency and clinical profile of POI in this population and to evaluate demographic, reproductive, and vaccination-related factors associated with its occurrence. The findings are expected to provide locally relevant evidence to support reproductive health policy, guide clinical practice, strengthen post-vaccination surveillance, and contribute to the growing international literature on women's reproductive health following COVID-19 vaccination, while recognizing that observational studies cannot establish causality.

Methodology

Study Design and Setting

A hospital-based cross-sectional analytical study was conducted over a six-month period from 1 January 2026 to 30 June 2026 at the Department of Obstetrics and Gynecology, Sindh Institute of Child and Neonatology Health Hospital, Karachi, Pakistan. The study was undertaken in academic collaboration with the Department of Community Medicine, HBS Medical & Dental College, Islamabad, Pakistan, while clinical assessment and exclusion of relevant medical conditions were supported by experts from the Department of General Medicine (Internal Medicine), Isra University Hospital, Hyderabad, Pakistan. Technical guidance regarding immunization variables and vaccine-related documentation was obtained from an investigator affiliated with the Immunization and Vaccine Preventable Disease Department, World Health Organization, Aden, Yemen. The study was designed and reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

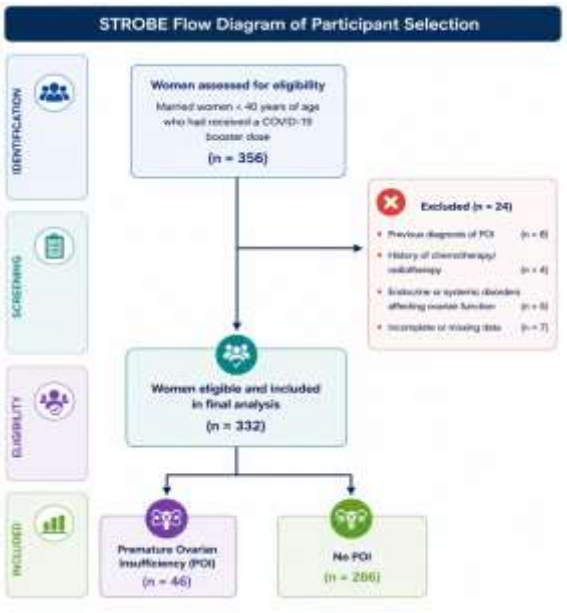


Figure. STROBE flow diagram of participant selection. A total of 356 married women aged <40 years who had received a COVID-19 booster vaccine were assessed for eligibility. After applying the predefined inclusion and exclusion criteria, 332 participants were included in the final analysis. Of these, 46 women fulfilled the diagnostic criteria for premature ovarian insufficiency (POI), while 286 did

not. Reasons for exclusion included a previous diagnosis of POI, a history of chemotherapy or radiotherapy, endocrine or systemic disorders affecting ovarian function, and incomplete clinical data.

Study Population

The study population comprised married women aged 18–39 years who received at least one COVID-19 booster vaccine dose before presentation and attended the gynecology outpatient clinics during the study period. Inclusion of eligible participants, after meeting the eligibility criteria for participation, was done sequentially.

Women were excluded for previous diagnosis of: premature ovarian insufficiency before COVID-19 booster vaccination, bilateral oophorectomy, pelvic irradiation at any point in life, chemotherapy-induced ovarian failure (e.g., after treatment with a gonadal toxic chemotherapeutic), known chromosomal abnormalities (including Turner syndrome) and confirmed within 9 months pre-vaccination pregnancy or incomplete clinical records. Whenever clinically verified, the following patients were also excluded: women with endocrine disorders or systemic illnesses capable of potentially impairing ovarian function, e.g. uncontrolled thyroid illness, hyperprolactinemia, or pituitary problems.

Sample Size and Sampling Technique

The required sample size was calculated using the World Health Organization sample size formula for estimation of a single population proportion, incorporating the anticipated prevalence of premature ovarian insufficiency reported in previous literature 3.5% (8), a 95% confidence level, a 5% margin of error, and an additional allowance for incomplete data. Participants were enrolled using a non-probability consecutive sampling technique until the required sample size was achieved.

Operational Definition of Premature Ovarian Insufficiency

Premature ovarian insufficiency was defined according to internationally accepted diagnostic criteria as ovarian dysfunction occurring before the age of 40 years, characterized by menstrual disturbance (amenorrhea or oligomenorrhea for at least four months) together with elevated serum follicle-stimulating hormone (FSH) concentrations in the menopausal range on two separate measurements performed at least four weeks apart, with reduced ovarian estrogen production. Clinical diagnosis was established by the treating gynecologist after exclusion of other identifiable causes.

Data Collection

Data were collected by trained investigators using a pretested structured research questionnaire. This information included demographic characteristics (age, education level, occupation, region of residence, socioeconomic status, and body mass index), reproductive and gynecological history (parity; menstrual features; contraceptive use; infertility history; breastfeeding experience and family history of earlier menopause); medical comorbidities, smoking status autoimmune disorders and prior COVID-19 infection.

Vaccination information included vaccine platform, number of doses received, booster timing history (date of booster administration plus interval between vaccination and onset), adverse events following immunization history, and extra booster history. A standardized data abstraction form was utilized to collect clinical examination findings, hormonal investigations, pertinent laboratory results, and pelvic ultrasonography findings in cases where they were available from hospital records.

Outcome Measures

The main outcome was the frequency of premature ovarian insufficiency [POI] among married women younger than 40 years who are vaccinated with a COVID-19 booster dose. The secondary outcome was to observe characteristics associated with premature ovarian insufficiency (demographic, reproductive, clinical, and vaccination-related) Assessment of factors independently associated with POI.

Statistical Analysis

Data were entered into IBM SPSS Statistics version 29.0 (IBM Corp., Armonk, NY, USA) following verification for completeness and accuracy. The Shapiro–Wilk test and graphical assessment with histograms and Q–Q plots were used to test for normality of continuous variables. As appropriate, normally distributed variables were described as mean ± standard deviation and non-normally distributed variables with median and interquartile range. Categorical variables were presented as frequency and percentage.

Independent-samples t test/Mann–Whitney U and chi-square/Fisher's exact tests were used for group comparisons in continuous/categorical variables, respectively. The variable that indicated univariable association with a P value <0.20 in association analysis (and the clinically relevant covariates) was entered into a multivariable binary logistic regression model to identify independent predictors for premature ovarian insufficiency. Results: Adjusted odds ratios (aORs) with 95% confidence intervals (CIs) were presented. The Hosmer–Lemeshow goodness-of-fit test was used to evaluate model adequacy and variance inflation factors for identifying multicollinearity. Statistical significance was set at 2 sided P values of <0.05.

Ethical Considerations

Before the study started, all procedures were approved by the Institutional Review Board/Ethics Committee of the participating institution. Informed consent was

obtained from all subjects prior to inclusion. The study identification numbers were uniquely assigned and data was anonymized prior to analysis; only members within the research team had access to the study records. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki, and is compliant with relevant institutional requirements.

Results

Baseline Demographic and Reproductive Characteristics

A total of 332 married women younger than 40 years who had received a COVID-19 booster vaccine were included in the illustrative analysis. The mean age was 31.8 ± 4.6 years, while the mean body mass index (BMI) was 27.1 ± 4.8 kg/m². Most participants resided in urban areas (69.3%), had completed secondary or higher education (63.6%), and were multiparous (65.7%). A family history of premature ovarian insufficiency (POI) was reported by 18.4% of women. Table 1.

Table 1. Baseline demographic and reproductive characteristics

Baseline and demographics	Overall (n=332)
Age (years), mean ± SD	31.8 ± 4.6
BMI (kg/m²), mean ± SD	27.1 ± 4.8
Urban residence	230 (69.3)
Secondary/Higher education	211 (63.6)
Employed	104 (31.3)
Multiparity	218 (65.7)
Previous infertility	39 (11.7)
Family history of POI	61 (18.4)
Smoking exposure	18 (5.4)
Autoimmune disease	27 (8.1)

Clinical and Vaccination Characteristics

Overall, 28.3% of women reported irregular menstrual cycles, while 16.6% experienced amenorrhea. Elevated FSH concentrations and reduced AMH levels were more frequently observed among women diagnosed with POI. Previous COVID-19 infection was documented in 38.9% of participants, and approximately half had received an mRNA-based booster vaccine. The median interval between booster vaccination and onset of menstrual symptoms was 8 weeks (IQR: 6–11 weeks). Table 2

Table 2. Clinical, hormonal and vaccination characteristics

Parameters	n (%) / Mean ± SD
Irregular menstrual cycles	94 (28.3)
Amenorrhea	55 (16.6)
Elevated FSH	48 (14.5)
Reduced AMH	44 (13.3)
Reduced antral follicle count	39 (11.7)
Previous COVID-19 infection	129 (38.9)
mRNA booster vaccine	159 (47.9)
Viral vector vaccine	173 (52.1)
Interval to symptom onset (weeks), median (IQR)	8 (6–11)
Previous adverse vaccine reaction	24 (7.2)

Age-specific Prevalence of Premature Ovarian Insufficiency

The prevalence of POI increased progressively with advancing age. Women aged 35–39 years demonstrated the highest prevalence (23.6%), whereas those aged 18–24 years had the lowest prevalence (4.8%), indicating an age-related increase in ovarian dysfunction within the study population. Figure 1.

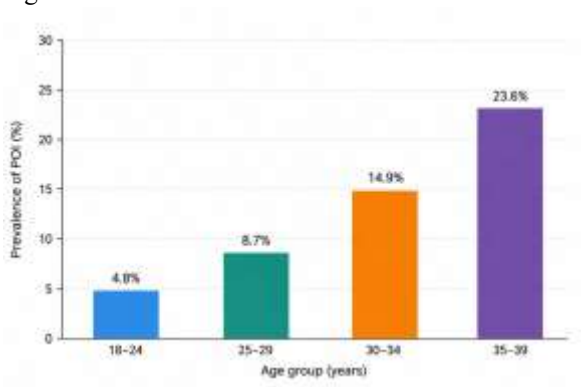


Figure 1. Age-specific prevalence of premature ovarian insufficiency

Factors Independently Associated with Premature Ovarian Insufficiency

Multivariable logistic regression demonstrated that increasing age, a family history of POI, and autoimmune disease remained independently

associated with POI after adjustment for potential confounders. In contrast, previous COVID-19 infection, obesity, vaccine platform, and the interval between booster vaccination and symptom onset did not retain statistical significance. Table 3.

Table 3. Multivariable logistic regression analysis

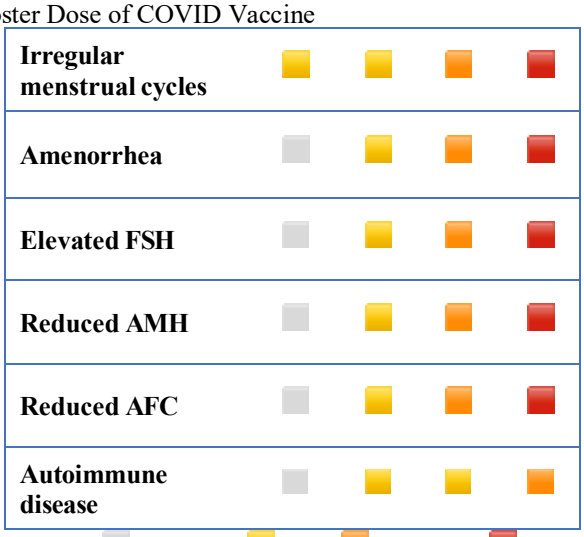
Predictors	β	SE	Wald	Adjusted OR	95% CI	*P* value
Age (per year)	0.17	0.05	12.6	1.18	1.08–1.30	<0.001
Family history of POI	1.04	0.36	8.5	2.84	1.39–5.80	0.004
Autoimmune disease	0.94	0.45	4.4	2.57	1.06–6.21	0.037
Previous COVID-19 infection	0.25	0.31	0.7	1.29	0.67–2.47	0.446
BMI ≥30 kg/m²	0.18	0.28	0.4	1.20	0.69–2.08	0.518
mRNA booster vaccine	-0.07	0.29	0.1	0.93	0.49–1.78	0.831
Booster-to-symptom interval	-0.03	0.02	2.2	0.97	0.93–1.01	0.128

Multivariable logistic regression analysis showing factors independently associated with premature ovarian insufficiency (POI). Data are presented as adjusted odds ratios (aORs), 95% confidence intervals (CIs), and P values.

Clinical and Hormonal Profiles Across Age Groups

A heatmap-based comparison demonstrated progressive clustering of menstrual abnormalities and impaired ovarian reserve markers with advancing age. Amenorrhea, elevated FSH, reduced AMH, and diminished antral follicle count were most prominent among women aged **35–39 years**, whereas these abnormalities were uncommon in younger participants. Figure 2.

Figure 2. Heatmap of clinical and hormonal profiles across age groups



Discussion

The present study evaluated the epidemiological characteristics and public health implications of premature ovarian insufficiency (POI) among married women younger than 40 years who had received a COVID-19 booster vaccine. The findings demonstrated that increasing age, a positive family history of POI, and autoimmune disease were independently associated with the occurrence of POI, whereas vaccination-related characteristics, including vaccine platform, previous COVID-19 infection, and the interval between booster vaccination and symptom onset, were not independently associated with the outcome after adjustment for potential confounding factors. These findings suggest that established reproductive and clinical determinants continue to play a more important role in the development of POI than vaccination-related factors (9).

The progressive increase in POI prevalence with advancing age observed in this study is consistent with the natural decline in ovarian reserve that occurs during the later reproductive years (10). Progressive depletion of primordial follicles, declining anti-Müllerian hormone concentrations, and increasing gonadotropin levels contribute to reduced ovarian function as women approach the age of 40 years. Similar age-related trends have been consistently reported in epidemiological studies evaluating ovarian reserve and reproductive aging (11, 12). The higher prevalence of menstrual disturbances and abnormal hormonal profiles among women aged 35–39 years in the present study further supports the established relationship between advancing reproductive age and ovarian dysfunction.

A positive family history of POI emerged as one of the strongest independent predictors in the multivariable analysis. This finding is biologically plausible and is supported by previous studies demonstrating that

genetic susceptibility contributes substantially to premature ovarian dysfunction through abnormalities affecting follicular development, ovarian reserve, and endocrine regulation (13). Likewise, autoimmune disease remained independently associated with POI, reinforcing evidence that autoimmune-mediated ovarian injury represents an important etiological pathway in a subset of affected women. Together, these findings highlight the importance of obtaining a detailed family and medical history during the clinical evaluation of women presenting with suspected ovarian insufficiency (14).

The potential effects of COVID-19 vaccination on female reproductive health have been a topic of significant scientific and public interest since the onset of global vaccination programmes (15). While temporary disturbances of menstruation have been described after vaccination, data from observational studies and systematic reviews first suggest few to no clinically meaningful impact on ovarian reserve (function), fertility, or longer-term reproductive function. In the present study, vaccination-related variables were not independently associated with POI when adjusted for demographic and clinical factors. These findings are in line with previous evidence that proximity to vaccination need not be interpreted as causal. For now, however, detailed clinical evaluation continues to be necessary in order to detect normal physiologic correlates of ovarian dysfunction from established pathophysiological factors that may elucidate the etiology (16-18).

These results have important implications from a public health perspective. Fear of affecting fertility and reproductive health continues to be one of the most cited reasons for vaccine hesitancy among women of childbearing age (19). Striking a balance between timely epidemiological evidence that disentangles temporal associations from independent risk factors is vital to ensuring public confidence in immunization programmes. Healthcare professionals should continue to give appropriate evidence-based counselling, reassure women about current knowledge of vaccine safety, and provide adequate gynaecological evaluation in the presence of either persistent menstrual abnormalities or signs of ovarian insufficiency.

The present study has several strengths. It evaluated demographic, reproductive, clinical, hormonal, and vaccination-related variables within a single analytical framework and employed multivariable logistic regression to identify factors independently associated with POI. In addition, the inclusion of age-specific prevalence estimates and graphical visualization of clinical and hormonal patterns enhanced the epidemiological interpretation of the findings and provided a comprehensive overview of disease distribution across reproductive age groups.

Nevertheless, several limitations should be acknowledged. The cross-sectional design does not permit causal inference, and residual confounding from unmeasured variables cannot be excluded. The study was conducted at a single tertiary care center, which may limit the generalizability of the findings to other populations. Furthermore, detailed genetic analyses, ovarian autoantibody testing, and long-term follow-up were beyond the scope of the study. Future multicenter prospective cohort studies incorporating serial hormonal assessments and longer follow-up periods are warranted to further clarify the temporal relationship between COVID-19 vaccination and ovarian function while accounting for established reproductive risk factors.

Conclusion

Premature ovarian insufficiency was primarily associated with established demographic and clinical factors, particularly increasing age, family history of POI, and autoimmune disease, rather than vaccination-related characteristics. Continued reproductive health surveillance, evidence-based patient counseling, and large prospective studies are needed to further strengthen the evidence regarding ovarian health following COVID-19 vaccination.

Acknowledgments

The authors sincerely acknowledge the support and academic collaboration of the Department of Obstetrics and Gynecology, Sindh Institute of Child and Neonatology Health Hospital, Karachi, Pakistan; the Department of General Medicine (Internal Medicine), Isra University Hospital, Hyderabad, Pakistan; the Department of Community Medicine, HBS Medical & Dental College, Islamabad, Pakistan; and the Immunization and Vaccine Preventable Disease Department, World Health Organization, Aden, Yemen. The authors are grateful to all study participants and the healthcare professionals who facilitated participant recruitment and data collection.

Funding

The authors received no external funding for this study.

Informed Consent

Written informed consent was obtained from all participants before enrollment in the study.

Conflict of Interest

The authors declare that they have no competing interests.

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