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The impact of long COVID on menstrual health, ovarian reserve, and pregnancy outcomes: A prospective matched cohort study from north-east India

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Abstract

Introduction: Long COVID, or Post-Acute Sequelae of SARS-CoV-2 (PASC), is a multi-systemic disorder affecting a substantial proportion of COVID-19 survivors. The angiotensin-converting enzyme 2 (ACE2) receptor, the primary entry point for SARS-CoV-2, is highly expressed in reproductive tissues, suggesting a potential direct impact on female reproductive health. Data on this association, particularly from diverse ethnic populations like those in North-East India, is scarce.

Objectives: To conduct a prospective, matched cohort study investigating the effects of Long COVID on menstrual cycle regularity, biomarkers of ovarian reserve, and clinical pregnancy outcomes in a population of women from North-East India.

Methods: A total of 316 women (18-45 years) with confirmed prior SARS-CoV-2 infection were recruited from three tertiary-care centres across North-East India. Participants were stratified into two cohorts: a Long COVID group (n=158) and a matched control group of post-COVID individuals without Long COVID symptoms (n=158). Matching was based on age (±2 years), body mass index (BMI ±2 kg/m²), and parity. Comprehensive assessment included structured interviews for menstrual history, biochemical analysis of serum Anti-Müllerian Hormone (AMH), Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), prolactin, and thyroid-stimulating hormone (TSH), and prospective follow-up of pregnancy outcomes over 12 months.

Results: The prevalence of menstrual irregularities was significantly higher in the Long COVID cohort compared to controls (55.1% vs. 28.5%; adjusted odds ratio [aOR] 3.12, 95% CI 1.95-4.98, $p<0.001$). Women with Long COVID exhibited statistically significant lower median AMH levels (2.1 ng/mL [IQR: 1.4-3.0] vs. 2.8 ng/mL [IQR: 1.9-3.6]; p=0.01). The cumulative incidence of pregnancy at 12 months was lower in the Long COVID group (62% vs. 78%; Hazard Ratio 0.65, 95% CI 0.48-0.89, p=0.02). Pregnancies in the Long COVID cohort were associated with higher rates of preterm birth (17.8% vs. 6.9%; p=0.03) and gestational hypertension (15.6% vs. 5.2%; p=0.04).

Conclusions: This study provides compelling evidence from a unique ethnic population that Long COVID is significantly associated with menstrual dysfunction, diminished ovarian reserve as indicated by reduced AMH, reduced fecundability, and an elevated risk of adverse obstetric outcomes. These findings necessitate increased clinical vigilance and dedicated reproductive health counselling for women recovering from COVID-19.

Keywords: Long COVID, post-acute COVID-19 syndrome, menstrual cycle, ovarian reserve, anti-müllerian hormone, fertility, pregnancy outcome, preterm birth, north-east India

Introduction

The global SARS-CoV-2 pandemic has transitioned from an acute crisis to a chronic public health challenge, with a significant focus on the long-term sequelae of the infection, collectively termed Long COVID or Post-Acute Sequelae of SARS-CoV-2 infection (PASC) [1]. The World Health Organization defines Long COVID as a condition occurring in individuals with a history of probable or confirmed SARS-CoV-2 infection, with symptoms that persist for at least 12 weeks and cannot be explained by an alternative diagnosis [2]. These symptoms, which include debilitating fatigue, cognitive impairment, dyspnoea, and autonomic dysfunction, affect multiple organ systems and impair quality of life [3].

A critical yet under-researched aspect of Long COVID is its potential impact on female reproductive health. The pathophysiological rationale for this lies in the mechanism of viral entry. SARS-CoV-2 gains entry into human cells via the angiotensin-converting enzyme 2 (ACE2) receptor, which is abundantly expressed in key reproductive tissues, including the

ovaries (particularly in granulosa and luteal cells), the uterus, and the placenta [4, 5]. The binding of the virus to ACE2 can disrupt the renin-angiotensin system (RAS) balance within these tissues, which is crucial for folliculogenesis, steroidogenesis, and maintaining endometrial receptivity [6]. Furthermore, the systemic hyperinflammatory state and vascular endothelial dysfunction characteristic of severe COVID-19 could have lasting effects on the hypothalamic-pituitary-ovarian (HPO) axis and placental function [7].

Preliminary studies and patient-reported surveys have indicated a high prevalence of menstrual disturbances including changes in cycle length, menstrual volume, and intermenstrual spotting following both acute COVID-19 and in Long COVID [8, 9]. More concerning are reports suggesting a potential decline in ovarian reserve, as measured by serum Anti-Müllerian Hormone (AMH) levels, although findings remain contested [10, 11]. In pregnancy, SARS-CoV-2 infection has been associated with histopathological changes in the placenta, such as perivillous fibrin deposition and chronic histiocytic intervillositis, leading to an increased risk of obstetric complications like preeclampsia and preterm birth [12, 13]. However, it remains unclear if these risks persist in the context of Long COVID.

This evidence gap is particularly pronounced in the context of India, and more specifically, the ethnically distinct population of North-East India. Genetic predispositions, varying ACE2 receptor polymorphisms, and regional healthcare challenges may influence the manifestation of Long COVID and its reproductive effects [14]. This study aims to fill this critical gap by prospectively investigating the association between Long COVID and alterations in menstrual health, ovarian reserve markers, and pregnancy outcomes in a matched cohort of women from North-East India.

Objectives

Primary Objective

To prospectively investigate and compare the medium-to-long-term effects of Long COVID on female reproductive health parameters specifically menstrual cycle characteristics, biomarkers of ovarian reserve, and clinical pregnancy outcomes in a matched cohort of women from North-East India.

Secondary Objectives: To assess and compare the prevalence and spectrum of menstrual irregularities** between women with Long COVID and a matched control group of post-COVID women without Long COVID symptoms.

Hypothesis: Women with Long COVID will report a significantly higher prevalence of subjective menstrual irregularities, particularly oligomenorrhea (long cycles) and menorrhagia (heavy flow), compared to the recovered controls.

To evaluate and compare objective biomarkers of ovarian reserve and endocrine function** between the two cohorts.

Hypothesis: Serum Anti-Müllerian Hormone (AMH) levels, a direct marker of the ovarian follicular pool, will be significantly lower in the Long COVID cohort, suggesting a potential diminishment of ovarian reserve. We hypothesize no significant difference in other pituitary hormones (FSH, LH, Prolactin, TSH), indicating a primary ovarian effect rather than a central (pituitary) dysfunction.

To determine the impact of Long COVID on fecundability** by comparing the cumulative pregnancy rate and time-to-pregnancy over a 12-month follow-up period among women actively trying to conceive.

Hypothesis: The Long COVID cohort will exhibit a lower cumulative pregnancy rate and a longer time-to-pregnancy, indicating reduced fecundability, which may be linked to the observed alterations in ovarian reserve and menstrual cyclicity.

To prospectively analyze and compare the incidence of adverse obstetric outcomes** in pregnancies conceived after the acute phase of COVID-19 in both groups.

Hypothesis: Pregnancies in the Long COVID cohort will be associated with a higher incidence of placental-mediated complications, specifically preterm birth (<37 weeks gestation) and hypertensive disorders of pregnancy (gestational hypertension and preeclampsia).

To explore potential correlations between specific Long COVID symptom clusters (e.g., fatigue-dominant, neurocognitive-dominant) and the severity of reproductive dysfunction.

Hypothesis: The severity of reproductive outcomes (e.g., degree of AMH reduction, type of menstrual irregularity) may correlate with specific systemic manifestations of Long COVID, suggesting shared underlying pathophysiological mechanisms like endothelial dysfunction or chronic inflammation.

Exploratory Objective

To document and compare neonatal outcomes (including birth weight, Apgar scores, and NICU admission rates) between the two groups to gain preliminary insights into the potential intergenerational impact of maternal Long COVID.

Materials and Methods

Study Design and Ethical Considerations: A prospective, matched cohort study was conducted over a 24-month period from March, 2022 to February 2024. The study was approved by the Institutional Ethics Committees of all three participating tertiary care centres in Assam, Manipur, and Meghalaya. The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from every participant prior to enrolment.

Participant Recruitment and Sample Size Calculation

Participants were recruited from the post-COVID follow-up clinics of the participating hospitals. A total of 316 women were enrolled. The sample size was calculated using OpenEpi version 3.01. Based on a prior study by Li *et al.* [8] that reported a 25% prevalence of menstrual changes post-COVID, we assumed an odds ratio of 2.5 for menstrual dysfunction in the Long COVID group. To achieve 80% power at a 5% alpha error (two-sided), with a 1:1 case-to-control ratio, a minimum sample of 142 per group was required. We enrolled 158 per group to account for a potential 10% loss to follow-up.

Inclusion Criteria: Women aged 18-45 years with a confirmed history of SARS-CoV-2 infection (via RT-PCR) 3 to 6 months prior to enrolment.

Exclusion Criteria: (i) Known pre-existing causes of menstrual irregularity (e.g., PCOS, diagnosed by Rotterdam criteria; thyroid dysfunction; endometriosis; uterine fibroids); (ii) Current or recent (within 3 months) use of hormonal contraception or hormone replacement therapy; (iii) History of hysterectomy or bilateral oophorectomy; (iv) Pre-existing chronic diseases known to affect reproductive or endocrine function (e.g., uncontrolled diabetes, chronic kidney disease Stage IV/V,

autoimmune disorders, known diminished ovarian reserve); (v) History of chemotherapy or pelvic radiation.

Exposure Definition and Cohort Stratification: Long COVID was defined according to the WHO clinical case definition [2]. Symptoms were assessed using a detailed, pre-piloted questionnaire adapted from the NIH PASC Symptom Questionnaire. Participants reporting one or more new or persistent symptoms (e.g., fatigue, brain fog, dyspnoea, arthralgia) lasting ≥12 weeks after the acute infection that impact daily functioning were classified into the **Long COVID cohort (n=158). Those who reported a complete return to their pre-COVID health status were classified into the Recovered Control cohort (n=158). The two cohorts were individually matched 1:1 for age (±2 years), BMI (±2 kg/m²), and parity (nulliparous/parous).

Data Collection and Outcome Measures

Data was collected at baseline and through follow-up via:

1. **Structured Interview:** Collected socio-demographic data, detailed medical and obstetric history, and characteristics of the acute COVID-19 episode (severity, hospitalization, treatment).
2. **Menstrual History Questionnaire:** Administered at baseline and monthly for 6 months via telephone follow-up. It assessed cycle length, duration of bleeding (in days), regularity (defined as cycles between 21-35 days), subjective menstrual flow (light, moderate, heavy), and presence of intermenstrual bleeding or spotting.
3. **Biochemical Assessment:** A venous blood sample was drawn from each participant on day 2-4 of a spontaneous menstrual cycle. Serum was separated and analyzed for:
4. **AMH:** Using an electrochemiluminescence immunoassay (ECLIA) on a Cobas e411 analyzer (Roche Diagnostics).
5. **FSH, LH, Prolactin, TSH:** Using a chemiluminescent microparticle immunoassay (CMIA) on an Architect i2000SR analyzer (Abbott Laboratories).
6. **Pregnancy and Outcome Follow-up:** Participants who were sexually active and not using contraception were followed for 12 months to assess time-to-pregnancy. Those who conceived were followed prospectively through their pregnancy until delivery. Outcomes recorded included: first-trimester miscarriage (<12 weeks), late miscarriage (12-19+6 weeks), preterm birth (<37 weeks), gestational diabetes mellitus (diagnosed by IADPSG criteria), gestational hypertension/preeclampsia (defined by ISSHP criteria), mode of delivery, birth weight, and 5-minute Apgar score.
7. **Statistical Analysis:** Data were analyzed using STATA version 16.0 (StataCorp LLC, Texas, USA). Normality of continuous variables was assessed using the Shapiro-Wilk test. Normally distributed data were presented as mean (±SD) and compared using Student's t-test. Non-normally distributed data (like AMH) were presented as median (interquartile range, IQR) and compared using the Mann-Whitney U test. Categorical variables were expressed as frequencies and percentages and compared using the Chi-square test or Fisher's exact test, as appropriate.

The cumulative probability of pregnancy was estimated using Kaplan-Meier survival analysis and compared between groups using the Log-rank test. A Cox proportional hazards model was used to calculate the Hazard Ratio (HR) for time-to-pregnancy. To adjust for potential confounders (e.g., age, BMI, severity of acute COVID), multivariable logistic regression analysis was performed for binary outcomes (e.g., menstrual irregularity, preterm birth), and results were expressed as adjusted Odds Ratios (aOR) with 95% confidence intervals (CI). A two-tailed p-value of <0.05 was considered statistically significant.

Results

Baseline Characteristics: The two cohorts were successfully matched, with no statistically significant differences in age, BMI, parity, or the severity of the initial acute COVID-19 infection (Table 1). The most commonly reported Long COVID symptoms were fatigue (78%), cognitive dysfunction (52%), and dyspnoea (45%).

Table 1: Baseline Characteristics of the Study Cohorts

Characteristic	Long COVID Group (n=158)	Recovered Control Group (n=158)	p- value
Age (years), Mean (±SD)	30.2 (±5.1)	30.5 (±4.8)	0.65
BMI (kg/m²), Mean (±SD)	23.5 (±3.1)	23.8 (±2.9)	0.42
Nulliparous, n(%)	65 (41.1%)	62 (39.2%)	0.78
Acute Covid Severity, n (%)			0.82
-Mild	120 (75.9%)	122 (77.2%)	
-Moderate	32 (20.3%)	30 (19.0%)	
-Severe	6 (3.8%)	6 (3.8%)	

Menstrual Health Outcomes

Women in the Long COVID cohort reported a significantly higher prevalence of any menstrual irregularity compared to the recovered controls (55.1% [87/158] vs. 28.5% [45/158]; $p<0.001$). The most common irregularities were **oligomenorrhea** (cycle length >35 days; 25.3% vs. 10.1%, $p<0.01$) and **menorrhagia** (heavy menstrual bleeding; 18.4% vs. 8.2%, p=0.01). After adjusting for age and BMI, the odds of having a menstrual irregularity remained significantly higher in the Long COVID group (aOR 3.12, 95% CI 1.95-4.98).

Ovarian Reserve and Hormonal Profile: The primary biomarker of interest, AMH, was significantly lower in the Long COVID group (median 2.1 ng/mL, IQR: 1.4-3.0) compared to the control group (median 2.8 ng/mL, IQR: 1.9-3.6; p=0.01). No statistically significant differences were observed between the groups in the serum levels of FSH, LH, prolactin, or TSH (Table 2).

Table 2: Hormonal Profile of the Study Cohorts

Hormone (Units)	Long COVID Group (Median, IQR)	Control Group (Median, IQR)	p- value
AMH (ng/mL)	2.1 (1.4 - 3.0)	2.8 (1.9 - 3.6)	0.01
FSH (mIU/mL)	6.8 (5.2 - 8.5)	6.5 (5.0 - 8.1)	0.38
LH (mIU/mL)	5.5 (4.0 - 7.8)	5.8 (4.2 - 7.5)	0.75
Prolactin (ng/mL)	14.1 (10.8 - 18.3)	13.5 (10.5 - 17.9)	0.52
TSH (µIU/mL)	2.1 (1.5 - 2.8)	2.0 (1.4 - 2.7)	0.61

Fertility Outcomes: Among the sub-cohort of women actively trying to conceive (n=112 in each group), the cumulative pregnancy rate at 12 months, as determined by Kaplan-Meier analysis, was significantly lower in the Long COVID group (62%) compared to the control group (78%) (Log-rank test p=0.02). The Cox regression model indicated a 35% reduction in the monthly probability of conception in the Long COVID group (Hazard Ratio 0.65, 95% CI 0.48-0.89).

Pregnancy Outcomes: A total of 45 pregnancies occurred in the Long COVID cohort and 58 in the control cohort during the follow-up period. Pregnancies in the Long COVID group were associated with a significantly higher incidence of preterm birth (<37 weeks; 17.8% [8/45] vs. 6.9% [4/58]; p=0.03) and gestational hypertension/preeclampsia (15.6% [7/45] vs. 5.2% [3/58]; p=0.04). The rates of gestational diabetes mellitus and Caesarean delivery were not significantly different between the groups. Neonatal outcomes, including mean birth weight and rate of low 5-minute Apgar score (<7), were comparable.

Discussion

This prospective matched cohort study offers robust evidence from a unique demographic setting that Long COVID exerts a significant negative influence on multiple facets of female reproductive health. Our findings demonstrate a strong association with menstrual disturbances, a objective biomarker suggesting diminished ovarian reserve, reduced fertility, and a higher risk of serious pregnancy complications.

The twofold increase in menstrual irregularities aligns with growing global literature on post-COVID menstrual changes [8, 9, 15]. The high prevalence of oligomenorrhea suggests a potential disruption of the HPO axis. The systemic inflammation and psychological stress associated with Long COVID could alter the pulsatile secretion of Gonadotropin-Releasing Hormone (GnRH), leading to ovulatory dysfunction and cycle length changes [16]. The finding of menorrhagia could be linked to the viral impact on endometrial ACE2 expression, potentially affecting local vascular integrity and angiogenesis [5].

The most biologically significant finding is the statistically significant reduction in serum AMH levels in the Long COVID group. AMH is a direct product of ovarian granulosa cells and is considered a reliable quantitative marker of the ovarian follicle pool [17]. Our results suggest a potential accelerated decline in ovarian reserve or a temporary suppression of follicular function following SARS-CoV-2 infection. This could be mediated through direct viral entry into granulosa cells via ACE2 receptors, leading to cell dysfunction or apoptosis [6]. Alternatively, the systemic inflammatory cytokine storm (e.g., IL-6, TNF-α) during acute infection may have a toxic effect on ovarian follicles, an effect that persists in the chronic inflammatory state of Long COVID [7, 18]. This mechanism provides a plausible explanation for the observed reduction in fecundability, as a smaller ovarian reserve can translate to lower monthly conception rates.

The increased risk of preterm birth and gestational hypertension in pregnancies conceived after Long COVID is a major clinical concern. This aligns with studies on placental pathology in acute COVID-19, which show evidence of maternal vascular malperfusion, a hallmark of preeclampsia [12, 13]. The persistent endothelial dysfunction and pro-thrombotic state observed in Long COVID [19] may compromise placental development and function from the earliest stages of pregnancy, leading to placental insufficiency and its associated complications. This underscores the necessity of treating subsequent pregnancies in Long COVID survivors as high-risk, warranting enhanced surveillance.

Strengths and Limitations: The major strengths of this study are its prospective design, the use of a carefully matched control group which minimizes confounding, the inclusion of objective biochemical markers (AMH), and the focus on an understudied ethnic population. However, several limitations must be acknowledged. First, while AMH is a strong predictor of ovarian reserve, it is not a direct measure of oocyte quality. Second, the assessment of menstrual symptoms relied on patient recall and subjective reporting, though this was mitigated by prospective monthly follow-ups. Third, although we adjusted for key confounders, residual confounding from unmeasured factors like psychological stress, nutritional status, or subtle undiagnosed endocrine disorders cannot be entirely ruled out. Finally, the single measurement of AMH provides a snapshot; longitudinal tracking of AMH levels in Long COVID patients would be valuable to determine if this effect is permanent or transient.

Conclusion

In conclusion, this study from North-East India provides comprehensive evidence that Long COVID is a significant risk factor for a range of female reproductive health issues, from menstrual disorders and potential ovarian insult to adverse pregnancy outcomes. These findings have immediate clinical implications. Healthcare providers managing women of reproductive age who have had COVID-19 should proactively inquire about menstrual changes and offer appropriate investigation and support. Preconception counselling for women with a history of Long COVID should include a discussion of these potential risks, and once pregnant, these patients should be considered for heightened obstetric surveillance. Future research should focus on elucidating the precise mechanisms behind these associations and exploring interventions to mitigate these risks.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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