Vaccine: X 19 (2024) 100501

Contents lists available at ScienceDirect

Vaccine: X

journal homepage: www.elsevier.com/locate/jvacx

The association of COVID-19 vaccination and menstrual health: A period-tracking app-based cohort study

Malini Ramaiyer [a,1], Malak El Sabeh [b,1], Jiafeng Zhu [c,d], Amanda Shea [e], Dorry Segev [f], Gayane Yenokyan [c], Mostafa A. Borahay [g,*]

[a] Johns Hopkins University School of Medicine, Baltimore, MD, United States
[b] Department of Gynecology and Obstetrics, Baylor College of Medicine, Houston, TX, United States
[c] Johns Hopkins Biostatistics Center, Johns Hopkins School of Public Health, Baltimore, MD, United States
[d] Department of Preventive Medicine, Northwestern University, Feinberg School of Medicine, Chicago, IL, United States
[e] Head of Science, Clue by BioWink GmbH, Berlin, Germany
[f] Department of Surgery, New York University, New York, NY, United States
[g] Department of Gynecology and Obstetrics, Johns Hopkins University, Baltimore, MD, United States

ARTICLE INFO

Keywords:
COVID-19 vaccine
Coronavirus
Menstrual cycle
Menstruation
Women's health

ABSTRACT

Background: In initial COVID-19 clinical trials, menstrual health was not formally monitored, yet anecdotal reports of menstruation changes surfaced on social media. This study aims to assess the association between COVID-19 vaccines and menstruation using Clue, a period-tracking application.

Study design: A survey assessing demographics, menstrual health, stress levels, and COVID-19 vaccination was sent to Clue users between 12/7/2021 and 2/9/2022. Inclusion criteria were (1) 18 years or older (2) currently menstruating (3) not pregnant or breastfeeding since 1/2020. Menstrual data was collected for each participant. Users with cycle lengths more than 90 days were excluded. Cycle lengths were calculated for the 6-month average pre-vaccination (PRIOR), the cycle during which vaccination was administered (DURING), the cycle following DURING (AFTER1), and the cycle following AFTER1 (AFTER2). For periods, individuals were stratified based on whether vaccination was received during their menstrual period (DURING). Period lengths were additionally calculated for the 6-month average pre-vaccination (PRIOR), the first period following vaccination (AFTER1), and the period following AFTER1 (AFTER2). For unvaccinated participants, an index date (4/1/2022) was used to similarly designate menstrual cycles and periods. For each participant, cycle length changes for DURING, AFTER1, and AFTER2 compared to PRIOR were determined. Student's *t*-test compared the mean of these changes between vaccinated and unvaccinated groups.

Results: Of 7,559 participants, 6,897 (91 %) were vaccinated. Compared to PRIOR, individuals vaccinated during their menstrual period demonstrated a statistically significant increase in the DURING period length, but not AFTER1 ($p = 0.463$) and AFTER2 ($p = 0.692$). No statistically significant changes were observed in period lengths of those vaccinated in between periods or in cycle lengths overall.

Conclusion: A small but statistically significant change in period length was observed only in individuals vaccinated for COVID-19 during their menstrual period. Providers can better counsel menstruating individuals to reduce vaccine misinformation.

Introduction

The large roll-out of the COVID-19 vaccines was accompanied by anecdotal evidence of unexpected menstrual irregularities following vaccination. Menstruating individuals on social media platforms reported changes in their menstrual cycle with most reporting heavier and/or longer cycles [1,2]. Unfortunately, the initial clinical trials for the Pfizer, Moderna, Johnson and Johnson, and Oxford-AstraZeneca

* Corresponding author at: Department of Gynecology & Obstetrics, Johns Hopkins University School of Medicine, 720 Rutland Ave, Baltimore, MD 21205, United States.
E-mail address: mboraha1@jhmi.edu (M.A. Borahay).
[1] These authors contributed equally.

https://doi.org/10.1016/j.jvacx.2024.100501
Received 26 January 2024; Received in revised form 13 May 2024; Accepted 17 May 2024
Available online 18 May 2024

vaccines did not evaluate menstrual health [3–6]. To date, the U.S. Vaccine Adverse Event Reporting System (VAERS), a national early warning system to detect possible safety problems in U.S. licensed vaccines, has recorded only a small number of menstrual-related adverse events among more than 100 million individuals who menstruate and have been vaccinated [7]. The reported events include irregular menses, menstrual disorder, and delayed menstruation.

Regular menstruation is considered an indicator of whole-body health, and subtle changes in health can lead to noticeable fluctuations in the regular cycle. There is limited data on the effect of vaccines on menstrual health. The earliest study was in 1913, and Typhoid vaccine was found to be associated with menstrual cycle irregularities in a cohort of nurses [8]. A more recent large data study on HPV vaccine post-vaccination symptoms found a statistically increased age-adjusted odds of hospital visits for abnormal amount of menstrual bleeding and irregular menstruation [9]. In recent years since COVID-19 vaccination release to the public, there has been a tremendous effort to investigate the association between vaccination and menstrual cycle length, yet the conclusions have been varied in proportions of individuals experiencing changes to menstruation [10–12]. Some studies have used period-tracking applications to prospectively collect menstrual cycle data and survey users on COVID-19 vaccination status. Edelman et al evaluated whether COVID-19 vaccination is associated with menstrual cycle disturbances in the USA using the digital fertility-awareness application "Natural Cycles" and found that the users experienced small variations in cycle length [13]. Those who were vaccinated had a statistically significant difference in cycle length, but the change was less than 1 day, which is not considered clinically significant [13]. Studies using other mobile applications, including the Apple Research App and Clue, have also observed minor changes to menstrual cycle length among users in the US [14–16]. In this study, we aimed to contribute to the growing literature on this topic, by analyzing changes in menstrual period length in addition to menstrual cycle length and investigating whether any observed changes post-vaccination are persistent. This study analyzed prospectively collected data from users in the USA, Canada, Australia, and the United Kingdom using a period tracking application, Clue, to assess the effect of the four COVID-19 vaccines on the menstrual cycle length and period length as reported by app users.

Material and methods

Study population

We conducted a retrospective cohort study with de-identified self-tracked data collected prospectively through Clue by BioWink, one of the most popular period-tracking applications used worldwide. Johns Hopkins Institutional Review Board approved the protocol. Users of this application voluntarily track their menstrual cycles and consent to the use of their de-identified data for research when they create their Clue account. Clue data has been previously validated [17–20]. To collect information on the English-speaking users' vaccination status, Clue users were asked at random through an in-app pop-up message if they are interested in participating in the study. Users in the United States, Canada, United Kingdom, and Australia who agreed to participate received a survey between December 7, 2021 and February 9, 2022. Completing the survey was voluntary. Each Clue user is assigned a "Clue ID" when they create their account for the first time. These IDs were used to link their uploaded menstrual period data to their survey answers. The process of collecting the survey data was entirely performed by the Clue App team, who securely transmitted the collected data to the research team for analysis.

Menstrual and other data

The survey collected users' Clue ID, demographics (i.e. age, race), menstrual health, COVID-19 infection, Perceived Stress Scale scores, and COVID-19 vaccination (type and timing). The survey did not contain any identifying information (i.e. date of birth, name). To complete the survey, users were 18 years or older, had menstrual periods, and had not been pregnant or breastfeeding since January 2020.

Per the Clue App, cycles start on the first day of a period and end on the day before the following period. Certain types of bleeding are not identified as a period by the application such as "spotting" and bleeding starting earlier than day 10 of the cycle. Cycle data provided by Clue ranged from January 2019 to January 2022.

We categorized the participants' age groups (age at the start of the first cycle included in the study) as 18–24, 25–29, 30–34, 35–39, or above 40 years old. Race was reported as Asian, Black, White or Other (Middle Eastern or North African, Native Hawaiian or Pacific Islander, Gypsy, First Nations, Aboriginal, and those who preferred not to report). Ethnicity was reported as Hispanic or Non-Hispanic. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared and categorized as underweight or normal weight, overweight, or obese.

To evaluate stress among participants, we included in the survey the "Perceived Stress Scale" which is a validated psychological tool to assess perceived stress [21]. This tool contains 10 items which participants score from 0 to 4 for frequency. The questions ask about feelings and thoughts of individuals for the past month in order to measure individual stress levels. Scores from 0 to 13 are low stress, 14–26 are moderate stress, and 27–40 are high stress [21].

Inclusion and exclusion criteria

As performed in previous studies using period-tracking applications for menstrual cycle data [22], we excluded users who had cycle lengths longer than 90-day cycles as this can result from users forgetting to log in their periods, which can lead to a falsely long cycle. We included those who were vaccinated with Pfizer-BioNTech, Moderna, Johnson & Johnson, and Oxford/AstraZeneca. We excluded those who did not remember the vaccine type and those who were vaccinated with Sinopharm due to their low number in the cohort ($N = 2$). We also excluded vaccinated users who did not have any reported cycles before and/or after their vaccination. Since we are comparing vaccinated to unvaccinated individuals, we only included unvaccinated individuals who reported cycles around the same time when most of the vaccinated users received their vaccine (Index vaccination date of April 1st, 2021), and we compared their cycles before April 1, 2021, to the cycles after this date. We excluded unvaccinated users if their menstrual cycles did not overlap with this index date. We excluded those who responded "Yes" to using a form of contraception, as hormonal contraceptives may affect cycle and period length. Fig. 1 summarizes the flowchart of the study population.

Timepoint definitions

Fig. 2 represents the time points defined for analysis based on individuals' menstrual cycles, menstrual periods, and vaccination/index vaccination date. For the unvaccinated individuals, the index vaccination date was treated as if it was the date the vaccine was administered. For each individual in the cohort, the pre-vaccination cycle and period length baseline were determined using the 6-month average of menstrual cycle and period lengths prior to vaccination (PRIOR).

For analysis of changes to menstrual cycle length, as shown in Fig. 2, the DURING menstrual cycle was defined as the cycle during which the first dose of the vaccine was administered. AFTER1 is the first cycle following the DURING cycle, and AFTER2 is the cycle following AFTER1.

For analysis of changes to menstrual period length, individuals were separated based on whether vaccination was received during their menstrual period. As such, the DURING period is defined as the period during which the first dose of the vaccine was administered. For

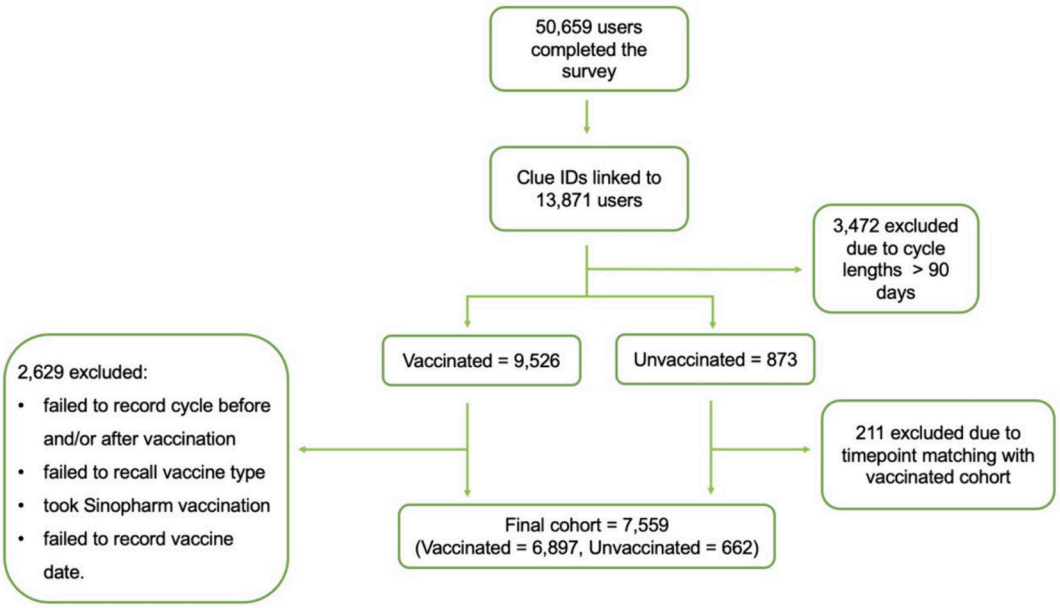

Fig. 1. Flowchart of study population.

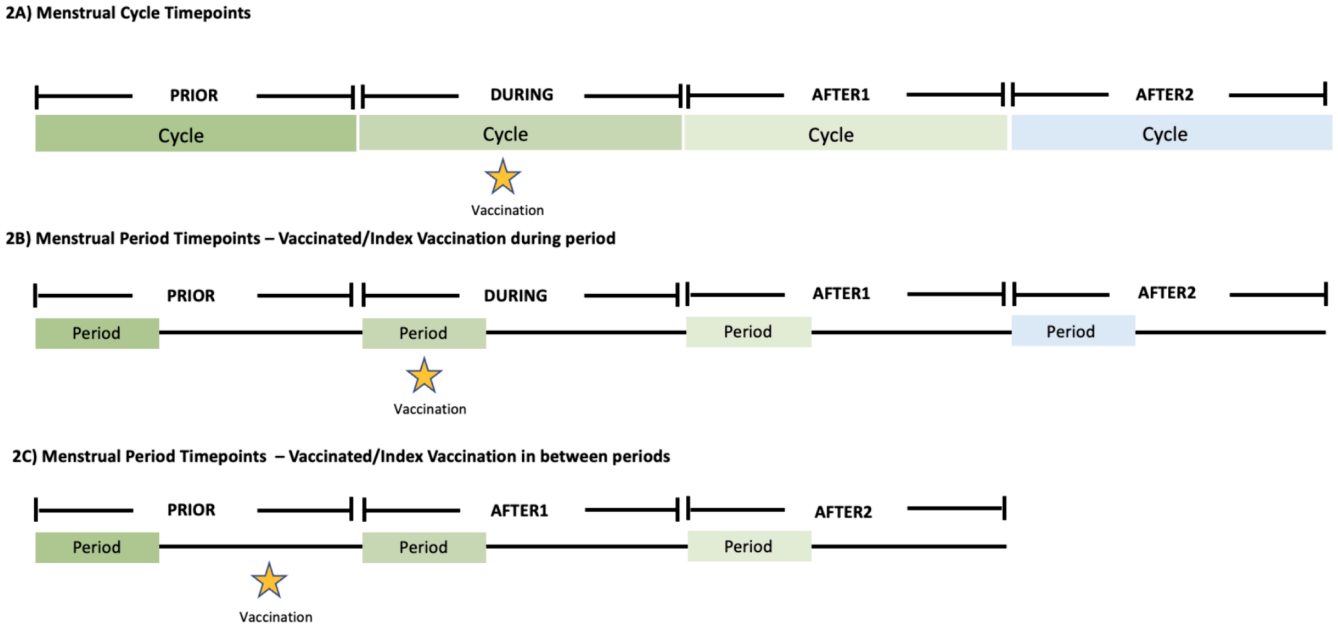

Fig. 2. Study timeline based on vaccine timepoint. 2A. "Prior" refers to the 6 month average of menstrual cycles before the vaccine administration. "During" refers to cycle during which fall in the time window during which the vaccine was received. "After1" in the figure refer to the first cycle following vaccination. "After2" refers to the second cycle following vaccination. 2B. Time points for individuals whose vaccination or index vaccination occurred during recorded menstrual periods. "Prior" refers to the 6 month average of periods before the vaccine administration. "During" refers to period during which fall in the time window during which the vaccine was received. "After1" in the figure refer to the first period following vaccination. "After2" refers to the second period following vaccination. 2C Time points for individuals whose vaccination or index vaccination occurred in between recorded menstrual periods. "Prior" refers to the 6 month average of periods before the vaccine administration. "After1" in the figure refer to the first period following vaccination. "After2" refers to the second period following vaccination.

individuals who were vaccinated in between periods, there is no DURING period. For all individuals in the vaccinated cohort, AFTER1 is the period that starts after the first dose of the vaccine was administered, and AFTER2 is the period that follows the AFTER1 period.

Statistical analysis

Our primary outcome was changes in cycle length before and after the administration of the vaccination first dose. Secondary outcome was changes in period length. First, we checked data for missing variables and grouped individuals based on vaccination status as "vaccinated" or "unvaccinated". Then, we performed descriptive analysis of age, race, country, education, BMI, method of contraception, stress level, tobacco use, and the vaccine type (only for those with vaccine). We used a Chi-Squared test for comparing vaccinated and unvaccinated individuals by age, BMI, education, and birth control method, and a Fisher's exact test to compare vaccinated and unvaccinated groups by race and country, due to small subgroup size. Student's *t*-test was used to compare results

from the Perceived Stress Scale between vaccinated and unvaccinated individuals.

For each individual in the vaccinated group, post-vaccination changes in cycle and period length were calculated at three timepoints (DURING, AFTER1, AFTER2) compared to pre-vaccination baseline (PRIOR). The DURING period was only calculated for individuals who were vaccinated during menstrual period. In the unvaccinated group, similar calculations were performed based on an index date of April 1, 2021, an arbitrary date selected around when COVID-19 vaccines became available to the public. The mean of these post-vaccination changes was subsequently calculated for the vaccinated and unvaccinated groups. For each timepoint after vaccination (DURING, AFTER1, AFTER2), Student's *t*-test was used to compare changes in cycle and period length between the vaccinated and unvaccinated groups. To analyze changes in menstrual period length of individuals before and after vaccination, vaccinated individuals were divided into two subgroups: those vaccinated during their period, and those vaccinated in between their periods. Similarly, as the control group, unvaccinated individuals were stratified as: individuals with the index vaccination date occuring during their recorded periods and individuals with the index vaccination date occuring in between their recorded periods. Statistical analysis was completed using software R 4.2.2.

Results

50,659 users completed the survey through the in-app pop up message. As individuals completed surveys, their survey data was linked through their Clue IDs to their cycle data logged in Clue. Uploaded cycle data was retrieved by the Clue application team. The Clue team experienced a technical issue, in which the tool that collected survey data stopped collecting the Clue IDs of users to link their cycle data. Due to this technical issue, survey and cycle data was only collected for users in the timeframe outside of this error occurrence. Clue IDs could only be linked to 13,871 users' survey data, limiting our analysis to 13,871 users. Of these participants, 3,472 participants were excluded due to cycle lengths greater than 90 days (as previously mentioned, out of caution due to the possibility of users forgetting to log bleeding into the App which can lead to falsely long cycles). This left 9,526 (91.6 %) who were vaccinated and 873 who were unvaccinated. Of the vaccinated individuals, 2,629 were excluded due to a variety of reasons, including: no record of at least one cycle before and/or after vaccination, not remembering the vaccine type, took Sinopharm vaccination (very small number), or failed to record the vaccination date. Of the unvaccinated, 211 were excluded if they did not have cycles recorded in the same timeframe as vaccinated individuals (timepoint matching). The final cohort was composed of 7,559 individuals, of which 6,897 were vaccinated and 662 were unvaccinated. Fig. 1 presents the flowchart of the study population.

Characteristics of the study population

The majority of the participants who were vaccinated received the Pfizer-BioNTech COVID-19 vaccine (59.3 %). Moderna was the second common, comprising 28 % of vaccinated individuals. Over 70 % of the overall cohort identified as White. While 70 % of individuals were from the USA, 24 % of participants were from the UK. 69 % of participants denied use of tobacco and 76 % of participants did not use any contraception. Table 1 summarizes the characteristics of the study population. The overall cohort was approximately equally split across age groups, except for those ages 18–24, which made up 29 % of the overall cohort.

There were a few statistically significant demographic differences between unvaccinated and vaccinated individuals. The unvaccinated cohort is relatively younger than the vaccinated cohort ($p = 0.001$). In terms of race, the unvaccinated group was composed of less Asian individuals, compared to the vaccinated cohort ($p < 0.001$). The proportion of individuals from the USA are 12 % higher in the unvaccinated cohort than the vaccinated cohort ($p < 0.001$). Furthermore, the unvaccinated cohort is less educated with 25 % completing undergraduate

Table 1
Demographics and Characteristics of Study Population.

Variable		Overall (N = 7559)	Vaccinated % (N = 6897)	Unvaccinated (N = 662)	P-value**
Age	18–24	2192 (29 %)	1960 (28 %)	232 (35 %)	0.001
	25–29	1430 (19 %)	1307 (19 %)	123 (19 %)	
	30–34	1479 (20 %)	1366 (20 %)	113 (17 %)	
	35–39	1146 (15 %)	1041 (15 %)	105 (16 %)	
	40+	1307 (17 %)	1219 (18 %)	88 (13 %)	
Race	Asian	445 (6 %)	439 (6 %)	6 (1 %)	<0.001
	Black	436 (6 %)	382 (6 %)	54 (8 %)	
	Hispanic	515 (7 %)	469 (7 %)	46 (7 %)	
	White	5905 (78 %)	5387 (78 %)	518 (78 %)	
	Other	258 (3 %)	220 (3 %)	38 (6 %)	
Country	Australia	388 (5 %)	371 (5 %)	17 (3 %)	<0.001
	Canada	42 (1 %)	41 (1 %)	1 (0 %)	
	UK	1805 (24 %)	1696 (25 %)	109 (16 %)	
	USA	5324 (70 %)	4789 (69 %)	535 (81 %)	
Education	High school or less	733 (10 %)	584 (9 %)	149 (23 %)	<0.001
	Some college	2007 (26 %)	1723 (25 %)	284 (43 %)	
	Undergraduate	2996 (40 %)	2830 (41 %)	166 (25 %)	
	Postgraduate	1814 (24 %)	1753 (25 %)	61 (9 %)	
BMI	Less or normal	3254 (48 %)	2952 (48 %)	302 (49 %)	0.217
	Overweight	1599 (23 %)	1473 (24 %)	126 (21 %)	
	Obesity	1947 (29 %)	1765 (28 %)	182 (30 %)	
Contraception use	No	5766 (76 %)	5234 (76 %)	532 (80 %)	0.011
	Not sure	1793 (24 %)	1663 (24 %)	130 (20 %)	
Stress	Mean	7	7.03	6.63	<0.001
	SD	1.75	1.66	2.48	
Smoking tobacco	No	5241 (69 %)	4831 (70 %)	410 (62 %)	<0.001
	Yes	2318 (31 %)	2066 (30 %)	252 (38 %)	
Type	J&J	349 (5 %)			
	Moderna	1909 (28 %)			
	Oxford-Astrazeneca	530 (8 %)			
	Pfizer-BioNTech	4109 (59 %)			

** We used Chi-squared test for Age, Race, Education, BMI, and Method, Fisher's exact test for Country, and *t*-test for Stress scale.

compared to 41 % in the vaccinated cohort ($p < 0.001$). Additionally, the prevalence of postgraduate class is 16 % higher in the vaccinated cohort. Interestingly, unvaccinated group reported lower stress scores ($p < 0.001$) and more tobacco use ($p < 0.001$).

Cycle length

The average cycle lengths for PRIOR, DURING, AFTER1, and AFTER2 can be found in Table 2. In the DURING compared to PRIOR cycle, a mean change of 1.07 days in vaccinated and 1.063 days in the unvaccinated was observed (p = 0.984). Likewise, comparing AFTER1 to baseline of PRIOR, a mean change of −0.161 days in the vaccinated group and 0.193 days in the unvaccinated group was observed, but it was not statistically significant (p = 0.234). Similarly, comparing AFTER2 to baseline of PRIOR, a mean change of −0.061 days in the vaccinated group and −0.102 days in the unvaccinated group was observed, which was not statistically significant (p = 0.887) (Table 3). These findings are presented in Fig. 3.

Period length

The average period lengths for PRIOR, DURING, AFTER1, and AFTER2 can be found in Table 2.

Analysis of individuals who were vaccinated during their menstrual period

In the DURING compared to PRIOR period, a mean change of 0.434 days in vaccinated and 0.051 days in the unvaccinated was observed, which was statistically significant (p = 0.014). However, comparing AFTER1 to baseline of PRIOR, a mean change of −0.017 days in the vaccinated group and 0.078 days in the unvaccinated group was observed, but it was not statistically significant (p = 0.463). Similarly, comparing AFTER2 to baseline of PRIOR, a mean change of −0.108 days in the vaccinated group and −0.033 days in the unvaccinated group was observed, which was not statistically significant (p = 0.692) (Table 3). These findings are presented in Fig. 4.

Analysis of individuals who were vaccinated between their menstrual periods

Comparing AFTER1 to baseline of PRIOR, a mean change of 0.009 days in the vaccinated group and 0.036 days in the unvaccinated group was observed (p = 0.635). Similarly, comparing AFTER2 to baseline of PRIOR, a mean change of −0.025 days in the vaccinated group and −0.055 days in the unvaccinated group was observed (p = 0.633) (Table 3).). These findings are presented in Fig. 5.

Stress levels

The Perceived Stress Scale mean score of the overall cohort was 7/40. The vaccinated had a mean score of 7.03 and the unvaccinated had a mean score of 6.63 (p=<0.001). A score from 0 to 13 is considered low stress.

Table 2
Average pre-vaccination baseline (Prior) and post-vaccination (During, After1, After2) cycle and period length for vaccinated and unvaccinated individuals.

	Prior	During	After1	After2
Vaccinated				
Avg cycles length	30.0 (7.29)	31.1 (8.68)	29.8 (7.04)	29.9 (7.08)
Avg period length (Individuals vaccinated during period)	4.63 (1.83)	5.06 (2.84)	4.61 (1.98)	4.52 (1.83)
Avg period length (Individuals vaccinated in between periods)	4.20 (1.76)	–-	4.21 (1.75)	4.18 (1.80)
Unvaccinated				
Avg cycles length	29.7 (7.12)	30.7 (8.52)	29.9 (7.47)	29.6 (7.13)
Avg period length (Index vaccination during period)	4.32 (1.66)	4.37 (1.56)	4.38 (1.46)	4.28 (1.63)
Avg period length (Index vaccination in between periods)	4.17 (1.72)	–-	4.21 (1.62)	4.12 (1.68)

Table 3
Post-vaccination changes in menstrual cycle length and period length(During, After1, After2) compared to pre-vaccination baseline(PRIOR).

		Vaccinated	Unvaccinated	P-value
Mean change in Cycle Length	During	1.07	1.063	0.984
	After1	−0.161	0.193	0.234
	After2	−0.061	−0.102	0.887
Mean change in Period Length(Vaccinated/index vaccination during period)	During	0.434	0.051	0.014*
	After1	−0.017	0.078	0.463
	After2	−0.108	−0.033	0.692
Mean change in Period Length (Vaccinated/index vaccination in between periods)	After1	0.009	0.036	0.635
	After2	−0.025	−0.055	0.633

Note: The p values reported are from *t*-test of the means at each time point. * indicates statistical significance.

Discussion

Menstrual data from 7,559 menstruating individuals demonstrated that the COVID-19 vaccination is associated with statistically significant change in the length of the period during which vaccination was received. These changes are only observed if vaccination is administered during a concurrent menstrual period. There were no statistically significant changes in the cycle length following the vaccination. While these changes were statistically significant, the observed clinical difference would amount to a few hours, and it is doubtful that menstruating individuals will notice this change. This knowledge allows providers to reinforce the safety of the vaccine and reduces hesitancy about perceived effects on menstrual health. With these findings, providers may better counsel and inform menstruating patients about limited changes that may occur in individuals immediately after they receive vaccination, if they receive the vaccine while menstruating.

Menstrual irregularities have been observed in conjunction with vaccine administration as early as 1913, when a group of nurses received the Typhoid vaccine. However, throughout history, the effect of vaccines on menstruation in general have not been well documented. This is evidenced by the clinical trials for COVID-19 vaccines, as menstrual irregularities were not monitored as adverse events for individuals [13].

This study builds upon the recent effort in research to determine the effect of COVID-19 vaccine on menstruation. Several other studies have utilized period-tracking mobile applications, including Clue, to collect menstrual cycle and period data. Using the Natural Cycles, Edelman et al previously evaluated the effect of COVID-19 vaccine on length of menstrual cycles and periods of individuals in the USA, and found that COVID-19 vaccination was associated with a small change in cycle length but not period length [13]. Using Natural Cycles as well, Darney et al surveyed users on changes to number of heavy bleeding days to investigate the association between COVID-19 vaccine and menstruation, and determined that vaccinated individuals reported more heavy bleeding days after their first vaccination dose [14]. Similarly, Gibson et al used the Apple's Women Health Study, a longitudinal mobile-application-based cohort of people in the U.S. with manually logged menstrual cycles, and found that COVID-19 vaccination was associated with a small and temporary change in cycle length [15]. Finally, our study builds off the work of Alvergne et al who used Clue as well and determined that infection with COVID-19 or COVID-19 vaccination was associated with small, temporary changes in cycle length [16]. These studies using period-tracking applications build upon a body of literature using cross-sectional data to demonstrate minor changes to menstrual cycles associated with COVID-19 vaccination or COVID-19

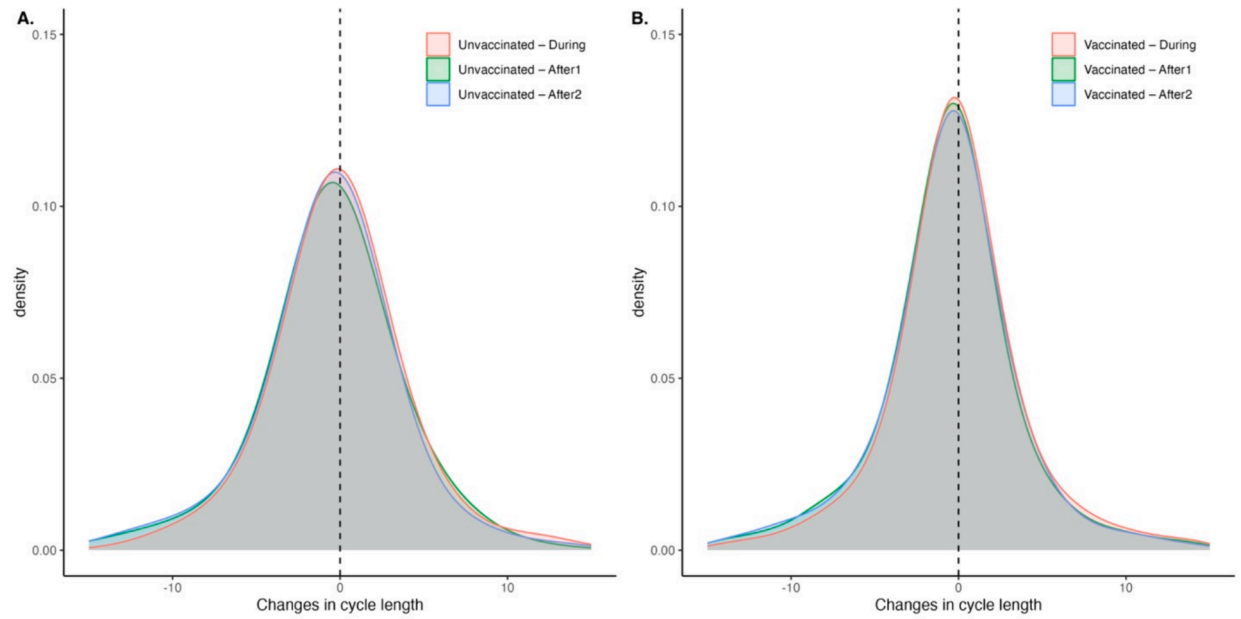

Fig. 3. 3A. Frequency histogram of changes in length of the cycle for unvaccinated group using index date (4/1/2021) as a control for vaccination. Cycle length changes are comparing the cycle during which index date occured (DURING), the first cycle after index date (AFTER1), and the second cycle after index date (AFTER2) compared to the 6-month average cycle length prior to vaccination. 3B. Among vaccinated group, frequency histogram of changes in length of the cycle during which vaccination is received (DURING), the first cycle after vaccination (AFTER1), and the second cycle after vaccination (AFTER2) compared to the 6-month average cycle length prior to vaccination.

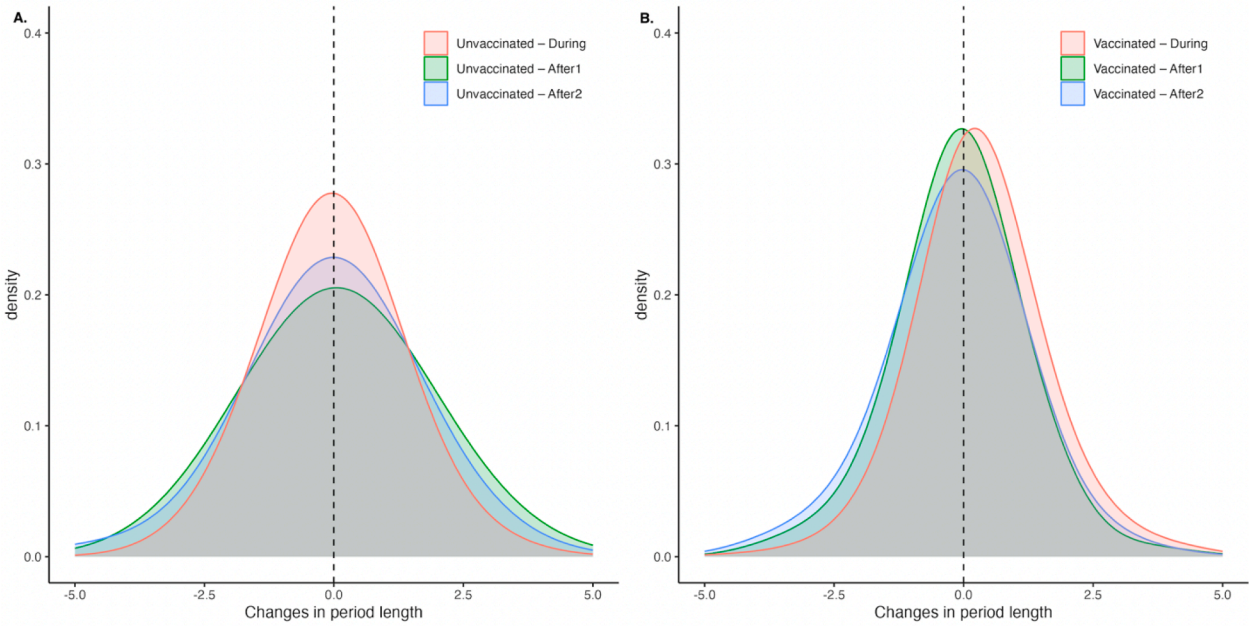

Fig. 4. 4A. Frequency histogram of changes in length of the period for unvaccinated group using index date (4/1/2021) as a control for vaccination. Period length changes are comparing the period during which index date occurred (DURING), the first period after index date (AFTER1), and the second period after index date (AFTER2) compared to the 6-month average of period lengths prior to index date. 4B. Among vaccinated group, frequency histogram of changes in length of the period during which vaccination is received (DURING), the first period after vaccination(AFTER1), and the second period after vaccination(AFTER2) compared to the 6-month average of period lengths prior to vaccination date.

infection [23–27]. While this study did not observe changes to cycle length, we expanded the scope of the literature by examining menstrual period length and similarly found that the observed statistically significant change in menstrual period length was neither clinically significant nor persistent past the first cycle post-vaccination. Additionally, this study adds to the literature by recruiting from regions outside of the USA, including UK, Australia, and Canada.

Clinical Implications

Menstrual irregularities are an important indicator of health for individuals who menstruate, as changes in the length or flow have been associated with depression, anxiety, and anemia [28,29]. Understanding the effect of vaccines on menstruation can help healthcare providers inform recipients on what changes they can expect post-vaccination.

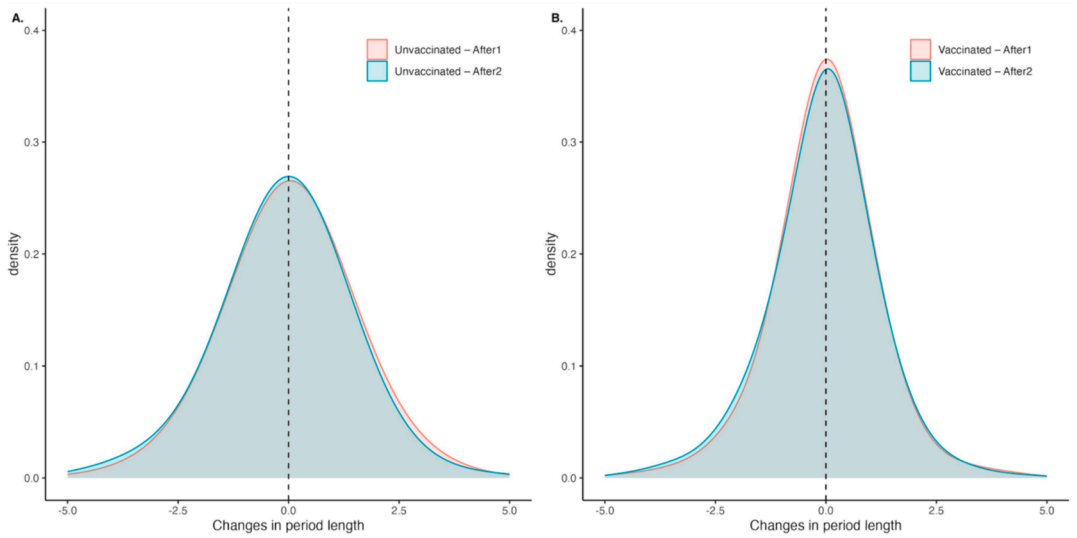

Fig. 5. 5A. Frequency histogram of changes in length of the period for unvaccinated group using index date (4/1/2021) as a control for vaccination. Period length changes the first period after index date (AFTER1) and the second period after index date (AFTER2) compared to the 6-month average of period lengths prior to index date. 5B. Among vaccinated group, frequency histogram of changes in length of the first period after vaccination (AFTER1) and the second period after vaccination (AFTER2) compared to the 6-month average of period lengths prior to vaccination date.

Furthermore, the spread of anecdotes about menstrual irregularities caused by vaccination amongst individuals has the potential to spread and exacerbate vaccine hesitancy. The demographic differences observed between unvaccinated and vaccinated cohorts is notable, as providers can better understand which individuals are more hesitant about COVID-19 and its potential side effects. Our unvaccinated cohort was younger, less educated, and disproportionately from the USA — this information can be used to inform educational efforts to reduce vaccine hesitancy. With the findings of this study, it is important on a public health level for providers clarify the picture [28].

Research implications

Further understanding the immune microenvironment of the endometrium may further elucidate the effect of COVID-19 vaccine on menstruation. The endometrium is enriched with immune cells, including uterine-specific natural killer (NK) cells, macrophages, as well as lymphoid aggregates containing B cells and CD8 + T cells [30]. The composition of this immune microenvironment varies throughout the menstrual cycle, as pro-inflammatory mechanisms relate to the initiation of menstruation and anti-inflammatory mechanisms relate to the conclusion of menstruation [30,31]. The NK cells specific to the endometrium are CD3- CD56 bright CD16-, compared to CD3- CD56 dim CD16 + in the peripheral blood. These cells are observed to increase after ovulation and in the days prior to menstruation [30]. The increase in leukocytes before menstruation results in the release of proteases, chemokines, and cytokines, such as IL-1 and TNFa, which ultimately produce and activate matrix metalloproteinases. The tissue breakdown caused by metalloproteinases is a hallmark of menstruation [31,32]. It is not clear how the recruitment of leukocytes and metalloproteinases is regulated; however, recent studies have isolated specific chemokines responsible for the increase in leukocytes leading to menstruation [33]. We hypothesize that the COVID-19 vaccination may modulate these chemokine levels, leading to changes in menstrual cycle and period length post-vaccination. To further investigate this effect, our group is conducting an additional study on the effect of COVID-19 vaccine on endometrium. Menstrual effluent will be examined for inflammatory and endocrine changes in the endometrium before and after vaccination. We hope to identify the specific mechanisms which may be implicated in the associated cycle and period changes post-vaccination.

Strengths and limitations

Limitations to this study include low diversity, as most participants were White. There is potential selection bias by recruiting users of the Clue app, as period-tracking applications may be used among the more technologically savvy individuals. Furthermore, due to the technical issue in linking Clue IDs with survey participants, there may have been a selection bias in included participants. However, the in-app messages to users were sent at random and those excluded were not based on user characteristics, but based on the timepoint at which the technical error occurred. Therefore, it is unlikely that this caused a selection bias in this cohort. The limitations of using Clue data are balanced with the strengths of the application in accessing thousands of individuals worldwide and collecting day to day data would be otherwise difficult on an individual basis.

Conclusion

Menstrual health is a vital contributor to an individual's wellbeing. Informing patients that vaccines may change period lengths would be helpful for vaccine recipients to know beforehand. Future studies should investigate changes to heaviness of flow as well as pain to elucidate the complete picture for individuals who menstruate. Menstrual health should be included in initial trials of future vaccines.

Funding

This work was supported, in part, by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) grant 3R01HD094380-04S1.

CRediT authorship contribution statement

Malini Ramaiyer: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Malak El Sabeh:** Writing – original draft, Methodology, Data curation, Conceptualization. **Jiafeng Zhu:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Amanda Shea:** Writing – review & editing, Formal analysis. **Dorry Segev:** Writing – review & editing, Formal analysis. **Gayane Yenokyan:** Writing – review & editing, Formal analysis, Data curation. **Mostafa A. Borahay:** Writing – review &

editing, Supervision, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Mostafa Borahay reports financial support was provided by National Institute of Child Health and Human Development. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

References

[1] McShane J. Can the vaccine make your period worse? These women say yes. The Lily n.d.

[2] Lu-Culligan A, Epstein RH. Opinion | No. N Y Times: We Don't Know if Vaccines Change Your Period; 2021.

[3] Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet 2021;397:99–111. https://doi.org/10.1016/S0140-6736(20)32661-1.

[4] Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N Engl J Med 2021;384:403–16. https://doi.org/10.1056/NEJMoa2035389.

[5] Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine | NEJM n.d. https://www.nejm.org/doi/full/10.1056/nejmoa2034577 (accessed January 5, 2023).

[6] Sadoff J, Le Gars M, Shukarev G, Heerwegh D, Truyers C, de Groot AM, et al. Interim Results of a Phase 1–2a Trial of Ad26.COV2.S Covid-19 Vaccine. N Engl J Med 2021;384:1824–35. https://doi.org/10.1056/NEJMoa2034201.

[7] NOT-HD-21-035: Notice of Special Interest (NOSI) to Encourage Administrative Supplement Applications to Investigate COVID-19 Vaccination and Menstruation (Admin Supp Clinical Trial Optional) n.d. https://grants.nih.gov/grants/guide/notice-files/NOT-HD-21-035.html (accessed January 5, 2023).

[8] Lamb ARE. With prophylactic typhoid vaccination: its effect on menstruation. Arch Intern Med 1913;XII:565–77. https://doi.org/10.1001/archinte.1913.00070050082008.

[9] Suzuki S, Hosono A. No association between HPV vaccine and reported post-vaccination symptoms in Japanese young women: Results of the Nagoya study. Papillomavirus Res Amst Neth 2018;5:96–103. https://doi.org/10.1016/j.pvr.2018.02.002.

[10] Alahmadi AM, Aljohani AH, Fadhloun RA, Almohammadi AS, Alharbi DF, Alrefai LS. The Effect of the COVID-19 Vaccine on the Menstrual Cycle Among Reproductive-Aged Females in Saudi Arabia. Cureus n.d.;14:e32473. Doi: 10.7759/cureus.32473.

[11] Farland LV, Khan SM, Shilen A, Heslin KM, Ishimwe P, Allen AM, et al. COVID-19 vaccination and changes in the menstrual cycle among vaccinated persons. Fertil Steril 2023;119:392–400. https://doi.org/10.1016/j.fertnstert.2022.12.023.

[12] Qazi TB, Dkhar SA, Quansar R, Khan SMS. Impact of COVID-19 vaccination on menstrual cycle in women of reproductive age. Int J Gynaecol Obstet Off Organ Int Fed Gynaecol Obstet 2023. https://doi.org/10.1002/ijgo.14822.

[13] Edelman A, Boniface ER, Benhar E, Han L, Matteson KA, Favaro C, et al. Association Between Menstrual Cycle Length and Coronavirus Disease 2019 (COVID-19) Vaccination: A U.S. Cohort Obstet Gynecol 2022;139:481–9. https://doi.org/10.1097/AOG.0000000000004695.

[14] Darney BG, Boniface ER, Van Lamsweerde A, Han L, Matteson KA, Cameron S, et al. Impact of coronavirus disease 2019 (COVID-19) vaccination on menstrual bleeding quantity: An observational cohort study. BJOG Int J Obstet Gynaecol 2023;130:803–12. https://doi.org/10.1111/1471-0528.17471.

[15] Gibson EA, Li H, Fruh V, Gabra M, Asokan G, Jukic AMZ, et al. Covid-19 vaccination and menstrual cycle length in the Apple Women's Health Study. Npj Digit Med 2022;5:1–8. https://doi.org/10.1038/s41746-022-00711-9.

[16] Alvergne A, Boniface E, Darney B, Shea A, Weber K, Ventola C, et al. Associations Among Menstrual Cycle Length, Coronavirus Disease 2019 (COVID-19), and Vaccination. Obstet Gynecol 2024;143:83. https://doi.org/10.1097/AOG.0000000000005343.

[17] Pierson E, Althoff T, Leskovec J. Modeling Individual Cyclic Variation in Human Behavior. Proc Int World-Wide Web Conf Int WWW Conf 2018;2018:107–16. https://doi.org/10.1145/3178876.3186052.

[18] Li K, Urteaga I, Wiggins CH, Druet A, Shea A, Vitzthum VJ, et al. Characterizing physiological and symptomatic variation in menstrual cycles using self-tracked mobile-health data. Npj Digit Med 2020;3:1–13. https://doi.org/10.1038/s41746-020-0269-8.

[19] Rodriguez EM, Thomas D, Druet A, Vlajic-Wheeler M, Lane KJ, Mahalingaiah S. Identifying Women at Risk for Polycystic Ovary Syndrome Using a Mobile Health App: Virtual Tool Functionality Assessment. JMIR Form Res 2020;4:e15094.

[20] Liu B, Shi S, Wu Y, Thomas D, Symul L, Pierson E, et al. Predicting pregnancy using large-scale data from a women's health tracking mobile application. Proc Int World-Wide Web Conf Int WWW Conf 2019;2019:2999–3005. https://doi.org/10.1145/3308558.3313512.

[21] Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J Health Soc Behav 1983;24:385–96.

[22] Nguyen BT, Pang RD, Nelson AL, Pearson JT, Benhar Noccioli E, Reissner HR, et al. Detecting variations in ovulation and menstruation during the COVID-19 pandemic, using real-world mobile app data. PLoS One 2021;16:e0258314.

[23] Baena-García L, Aparicio VA, Molina-López A, Aranda P, Cámara-Roca L, Ocón-Hernández O. Premenstrual and menstrual changes reported after COVID-19 vaccination: The EVA project. 17455057221112237 Womens Health 2022;18. https://doi.org/10.1177/17455057221112237.

[24] Alvergne A, Kountourides G, Argentieri MA, Agyen L, Rogers N, Knight D, et al. A retrospective case-control study on menstrual cycle changes following COVID-19 vaccination and disease. iScience 2023;26. Doi: 10.1016/j.isci.2023.106401.

[25] Bentov Y, Beharier O, Moav-Zafrir A, Kabessa M, Godin M, Greenfield CS, et al. Ovarian follicular function is not altered by SARS–CoV-2 infection or BNT162b2 mRNA COVID-19 vaccination. Hum Reprod 2021;36:2506–13. https://doi.org/10.1093/humrep/deab182.

[26] Ljung R, Xu Y, Sundström A, Leach S, Hallberg E, Bygdell M, et al. Association between SARS-CoV-2 vaccination and healthcare contacts for menstrual disturbance and bleeding in women before and after menopause: nationwide, register based cohort study. BMJ 2023;381:e074778.

[27] Marcelino AC, Fim AB, da Cunha PP, Monteiro I, Darney BG, Bahamondes L. Association between COVID-19 and vaccination on menstrual cycle. Int J Gynecol Obstet 2024;164:571–7. https://doi.org/10.1002/ijgo.15200.

[28] Sharp GC, Fraser A, Sawyer G, Kountourides G, Easey KE, Ford G, et al. The COVID-19 pandemic and the menstrual cycle: research gaps and opportunities. Int J Epidemiol 2021;51:691–700. https://doi.org/10.1093/ije/dyab239.

[29] Toffol E, Koponen P, Luoto R, Partonen T. Pubertal timing, menstrual irregularity, and mental health: results of a population-based study. Arch Womens Ment Health 2014;17:127–35. https://doi.org/10.1007/s00737-013-0399-y.

[30] Agostinis C, Mangogna A, Bossi F, Ricci G, Kishore U, Bulla R. Uterine Immunity and Microbiota: A Shifting Paradigm. Front Immunol 2019;10:2387. https://doi.org/10.3389/fimmu.2019.02387.

[31] Salamonsen LA, Lathbury LJ. Endometrial leukocytes and menstruation. Hum Reprod Update 2000;6:16–27. https://doi.org/10.1093/humupd/6.1.16.

[32] Salamonsen A, Woolley DE. Menstruation: induction by matrix metalloproteinases and inflammatory cells. J Reprod Immunol 1999;44:1–27. https://doi.org/10.1016/S0165-0378(99)00002-9.

[33] Jones RL, Hannan NJ, Kaitu'u TJ, Zhang J, Salamonsen LA. Identification of chemokines important for leukocyte recruitment to the human endometrium at the times of embryo implantation and menstruation. J Clin Endocrinol Metab 2004;89:6155–67. https://doi.org/10.1210/jc.2004-0507.