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RESEARCH ARTICLE

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Impact of coronavirus disease 2019 (COVID-19) vaccination on menstrual bleeding quantity: An observational cohort study

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Abstract

Objective: To assess whether coronavirus disease 2019 (COVID-19) vaccination impacts menstrual bleeding quantity.
Design: Retrospective cohort.
Setting: Five global regions.
Population: Vaccinated and unvaccinated individuals with regular menstrual cycles using the digital fertility-awareness application Natural Cycles°.
Methods: We used prospectively collected menstrual cycle data, multivariable longitudinal Poisson generalised estimating equation (GEE) models and multivariable multinomial logistic regression models to calculate the adjusted difference between vaccination groups. All regression models were adjusted for confounding factors.
Main outcome measures: The mean number of heavy bleeding days (fewer, no change or more) and changes in bleeding quantity (less, no change or more) at three time points (first dose, second dose and post-exposure menses).
Results: We included 9555 individuals (7401 vaccinated and 2154 unvaccinated). About two-thirds of individuals reported no change in the number of heavy bleeding days, regardless of vaccination status. After adjusting for confounding factors, there were no significant differences in the number of heavy bleeding days by vaccination status. A larger proportion of vaccinated individuals experienced an increase in total bleeding quantity (34.5% unvaccinated, 38.4% vaccinated; adjusted difference 4.0%, 99.2% CI 0.7%–7.2%). This translates to an estimated 40 additional people per 1000 individuals with normal menstrual cycles who experience a greater total bleeding quantity following the first vaccine dose' suffice. Differences resolved in the cycle post-exposure.
Conclusions: A small increase in the probability of greater total bleeding quantity occurred following the first COVID-19 vaccine dose, which resolved in the cycle after the post-vaccination cycle. The total number of heavy bleeding days did not differ by vaccination status. Our findings can reassure the public that any changes are small and transient.

KEYWORDS
bleeding quantity, COVID-19 vaccination, menstrual cycle, menstruation

1 | INTRODUCTION

Menstrual cycles are considered a sign of overall health, a 'vital sign' according to the US National Institutes of Health (NIH) and medical societies.[1,2] Experiencing an unexpected change in menstruation can cause concern and even alarm. Public and media reports about a possible link between coronavirus disease 2019 (COVID-19) vaccination and menstrual disturbances have highlighted the lack of evidence to respond to such concerns.[3,4] Menstrual outcomes were not included in COVID-19 vaccine trials,[5–8] limiting the ability of the manufacturers, public health agencies and clinicians to respond to questions about the impact of the vaccine on menstrual health. There are biologically plausible ways in which a vaccine-elicited immune response could cause menstrual changes: cytokine production may transiently interfere with the hypothalamic–pituitary–ovarian axis, which drives the menstrual cycle,[9–12] and/or the activation of local immune cells in the endometrium could impact tissue repair at this site, potentially increasing menstrual bleeding.[13,14] However, individuals naturally experience inherent and normal variations in menstrual cycle duration and bleeding patterns,[15,16] making it challenging to isolate COVID-19 vaccination as a cause.

A growing body of evidence demonstrates that COVID-19 vaccination is associated with a small (less than 1 day) increase in cycle length but with no change in the duration of menses.[17–21] Other disturbances, such as bleeding quantity or menstrual symptoms, are less well studied. Retrospective studies have identified that changes in bleeding quantity may occur with vaccination, but these studies are not designed to determine whether vaccination is the main factor associated with these changes, as they lack a comparison group and use retrospective self-reported data.[22–24]

The objective of this study is to estimate the association of COVID-19 vaccination on menstrual bleeding quantity among individuals with normal menstrual cycles (i.e. cycle lengths of 24–38 days with menses of ≤8 days). We examine changes in the number of heavy bleeding days and in total bleeding quantity using data from a retrospective cohort study using prospectively collected menstrual cycle data and an unvaccinated comparison group.

2 | METHODS

We conducted a retrospective cohort study using vaccination and menstrual cycle data from individuals using the fertility awareness application Natural Cycles° (Natural Cycles°, New York, NY, USA). Cycle data ranged from October 2020 to May 2022; initial COVID-19 vaccinations were received between January 2021 and April 2022. Individuals using Natural Cycles° prospectively track their physiological data as a non-hormonal pregnancy prevention or planning method, with no concurrent use of any hormonal contraceptive method. Details on variables tracked by Natural Cycles° are reported elsewhere.[15] Users may consent to the use of their de-identified data for research upon registering in the app, and may remove their consent at any time. Individuals who had consented to the use of their data for research purposes (72% of all app users) were sent an in-app message between 7 May and 27 October 2021 explaining that their data could be used for studies about the COVID-19 vaccine and requesting their vaccination status (yes/no), and if yes, vaccine dates (day, month, year) and vaccine brand. To be eligible for study inclusion, individuals must have given consent for the use of their anonymised data and reported their vaccination information. We included individuals aged 18–45 years who were at least three cycles post-pregnancy or from the use of hormonal contraception, were not menopausal by self-report and who had a normal pre-vaccination menstrual cycle (average length of 24–38 days and menses duration of ≤8 days).

Every individual contributed menses data from a minimum of four consecutive cycles. For vaccinated individuals, we included the three menses immediately prior to vaccination, and at least the menses associated with the first vaccine dose (designated as 'first-dose menses'). If available, we also included menses data from the cycles associated with the second vaccine dose ('second-dose menses'), as well as the cycle and menses following vaccination ('post-exposure menses') to assess the potential resolution of changes. If an individual was vaccinated during their menses, that menses was designated as the 'first-dose menses' or 'second-dose menses', respectively; if they were vaccinated after the completion of their menses, the following menses was designated as the 'first- or second-dose menses'. For unvaccinated individuals (control group), we included menses data from four to six cycles from a similar time period, depending on the volume of data recorded, to serve as the notional pre-vaccination period, first-dose menses, second-dose menses and post-exposure menses. Individuals without data beyond menses associated with the first vaccination dose were excluded from the analyses of later time points.

Individuals using the Natural Cycles° application can choose whether or not they want to track their menstrual 'flow' or menstrual bleeding quantity; it is not a required variable and thus many individuals have no or incomplete data for menstrual bleeding quantity. We excluded individuals with more than 1 day of missing bleeding quantity data during the three pre-vaccination menses or during the three post-vaccination menses of interest. Thus, in total, individuals were allowed up to 4 days of missing bleeding quantity data to be included in the final data set: one during the pre-vaccination period and one for each of the post-vaccination menses analysed.

Our primary exposure was COVID-19 vaccination, as reported by individuals using the Natural Cycles° application: individuals recorded their vaccination date(s) or confirmed their unvaccinated status. We also classified vaccinated individuals by the mechanism of action of the vaccine that they received: mRNA (Pfizer-BioNTech and Moderna), adenovirus vector (Astrazeneca, Johnson & Johnson/Janssen, Covishield and Sputnik) and inactivated virus (Covaxin,

Sinopharm and Sinovac). One individual who received the protein subunit-mediated Novavax vaccine could not be classified with the other vaccine mechanisms, and was therefore grouped with individuals with unspecified vaccine brand.

Individuals report their daily bleeding quantity as 'spotting', 'light', 'medium' or 'heavy'. We assessed menstrual bleeding quantity in two ways: the number of heavy bleeding days and the total bleeding quantity, i.e. the ordinal sum of bleeding scores with spotting scored as 1, light scored as 2, medium scored as 3, and heavy scored as 4. For each post-vaccination menses (first-dose menses, second-dose menses and post-exposure menses), we calculated the within-individual change from the median of the three pre-vaccination menses. We then categorised the change in the number of heavy bleeding days as fewer days, no change or more days, and categorised the change in total bleeding quantity as less bleeding, no change or more bleeding. We chose to categorise these changes rather than analyse them as numeric because the units of the total bleeding quantity outcome are not interpretable, and no clinical meaning exists for a fractional unit for either outcome. We therefore had two outcomes measured at three time points each (the first-dose menses, second-dose menses and post-exposure menses): (1) the change in number of heavy bleeding days; and (2) the change in total bleeding quantity. We used a Bonferroni-adjusted significance level of 0.008 to account for multiple comparisons for these six total outcomes and thus report 99.2% confidence intervals. No relevant core outcome set exists or is in development to address menstrual changes, and therefore none were used for these analyses.

We included sociodemographic information collected by Natural Cycles° using in-app messages. Individuals using the app are required to input some demographic variables (such as age and country of residence) and other data are optional (such as body mass index, BMI), whereas additional sociodemographic data are supplied voluntarily as part of research requests and are thus not supplied by all app users. As such, some sociodemographic variables have a large volume of non-ignorable missing data (for details on 'missingness', see Table S1). As a result, we included 'missing' as a category in multivariable analyses. We classified age at the beginning of the first cycle: 18–24, 25–29, 30–34, 35–39 or 40–44 years. Individuals reported their race and ethnicity as Asian, black, Hispanic, Middle Eastern or North African, Native Hawaiian or Pacific Islander, or white. As a result of the small sample sizes, we collapsed race and ethnicity categories into a binary variable for multivariable analyses. We categorised global region as UK and Channel Islands, Europe, USA and Canada, Australia and New Zealand or other. Individuals from Sweden made up the majority of users in Europe (51%), whereas individuals from Brazil made up the majority of users in the 'other' category (62%). We grouped BMI into underweight, normal weight, overweight and obese categories, and collapsed the underweight and normal weight groups for multivariable analyses because of the small sample sizes. We also included parity (nulliparous vs parous), education (an undergraduate or higher degree vs less education) and relationship status (in a steady relationship or not).

The Oregon Health & Science University Institutional Review Board (study no. 00023204, approved 6 August 2021) and the UK's Reading Independent Ethics Committee (study no. 230721, approved 23 July 2021) approved the study protocol. De-identified data were used under a data use agreement with Natural Cycles°. No members of the public were directly involved in this analysis, although the research was developed in response to public reports of menstrual disturbances following COVID-19 vaccination.

2.1 | Analysis

We examined sociodemographic characteristics of the study sample, by vaccination status and overall. We compared the changes in the number of heavy bleeding days and total bleeding quantity for the first-dose, second-dose and post-exposure menses using Pearson's chi-square test, adjusting the *p*-values to reflect our significance level of 0.008. We then created histograms of the raw, uncategorised change in number of heavy bleeding days and total bleeding quantity by vaccination group and tabulated the change in heavy bleeding days. We developed multivariable longitudinal Poisson general estimating equation (GEE) models for all three heavy bleeding day outcomes. GEE models included an offset for duration of menses, and an interaction between time (pre-/post-vaccination) and vaccination status to determine the effect of vaccination, i.e. the adjusted difference in the change in number of heavy bleeding days between vaccination groups. We then plotted the predicted number of heavy bleeding days before and after vaccination for both groups. For the three total bleeding quantity outcomes, we developed multivariable multinomial logistic regression models using no change as the base outcome, then calculated the adjusted predicted probability of each outcome (less bleeding, no change or more bleeding) for each vaccination group and the adjusted difference between groups. All regression models were adjusted for age, race and ethnicity, parity, BMI, education, relationship status and global region. Models for the second-dose menses and post-exposure menses were also adjusted for time between the first and second vaccine doses.

We conducted nine sensitivity analyses to confirm our results. First, we compared the sociodemographic characteristics of individuals included in the study with those who were excluded for missing bleeding quantity data. Second, we compared the change in the number of heavy bleeding days and total bleeding quantity for the first- and second-dose menses by vaccine mechanism of action (mRNA, adenovirus vector or inactivated virus), by type of mRNA vaccine and by timing of vaccination (during menses vs after menses). Third, although the data did not meet the missing-at-random assumption required for imputation techniques, we completed 500 iterations of imputation

and weighting with covariate balancing propensity scores using bootstrapped standard errors to confirm that our findings were not biased by missing data or by differences in the characteristics of the vaccination groups. Fourth, we excluded any individuals with polycystic ovary syndrome (PCOS), endometriosis or thyroid disorder (n = 454). Fifth, we excluded any individual who used emergency contraception during the study period (n = 472). Sixth, we excluded individuals with any pre-vaccination cycles outside the normal length of 24–38 days (n = 1420). Seventh, we excluded individuals who had received both vaccine doses since their previous menses (n = 422). Eighth, we excluded individuals with any missing bleeding quantity data during the pre-vaccination menses, or first-dose, second-dose or post-exposure menses (n = 4090 for first-dose menses, n = 3050 for second-dose menses and n = 3020 for post-exposure menses). Finally, we included individuals with pre-vaccination menses durations of 9 or 10 days (who had previously been excluded; n = 93). We used Stata 15.1 (StataCorp LLC, College Station, TX, USA) for all analyses.

3 | RESULTS

Out of 42 095 users, 9555 individuals (7401 vaccinated and 2154 unvaccinated) representing 229 320 menses met the inclusion criteria (Figure 1). The overall cohort was under the age of 35 years (80.4%), nulliparous (77.4%), had at least a college degree (67.8%) and was in a steady relationship (69.7%) (Table 1). Most individuals were located in the UK (32.4%), Europe (31.2%) or the USA and Canada (30.1%). Among vaccinated individuals, the majority received mRNA vaccines: 66.7% Pfizer-BioNTech and 17.9% Moderna. Vaccinated individuals were more likely to have at least an undergraduate degree (71.4%, compared with 55.3% among unvaccinated individuals). The characteristics of individuals included in the study were similar to those excluded for missing bleeding quantity data (Table S2); the majority of participants excluded for missing bleeding quantity data were excluded for missing data in the pre-vaccination period (8851 out of 10 325 excluded, 86%; data not shown), and thus a baseline could not be established. The sample size for the vaccinated group was considerably smaller for the second-dose menses (n = 5288; Figure 1) for a variety of reasons: some individuals (2.0% of vaccinated) received the single-dose Johnson & Johnson/Janssen vaccine; some did not receive or did not record information about their second dose (22.6%); and some (3.1%) received their second dose after their last cycle of tracked data.

The unadjusted change in the number of heavy bleeding days reported did not differ between the vaccinated and unvaccinated control group for any of the post-vaccination menses (Figure S1; Tables 2 and S3). Regardless of vaccination status (actual or notional) for the first-dose menses, second-dose menses and post-exposure menses, approximately 60% of individuals experienced no change, whereas approximately 20% experienced fewer heavy bleeding days and 20% experienced more heavy bleeding days. After adjustment in multivariable models, there were still no significant differences by vaccination status during the first- and second-dose menses (Figure 2).

When examining the change in total bleeding quantity pre- and post-vaccination (less bleeding, no change or more bleeding), we found differences between the vaccinated and unvaccinated control groups (Figure S2; Table 2). Vaccinated individuals were more likely to report more bleeding overall than their unvaccinated counterparts during both the first-dose menses (38.3% vs 34.8%, p = 0.027) and the second-dose menses (39.8% vs 35.5%, $p < 0.001$). The proportion of individuals who experienced no change in total bleeding quantity was roughly 18% for both vaccinated and unvaccinated groups at both time points. The differences between groups were no longer statistically significant in the post-exposure cycle. After adjustment in multivariable models, the predicted probability of experiencing more total bleeding quantity was higher in the vaccinated group compared with the unvaccinated control group: 4.0% higher (99.2% CI 0.7%–7.2%) for the first-dose menses and 3.8% higher (99.2% CI 0.2%–7.3%) for the second-dose menses (Table S4). This translates to approximately 40 additional people per 1000 individuals with normal cycles who will experience a greater total bleeding quantity as a result of vaccination. Again, this difference was no longer significant in the post-exposure menses: 2.8% higher for vaccinated individuals (99.2% CI −0.8%–6.3%; data not shown).

We found no major differences in the change in number of heavy bleeding days or the total bleeding quantity when comparing individuals who received an mRNA vaccine with individuals who received an adenovirus vector vaccine during either the first- or second-dose menses (Figures S3 and S4), and there were no differences in any bleeding outcome between the two mRNA vaccines (data not shown). The number of individuals who received an inactivated virus (n = 44 for first-dose menses and n = 31 for second-dose menses) was too small to draw conclusions. We also found no meaningful differences in our outcomes when examined by timing of vaccination (during menses vs after completion of menses; Figures S5 and S6).

The sensitivity analysis imputing missing data and weighting the sample to balance vaccination groups on all sociodemographic variables did not alter our unadjusted or adjusted results in a meaningful way (Table S5). Nor did the sensitivity analyses excluding individuals: (1) with PCOS, endometriosis or thyroid disorders; (2) who used emergency contraception; (3) with pre-vaccination cycle lengths outside the normal range; (4) who received two vaccine doses prior to a single menses; or (5) with any missing bleeding quantity data. The final sensitivity analysis including individuals with slightly longer durations of pre-vaccination menses (9–10 days) also did not change the results.

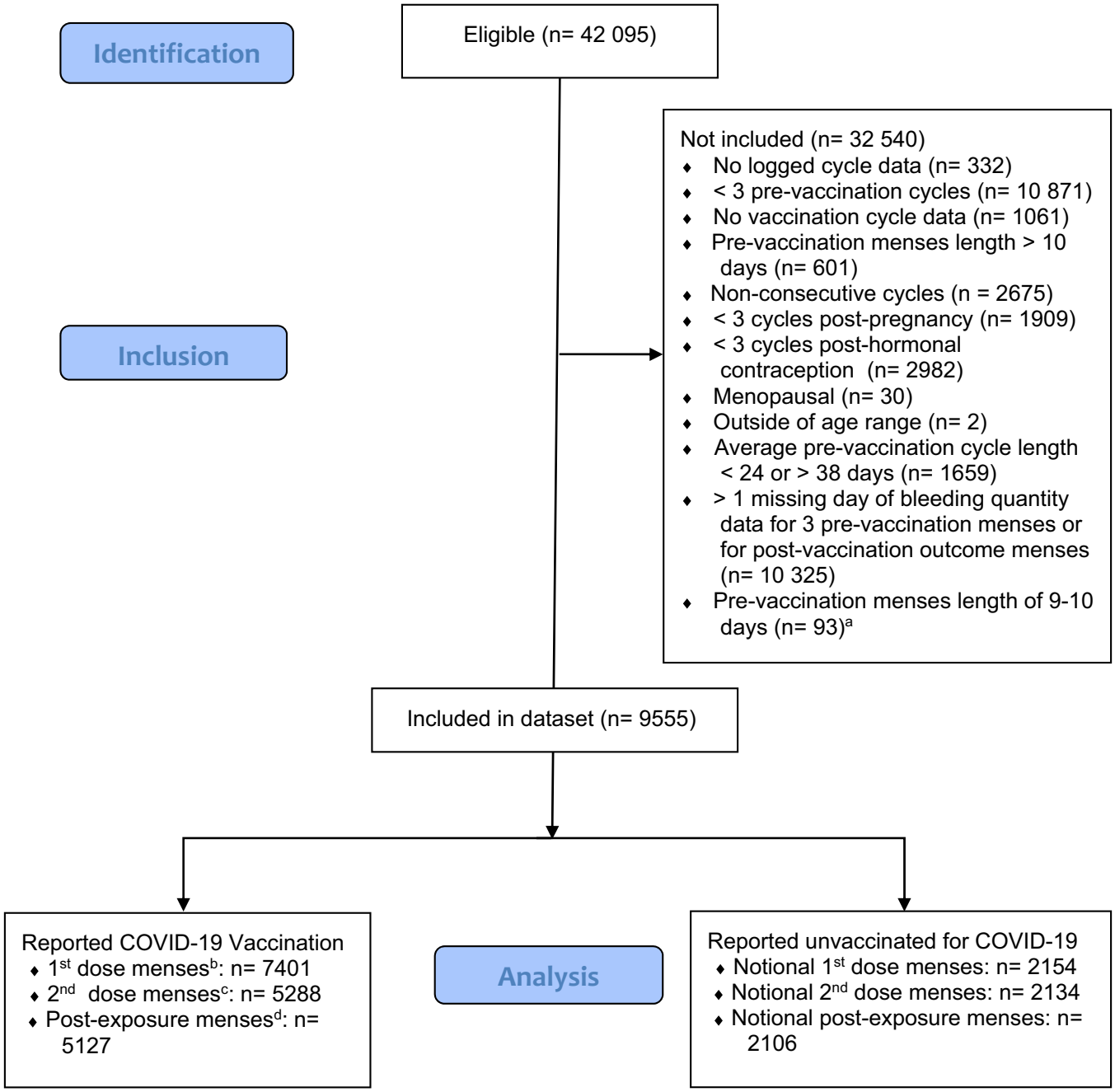

FIGURE 1 STROBE flow diagram. [a]Individuals with pre-vaccination menses of 9–10 days in duration were excluded from all primary analyses but were then later included for a sensitivity analysis. [b]1st dose menses indicates the menses during or immediately following receipt of the first COVID-19 vaccine dose. [c]2nd dose menses indicates the menses during or immediately following receipt of the second COVID-19 vaccine dose. [d]Post-exposure menses indicates the menses of the cycle following the second-dose menses

4 | DISCUSSION

4.1 | Main findings

We used prospectively tracked menstrual cycle bleeding data to assess changes in the number of heavy bleeding days and total bleeding quantity (menstrual 'flow') in individuals who received COVID-19 vaccination, as compared with an unvaccinated control group. Overall, about two-thirds of individuals reported no change in the number of heavy bleeding days tracked, regardless of vaccination status. After adjusting for confounding factors, we found no significant differences in the number of heavy bleeding days by vaccination status. However, a larger proportion of vaccinated individuals experienced an increase in total bleeding quantity. Of the unvaccinated individuals, 34.5% experienced a greater bleeding quantity following the first vaccine dose, compared with 38.4% among the vaccinated individuals: representing an adjusted increase of 4.0% (99.2% CI 0.7%–7.2%) in the probability of a greater bleeding quantity following the first vaccine

TABLE 1 Study participant characteristics (n = 9555), by vaccination status

Characteristic	Unvaccinated	Vaccinated	Overall	*p*
n	2154	7401	9555	
Age group (years)				
18–24	423 (19.6)	745 (10.1)	1168 (12.2)	<0.001
25–29	724 (33.6)	2571 (34.7)	3295 (34.5)	
30–34	611 (28.4)	2609 (35.3)	3220 (33.7)	
35–39	296 (13.7)	1109 (15.0)	1405 (14.7)	
40–44	100 (4.6)	367 (5.0)	467 (4.9)	
BMI category				
Underweight	52 (2.4)	196 (2.7)	248 (2.6)	0.004
Normal weight	951 (44.2)	3468 (46.9)	4419 (46.3)	
Overweight	277 (12.9)	993 (13.4)	1270 (13.3)	
Obese	123 (5.7)	486 (6.6)	609 (6.4)	
No data	751 (34.9)	2258 (30.5)	3009 (31.5)	
Race and ethnicity				
Asian	7 (0.3)	73 (1.0)	80 (0.8)	<0.001
Black	46 (2.1)	54 (0.7)	100 (1.1)	
Hispanic	47 (2.2)	145 (2.0)	192 (2.0)	
Middle Eastern or North African	7 (0.3)	19 (0.3)	26 (0.3)	
Native Hawaiian or Pacific Islander	3 (0.1)	15 (0.2)	18 (0.2)	
Non-Hispanic white	829 (38.5)	2926 (39.5)	3755 (39.3)	
No data	1215 (56.4)	4169 (56.3)	5384 (56.4)	
Parity				
Nulliparous	1513 (70.2)	5882 (79.5)	7395 (77.4)	<0.001
Parous	332 (15.4)	824 (11.1)	1156 (12.1)	
No data	309 (14.4)	695 (9.4)	1004 (10.5)	
Education				
Less than college degree	548 (25.4)	1093 (14.8)	1641 (17.2)	<0.001
Completed college degree	1190 (55.3)	5284 (71.4)	6474 (67.8)	
No data	416 (19.3)	1024 (13.8)	1440 (15.1)	
Relationship status				
Not in steady relationship	313 (14.5)	962 (13.0)	1275 (13.3)	<0.001
In steady relationship	1423 (66.1)	5236 (70.8)	6659 (69.7)	
No data	418 (19.4)	1203 (16.3)	1621 (17.0)	
Geographic region				
UK	521 (24.2)	2574 (34.8)	3095 (32.4)	<0.001
Europe	519 (24.1)	2466 (33.3)	2985 (31.2)	
USA and Canada	931 (43.2)	1941 (26.2)	2872 (30.1)	
Australia and New Zealand	152 (7.1)	222 (3.0)	374 (3.9)	
Other	31 (1.4)	198 (2.7)	229 (2.4)	
Vaccine type				
Pfizer	0 (0)	4938 (66.7)	4938 (51.7)	N/A[a]
Moderna	0 (0)	1322 (17.9)	1322 (13.8)	
Johnson & Johnson	0 (0)	151 (2.0)	151 (1.6)	
Astrazeneca/adenovirus vector	0 (0)	650 (8.8)	650 (6.8)	
Whole/inactivated virus	0 (0)	44 (0.6)	44 (0.5)	
Unspecified[b]	0 (0)	296 (4.0)	296 (3.1)	
Unvaccinated	2154 (100)	0 (0)	2154 (22.5)	

Note: *p*-values represent differences by vaccination status using Pearson's chi-square test.

[a]No statistical test performed.

[b]Unspecified group contains one individual who received the protein subunit-based Novavax vaccine.

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TABLE 2 Changes in number of heavy bleeding days from the median of three pre-vaccination menses to the first-dose menses, second-dose menses and post-exposure menses, by vaccination status

Outcome	Difference from pre-vaccination to:	Category	Unvaccinated	Vaccinated	p[a]
Number of heavy bleeding days	First-dose menses[b]	*n*	2154	7401	1.000
		Fewer heavy days	420 (19.5)	1371 (18.5)	
		No change	1304 (60.5)	4630 (62.6)	
		More heavy days	430 (20.0)	1400 (18.9)	
	Second-dose menses[c]	*n*	2134	5288	1.000
		Fewer heavy days	390 (18.3)	916 (17.3)	
		No change	1299 (60.9)	3270 (61.8)	
		More heavy days	445 (20.9)	1102 (20.8)	
	Post-exposure menses[d]	*n*	2106	5127	1.000
		Fewer heavy days	400 (19.0)	908 (17.7)	
		No change	1282 (60.9)	3197 (62.4)	
		More heavy days	424 (20.1)	1022 (19.9)	
Total bleeding quantity	First-dose menses[b]	*n*	2154	7401	0.027
		Less quantity	1013 (47.0)	3208 (43.4)	
		No change	392 (18.2)	1356 (18.3)	
		More quantity	749 (34.8)	2837 (38.3)	
	Second-dose menses[c]	*n*	2134	5288	<0.001
		Less quantity	983 (46.1)	2161 (40.9)	
		No change	393 (18.4)	1020 (19.3)	
		More quantity	758 (35.5)	2107 (39.8)	
	Post-exposure menses[d]	*n*	2106	5127	0.157
		Less quantity	973 (46.2)	2192 (42.8)	
		No change	369 (17.5)	969 (18.9)	
		More quantity	764 (36.3)	1966 (38.4)	

Note: Data are *n* (%).

[a]*p*-values are adjusted to account for six comparisons; values below 0.05 represent statistically significant differences.

[b]First-dose menses indicates the menses during or immediately following receipt of the first COVID-19 vaccine dose.

[c]Second-dose menses indicates the menses during or immediately following receipt of the second COVID-19 vaccine dose.

[d]Post-exposure menses indicates the menses of the cycle following the second-dose menses.

dose in multivariable analyses. For the second dose, 35.9% of the unvaccinated individuals compared with 39.7% of the vaccinated individuals experienced a greater bleeding quantity following vaccination: a 3.8% higher (99.2% CI 0.2%–7.3%) adjusted probability for the second-dose menses in multivariable analyses. This translates into an estimated additional 40 people per 1000 individuals with normal cycles (the difference between 342/1000 unvaccinated and 384/1000 vaccinated) who will experience a greater total bleeding quantity with the first vaccine dose as a result of vaccination. The bleeding quantity recorded by vaccinated and unvaccinated groups no longer differed by the time of the post-exposure cycle.

4.2 | Strengths and limitations

The strengths of this study include a large global sample with geographic diversity and prospectively tracked bleeding data: users tracked bleeding quantity each day (vs the retrospective self-reported perceptions of changes in bleeding that were previously reported). Our outcome measures used counts of heavy bleeding days and an ordinal measure of total bleeding quantity, which are less subjective than retrospective reports of 'more' or 'less' bleeding (as previously reported). We also include an unvaccinated control group that allows us to isolate the relationship of vaccination and menstrual bleeding quantity.

The limitations of our study include a lack of complete sociodemographic data to fully understand the racial/ethnic and gender-identity diversity in our sample. Our study population is also of lower BMI and higher education than is found in general populations. Further, our sample is global, but the USA, UK and Europe are over-represented. To isolate the association of the COVID-19 vaccine and menstrual bleeding changes, our study population includes individuals with normal menstrual characteristics pre-vaccine. Future studies should focus on key subgroups, including contraceptive users, menopausal individuals, people using gender-affirming hormonal therapy and those with non-clinically normal cycles

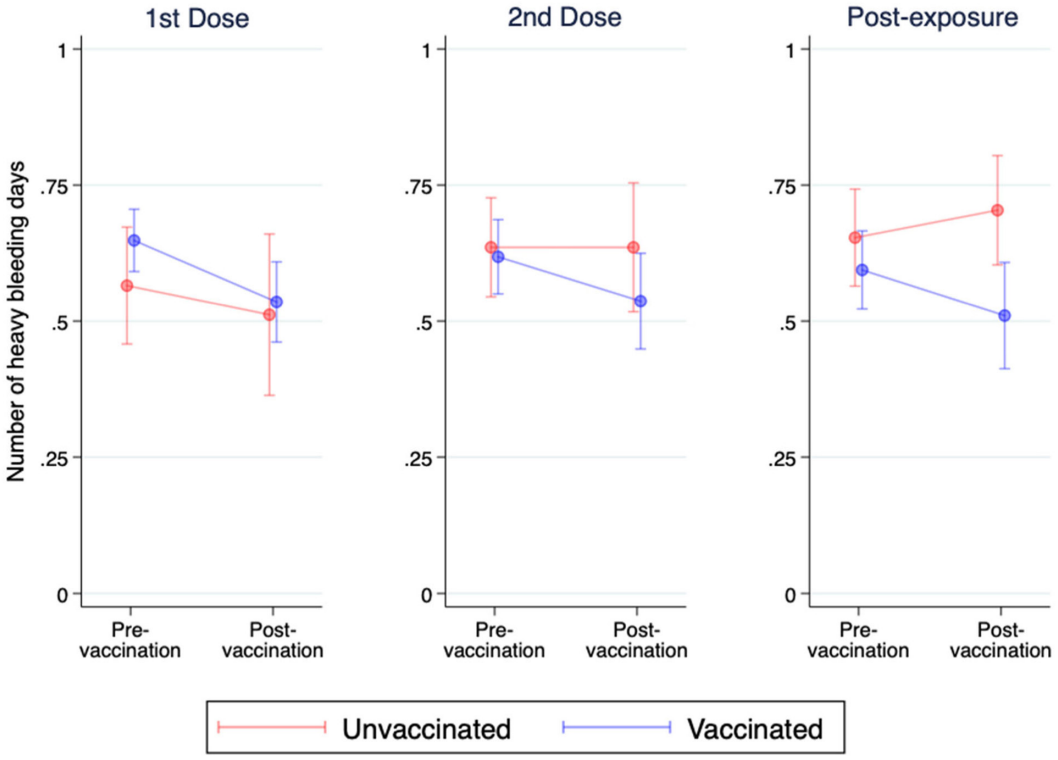

FIGURE 2 Predicted number of heavy bleeding days for the median of three pre-vaccination menses and the first-dose (left), second-dose (centre) and post-exposure (right) menses. Estimates are from longitudinal GEE models with an offset for menses duration, an interaction between vaccination status and pre-/post-vaccination timing, and adjusted for age, BMI, educational attainment, parity, relationship status, global region and time between doses (second dose and post-exposure only). Unvaccinated users are shown in red, vaccinated users are shown in blue and error bars represent the 99.2% CIs. First- and second-dose menses indicate the menses during or immediately following receipt of the first and second vaccine dose, respectively. Post-exposure menses indicates the menses of the cycle following the second-dose menses

or menses at baseline. Our sample may also be subject to selection bias if individuals who experienced bleeding changes were more likely to provide information on their vaccination status than individuals with no changes. However, the existence of a sizable unvaccinated cohort suggests that our study population is willing to contribute to menstrual cycle research, regardless of the potential impact of the vaccine.

We recognise that more questions remain regarding different aspects of menstrual bleeding, but we are limited by the variables collected within the tracking application. On the one hand, the app utilises the gold standard for real-time menstrual cycle tracking and identifying heavy bleeding, which is based on an individual's self-assessment.[16] On the other hand, the units of our ordinal measure of total bleeding quantity have no real numerical interpretation and are not necessarily comparable with the self-reported outcomes of 'more' or 'less' bleeding reported previously. However, this outcome enables us to note whether a change in bleeding quantity or 'menstrual flow' has occurred or not in relation to vaccine exposure, which is a critical knowledge gap and an important outcome of documented interest to the public. Additionally, individuals are not required to track bleeding quantity data in the app, which limited our sample size. We examined the 'missingness' of the data between included and excluded individuals and found no changes in our results. We have no measure of COVID-19 disease, which may impact bleeding patterns,[25–27] and vaccination status is self-reported and not verified, although we do have dates and vaccine brands, and the self-report of vaccine status and timing has been shown to be a good surrogate for clinical data.[28]

4.3 | Interpretation in light of other evidence

A study using the retrospective recall of changes in bleeding quantity following COVID-19 vaccination and no comparison group found that 42% of those reporting regular menstrual cycles prior to vaccination reported experiencing heavier bleeding following vaccination, relative to their bleeding before vaccination.[24] Another study also using retrospective recall of menstrual changes but including an unvaccinated pre-/post-comparison group (surveyed individuals were asked about outcomes before and after vaccination) found that menstrual disturbances did follow vaccination. However, this study also identified that more than one-third of their total participants (vaccinated and control) experienced disturbances even prior to vaccination or for controls over a similar time period.[22] This finding is consistent with our results that over one-third of individuals, regardless of vaccination status, had a greater bleeding quantity. A retrospective study without an unvaccinated comparison group reported that menstrual changes

were more frequent following the second dose than the first dose;[23] another found no differences in menstrual flow.[18]

Our study highlights the importance of an unvaccinated comparator group: bleeding variability was high, regardless of receiving a vaccine. With the inclusion of an unvaccinated control group and accounting for confounding factors, we found no differences in the number of heavy bleeding days by vaccination status, but we did find that a small but significantly larger proportion of vaccinated individuals experienced a greater total bleeding quantity in the cycle of their first- and second-vaccine doses, compared with unvaccinated individuals. This difference was resolved by the time of the cycle following exposure to COVID-19 vaccination. Previous research using the Natural Cycles° app data found a small increase in cycle length and no difference in menses length associated with COVID-19 vaccination.[20,21] This study adds to the growing body of evidence of time-limited menstrual disturbances associated with COVID-19 vaccination at a population level of reproductive-aged individuals with previously regular menstrual cycles.

The impact of COVID-19 disease on menstrual disturbances in unvaccinated individuals is less well defined, which is the relevant counterfactual for those hesitant to vaccinate themselves or family members because of concerns about menstrual disturbances. Changes in menstrual cycles were reported by 16% of a cohort who had COVID-19 disease prior to the availability of vaccines (2020), compared with those who had COVID-19 disease in 2020 and reported no change in menstrual cycles. In two studies carried out prior to the availability of vaccines, 16% or 28% of menstruating people with COVID-19 disease reported menstrual changes, with changes associated with more severe disease.[25, 26] In a cohort of people with long COVID, more than 30% of menstruating participants reported some kind of menstrual disturbance.[27] Those who reported menstrual changes also reported more severe COVID-19 symptoms.[25] Finally, COVID-19 disease carries serious morbidity and mortality risks to unvaccinated individuals, which must be considered when discussing concerns about menstrual disturbances and vaccination.

The development of core outcome sets and regulatory support to include these core outcomes in future vaccine development studies would aid in providing foundational information on a critical patient-reported outcome.

In conclusion, we find a small but statistically significant increase (4.0%) in the adjusted probability of a greater total bleeding quantity following the first COVID-19 vaccine dose, compared with an unvaccinated comparison group. This difference translates into an estimated additional 40 people per 1000 individuals with normal cycles experiencing a greater bleeding quantity post-vaccine that is resolved by the time of the post-vaccination cycle. We found no difference in the total number of heavy bleeding days by vaccination status. Our findings can inform patients and help providers in counselling about what to expect following a COVID-19 vaccination.

AUTHOR CONTRIBUTIONS

AE conceived of the study and secured funding. AE, ERB, and BGD developed the analysis plan and conducted analyses. BGD and ERB drafted the manuscript. AE, EB, JTP, AVL, KAM, SC, LH, JA, and VM provided substantive intellectual contributions to the manuscript.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from NaturalCycles and were used under a data use agreement. Restrictions apply to the availability of these data.

ETHICS APPROVAL

The OHSU Institutional Review Board approved the protocol. De-identified data were used under a data-use agreement with Natural Cycles° and from the Reading Independent Ethics Committee.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Additional supporting information can be found online in the Supporting Information section at the end of this article.

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