Pre-specified analysis plan reviewed and confirmed
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Study purpose statement: The purpose of this study is to determine the potential impact of the COVID-19 vaccine on menstrual cycle length and menses length by comparing these outcomes in vaccinated and unvaccinated Natural Cycles app users during the cycles when the first and second doses of vaccine were received, as well as the cycle following the second dose.

Primary Outcome	1. Mean within-user difference in cycle length between 3 pre-vaccination cycle average and 1st dose vaccination cycle
Secondary Outcomes	2. Mean within-user difference in cycle length between 3 pre-vaccination cycle average and 2nd dose vaccination cycle 3. Proportion of users with an 8 day or larger change in cycle length during 1st dose vaccination cycle 4. Proportion of users with an 8 day or larger change in cycle length during 2nd dose vaccination cycle 5. Mean within-user difference in menses length between 3 pre-vaccination cycle average and 1st dose vaccination cycle 6. Mean within-user difference in menses length between 3 pre-vaccination cycle average and 2nd dose vaccination cycle 7. Mean within-user difference in cycle length between 3 pre-vaccination cycle average and cycle following the 2nd dose vaccination cycle (“post-vaccine cycle” or cycle without vaccine exposure) • We will apply a Bonferroni correction to the 7 primary and secondary analyses in order to adjust for multiple comparisons, lowering the alpha level to 0.007 for each analysis.
Independent variable	• COVID-19 vaccination status: vaccinated (received at least first dose) versus unvaccinated o For unvaccinated users, we will designate the first three cycles of consecutive data (median start date January 2021) as the “pre-vaccination” period. We will designate cycles 4 and 5 as the “1st dose” and “2nd dose” vaccination cycles, respectively (equivalent to all users in the dataset), and cycle 6 as the “post-vaccine cycle”
Inclusion criteria	• Ages 18-45 • Logged 3 consecutive pre-vaccination cycles and at least one full cycle following 1st dose vaccination, or at least 4 consecutive cycles in 2021 if unvaccinated. All consecutive logged cycles from 3 pre-vaccination cycles through the “clean cycle” following the 2nd dose vaccination cycle will be included. • Consecutive logged cycles from 3 pre-vaccination cycles to at least 2nd dose vaccination cycle, if 2nd dose is logged. Unvaccinated users without five consecutive logged cycles will be excluded from 2nd dose analyses. • Logged vaccination data or verified unvaccinated status • At least 3 cycles post-pregnancy or use of hormonal birth control • Average cycle length of 24-38 days in 3 cycles pre-vaccination/ first 3 cycles if unvaccinated • Not menopausal (individuals that note menopausal symptoms)

Power Calculation	• Assuming a sample size at least as large as the included US cohort (2,403 vaccinated/1,556 unvaccinated users) and a significance level of 0.007, we will have more than 99% power to detect a 1-day difference in cycle length change and a 0.5-day difference in menses length change, by vaccination status, with a two-sided two-sample t-test. We will also have more than 80% power to detect a 2.7% difference in the percentage of users experiencing an 8 day or larger change in cycle length, by vaccination status, assuming 4.3% in unvaccinated users (previously observed in the US cohort) with Pearson's chi-squared test.

Planned analyses	• Describe sample characteristics by vaccination status
	• Two-sided two-sample t-tests of cycle and menses length changes, by vaccination status
	• Pearson's chi-squared test of proportion of users with an 8 day or larger change in cycle length, by vaccination status
	• Longitudinal mixed effects models with random effects at the user level
	  ○ Outcome: cycle length or menses length
	  ○ Time variable: pre-/post-vaccination
	  ○ Independent variable: vaccination status
	  ○ Interaction between pre-/post-vaccination and vaccination status
	  ○ Potential confounders: age, race/ethnicity, parity, BMI, education, relationship status, country or global region, time between 1st and 2nd dose (outcomes 2, 6 & 7)

Sensitivity analyses	• Exclude users with diagnoses of PCOS, endometriosis, or thyroid disorders • Exclude users with any recorded emergency contraception use during study cycles • Exclude users with any cycles outside of 24-38-day range in 3 cycles pre-vaccination • Stratify by number of doses obtained in a single cycle (one vs. two) • Stratify by country or global region, depending on data distribution • Balance distributions of confounders between vaccination groups using covariate balancing propensity score (CBPS) weighting
Anticipated problems and alternative strategies	• If we do not have enough confirmed unvaccinated users to serve as an adequate control group, we will restrict analysis to vaccinated users, looking at the average change in cycle or menses length using two-sided one-sample t-tests (null hypothesis = no change in length) and mixed effects models without vaccination status or interaction terms. • We will use multiple imputation for any confounders with >5% missing data that meet the Missing at Random (MAR) assumption. If missingness is low, we will use complete case analysis (with a sensitivity analysis). If missingness is non-ignorable we will model missingness as a category and conduct a sensitivity analysis using imputation followed by CBPS weighting with bootstrapped standard errors. • If too few users have logged 3 cycles pre-vaccination, we will use 2 cycles instead. • We anticipate having limited cycle data following the 2nd dose vaccination cycle, but if possible, we will replace outcome 7 with the difference in cycle length change for 2 or even 3 cycles post-2nd dose vaccination cycle. • If many users are missing the "post-vaccine" cycle following the 2nd dose, we will perform Outcome 7 analyses with

	the understanding that we may not be powered to detect differences by vaccination status, and will assess potential differences between the samples for the 2nd dose vaccination cycle and for the "clean cycle".