Original Research

Longitudinal Epigenetic Aging in Pregnancy and Associations With Adverse Outcomes

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OBJECTIVE: To understand the relationship between pregnancy and epigenetic aging estimated by DNA methylation "clocks," which offers a molecular measure of biologic aging.

METHODS: This was a prospective cohort study of nulliparous women (age 18–50 years) seeking obstetric (pregnant 10–14 weeks) or gynecologic (nonpregnant) care in 2020–2021. Blood was collected at enrollment (time 1) and postpartum day 1 (pregnant, time 2) or 7 months later (nonpregnant, time 2). Epigenetic age was measured with 11 established clocks from Illumina EPIC 2 arrays. Within-person changes in epigenetic age were compared with mixed-effects linear regression models adjusted for confounders and interval duration (days). Results were scaled per 200-day interval. *P* values were corrected for multiple testing. Multivariable logistic regression explored associations between first-trimester epigenetic age and a composite of potentially immune-mediated complications (hypertensive disorders, gestational diabetes mellitus, preterm birth before 37 weeks of gestation, and small-for-gestational-age birth weight) adjusted for age and body mass index (BMI) higher than 30 at time 1.

RESULTS: In total, 75 women enrolled; 45 (60.0%) were pregnant, and 61 (81.3%) completed the study. Pregnant women exhibited significant within-person epigenetic age acceleration compared with nonpregnant women in six clocks (Hannum, PhenoAge, GrimAge, GrimAge2, Stem Cell Division, DunedinPACE). Additional epigenetic age acceleration per 200 days in the pregnant cohort ranged from 1.58 years (Hannum, 95% CI, 0.45–2.72, $P=.01$) to 5.28 years (PhenoAge, 95% CI, 2.97–7.61, $P<.01$). Each additional year of first-trimester GrimAge2 increased odds of the composite of pregnancy complications by 36% (adjusted odds ratio [aOR] 1.36, 95% CI, 1.01–1.84), while chronologic age (in continuous years) showed no association (aOR 1.00, 95% CI, 0.83–1.21).

CONCLUSION: Pregnancy accelerated within-person epigenetic aging by up to 5.3 years. Older first-trimester GrimAge2, but not chronologic age, was associated with a composite of pregnancy complications. These findings suggest that gestation may influence biologic aging and support further investigation into epigenetic age as a potential marker of pregnancy health.

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The United States is facing a maternal health crisis, with mortality rates far exceeding those in other high-income countries.[1,2] This is exacerbated by a sharp rise in pregnancies occurring in women aged 40 years or older, a demographic in which there has been a 194% increase in birth rates since 1989.[3] Although older age is a well-established risk factor for pregnancy complications, chronologic age alone

See related editorial on page 781.

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does not reliably predict adverse outcomes; some older women can have uncomplicated pregnancies, and younger women can have unexpected complications.[4,5] This raises the question of whether biologic, rather than chronologic, age better predicts pregnancy risk.[6,7]

Biologic aging, driven by cumulative molecular changes that increase disease vulnerability, is strongly linked to morbidity in nonpregnant adults.[7–12] One key marker of biologic aging is epigenetic aging, which measures alterations in DNA methylation patterns at sites related to the aging process.[8] These epigenetic modifications have been leveraged to compute epigenetic clocks. Three generations of clocks have been developed with distinct CpG sites and regression models to calculate epigenetic age: The first-generation clocks predict chronologic age from epigenetic modifications (eg, Horvath, Hannum), yielding results in years; the second-generation clocks use the epigenetic variations to predict mortality (eg, GrimAge) or morbidity (eg, PhenoAge), also usually yielding results in years but can vary dependent on what is being measured; and the third-generation clocks predict rates of aging (eg, DunedinPACE), yielding results in values relative to one (ie, faster vs slower pace of aging).[13–15] Although many clocks are being developed in the literature, this article focuses on 13 commonly used clocks that have been validated in other larger epidemiologic cohorts as they relate to mortality, morbidity, and lifestyle factors.

Recent studies suggest that pregnancy accelerates epigenetic aging, with potential deceleration several months postpartum.[6,16,17] These epigenetic aging changes, however, have not been directly compared with those in nonpregnant, nulliparous, reproductive-aged women over similar time periods. Because epigenetic clocks were developed in older, nonpregnant populations, understanding their relevance in pregnancy compared with a similarly aged referent group is critical. Moreover, although limited data suggest a link between accelerated epigenetic aging and pregnancy complications such as preterm birth, it is important that we gain a more comprehensive understanding of the aging process in pregnancy across all available epigenetic clocks.[18] Therefore, we aimed to quantify maternal epigenetic aging in nulliparous pregnant and nonpregnant women over similar time frames and to explore associations with pregnancy complications. We hypothesized that pregnancy, compared with the nonpregnant state, would accelerate epigenetic aging.

METHODS

We conducted a prospective cohort study of nulliparous pregnant and nonpregnant women aged 18–50 years who were seeking outpatient obstetric or well-woman gynecologic care at a single quaternary care institution between January 2020 and April 2021. We used convenience sampling to screen all potentially eligible patients from the clinics; patients were recruited from both obstetrics–gynecology specialist and maternal–fetal medicine subspecialty clinics. Pregnant women were recruited between 10 and 14 weeks of gestation; this window was selected to minimize loss to follow-up from early miscarriages and to ensure that all participants were in the first trimester at the time of the first blood sample. Women were eligible if they had no prior pregnancy continuing beyond 13 weeks of gestation and if they were fluent in English. Exclusion criteria, selected on the basis of known or theoretical associations with white blood cell composition or biomarkers of aging,[19–21] were current tobacco or cannabis use; history of solid-organ or blood malignancy; current anticoagulation with heparin or a derivative; signs of or treatment for an infection, illness, or injury (eg, active respiratory illness, use of antibiotics, or broken bone); surgical procedure in the preceding 4 weeks; multiple gestation; use of egg donation; known lethal fetal anomaly at enrollment; and plan for pregnancy termination. For nonpregnant women, an additional exclusion criterion was if they were planning to conceive in the next year. Our study was approved by the Stanford University IRB, and all participants provided written informed consent. STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines were followed.

The primary outcome was within-person change in epigenetic age over the study period, measured from maternal whole-blood DNA. Blood was collected at enrollment (time 1) for all participants and again either on postpartum day 1 for the pregnant cohort or approximately 7–8 months later in the nonpregnant cohort so that the study periods would be of similar duration in the pregnant and nonpregnant groups (time 2). Whole blood was collected in EDTA tubes and then aliquoted into Sardstedt SRS-72-694-006 tubes (seven per participant per timepoint) and frozen at −80°C within 4 hours. Samples were thawed together, and DNA was extracted with the Qiagen QIAamp 96 DNA blood kit following the manufacturer's instructions, after which the samples were measured on an Illumina EPIC 2 bead-chip array in a randomized order, ensuring that both timepoint samples for one person were on the same plate. Quality control was ensured from control runs and random duplicate samples across plates.

DNA methylation preprocessing, normalization, and analysis were conducted with R 4.4.1 (Appendix

1, available online at http://links.lww.com/AOG/E214, provides details). All available epigenetic clocks were initially included in the analysis.[22] The Horvath, Hannum, SkinBlood, PhenoAge, Yang (pcgtAge), GrimAge, and GrimAge2 clocks were calculated with the online clock calculator (https://dnamage.clockfoundation.org/, accessed July 22, 2024). The Lin, Weidner, Vidal-Bralo, and Zhang clocks were calculated by obtaining the predictor CpGs and coefficients from their respective publications, using the CpGs from the current data set, and calculating methylation values multiplied by the respective clock coefficients and summed to obtain their scores. DunedinPACE was calculated with DunedinPACE 0.99.0.[23]

The clocks provide results in various ways: 1) in years for Hannum, Horvath, Weidner, Vidal-Bralo, Lin, SkinBlood, Zhang, PhenoAge, GrimAge, and GrimAge2[11,13,14,24–28]; 2) in pace (DunedinPACE [Pace of Aging Calculated from the Epigenome], in which a value of 1 means biologic aging equals chronologic aging, a value less than 1 indicates that it is slower, and a value greater than 1 indicates that it is faster)[22,23]; or 3) in prediction of stem cell division as it relates to cancer risk, with a value of 0 indicating average methylation status of CpG sites related to stem cell division that are included in the model (Yang stem cell division rate).[29,30] For example, a chronologic age of 35, an epigenetic age of 40, and a DunedinPACE of 0.5 suggest that epigenetic age is greater than chronologic age but that the aging pace is slowing.

Sample size was determined from data for another biomarker of aging, leukocyte telomere length, as a proxy given that annual change data were more readily available in the literature and it was one of the intended epigenetic clock measurements.[31] We estimated the minimum sample size required for 80% power and a two-sided α of 0.05 and estimated effect sizes for the outcome of leukocyte telomere length shortening by 50% over the study period. Using these study parameters and allowing for a 25% dropout rate in the nonpregnant group and 10% dropout rate in the pregnant group, we calculated that the study required enrolling 53 nonpregnant and 46 pregnant participants per group.

Secondarily, we explored associations between epigenetic age in the first trimester (time 1) and subsequent complications in the pregnant cohort that are known to be related to advanced maternal chronologic age or could potentially be caused by underlying immune-mediated processes.[4,5,32] Because of the exploratory nature of the question and the small sample size, pregnancy complications were modeled as a composite of events that occurred after time 1: preeclampsia or gestational hypertension antenatally or intrapartum, gestational diabetes mellitus (GDM), small-for-gestational-age birth weight (SGA, birth weight below the 10th percentile for gestational age), and preterm birth before 37 weeks of gestation.[4,5,33] The GDM diagnoses were recorded as A1 or A2 GDM that developed any time between time 1 and time 2 and was not preexisting at the time of enrollment. At our institution, we perform a routine 24- to 28-week 1-hour glucose tolerance test followed by a 3-hour glucose tolerance test to make the diagnosis when the 1-hour glucose tolerance test result is elevated. In addition, early glucose tolerance tests are recommended for those who are at high risk for developing GDM because of clinical factors.

The exposure was pregnancy status, which was confirmed through medical record abstraction and participant report. Sociodemographic and clinical variables, including chronologic age, body mass index (BMI, calculated as weight in kilograms divided by height in meters squared) at enrollment (time 1), level of education, race and ethnicity as social constructs, and medical comorbidities (neurologic, psychiatric, thyroid, or autoimmune conditions; type 1 or 2 diabetes; chronic hypertension) were collected from medical record abstraction and participant self-report. Because of the small sample size, chronologic age was deemed the most important covariate to adjust for as a confounder in both models for the primary analysis and secondary subanalysis in the pregnant cohort.[12,16,17] For the secondary subanalysis, BMI at enrollment was included as a second potential confounder given the known associations with both pregnancy complications and epigenetic age and the likelihood that it would be a proxy for underlying chronic disease status.[34–36]

Participant characteristics were compared between the pregnant and nonpregnant cohorts with Wilcoxon rank-sum tests for continuous variables and the Fisher exact test for categorical variables. Quality control assessments were conducted for the epigenetic clocks, and only those with at least 80% of the necessary probes present in the data set were analyzed. Accuracy of epigenetic age prediction of chronologic age was calculated for all clocks and reported with correlation coefficients, root mean square deviations, and intraclass correlation coefficients.

For the primary outcome, within-person changes in epigenetic age over time were compared between the pregnant and nonpregnant groups with mixed-effects linear regression models adjusted for chronologic age as a confounder and statistical interaction between pregnancy status and days between measurements (model 1) with the lmer function in R. To enhance interpretability of the β coefficients for the interaction term, they were multiplied by the average duration (days) between time 1 and time 2 in the pregnant group (200 days). As a sensitivity analysis, immune cell type distribution was added as a covariate in a separate model.[37,38] Because the clocks account for tissue type, the sensitivity analysis adjusted for cell proportions was not the primary model because it can be overadjusted.[15] Lastly, epigenetic ages were compared between the pregnant and nonpregnant cohorts at each individual timepoint with an analysis of covariance using the function lm 4.4.1.[39] *P* values for aim 1 were corrected for multiple testing with the Benjamini–Hochberg method.

For the secondary substudy, the composite of pregnancy or delivery complications (hypertensive disorders, GDM, preterm birth before 37 weeks of gestation, and SGA birth weight) was compared across all of the first-trimester epigenetic ages from time 1 with logistic regression adjusted for age and BMI higher than 30 at time 1 as potential confounders.[8,10,12,40,41] Primary analyses were conducted with R 4.4.1 in RStudio 2023.06.0+421, and the secondary analysis was conducted with SAS Studio 2024.

RESULTS

In total, 305 women were screened; 250 were eligible and 75 (30.0%) enrolled and analyzed (Fig. 1). Of these, 45 were pregnant and 30 were not. Because of unprecedented research changes with the coronavirus disease 2019 (COVID-19) pandemic in 2020–2021 and one participant who withdrew after enrollment, enrollment was closed when the pregnant cohort reached 45 participants. Characteristics at enrollment were similar between groups; notably, chronologic ages were similar, and all in the pregnant cohort enrolled in the first trimester, giving similar time to postpartum measurements (Table 1). A total of 14 participants were lost to follow-up: of the 45 pregnant participants, 43 (95.6%) returned for time 2 compared with 18 of the nonpregnant participants (60.0%) (*P*=.02). Sociodemographic variables were similar

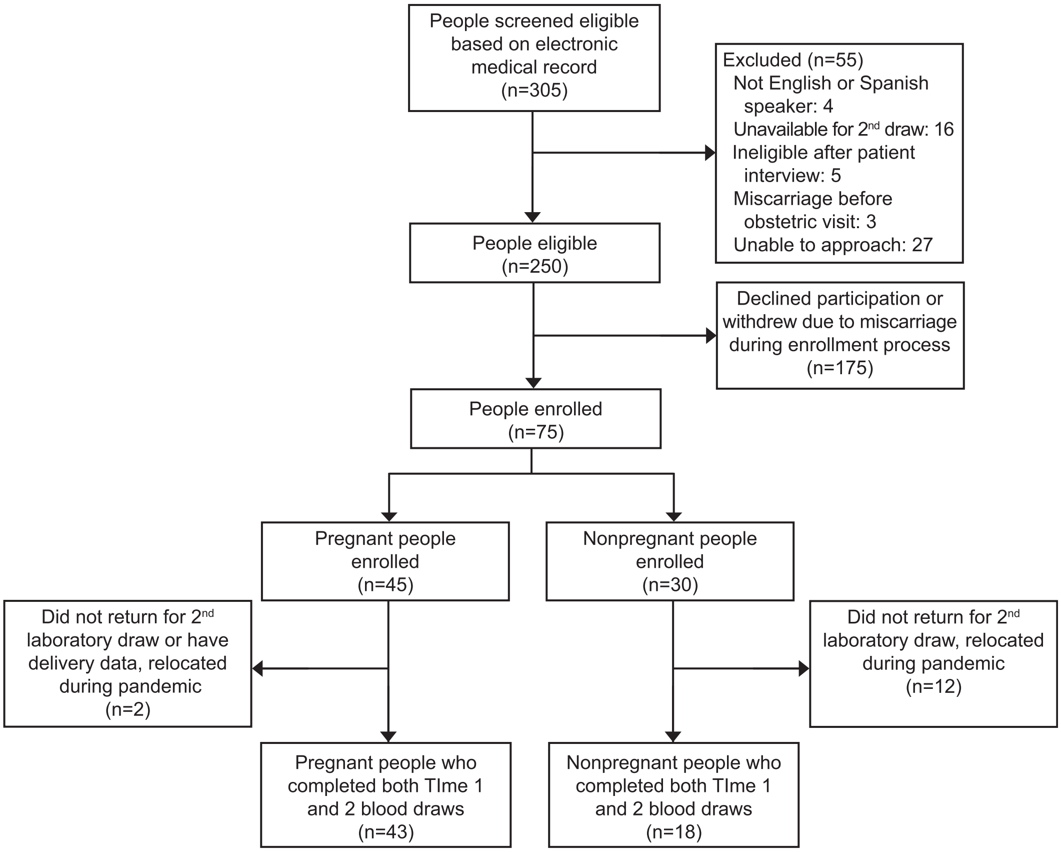

Fig. 1. CONSORT (Consolidated Standards of Reporting Trials) diagram.
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for participants who did and did not return for time 2 (Appendix 2, available online at http://links.lww.com/AOG/E214). The nonpregnant group had a longer follow-up interval between times 1 and 2 (nonpregnant median 232 days [quartiles 1 and 3, 205, 260 days], pregnant 198 days [quartiles 1 and 3, 187, 206 days], $P<.01$).

For the primary analysis, the average percentage of CpG loci detected for the DNA methylation analysis was 99.9%. Accuracy for all the epigenetic clocks in predicting chronologic age is presented in Appendix 3, available online at http://links.lww.com/AOG/E214. Of the 13 epigenetic clocks that were initially computed, 11 had adequate CpG coverage and were analyzed. Of the 75 participants enrolled, 61 had samples from both timepoints that were analyzed for within-person changes. There was significantly greater within-person epigenetic age acceleration in the pregnant than in the nonpregnant cohort for the Hannum, Stem Cell Division, PhenoAge, GrimAge, GrimAge 2, and DunedinPACE clocks over time (Table 2). The greatest increase in within-person epigenetic age acceleration was for PhenoAge and GrimAge2, with the pregnant group gaining 5.28 ($P<.01$) and 4.56 ($P<.01$) additional epigenetic years, respectively, over a 200-day interval compared with the nonpregnant group. This is demonstrated graphically in Figure 2, which demonstrates box plots for each epigenetic clock between the pregnant and nonpregnant cohorts at times 1 and 2. Results were similar in the sensitivity analysis adjusted for cell proportions and were most robust for GrimAge, GrimAge2, and DunedinPACE (Appendix 4, available online at http://links.lww.com/AOG/E214).

We also compared epigenetic age between the pregnant and nonpregnant groups cross-sectionally at each of the two timepoints. At time 1, the pregnant cohort had an older mean epigenetic age than the nonpregnant cohort (GrimAge $\beta=1.65$, $P<.01$; GrimAge2 $\beta=2.74$, $P<.01$; DunedinPACE $\beta=0.09$, $P<.01$; Table 3). At time 2, the pregnant cohort had

Table 1. Demographic Characteristics of Pregnant and Nonpregnant Study Participants, 2020–2021 (N=75)

Characteristic	Pregnant (n=45)	Nonpregnant (n=30)	*P**
Chronologic age (y)			
Time 1	30 (29, 33)	32 (27, 37)	.73
Time 2	31.5 (30, 34.5)	31.5 (28, 37)	.63
Race			.29
Asian or Pacific Islander	19 (42.2)	8 (26.7)	
Black	2 (4.4)	0	
White	21 (46.7)	19 (63.3)	
Other or unknown	3 (6.7)	3 (10.0)	
Hispanic ethnicity	5 (11.1)	2 (6.7)	.70
Highest level of education			.20
Completed high school	3 (6.7)	1 (3.3)	
Completed college	10 (22.2)	11 (36.7)	
Pursuing or completed higher degree	30 (66.7)	14 (46.7)	
Unknown or missing	2 (4.4)	4 (13.3)	
Median BMI at time 1 (kg/m^2)	22.6 (20.6, 24.3)	23.3 (21.7, 27.2)	.12
Comorbidity at time of enrollment			
None	24 (53.3)	15 (50.0)	.81
Neurologic or psychiatric	9 (20.0)	5 (16.7)	.77
Chronic hypertension	0	2 (6.7)	.16
Type 1 or 2 diabetes	1 (2.2)	0	1.00
Hypothyroidism or hyperthyroidism	5 (11.1)	1 (3.3)	.39
Other[†]	13 (28.9)	11 (36.7)	.61
Enrolled before COVID-19 shelter-in-place order[‡]	23 (51.1)	18 (60.0)	.49
Participants with both timepoint measurements completed	44 (97.8)	18 (60.0)	.02
Time between times 1 and 2 (d)	198 (187, 206)	232 (205, 260)	<.01
Spontaneous conception	39 (86.7)	—	—
Gestational age at enrollment (wk)	10 (10, 11)	—	—

BMI, body mass index; COVID-19, coronavirus disease 2019.

Data are median (quartile 1, quartile 3) or n (%) unless otherwise specified.

* Wilcoxon rank-sum test for continuous variables or Fisher exact test for categorical variables.

[†] Includes polycystic ovarian syndrome, asthma, α thalassemia trait, rheumatologic (Crohn disease, ulcerative colitis), hyperlipidemia, and chronic back pain.

[‡] March 17, 2020.

an older mean epigenetic age than the nonpregnant cohort for the same three clocks, in addition to lower stem cell division ($\beta=-0.01$, *P*<.01).

For the secondary analysis, 44 pregnant participants had a time 1 blood draw and outcome data available. All 11 epigenetic clocks were evaluated for their association with the composite of pregnancy complications. Seventeen participants (38%) experienced at least one of the composite events; nine (20%) developed a hypertensive disorder of pregnancy, six (13%) developed GDM, three (7%) had preterm birth before 37 weeks of gestation, and one (2%) had a neonate with SGA birth weight. Of the six participants in our study who developed GDM, the gestational age range of their diagnoses was 13 5/7 weeks to 26 5/7 weeks, with two of the six GDM diagnoses occurring before 24 weeks of gestation according to an early screen. For each additional year of epigenetic aging in the GrimAge2 clock at time 1, there was a 36% increase in the odds of the pregnancy complication composite (adjusted odds ratio 1.36, 95% CI, 1.01–1.84, Table 4]. In contrast, chronologic age was not associated with the pregnancy complication composite (adjusted odds ratio 1.00, 95% CI, 0.83–1.21).

DISCUSSION

Over a 7-month period, we found greater within-person epigenetic age acceleration in nulliparous pregnant compared with nulliparous nonpregnant women using six epigenetic clocks that reflect morbidity and mortality risk. First-trimester GrimAge2 epigenetic age was modestly associated with a composite of pregnancy complications. These findings contribute to growing evidence that pregnancy may influence biologic aging and highlight the potential value of early gestational epigenetic age as a predictor of pregnancy complications.

Epigenetic age acceleration has been linked to adverse health outcomes across the lifespan, including cardiovascular disease and mortality.[12,23,40] In our pregnant cohort, the clocks that most strongly reflected age acceleration–PhenoAge, GrimAge2, and DunedinPACE–were developed to predict morbidity and mortality.[42] Results remained consistent across multiple clocks and after adjustment for cell type composition, suggesting that the epigenetic changes may reflect more than the expected immune shifts of pregnancy. These data support and extend prior research such as the report by Poganik et al,[6] which also described reversible epigenetic aging acceleration in pregnancy.[16,17,31] One prevailing hypothesis is that maternal molecular resources are redirected toward fetal development at the expense of somatic maintenance, possibly leading to transient maternal epigenetic aging. After parturition, these maternal somatic cell effects may or may not revert to baseline.[6,43]

A key strength of our study is the inclusion of a nulliparous, similarly aged, nonpregnant comparison group. This helps to distinguish pregnancy-related changes from background aging in reproductive-aged women, addressing a gap in prior research.[12,37,38,40,44]

Table 2. Changes in Epigenetic Age for Pregnant and Nonpregnant Participants Over Time (n=61)

Epigenetic Clock (Output Type)	Pregnant Cohort's Epigenetic Age Difference/200 d[*]	95% CI	*P*[†]
Horvath (y)	−0.08	−1.54 to 1.39	.93
Hannum (y)	**+1.58**	**0.45–2.72**	**.01**
SkinBlood (y)	+0.38	−0.30 to 1.05	.36
Weidner (y)	+0.08	−1.65 to 1.80	.93
Lin (y)	−1.05	−3.00 to 0.91	.37
Zhang (y)	+0.09	−0.12 to 0.30	.46
Stem cell division rate[‡]	**−0.01**	**−0.01 to −0.01**	**<.01**
PhenoAge (y)	**+5.28**	**2.97–7.61**	**<.01**
GrimAge (y)	**+2.81**	**1.98–3.62**	**<.01**
GrimAge2 (y)	**+4.56**	**3.40–5.71**	**<.01**
DunedinPACE[‡]	**+0.16**	**0.12–0.19**	**<.01**

Bold indicates statistical significance.

[*] Mixed linear effects model (independent variable pregnancy status, dependent variable epigenetic age) adjusted for chronologic age at sample collection and pregnancy by timepoint interaction term. The β coefficients and 95% CIs are presented for the interaction term multiplied by 200 to convert effect estimate to be from 1-day intervals into a per 200-day interval, which was the approximate duration between samples in the pregnant cohort. This is presented as the "difference per 200 days."

[†] *P* values shown for interaction term between cohort (pregnant vs nonpregnant, referent) and timepoint, corrected for multiple testing with the Benjamini–Hochberg method.

[‡] The stem cell division rate and DunedinPACE clock outputs are not in years like the others. Yang stem cell division less than 0 indicates less methylation of sites involved with stem cell division and more than 0 indicates more methylation of these sites. DunedinPACE less than 1 indicates slower pace of epigenetic aging relative to biologic aging; DunedinPACE greater than 1 indicates faster epigenetic aging. See text for details.

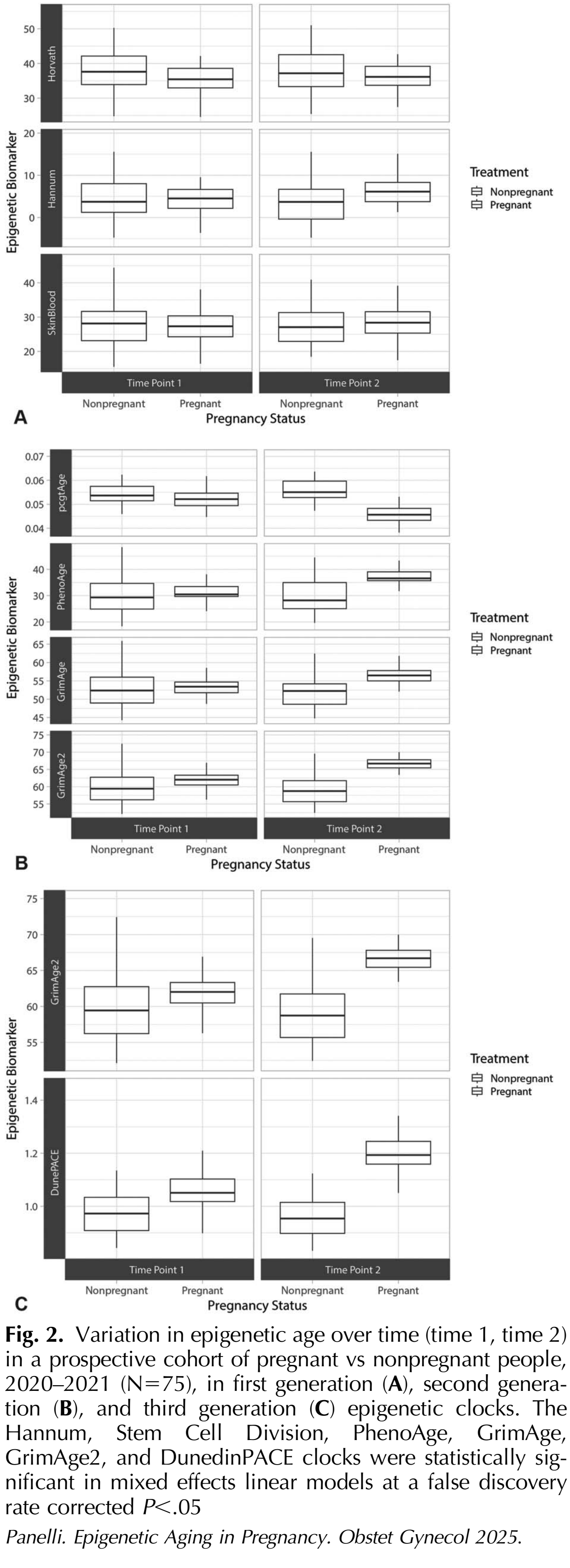

Fig. 2. Variation in epigenetic age over time (time 1, time 2) in a prospective cohort of pregnant vs nonpregnant people, 2020–2021 (N=75), in first generation (**A**), second generation (**B**), and third generation (**C**) epigenetic clocks. The Hannum, Stem Cell Division, PhenoAge, GrimAge, GrimAge2, and DunedinPACE clocks were statistically significant in mixed effects linear models at a false discovery rate corrected $P<.05$

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Although past cross-sectional studies have linked multiparity to older epigenetic age, few have assessed these patterns prospectively in a nulliparous cohort.[6,17,45] Furthermore, this notion would potentially contrast with the idea of postpartum epigenetic age deceleration, which has been noted in some studies; this may speak to variations in postpartum lifestyle habits, breastfeeding, sleep, and recovery that may affect epigenetic age over time. Our time 2 results, taken in the immediate postpartum period, would theoretically minimize the variability of these postpartum effects on aging, which is an important but distinct question.

Notably, we observed that older first-trimester GrimAge2 was more strongly associated with a composite of potentially immune-mediated[32,46] pregnancy complications than chronologic age. We should acknowledge that although some underlying pathways to these complications may be immune mediated, other pathways may not be (eg, cervical insufficiency for preterm birth or fetal constitutional growth potential for SGA birth weight). These results add to other literature in this area, such as work by Gascoigne et al[18] that linked some measures of accelerated maternal epigenetic aging to increased risk of preterm birth. In our cohort, epigenetic age acceleration was already underway early in gestation. It may be that early disruptions in immune tolerance are reflected by epigenetic aging changes before they manifest as the complications themselves.[41]

Our findings suggest the potential of first-trimester epigenetic age as a predictor of pregnancy complications, which may be of particular interest given increasing efforts toward personalized medicine approaches.[46] If validated, this could open the door to targeted, early interventions. Because epigenetic age is modifiable,[6,47] it may be possible to mitigate risk before these complications arise. Further research is needed to determine whether reversal of epigenetic aging before or early in pregnancy is feasible or beneficial. In addition, our results reinforce the concept of pregnancy as a physiologic "stress test," potentially revealing long-term vulnerability to chronic disease.[48]

Further quantification of the effects of the postpartum period and subsequent pregnancies on epigenetic aging over a person's lifespan is needed. The observed association between GrimAge2 and pregnancy complications warrants further investigation in a larger cohort to elucidate potential mechanisms. Some clocks (eg, Horvath) were less correlated with chronologic age in our sample, perhaps because their test and validation cohorts were older populations and

Table 3. Adjusted Differences in Epigenetic Age for the Pregnant and Nonpregnant Groups Separately at Enrollment (Time 1) and Follow-Up (Time 2) Visits, 2020–2021

	Epigenetic Age		Linear Regression Model*	
Epigenetic Clock	Pregnant	Nonpregnant	Epigenetic Age Difference (95% CI)	FDR-corrected *P*[†]
Time 1 (N=75)				
Horvath (y)	35.31±4.85	38.00±7.13	−1.41 (−3.19 to 0.37)	.25
Hannum (y)	4.31±3.92	5.15±6.67	0.41 (−0.89 to 1.70)	.65
SkinBlood (y)	27.46±4.47	28.59±7.61	0.35 (−0.76 to 1.45)	.65
Weidner (y)	44.53±4.97	46.44±6.53	−1.40 (−3.81 to 1.00)	.37
Lin (y)	20.23±5.93	20.00±7.93	1.48 (−0.78 to 3.73)	.33
Zhang (y)	55.29±1.66	55.99±2.81	−0.15 (−0.55 to 0.25)	.63
Yang stem cell[‡]	0.05±0.01	0.05±0.01	−0.00 (−0.01 to 0.00)	.34
PhenoAge (y)	31.15±5.37	30.15±8.04	2.27 (0.04–4.49)	.13
GrimAge (y)	53.43±3.08	52.91±6.09	**1.65 (0.69–2.60)**	**<.01**
GrimAge2 (y)	61.94±3.26	60.23±6.07	**2.74 (1.49–3.99)**	**<.01**
DunedinPACE	1.06±0.11	0.98±0.10	**0.09 (0.04–0.13)**	**<.01**
Time 2 (n=61)				
Horvath (y)	35.90±4.64)	37.84±7.93)	−1.33 (−3.21 to 0.54)	.24
Hannum (y)	5.99±4.26	4.40±6.66	**2.11 (0.43–3.79)**	**.03**
SkinBlood (y)	28.61±4.66	28.48±7.77	0.78 (−0.55 to 2.11)	.30
Weidner (y)	44.34±4.44	45.83±5.17	−1.50 (−4.05 to 1.05)	.30
Lin (y)	20.06±5.71	19.81±9.29	0.80 (−1.72 to 3.33)	.58
Zhang (y)	55.47±1.63	55.67±2.73	0.03 (−0.39 to 0.45)	.88
Yang stem cell[‡]	0.05±0.01	0.06±0.01	**−0.01 (−0.01 to −0.01)**	**<.01**
PhenoAge (y)	37.09±6.87	30.21±8.29	**7.64 (4.66–10.61)**	**<.01**
GrimAge (y)	56.68±3.57	52.86±6.03	**4.30 (3.16–5.45)**	**<.01**
GrimAge2 (y)	66.77±4.00	60.00±5.91	**7.23 (5.74–8.71)**	**<.01**
DunedinPACE[‡]	1.20±0.09	0.96±0.09	**0.24 (0.20–0.29)**	**<.01**

FDR, false discovery rate.

Data are mean±SD unless otherwise specified.

Bold indicates statistical significance.

* Mixed linear-effects model adjusted for chronologic age at sample collection. Presenting β coefficients as "difference" between pregnant and nonpregnant cohorts at each timepoint.

[†] *P* values were FDR corrected for multiple testing with the Benjamini–Hochberg method.

[‡] The stem cell division rate and DunedinPACE clock outputs are not in years like the others. Yang stem cell division less than 0 indicates less methylation of sites involved with stem cell division and more than 0 indicates more methylation of these sites. DunedinPACE less than 1 indicates slower pace of epigenetic aging relative to biologic aging; DunedinPACE more than 1 indicates faster epigenetic aging. See text for details.

included both men and women or because of the EPIC 2 chips (which can show age deviations with earlier clocks).[49] Our samples were run on the same array, and all comparisons were conducted within our cohort, which should minimize bias. Interrogation of differentially methylated regions toward the development of a pregnancy-specific clock is a natural progression of this work.

We should note several limitations. Because of the COVID-19 pandemic, more participants in the nonpregnant group were lost to follow-up and longer intervals between study visits, which may introduce selection bias; notably, characteristics of those who did and did not return for time 2 were similar. If anything, a longer time interval should have led the nonpregnant group to appear to age more and biased the results toward the null. Our secondary analysis of pregnancy complications used a composite of outcomes resulting from our small available sample size, increasing the possibility of residual confounding. We estimated the trajectory of epigenetic aging using maternal blood from postpartum day 1 rather than from the third trimester. Although we did this to maximize ability to detect measurable change over time in epigenetic biomarkers and to minimize participant burden, future research should evaluate how third-trimester epigenetic age compares with postpartum day 1 and whether this is affected by mode of delivery. Additional follow-up during and after pregnancy is needed to determine whether these patterns persist over time. We did not meet our intended sample size, which may have resulted in type 2 error.

Strengths of our study include the use of a similarly aged, nonpregnant, nulliparous comparison group and our rigorous data quality and longitudinal

Table 4. Association Between Epigenetic Age in the First Trimester and the Composite Adverse Pregnancy Outcome (n=44)

Clock	Composite Adverse Pregnancy Outcome*	*P*
Horvath	1.06 (0.90 to 1.24)	.48
Hannum	0.98 (0.79 to 1.23)	.87
PhenoAge	1.14 (0.98 to 1.33)	.10
SkinBlood	0.96 (0.73 to 1.27)	.78
Stem cell division[†]	—	.55
Lin	1.02 (0.89 to 1.17)	.77
Weidner	1.11 (0.97 to 1.28)	.13
DunedinPACE more than 1[†]	2.80 (0.63 to 12.52)	.18
Zhang	1.13 (0.53 to 2.39)	.76
GrimAge	1.33 (0.94 to 1.89)	.11
GrimAge2	**1.36 (1.01 to 1.84)**	**.04**

Data are adjusted odds ratio (95% CI) unless otherwise specified. Bold indicates statistical significance.

* Logistic regression with clock age as independent variable and with dependent variable as a composite of inflammatory-mediated pregnancy or delivery complications, including hypertensive disorders, gestational diabetes, preterm birth before 37 weeks, or small-for-gestational-age birth weight. Adjusted for maternal chronologic age and body mass index higher than 30 in the first trimester.

[†] Data not shown because of wide CI (less than 0.01, more than 999.99). DunedinPACE modeled as binary (1 or less vs more than 1) for the same reason.

design. We have also presented innovative findings concerning the relationship between maternal epigenetic aging and pregnancy complications that warrant further evaluation.

In conclusion, over 7 months, nulliparous pregnant women demonstrated up to 5 additional years of epigenetic aging compared with their similarly aged, nonpregnant counterparts. First-trimester epigenetic age from the GrimAge2 clock was associated with pregnancy complications. As pregnancies at older chronologic ages become increasingly common, these findings suggest a potential role for epigenetic clocks in further understanding pregnancy physiology and risk. Further research is needed to determine whether epigenetic aging is a modifiable target or a marker of underlying susceptibility to adverse outcomes in pregnancy.

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