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Preconception leukocyte telomere length and pregnancy outcomes among women with demonstrated fecundity

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STUDY QUESTION: Is preconception leukocyte telomere length associated with fecundability, pregnancy loss and live birth among women attempting natural conception with a history of 1–2 prior pregnancy losses?

SUMMARY ANSWER: Preconception leukocyte telomere length is not associated with fecundability, pregnancy loss or live birth.

WHAT IS KNOWN ALREADY: As women increasingly delay childbearing, accessible preconception biomarkers to predict pregnancy outcomes among women seeking natural conception could improve preconception counseling. Findings of small case–control or cross-sectional studies suggest that telomere attrition is associated with adverse pregnancy outcomes among women undergoing fertility treatment, but prospective studies in non-clinical populations are lacking.

STUDY DESIGN, SIZE, DURATION: Participants included 1228 women aged 18–40 years with a history of 1–2 prior pregnancy losses who were recruited at four university medical centers (2006–2012).

PARTICIPANTS/MATERIALS, SETTING, METHODS: Preconception leukocyte telomere length was measured at baseline using PCR and reported as a ratio (T/S) in relation to population-specific standard reference DNA. Women were followed for up to six cycles while attempting to conceive. Associations of telomere length with fecundability, live birth and pregnancy loss were estimated using discrete Cox proportional hazards models and log-binomial models.

MAIN RESULTS AND THE ROLE OF CHANCE: After adjustment for age, BMI, smoking and other factors, preconception telomere length was not associated with fecundability (Q4 vs Q1 FOR = 1.00; 95% CI = 0.79, 1.27), live birth (Q4 vs Q1 RR = 1.00; 95% CI = 0.85, 1.19), or pregnancy loss (Q4 vs Q1 RR = 1.12; 95% CI = 0.78, 1.62).

LIMITATIONS, REASONS FOR CAUTION: Telomere length was measured in leukocytes, which is an accessible tissue in women attempting natural conception but may not reflect telomere length in oocytes. Most women were younger than 35 years, limiting our ability to evaluate associations among older women. Participants had a history of 1–2 prior pregnancy losses; therefore, our findings may not be widely generalizable.

WIDER IMPLICATIONS OF THE FINDINGS: Despite prior research suggesting that telomere length may be associated with pregnancy outcomes among women seeking fertility treatment, our findings suggest that leukocyte telomere length is not a suitable biomarker of pregnancy establishment or maintenance among women attempting natural conception.

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Key words: telomere / fertility / reproductive aging / biologic aging / pregnancy loss / fecundity

Introduction

Female reproductive aging is characterized by the gradual decline of the quantity and quality of oocytes in the ovarian cortex, beginning *in utero* and ending at menopause (Broekmans *et al.*, 2007). The decline of the ovarian follicle pool confers greater risk of meiotic nondisjunction and embryonic aneuploidy, which in turn contributes to a lower likelihood of pregnancy and live birth, and higher risk of pregnancy loss (Te Velde and Pearson, 2002). The inability to conceive as desired can have psychological and financial consequences (Broekmans *et al.*, 2007) as infertility treatment is frequently costly, invasive and not always effective, particularly in the context of advanced maternal age (Smith *et al.*, 2015). Chronologic age is the only established biomarker of subfertility and infertility among women attempting natural conception (DeCherney and Berkowitz, 1982; Dunson *et al.*, 2004), but considerable interindividual variation in reproductive longevity exists, presumably related to genetic traits and lifestyle, environmental and social factors that slow or accelerate the reproductive and biologic aging process (Te Velde and Pearson, 2002; Gold, 2011). Biomarkers of ovarian reserve, such as anti-Müllerian hormone (AMH), reliably predict oocyte yield among women undergoing controlled ovarian hyperstimulation for IVF (Wu *et al.*, 2009), but have little utility in predicting pregnancy outcomes among women attempting natural conception (Zarek *et al.*, 2015, 2016; Steiner *et al.*, 2017). As women increasingly delay childbearing into the later reproductive years (Te Velde and Pearson, 2002), identifying accessible preconception biomarkers that improve prediction of pregnancy outcomes beyond chronologic age in non-clinical populations could enhance preconception counseling by providing women with a more individualized estimate of their likelihood pregnancy and live birth.

Telomeres, non-coding DNA regions that preserve genomic stability and ensure proper chromosome segregation, are hypothesized to play a role in the poor pregnancy outcomes associated with reproductive aging, and may offer promise as a potential preconception biomarker (Keefe *et al.*, 2006). The telomeres of oocytes remaining in the ovarian cortex during the later reproductive years are shorter owing to the accumulation of DNA damage from reactive oxygen species throughout the lifetime, which may contribute to the genetic abnormalities associated with subfertility and pregnancy loss (Keefe *et al.*, 2007; Shammas, 2011). Most, but not all (Keefe *et al.*, 2005; Hanna *et al.*, 2009; Cheng *et al.*, 2013; Czamanski-Cohen *et al.*, 2015; Morin *et al.*, 2018; Hanson *et al.*, 2021) epidemiologic data suggest that shorter telomere length is associated with infertility and poorer IVF treatment outcomes; however, studies among women attempting natural conception are lacking. Additional studies have reported shorter telomere length among women with recurrent pregnancy loss (Hanna *et al.*, 2009; Thilagavathi *et al.*, 2013) as well as higher parity (Gray *et al.*, 2014; Pollack *et al.*, 2018), suggesting that having more children may accelerate telomere attrition. However, inference from these studies is limited by cross-sectional design or modest sample sizes.

Considering the need for noninvasive preconception biomarkers and the lack of prospective data on preconception telomere length and pregnancy outcomes among women attempting natural conception, the objective of our study was to evaluate associations of preconception leukocyte telomere length, a biomarker that can be measured by standard blood collection, with fecundability, pregnancy loss and live birth among 1228 healthy reproductive-age women attempting pregnancy who had a history of 1–2 pregnancy losses.

Materials and methods

Study population

This was a secondary analysis of the Effects of Aspirin in Gestation and Reproduction (EAGeR) trial, a multi-center, block-randomized, double-blind, placebo-controlled clinical trial designed to determine the effect of preconception-initiated daily low-dose aspirin on reproductive outcomes in women with history of pregnancy loss (Schisterman *et al.*, 2013, 2014). The trial design and overall results have been described previously (Schisterman *et al.*, 2013).

Study participants were 1228 women attempting pregnancy, aged 18–40 years, with 1–2 prior pregnancy losses. Women with a known history of infertility treatment, pelvic inflammatory disease, tubal occlusion, endometriosis, anovulation and polycystic ovary syndrome, or uterine abnormality were excluded from participation. Participants were recruited at four clinical sites in the USA from 2007 to 2011 and followed for up to six menstrual cycles while attempting pregnancy and then throughout pregnancy for women who conceived. Baseline demographic characteristics and reproductive history information was obtained via questionnaire. Follow-up was completed by 1088 participants (89%).

Ethical approval

The institutional review board at each US study site (Salt Lake City, UT, USA; Denver, CO, USA; Buffalo, NY, USA; Scranton, PA, USA) and data coordinating center approved the trial protocol and all participants provided written informed consent prior to enrolling. The trial was registered with ClinicalTrials.gov, number NCT00467363.

Biomarker assessment

After enrollment in the EAGeR trial and prior to randomization, women provided blood samples in EDTA lavender-top tubes at the clinic, which were frozen and stored at −80°C until analysis (Tsai Lab, University of Minnesota, Minneapolis, MN, USA). Leukocyte DNA was extracted from buffy coat samples using the PureGene DNA Isolation reagents from Qiagen (Hilden, Germany). The concentration of DNA was determined using a NanoDrop Spectrophotometer and PicoGreen quantification assay provided by Molecular Probes (Eugene, OR, USA). The telomere repeat copy number to single gene copy

number (T/S) ratio was determined using a previously described method (McGrath et al., 2007), except in a 384-well format using a 7900HT Fast Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA). Leukocyte telomere length was measured in relation to a standard DNA reference as a T/S ratio in duplicate 1–2 times. Leukocyte telomere length was equal to the mean of the 2–4 T/S ratio measurements for each individual. The corresponding number of base pairs was calculated using the following formula: $3274 + 2413 \times$ (T/S ratio). In total, 1171 women in the study population had available leukocyte telomere length measurements.

Preconception AMH concentrations were measured in serum samples collected on Days 2–4 of the menstrual cycle using the GEN II ELISA™ assay (Beckman Coulter, Brea, CA, USA). All machine observed concentrations were used without substitution of concentration below the limits of detection (0.006 ng/ml) (Craciunas et al., 2015). The inter-assay laboratory coefficients of variation were 6.2% and 6.6% at mean concentrations of 8.9 and 3.1 ng/ml, respectively, for lyophilized manufacturer's controls and 6.3% for an in-house pooled serum control.

Pregnancy outcomes

Outcomes examined included time to hCG-detected pregnancy (in menstrual cycles), pregnancy loss and live birth. Urine hCG tests were conducted at home, on the expected day of menses, or in the clinic if the participant reported a missed menses. Tests were sensitive to 25 mIU/ml (Quidel Quickvue, Quidel Corp, San Diego, CA, USA). β-hCG was also measured from first-morning daily urine samples provided by participants on the last 10 days of their menstrual cycle during Cycles 1 and 2, and from spot urine samples collected at all end of cycle visits. Two assays (initial test: catalogue no. RIS0011R, BioVendor, Asheville, NC, USA; confirmatory test: catalogue no. 4221-16, Diagnostic Automation Inc., Calabasas, CA, USA), performed sequentially, detected very early pregnancies with higher sensitivity. Clinically confirmed intrauterine pregnancies were confirmed by ultrasound visualization at clinic visits during gestational weeks 6 and 7.

Pregnancy loss was defined as a positive pregnancy test at home or at the clinic site followed by the absence of a clinically confirmed pregnancy; or positive hCG from batched augmented urine samples followed by the absence of a positive at home or clinic pregnancy test; or a loss occurring after clinical ultrasound confirmation but prior to 20 weeks after the last menstrual period. Live birth was defined as a live-born infant after 23 weeks' gestation.

Statistical analysis

We first plotted the distribution of telomere length to assess normality. Using generalized linear models, we compared the distribution of sociodemographic characteristics, reproductive history and anthropometric measurements according to quartiles of telomere length. We addressed missing data on leukocyte telomere length and covariate data using multiple imputation with fully conditional specification and 10 replications. The telomere length was missing for 57 women (5%) and less than 8% of women had missing covariate data.

For all analyses, we modeled leukocyte telomere length in both its continuous form and in quartiles because established cut-points for telomere length are not available. We used discrete-time Cox proportional hazards regression to estimate fecundability odds ratios (FOR) and 95% CI per 1000 base-pairs or compared to the lowest quartile of leukocyte telomere length. Models accounted for left truncation (i.e. cycles attempting pregnancy before enrollment) and right-censoring. An FOR describes the probability of becoming pregnant in a given cycle, conditional on not being pregnant in the previous cycle. An FOR >1 indicates improved fecundability or shorter time to pregnancy, whereas an FOR <1 indicates reduced fecundability or longer time to pregnancy.

We utilized log-binomial models to estimate relative risks (RR) and 95% CI for pregnancy loss and live birth according to leukocyte telomere length. Models evaluating live birth included all women in the EAGeR population ($n = 1228$) and answer the question, 'Based on preconception telomere length, what is the likelihood of having a live-born baby?', which is presumably the question of greatest interest to a woman or clinician in the preconception period. Models evaluating pregnancy loss were limited to women who became pregnant, which could introduce selection bias if factors that affect selection into the pregnant group also affect pregnancy loss (Flannagan and Mumford, 2020). To address this potential bias, we employed inverse-probability weights to account for the selection bias imposed by conditioning on pregnancy (Cole and Hernan, 2008). The logistic regression models used to generate the inverse-probability weights included factors associated with pregnancy in this cohort, including age, BMI, number of previous losses, number of previous live births, smoking status, alcohol use, race, multivitamin use and income. In this context, the RRs for pregnancy loss estimated from inverse-probability weighted models can be interpreted as the association of preconception leukocyte telomere length and pregnancy loss that would be observed if, possibly counter to fact, all women in the EAGeR population had become pregnant. Estimates from these models answer the question, 'Based on preconception telomere length, what is the likelihood of a pregnancy loss, conditional on an hCG-detected pregnancy?' This would be a relevant question for a woman at a preconception visit, who may want to know her likelihood of pregnancy loss if she goes on to conceive.

We identified potential confounders using directed acyclic graphs (Shrier and Platt, 2008). In analyses, we specified three sets of models: unadjusted (Model 1); adjusted for BMI (continuous), number of previous losses (1 or 2), number of previous live births (0, 1, or 2), smoking status (smoker or non-smoker), alcohol use (drinker or non-drinker), race/ethnicity (non-Hispanic white or other), multivitamin use (yes or no) and income ($\geq$\$40 000 or $<$\$40 000) (Model 2); and adjusted for factors included in model 2 + age (continuous) (Model 3). We included models not adjusted for age (Model 2) to estimate the overall association of telomere length, as a marker of biological aging on pregnancy outcomes. In Model 3, which was adjusted for age, we estimated the associations of telomere length with pregnancy outcomes, independent of chronologic age (as a marker of the passage of time).

To determine whether associations may be limited to women at the older end of the age range in our population, we also stratified each of our analyses by age (<30 vs ≥ 30 years). Because race itself is not a cause, but rather a poor proxy for social determinants of health or genetic factors, we conducted sensitivity analyses in which we omitted race from multivariable models. To assess whether our use of multiple imputation to impute exposure data may have impacted our results, we also conducted sensitivity analyses limited to women with complete data on telomere length ($n = 1171$). In models examining pregnancy loss as an outcome, we additionally conducted sensitivity analyses that

Table I Baseline characteristics by mean preconception leukocyte telomere length among 1171 women in the EAGeR trial.

	Leukocyte telomere length[a,b]			
	Q1 (median = 0.75)	Q2 (median = 0.89)	Q3 (median = 0.99)	Q4 (median = 1.14)
N	279	304	290	298
Age, years	29.4 (5.0)	29.4 (4.7)	28.7 (4.7)	27.6 (4.6)
<30	157 (56)	175 (58)	180 (62)	217 (73)
30–34	80 (27)	91 (30)	76 (26)	58 (20)
35–37	14 (5)	17 (6)	17 (6)	12 (4)
38–40	24 (9)	16 (5)	15 (4)	9 (3)
BMI, kg/m^2	26.3 (6.3)	26.2 (6.5)	26.2 (6.4)	26.5 (6.6)
<18.5	14 (5)	5 (2)	8 (3)	10 (3)
18.5–24.9	129 (46)	160 (53)	140 (48)	138 (46)
25.0–29.9	66 (24)	66 (22)	72 (25)	73 (25)
>30.0	67 (24)	70 (23)	66 (23)	72 (24)
Race/ethnicity				
White	270 (97)	286 (94)	274 (94)	278 (93)
Non-white	9 (3)	18 (6)	16 (6)	20 (7)
Smoking status				
Never	240 (86)	263 (87)	255 (88)	258 (87)
Past	27 (10)	16 (5)	19 (7)	22 (7)
Current	10 (4)	21 (7)	15 (5)	16 (5)
Multivitamin use				
Yes	257 (92)	279 (92)	261 (90)	274 (92)
No	18 (7)	23 (8)	23 (8)	22 (7)
Alcohol use				
Never	181 (65)	200 (66)	195 (67)	196 (66)
Sometimes	84 (30)	92 (30)	88 (30)	96 (32)
Frequently	9 (3)	8 (3)	4 (1)	5 (2)
Exercise				
Low	72 (26)	69 (23)	82 (28)	81 (27)
Moderate	112 (40)	132 (43)	124 (43)	110 (37)
High	95 (34)	103 (34)	84 (29)	107 (36)
Income				
<$19 999	16 (6)	29 (10)	16 (6)	30 (10)
$20 000–$39 999	63 (23)	62 (20)	75 (26)	100 (34)
$40 000–$74 999	42 (15)	50 (17)	39 (14)	43 (14)
$75 000–$99 999	38 (14)	37 (12)	40 (14)	29 (10)
>$100 000	120 (43)	126 (42)	119 (41)	96 (32)
Number of previous live births				
0	128 (46)	137 (45)	119 (41)	159 (53)
1	97 (35)	110 (36)	113 (39)	102 (34)
2	54 (19)	57 (19)	58 (20)	37 (12)
Number of previous losses				
1	192 (69)	205 (67)	177 (61)	207 (70)
2	87 (31)	99 (33)	113 (34)	91 (31)
Anti-Müllerian hormone, ng/ml				
<1.00	38 (14)	34 (11)	24 (8)	20 (7)
1.00–3.5	152 (55)	148 (49)	136 (47)	148 (50)
>3.5	88 (32)	119 (39)	127 (44)	129 (43)

[a]Values are means ± SD or N (%).
[b]Quartile ranges are Q1: 0.10–0.83; Q2: 0.83–0.94; Q3: 0.94–1.05; Q4:1.05–1.51.
Data may not sum to 1171 due to missing data.
EAGeR, Effects of Aspirin in Gestation and Reproduction.

did not include an inverse-probability weight in the model. To facilitate comparison of the EAGeR population with previous cross-sectional data, which suggests that higher parity is associated with shorter telomeres (Pollack et al., 2018), we further explored the cross-sectional association of baseline parity and leukocyte telomere length in our study population using generalized linear models adjusted for age, BMI, smoking, race/ethnicity, income, education and age at menarche. All analyses were run using SAS v9.4 (SAS Institute, Cary, NC, USA).

Results

Among 1228 women in the EAGeR trial, average age at baseline was 28.7 years (SD = 4.8). Preconception leukocyte telomere length was normally distributed in the population with a mean T/S ratio of 0.94 (SD = 0.17; range = 0.43–1.51). At baseline, younger women and women who self-identified as non-Hispanic white race/ethnicity and reported lower alcohol consumption, lower income and fewer live births had longer telomere length, on average. Women with higher AMH also had slightly longer telomere length (Table I).

During follow-up, 797 women became pregnant, 188 experienced a pregnancy loss and 597 experienced a live birth. In unadjusted and adjusted analyses, telomere length was not associated with fecundability (Q4 vs Q1, Model 1 FOR = 0.96; 95% CI = 0.76, 1.20; Model 2 FOR = 1.03; 95% CI = 0.81, 1.30). Further adjustment for age did not materially change these associations (Q4 vs Q1, Model 3 FOR = 1.00; 95% CI = 0.79, 1.27) (Table II). Similarly, the telomere length was not associated with live birth (Q4 vs Q1, Model 3 RR = 1.00; 95% CI = 0.85, 1.19) or pregnancy loss (Q4 vs Q1, Model 3 RR = 1.12; 95% CI = 0.78, 1.62) in fully adjusted models (Table III). The telomere length was not associated with fecundability, live birth, or pregnancy loss in analyses stratified by age (≥30 vs <30 years) (Table IV). Results did not substantively change when race/ethnicity was omitted from Model 3 (FOR Q4 vs Q1 FOR = 0.99; 95% CI = 0.78–1.25; live birth Q4 vs Q1 RR = 0.99; 95% CI = 0.84–1.18; pregnancy loss Q4 vs Q1 RR = 1.08; 95% CI = 0.76–1.53). Findings were also unchanged in models limited to women with complete telomere length data (n = 1171; Model 3 Q4 vs Q1 FOR = 1.02; 95% CI = 0.81–1.29; live birth Q4 vs Q1 Model 3 RR = 1.01; 95% CI = 0.86–1.19; pregnancy loss Q4 vs Q1 Model 3 RR = 1.09; 95% CI = 0.75–1.56). Unweighted models for pregnancy loss produced similar estimates to the inverse-probability weighted models (Model 3 Q4 vs Q1 RR = 1.08; 95% CI = 0.74–1.58).

In unadjusted cross-sectional analyses that treated parity as the exposure and telomere length as the outcome, compared to nulliparous women those with one prior live birth had 0.65% (95% CI = −1.47, 2.77%) shorter telomeres and those with two prior live births had 3.68% (95% CI = 1.03, 6.32%) shorter telomeres. However, after adjusting for potential confounders in multivariable models, the mean differences comparing women with one prior live birth (0.01%; 95% CI = −2.12, 2.14%) and two prior live births (2.30%; 95% CI = −0.39, 4.99%) to nulliparous women were largely attenuated.

Discussion

As women delay childbearing in the later reproductive years, identifying biomarkers to improve the prediction of pregnancy outcomes beyond chronological age could greatly improve preconception counseling, particularly among women attempting natural conception. We hypothesized that leukocyte telomere length, as a marker of biologic aging, could serve as one such biomarker. However, in this prospective study of healthy women with a history of 1–2 pregnancy losses, we did not find leukocyte telomere length to be associated with fecundability, pregnancy loss, or live birth, overall or within strata of chronologic age. Although unadjusted models suggested shorter telomeres among women with higher parity, adjustment for age, BMI and other factors largely attenuated this association. Collectively, these findings do not support a role of leukocyte telomere length, an accessible marker of biological aging, in pregnancy establishment or maintenance in a non-clinical population of women attempting to conceive.

Telomere attrition has been hypothesized to play a role in the phenotypic manifestations of reproductive aging including subfertility and pregnancy loss through increased meiotic dysfunction (Keefe et al., 2006, 2007). According to the telomere theory of reproductive senescence, telomere attrition in oocytes likely occurs through at least two mechanisms (Keefe et al., 2006, 2007). First, during oogenesis, oocytes undergo several mitotic divisions that shorten telomeres before they enter meiosis in waves. The oocytes that enter meiosis last during oogenesis, which have undergone a greater number of replication cycles, are also the last to be ovulated in adult life (Polani and Crolla, 1991).

Table II Associations of mean leukocyte telomere length and fecundability in the EAGeR trial.

		Model 1[a]			Model 2[b]			Model 3[c]		
	N[d]	FOR	95% CI		FOR	95% CI		FOR	95% CI	
Per 1k base pairs	797	1.01	0.83	1.23	1.07	0.87	1.30	1.04	0.85	1.27
Quartile 1 (median = 0.75)	195	1	1	1	1	1	1	1	1	1
Q2 (0.89)	203	0.98	0.78	1.23	0.98	0.78	1.24	0.99	0.79	1.24
Q3 (0.99)	202	1.04	0.82	1.31	1.04	0.82	1.32	1.03	0.82	1.31
Q4 (1.14)	197	0.96	0.76	1.20	1.03	0.81	1.30	1.00	0.79	1.27

[a]Unadjusted model.
[b]Multivariable Model 2 adjusted for race/ethnicity, BMI, number of previous losses, number of previous live births, smoking, alcohol consumption, marital status, income and multivitamin use.
[c]Multivariable Model 3 adjusted for Model 2 covariates + age.
[d]Pregnancies.

Table III Associations of mean leukocyte telomere length with pregnancy loss and live birth in the EAGeR trial.

		Model 1[a]			Model 2[b]			Model 3[c]		
	N[d]	RR	95% CI		RR	95% CI		RR	95% CI	
Live birth										
Per 1k BP	597	1.03	0.89	1.19	1.04	0.91	1.20	1.02	0.89	1.18
Quartile 1	147	1	1	1	1	1	1	1	1	1
Q2	153	0.99	0.84	1.17	0.96	0.82	1.13	0.95	0.81	1.12
Q3	150	1.02	0.86	1.21	0.99	0.84	1.16	0.98	0.83	1.15
Q4	147	0.99	0.83	1.18	1.02	0.87	1.21	1.00	0.85	1.19
	N[e]	RR	95% CI		RR	95% CI		RR	95% CI	
Pregnancy loss[f]										
Per 1k BP	188	1.10	0.80	1.51	1.08	0.78	1.49	1.13	0.82	1.56
Quartile 1	43	1	1	1	1	1	1	1	1	1
Q2	49	1.04	0.71	1.50	1.01	0.70	1.47	0.99	0.69	1.44
Q3	49	1.13	0.78	1.63	1.11	0.77	1.61	1.11	0.77	1.61
Q4	47	1.10	0.76	1.59	1.07	0.74	1.54	1.12	0.78	1.62

[a]Unadjusted model.
[b]Multivariable Model 2 adjusted for race/ethnicity, BMI, number of previous losses, number of previous live births, smoking, alcohol consumption, marital status, income, multivitamin use.
[c]Multivariable Model 3 adjusted for Model 2 + age.
[d]Live births.
[e]Pregnancy losses.
[f]Models include inverse-probability weights to account for selection on pregnancy (n = 797).

Second, reactive oxygen species induce DNA damage that shortens telomeres in the years between oogenesis and ovulation (Liu *et al.*, 2002; Passos and von Zglinicki, 2005). The resulting shortened telomeres of oocytes present in the ovarian cortex during the later reproductive years may contribute to reduced chiasmata, spindle dysmorphology, disrupted chromosome recombination, and genetic abnormalities in embryos, leading to subfertility, pregnancy loss and lower likelihood of live birth.

Despite biological plausibility, epidemiologic studies on telomere length and reproductive outcomes are somewhat sparse and yield conflicting results. In the present study of healthy women attempting natural conception, we did not find any associations of preconception leukocyte telomere length with fecundability, pregnancy loss, or live birth. Several studies in clinical populations, however, reported associations of telomere length or telomerase, the polymerase responsible for telomere maintenance, with IVF treatment (Czamanski-Cohen *et al.*, 2015) and IVF outcomes, including oocyte quality (Cheng *et al.*, 2013), embryonic aneuploidy (Hanson *et al.*, 2021). embryo fragmentation (Keefe *et al.*, 2005; Cheng *et al.*, 2013) and clinical pregnancy (Wang *et al.*, 2014), as well as recurrent miscarriage (Hanna *et al.*, 2009; Thilagavathi *et al.*, 2013). For example, in a small case–control study (n = 30), women undergoing IVF had significantly shorter lymphocyte telomere length than healthy controls (Czamanski-Cohen *et al.*, 2015). In two other small studies among IVF patients, longer telomere length measured in oocytes and cumulus cells were associated with less embryo fragmentation and better oocyte quality (Keefe *et al.*, 2005; Cheng *et al.*, 2013). More recently, another study (n = 175) reported that shorter white blood cell, but not cumulus cell, telomere length was associated with embryonic aneuploidy (Hanson *et al.*, 2021). Likewise, women with recurrent miscarriage had shorter telomeres compared to healthy controls in two additional small case–control studies (Hanna *et al.*, 2009; Thilagavathi *et al.*, 2013). Granulosa cell telomerase activity, but not telomere length, was associated with a higher clinical pregnancy rate in another study of 76 women presenting for IVF (Wang *et al.*, 2014).

The findings of these studies cannot be directly compared with ours but imply that shorter telomeres may be detrimental to pregnancy outcomes. Because women in the EAGeR trial had demonstrated fecundity and were not seeking fertility treatment, many of the intermediate outcomes (e.g. oocyte and embryo quality) evaluated in previous studies are unobservable in a population of women attempting natural conception such as ours. We cannot rule out the possibility that leukocyte telomere length may be related to oocyte and embryo quality in healthy women, but any influence on these intermediate parameters does not translate to reduced fecundability or likelihood of live birth, or higher risk of pregnancy loss, according to our results.

In cross-sectional analyses, we also observed slightly shorter telomeres among women with baseline higher parity among participants of the EAGeR population, though this association was attenuated after adjustment. Similarly, Gray *et al.* reported shorter telomeres among postmenopausal women with higher parity in crude analyses; however, adjusted models were not presented (Gray *et al.*, 2014). Among women in the cross-sectional National Health and Nutrition Examination Survey (NHANES) study, leukocyte telomere length of parous women was ~6000 base-pairs shorter than nulliparous women after adjusting for age, BMI, smoking and other factors (Pollack *et al.*, 2018). Finally, a prospective study of 75 Mayan women reported a positive association of number of surviving children and leukocyte telomere length (Barha *et al.*,

Table IV Associations of mean leukocyte telomere length and pregnancy outcomes stratified by age in the EAGeR trial.

	<30 years				≥30 years			
		Model 3[a]				Model 3[a]		
Fecundability	N[b]	FOR	95% CI		N[b]	FOR	95% CI	
Quartile (Q)1	112	1	1	1	83	1	1	1
Q2	123	1.04	0.77	1.42	80	0.91	0.63	1.30
Q3	128	1.04	0.77	1.40	74	0.96	0.66	1.40
Q4	144	0.95	0.71	1.28	53	1.12	0.75	1.67
	N[c]	RR	95% CI		N[c]	RR	95% CI	
Live birth								
Q1	87	1	1	1	60	1	1	1
Q2	95	0.98	0.80	1.20	58	0.91	0.70	1.19
Q3	98	1.01	0.83	1.23	52	0.90	0.69	1.19
Q4	113	1.04	0.85	1.27	34	0.92	0.68	1.25
	N[d]	RR	95% CI		N[d]	RR	95% CI	
Pregnancy loss[e]								
Q1	22	1	1	1	21	1	1	1
Q2	28	1.25	0.71	2.20	21	0.86	0.49	1.50
Q3	28	1.36	0.78	2.37	21	1.11	0.64	1.91
Q4	28	1.22	0.71	2.11	19	1.21	0.69	2.13

[a]Model 3 adjusted for age, race/ethnicity, BMI, number of previous losses, number of previous live births, smoking, alcohol consumption, marital status, income, multivitamin use.
[b]Pregnancies.
[c]Live births.
[d]Pregnancy losses.
[e]Models include inverse-probability weights to account for selection on pregnancy.

2016). Differences in findings between our study population and others may relate, in part, to differences in the study populations themselves, particularly with respect to reproductive history and the underlying age distribution of participants. For example, the mean age of EAGeR participants at baseline was 28.7 years (range = 18.7–40.1), and only 12% were older than 35 years. In contrast, the age of women included in the NHANES analysis ranged from 20 to 44 years, with 37% older than 35 years. Furthermore, EAGeR women had a history of 1–2 pregnancy losses, which could be stressful events that shorten telomeres. Indeed, EAGeR participants had a shorter mean telomere length in our population (nulliparous women mean T/S ratio = 0.95; parous women mean T/S ratio = 0.93) than in NHANES women (nulliparous women mean T/S ratio = 1.17; parous women mean T/S ratio = 1.10) (Pollack *et al.*, 2018).

Strengths of our study include a large sample size and prospective design with preconception measurements of leukocyte telomere length. Furthermore, women enrolled in the EAGeR trial were healthy and had demonstrated fecundity, improving generalizability to women attempting pregnancy without the assistance of fertility treatment. Additionally, the collection of daily urine samples during active follow-up, coupled with augmented β-hCG testing, allowed for the earliest possible detection of pregnancies and pregnancy losses. Finally, follow-up in the EAGeR trial was high (89%), reducing the potential for selection bias arising from loss to follow-up.

Our study also has limitations. First, we quantified telomere length in leukocytes rather than oocytes or cumulus or granulosa cells, which are not accessible among women attempting natural conception. Measurement of granulosa cell, cumulus cell, or oocyte telomere length requires women to undergo egg retrieval, an invasive procedure involving ovarian stimulation, multiple monitoring visits and general anesthesia. In contrast, leukocyte telomere length can be measured non-invasively with a standard blood collection and would, therefore, be a potentially useful biomarker in women seeking unassisted conception. However, telomere length in leukocytes may not reflect telomere length in granulosa or cumulus cells (Lara-Molina *et al.*, 2020) or oocytes, which cannot be measured non-invasively, but may be the more relevant tissues of interest. Second, women in the EAGeR study were relatively young, limiting our ability to evaluate telomere length and pregnancy outcomes at the older end of the age range. Third, residual confounding in measured or unmeasured factors is possible, as in any observational study. Finally, our study sample included women with 1–2 prior pregnancy losses and excluded women with a history of infertility treatment and those with conditions associated with infertility, which importantly extends the literature by examining the relation between telomere length and pregnancy outcomes in a population of women with demonstrated fecundity. Nevertheless, these eligibility criteria may limit generalizability to women with a similar reproductive history, particularly considering the shorter telomere length in our population relative to the NHANES population (Pollack *et al.*, 2018). Relatedly, women in the EAGeR trial identified mostly as non-Hispanic white, limiting our ability to examine associations in other racial and ethnic groups. Because telomere length varies by race/ethnicity (Diez Roux *et al.*, 2009), likely resulting from different cumulative lifetime stressors that affect telomere length, and disparities in fertility and adverse pregnancy outcomes exist (Huddleston *et al.*, 2010; Mukherjee *et al.*, 2013), future studies should examine these relations in more diverse cohorts.

In conclusion, our findings do not support an association of preconception leukocyte telomere length with fecundability, pregnancy loss, or live birth. As such, leukocyte telomere length, a measure of biologic aging, is unlikely to be a useful preconception marker of reproductive outcomes among women attempting natural conception with a history of pregnancy loss.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Authors' roles

E.F.S., R.M.S. and S.L.M. designed and executed the study. A.C.P.-S. and S.L.M. conducted data analyses and drafted the manuscript. E.F.S., R.M.S., S.L.M., K.K., V.C.A., A.Z.P. and L.A.S. revised the manuscript and participated in the critical discussion of the intellectual content.

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Conflict of interest

The authors have no conflicts of interest to declare.

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