# **Reproduction predicts shorter telomeres and epigenetic age acceleration among young adult women**

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**Abstract**

Evolutionary theory predicts that energy invested into reproduction comes at the expense of somatic maintenance, thereby accelerating aging. Despite support from studies in human and non-human animals, mechanisms linking costs of reproduction (CoR) to senescence remains scarce. Pregnancy entails massive physiological and immunological shifts capable of accelerating aging through mitotic or non-mitotic cellular pathways - or both. Telomeres are repetitive DNA sequences that cap chromosomes and shorten with cell replication, and telomere length (TL) provides an index of mitotic age. DNA-methylation age (DNAmAge) is marker of non-mitotic cellular aging correlated with chronological age, but is accelerated in response to physiological and immunological stress. Shorter TL and accelerated DNAmAge both predict age-related morbidity and mortality. To elucidate mechanisms underpinning CoR, we examined the relationship between parity, TL (n=821), and DNAmAge (n=397) in young (20-22 year-old) Filipino women. TL decreased (p=0.016) and DNAmAge increased (p=0.011) with parity, an effect that was not contingent upon resource availability. Neither biomarker predicted subsequent fertility, supporting a causal effect of parity on aging (p>0.4 for both). Consistent with prior work, TL and DNAmAge were uncorrelated, but appear to represent distinct aspects of senescence, both exhibiting patterns of premature aging with reproduction among these young women.**Introduction**

Evolutionary theory predicts that energy expenditure in the form of reproductive effort comes at the expense of somatic maintenance and lifespan (1). Because resources are finite and selection favors early life fecundity over late life functional decline (2), reproduction is expected to carry a ‘cost’ in the form of mortality risk, senescence, and functional decline (3). Direct ‘costs of reproduction’ (CoR) have been demonstrated among animal models, whereby reproduction hastens senescence (4, 5). Conversely, selection for late life fecundity results in lifespan extension (6, 7). In humans, CoR has been predominantly studied through the use of historical datasets. This research suggests that increased reproductive effort is often associated with a shortening of lifespan (8–12, but see 13), and that these costs are more evident when resources are limited (14–16). However, most studies of CoR in humans are restricted to the use of mortality as the only outcome, and are unable to address the underlying biological processes through which CoR might lead to functional decline and mortality in humans.

Among women, CoR likely arise predominantly from pregnancy and lactation (41, 42). Human pregnancy is relatively ‘invasive’ and energetically demanding (ref.), and requires massive physiological and immunological modifications capable of accelerating senescence. Pregnancy induced cellular senescence could occur through either mitotic and non-mitotic pathways, or both (ref.). Mitotic senescence is commonly measured using telomere length (TL). Telomeres are non-coding DNA sequences that cap chromosomes, and are required for cell division and survival (17, 18). Telomere length shortens with cell division and chronological age, placing a limit on the number of cell divisions (19–21). Shorter TL, controlling for age, in turn predicts higher morbidity and mortality rates (22–25).

A powerful and emerging marker of non-mitotic senescence in human cells is epigenetic age (DNAmAge). DNAmAge in human (27) and non-human genomes (refs) is calculated from methylation at a species-specific subset of cytosine-guanine dyads (CpGs), and is strongly correlated with chronological age (26). Independent of a host of associated risk factors in humans, accelerated DNAmAge relative to chronological age is associated with elevated risks for morbidity and mortality (28–30). Vital to capitalizing on epigenetic age as a marker of non-mitotic senescence, accelerated DNAmAge predicts senescence and mortality independently of TL in living humans (31, 32), and independently of both TL and the DNA damage response in vitro (27, 31).

Human pregnancy could generate costs to female health and lifespan by shortening TL (mitotic age), accelerating DNAmAge (non-mitotic age), or both. During pregnancy, blood cells proliferate to compensate for fluid volume expansion (43, 44), and the female body shifts towards a pro-inflammatory but immunocompromised state (45–47). Data from cell culture, rodent based experiments, and clinical studies show that inflammation and infection increase cell proliferation and DNA damage, both expected to accelerate the pace of telomere shortening (48–55). Accelerated DNAmAge relative to chronological age has been observed in other pro-inflammatory contexts (40, 56), and with menopause (57), an important physiological and life history transition in human females. DNAmAge acceleration arising from menopause, whether naturally-occurring or surgically-induced, was attenuated by hormone therapy (57),m, suggesting that physiological and hormonal changes like those accompanying pregnancy could have profound effects on DNAmAge. While two studies have recently examined TL and pregnancy with mixed results (refs), none have attempted to test for CoR in humans using mitotic and non-mitotic measures of senescence simultaneously. While numerous studies do support CoR on human aging (ref. Ziomewics, Jasienska), examining multiple pathways of senescence simultaneously may be necessary.

Here, we test for human CoR using mitotic (TL) and non-mitotic (DNAmAge) measures of cellular senescence. We test three inter-related hypotheses in a relatively young cohort (age 20-22) of women in the Philippines. First, we ask whether pregnancy history increases mitotic or non-mitotic measures of cellular senescence, or both (H1). We also ask whether any associations between reproductive history and senescence are stronger among women of lower socioeconomic status, for whom resource constraints are highest (H2). Finally, we test a more causal effect of pregnancy on TL and DNAmAge by examining the effect of both measures on the number of pregnancies over the subsequent 4 years (H3).

**Results**

Our sample consisted of relatively young women (21.7 ± 0.4 years old) with a range of reproductive histories. While women who had never been pregnant formed the largest group (n=507; 61.7%), women having experienced one (n=174; 21.2%), two (n=102; 12.4%), and three (n=28; 3.4%) pregnancies were well represented. A small subset of women had experienced four (n=7; 0.8%) and five (n=3; 0.4%) pregnancies. Because our models did not differ qualitatively when grouping women with three or more pregnancies, we retained the more conservative models using the full natural variation in parity in our analyses. DNAmAge among our sample was slightly accelerated relative to chronological age, while age-corrected TL was longer than expected (). Consistent with their independent roles in cellular senescence, DNAmAge and TL were uncorrelated with each other including () or not including () chronological age corrections.

Both mitotic (TL) and non-mitotic (DNAmAge) measures of cellular senescence increased with the number of pregnancies in a woman’s reproductive history (H1). The effects of parity on cellular senescence were also relatively robust – in nested models controlling for a range of potential confounders, effects sizes for pregnancy number remained stable or increased (). Each additional pregnancy was associated with between 0.34-3.67 years of telomere aging, and between 0.29-0.63 years of DNAmAge acceleration (). Effect sizes and p-values were largely unchanged when women with three or more pregnancies were grouped and analyzed together (). In contrast with reproductive history, being currently pregnant was associated with a decrease in DNAmAge. This effect attenuated the influence of parity on DNAmAge, evident from the considerable increase in the effect size of parity on DNAmAge in models 7 and 8.

Contrary to our prediction, the effect of parity on our measures of cellular senescence did not differ among women of low socioeconomic status, for whom resource constraints are highest (TL: p=0.35; DNAmAge: p=0.37). Furthermore, neither measure of cellular senescence at the time of measurement (2005) predicted the number of pregnancies over the subsequent four years (2005-2009), whether or not we controlled for baseline parity in 2005 ().

**Discussion**

TL and DNAmAge, measures of mitotic and non-mitotic cellular senescence, respectively, were both associated with reproductive history in our sample of young women. The senescent effect of parity was relatively robust to a number of potential confounders, and did not appear to be mediated by constraints imposed by socioeconomic status. Moreover, neither measure was predictive of parity over the subsequent 4 years, supporting a causal effect of pregnancy on cellular senescence, rather than vice versa.

Although consistent with theoretical predictions and non-human animal work, this is the first study to our knowledge to examine both mitotic and non-mitotic measures of cellular senescence in humans. While parity predicted senescence for both TL and DNAmAge in our study, several recent studies of CoR in women did not find such a relationship using TL alone. The first, conducted among 75 Guatamalan Maya women, reported a positive association between TL and number of surviving offspring over a 13-year time period (58). TL in that study was determined using a combination of saliva- and buccal-derived DNA samples, which unfortunately have not been consistently associated with chronological age (67–69). Furthermore, two separate measures of TL in that study were uncorrelated within individuals between the two timepoints, making comparisons between these findings and our own blood-derived TL findings difficult. Contrasting our findings and those of Barha et al., a study among 620 participants of the US-based CARDIA study found no evidence for an effect of parity on TL in either direction (59). The reasons for such mixed findings are unclear, but may relate to pronounced differences in the age ranges and socio-ecological conditions in the different populations under investigation. TL attrition occurs more rapidly at younger ages (71), suggesting that any impacts of reproduction on TL shortening could be amplified among young women, especially if reproduction overlaps with late stages of somatic growth (1, 72). Whether or not the women in our study will ‘recover’ for these measures of cellular senescence remains an open question.

While we found significant evidence for an effect of parity on both TL and DNAmAge, both appear to reflect different biological pathways linking reproductive effort with somatic senescence. Congruent with this interpretation is the observation that TL and DNAmAge measured in the same individuals have been independently associated with aging and mortality in prior studies (32, 73), and capture distinct dimensions of cellular senescence (31). Accordingly, TL and DNAmAge acceleration were not associated with each other in this study. Also notable is our finding that DNAmAge was slightly elevated relative to chronological age at Cebu, while TLs were longer and show evidence for a slightly slower attrition rate relative to other populations (Table S1). Accelerated TL attrition – a measure of ‘mitotic age’ that is modified directly by cellular division – could stem from factors that modify cellular proliferation rates, such as the elevated inflammation, blood cell production, and cell-turnover rates, all of which that characterize pregnancy in this and other samples (74).

In contrast to TL, DNAmAge is not considered a marker of mitotic age, as it is associated with chronological age even in immortal, non-dividing, and non-proliferative tissues and cells (27). Although the biological significance of DNAmAge is unknown, it is hypothesized to reflect the integrity of an epigenetic maintenance system, itself responsible for maintaining dynamic regulatory stability within cells (27). In light of the relationship between DNAmAge and epigenetic maintenance, our findings are consistent with the prediction that reproduction comes at the expense of ‘maintenance’ – in this case at the scale of cellular regulatory maintenance. Exactly how parity might lead to DNAmAge acceleration is unclear, but could involve tradeoffs between protein homeostasis and epigenetic control arising from immune activation or the buffering of oxidative stress (75–78). Indeed, cumulative changes in immune cell composition during pregnancy also likely contribute to DNAmAge acceleration with parity, although the measure of DNAmAge used here is remarkably robust across tissue types (27). Nevertheless, the fact that the functionally-distinct measures of TL and DNAmAge show similar associations with parity provides strong support for our prediction that reproduction accelerates senescence, at least among the young adult women represented by our sample.

Contrary to our prediction that the costs of reproduction would be greatest among individuals with limited resources (14–16), we found no evidence for an interaction between parity and SES in models predicting either TL or DNAmAge acceleration. While women in low SES conditions in our study very likely experience constraints in time, energy, or nutrient availability, it is still unclear to what extent SES adequately captures limitations in the resources most relevant to CoR. Given the relatively young age of the participants, however, it is possible that any moderating effect of resource limitations will only emerge at more advanced ages. SES in this population may also index factors other than resource availability that contribute to accelerated aging, such as less healthful diets or decreases in physical activity, although neither TL or DNAmAge were associated with SES in our models.

Importantly, neither measure of cellular senescence obtained in 2005 predicted parity over the subsequent 4 years (2005-2009). This argues against a life-history framework whereby ‘pace-of-living’ as captured by TL and DNAmAge is itself predictive of future fecundity. Intriguingly, currently pregnant women exhibited significantly ‘younger’ DNAmAge. These results could reflect the suite of immunological and physiological shifts that occur during pregnancy, such as the protective effects of estrogens on DNAmAge (57, 63)REMOVE 63 . Notably, pregnancy status may be an important confounder to include in future studies investigating the costs of reproduction in women.

Our findings, though relatively robust, should be considered in the context of several limitations inherent in our study. While we attempt to control for socio-ecological factors that could affect both parity and our biomarkers of aging, confounding arising from differences in health and/or resources remains a possibility. Although there was no strong evidence for confounding effects in our data, confounding may explain the slight decrease in effect size of parity between models 1 and 2 (TL) and 5 and 6 (DNAmAge). Future studies employing a longitudinal measures of TL and DNAmAge acceleration would minimize the potential effects of such confounders (79), while modeling lactation and other indices for reproductive effort might provide a more accurate estimate of the CoR (66, 80). Finally, the women in this study all fell within a relatively narrow age range during young adulthood (20-22 years old). Because both TL and DNAmAge ‘age’ more rapidly early in adulthood (27, 71), it is possible that both measures are particularly sensitive to reproduction at this time. Multiple measures of TL and DNAmAge for each individual that capture the ‘pace’ of mitotic and non-mitotic cellular senescence over time will again prove important to resolving such effects.

In sum, our study suggests that parity is associated with shorter telomeres and epigenetic age, measures of mitotic and non-mitotic aging, respectively, among the young women in our sample. The consistent relationships between parity and aging in two distinct pathways—one reflecting cellular turnover and genomic stability, and the second a marker of epigenomic stability—support a cost of reproduction from pregnancy in humans.

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**Supplementary Information**

**Table S1. Comparison of TL across ethnicities.** qPCR measured TL does not permit comparisons across populations, but 190 samples from the population for which the samples from this paper come from have had southern blot TL measured (Eisenberg et al. 2015). The average age of this sample is 35.96 (± 15.83, range 21.02-68.33), with an average TL of 7.86 kb (± 0.76), and an estimated age related decline in TL of 21.98 bp/year (95% CI 15.81-28.15). This age related decline is non-significantly less than that found in other populations (African=27.7 bp/year; African Americans = 25.6 bp/year; Europeans = 27.3 bp/year) (Hansen et al. 2016). These three populations from Hansen et al. (2016) have an average age of 43.25. Adjusting the Cebu population TL for the 7.29 years younger they are by assuming the observed 21.98 bp/year attrition rates yields an interpolated TL of 7.70 kb if the mean age was 43.25, the same as the samples in Hansen et al. (2016). This suggests that the TL in Cebu is longer than that of European and African Americans, but slightly shorter than that of Africans (Table S1).

|  |  |  |  |
| --- | --- | --- | --- |
| **Ethnicity (N)** | **% Female** | **Age (years)** | **LTL (kb)** |
| Europeans (90)\* | 63.3 | 43.9 (18-78) | 7.21 (5.39-9.42) |
| African Americans (97)\* | 67.1 | 42.9 (21-79) | 7.45 (5.55-9.16) |
| Africans (100)\* | 68.0 | 43.0 (18-79) | 7.85 (5.64-10.13) |
| Cebu, Philippines (190) | 50.3 | 43.3~ | 7.70 (6.10-9.21) |

\* from Hansen MEB, et al. (2016) Shorter telomere length in Europeans than in Africans due to polygenetic adaptation. *Human Molecular Genetics*.

†from Eisenberg DT, Kuzawa CW, Hayes MG (2015) Improving qPCR telomere length assays: Controlling for well position effects increases statistical power. *Am J Hum Biol* 27(4):570–5.

~ interpolated to match mean age of other populations - see above text

**Table S2. Full model estimates for parity and telomere length and epigenetic age (DNAmAge) among young women in the Philippines.**

|  | **Telomere Length~** | | | | **DNAmAge~** | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) |
| **Age** | -0.047 | -0.028 | -0.027 | -0.028 | 0.485 | 0.667 | 0.629 | 0.618 |
|  | p = 0.004\*\* | p = 0.080+ | p = 0.086+ | p = 0.079+ | p = 0.293 | p = 0.157 | p = 0.179 | p = 0.188 |
| **No.Pregnancies** | -0.013 | -0.012 | -0.016 | -0.018 | 0.363 | 0.326 | 0.437 | 0.491 |
|  | p = 0.030\* | p = 0.044\* | p = 0.016\* | p = 0.010\*\* | p = 0.026\* | p = 0.049\* | p = 0.011\* | p = 0.007\*\* |
| **PC1** |  | -0.426 | -0.437 | -0.438 |  | -11.623 | -10.481 | -10.457 |
|  |  | p = 0.060+ | p = 0.054+ | p = 0.054+ |  | p = 0.084+ | p = 0.117 | p = 0.118 |
| **PC2** |  | -0.155 | -0.144 | -0.131 |  | 5.098 | 4.679 | 4.207 |
|  |  | p = 0.499 | p = 0.530 | p = 0.568 |  | p = 0.458 | p = 0.494 | p = 0.540 |
| **PC3** |  | 0.013 | 0.002 | 0.008 |  | 8.510 | 9.075 | 8.853 |
|  |  | p = 0.955 | p = 0.992 | p = 0.972 |  | p = 0.202 | p = 0.172 | p = 0.183 |
| **PC4** |  | -0.024 | -0.015 | -0.022 |  | 11.991 | 11.495 | 11.551 |
|  |  | p = 0.917 | p = 0.949 | p = 0.922 |  | p = 0.069+ | p = 0.080+ | p = 0.078+ |
| **PC5** |  | -0.252 | -0.251 | -0.244 |  | -14.123 | -14.441 | -14.157 |
|  |  | p = 0.277 | p = 0.279 | p = 0.291 |  | p = 0.023\* | p = 0.020\* | p = 0.022\* |
| **PC6** |  | -0.293 | -0.304 | -0.294 |  | -4.002 | -3.125 | -3.748 |
|  |  | p = 0.205 | p = 0.189 | p = 0.204 |  | p = 0.548 | p = 0.637 | p = 0.574 |
| **PC7** |  | -0.497 | -0.529 | -0.531 |  | 18.513 | 19.744 | 20.019 |
|  |  | p = 0.034\* | p = 0.025\* | p = 0.024\* |  | p = 0.008\*\* | p = 0.005\*\* | p = 0.004\*\* |
| **PC8** |  | 0.426 | 0.428 | 0.433 |  | 3.987 | 3.796 | 3.691 |
|  |  | p = 0.062+ | p = 0.061+ | p = 0.058+ |  | p = 0.534 | p = 0.552 | p = 0.563 |
| **PC9** |  | -0.457 | -0.476 | -0.481 |  | -1.730 | -1.521 | -1.534 |
|  |  | p = 0.042\* | p = 0.035\* | p = 0.033\* |  | p = 0.789 | p = 0.813 | p = 0.812 |
| **PC10** |  | 0.559 | 0.548 | 0.541 |  | -2.701 | -1.976 | -1.672 |
|  |  | p = 0.020\* | p = 0.022\* | p = 0.024\* |  | p = 0.690 | p = 0.769 | p = 0.804 |
| **SES-score** |  | -0.006 | -0.006 | -0.004 |  | -0.180 | -0.190 | -0.271 |
|  |  | p = 0.140 | p = 0.158 | p = 0.395 |  | p = 0.146 | p = 0.123 | p = 0.075+ |
| **Urbanicity-score** |  | 0.002 | 0.002 | 0.003 |  | 0.015 | 0.016 | 0.015 |
|  |  | p = 0.00000\*\* | p = 0.00000\*\* | p = 0.00000\*\* |  | p = 0.270 | p = 0.260 | p = 0.275 |
| **Currently Pregnancy** |  |  | 0.038 | 0.038 |  |  | -1.223 | -1.211 |
|  |  |  | p = 0.080+ | p = 0.079+ |  |  | p = 0.018\* | p = 0.019\* |
| **No.Pregnancies\*SES-score** |  |  |  | -0.004 |  |  |  | 0.111 |
|  |  |  |  | p = 0.354 |  |  |  | p = 0.365 |
| **Intercept** | 1.816 | 1.315 | 1.303 | 1.314 | 14.818 | 10.319 | 11.177 | 11.422 |
|  | p = 0.00000\*\* | p = 0.0002\*\* | p = 0.0002\*\* | p = 0.0002\*\* | p = 0.138 | p = 0.318 | p = 0.276 | p = 0.266 |
| Observations | 824 | 824 | 824 | 824 | 397 | 397 | 397 | 397 |
| R2 | 0.017 | 0.080 | 0.083 | 0.084 | 0.016 | 0.075 | 0.089 | 0.091 |
| Adjusted R2 | 0.015 | 0.064 | 0.066 | 0.066 | 0.011 | 0.041 | 0.053 | 0.052 |
| Residual Std. Error | 0.161 (df = 821) | 0.157 (df = 809) | 0.157 (df = 808) | 0.157 (df = 807) | 3.165 (df = 394) | 3.117 (df = 382) | 3.098 (df = 381) | 3.098 (df = 380) |
| F Statistic | 7.089\*\* (df = 2; 821) | 5.007\*\* (df = 14; 809) | 4.892\*\* (df = 15; 808) | 4.639\*\* (df = 16; 807) | 3.220\* (df = 2; 394) | 2.214\*\* (df = 14; 382) | 2.475\*\* (df = 15; 381) | 2.371\*\* (df = 16; 380) |

**Table S3. Predictive effect of Telomere Length (TL) or Epigenetic Age (DNAmAge) on parity over the subsequent 4 years in young women in the Philippines.** Models derived from Poisson generalized linear regression meeting assumptions of equidispersion.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Parity 2005-2009** | | | |
|  | **Age Adjusted TL~** | | **Age Adjusted DNAmAge**~ | |
|  | (1) | (2) | (3) | (4) |
| Measurement time bt. 2005-2009 (Days) | -0.003 | -0.003 | -0.002 | -0.002 |
|  | p = 0.004\*\* | p = 0.009\*\* | p = 0.058+ | p = 0.068+ |
| Parity in 2005 |  | 0.252 |  | 0.123 |
|  |  | p = 0.000\*\* |  | p = 0.016\* |
| Age Adjusted Telomere Length in 2005 | 0.059 | 0.148 |  |  |
|  | p = 0.835 | p = 0.601 |  |  |
| Age Adjusted DNAmAge in 2005 |  |  | -0.011 | -0.016 |
|  |  |  | p = 0.483 | p = 0.325 |
| Intercept | 4.457 | 3.777 | 3.460 | 3.265 |
|  | p = 0.006\*\* | p = 0.022\* | p = 0.062+ | p = 0.082+ |
| Observations | 743 | 743 | 397 | 397 |
| Log Likelihood | -836.740 | -818.205 | -485.276 | -482.433 |
| Akaike Inf. Crit. | 1,679.481 | 1,644.411 | 976.552 | 972.866 |
| *Note:* | +p<0.1;\*p<0.05;\*\*p<0.01;\*\*\*p<0.001 | | | |