OBSTETRICS

The telomere gestational clock: increasing short telomeres at term in the mouse

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BACKGROUND: The biologic mechanism(s) regulating the length of gestation are currently poorly understood. After peaking at the blastocyst stage, the average telomere lengths have been reported to shorten during the remainder of gestation in the placenta and fetal membranes in both human and mouse pregnancies, thereby providing a potential countdown biologic clock. These previous studies have reported changes in the average telomere lengths, whereas it has now been shown that the shortest telomeres, not the average telomere lengths, are the mediators of telomere dysfunction which limits cellular survival and results in aging.

OBJECTIVE: These studies sought to assess for the first time a significant increase in short telomeres in the fetal membrane and placental tissue near the end of pregnancy in the mouse.

STUDY DESIGN: Placental and fetal membrane tissues were harvested from timed-pregnant CD-1 mice on gestational days 14—18 prior to the onset of parturition. Telomere lengths were determined for 30 DNA samples (5 each for gestational days 14, 16, and 18 from placentas and fetal membranes) using a commercial high-throughput quantitative fluorescence in situ hybridization technique. Quantitative measurements of representative short telomeres (ie, 3 kb and 5 kb telomere fragments) were performed for 29—30 DNA samples (4—7 each for gestational days 14, 15, 16, 17, and 18 from placentas, fetal membranes, and maternal liver) using a real-time quantitative polymerase chain reaction modification of the classic telomere restriction fragment technique.

RESULTS: The median telomere lengths of fetal membrane tissue decreased from gestational days 14—18 (18,705—16,364 kb) and were significantly shorter than telomeres in placental tissue ($P < .05$). Representative histograms for the distribution of telomere lengths in mouse fetal membranes (as shown in the Figure) confirm a curve skewed to the left (toward shorter telomere lengths).The relative quantity of the representative short telomeres (ie, 3 kb and 5 kb fragments) increased significantly as gestation progressed in both placenta and fetal membrane tissue. In gestational day 18 fetal membranes, the relative quantity of 3 kb and 5 kb telomeres increased 5.5-fold and 9.3-fold compared with gestational day 14 tissues ($P < .05$). In placental tissue the relative quantity of 3 kb and 5 kb telomeres increased 9.3-fold and 7.8-fold compared with gestational day 14 tissues ($P < .05$). Studies performed using adult liver tissue demonstrated little variation of the representative short telomeres and no significant difference between the nonpregnant and pregnant samples.

CONCLUSION: These mouse studies have demonstrated that the distribution of telomere lengths in fetal membrane and placental tissues are skewed toward shorter lengths and that the quantity of representative short telomeres increase significantly prior to parturition. The telomere gestational clock is a novel hypothesis supported by several preliminary mouse studies and interesting associations in human pregnancies between maternal conditions and telomere lengths. (eg, stress, education, pollution, neighborhood quality, and race). As such, the current hypothesis generating study provides a foundation for future research regarding the potential role for a telomere-based biologic clock that determines gestational length in human and other mammalian pregnancies.

Key words: biologic clock, duration of pregnancy, parturition (labor), placental aging, pregnant CD-1 mouse, telomerase, inflammation

The cellular and molecular mechanism(s) determining the length of gestation and the timing for the onset of labor (parturition) remain poorly understood. Although much is known regarding the contributions of estrogens, progesterone, corticosteroids, prostaglandins, and oxytocin in regard to the onset of parturition, none of these endocrine observations appears to provide an explanation for the biologic clock that defines the length of pregnancy or the timing for the onset of labor (for a review of the endocrine events during parturition, see Christiaens et al[1]). Even in rodents, in which the role of prostaglandin-induced luteolysis leading to the fall in progesterone at term has been well described,[2] the biologic clock triggering the increase in prostaglandins remains unknown.

Progressive telomere shortening has been observed to be a key component in the multifaceted biologic clock that determines overall life span in the adult[3,4]; therefore, it is biologically plausible that telomere shortening could also serve a similar role in determining the life span of gestational tissues (ie, the placenta and fetal membranes) and their subsequent aging at term.

In recent reviews,[5,6] Phillippe has described a substantial body of published evidence supporting a telomere gestational clock hypothesis. Although several previous publications have described a possible link between telomere shortening and the onset of parturition, this hypothesis is novel because it proposes for the first time a well-defined signaling pathway between the shortening of telomeres in the placenta and fetal membranes leading to apoptosis of the cells in these tissues, the release of increasing amounts of cell-free DNA as these cells undergo apoptosis, and the subsequent stimulation of a proinflammatory response mediated by cell-free DNA activation of Toll-like receptor 9 (TLR9). Thus, this hypothesized telomere-activated signaling pathway provides a possible countdown biologic clock mechanism that could stimulate the proinflammatory events

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AJOG at a Glance

Why was this study conducted?

Based on the functional importance of short telomeres in regard to cellular senescence and aging, the studies described in this report sought to assess for the first time the relative increase in short telomeres and their modulation in gestational tissues during late pregnancy in the mouse.

Key findings

Quantitative fluorescence in situ hybridizations studies were performed to determine the distribution of the telomere sizes and a quantitative polymerase chain reaction modification of the classic telomere restriction fragment assay was used to quantify the increase in short telomeres in DNA extracted from mouse placentas and fetal membranes.

What does this add to what is known?

These observations demonstrating a significant increase in representative short telomeres in gestational tissues provide support for the hypothesis that increasing short telomeres at term potentially function as a biologic clock for parturition.

that have been well described to result in parturition (for more information regarding the role of inflammation during parturition, see the reviews by Nadeau-Vallee et al,[7] Romero et al,[8] and Christiaens et al[1]).

The lengths of the telomeres on the chromosomes of the conceptus are reset to their genetic and environmentally determined maximal lengths early during embryonic development.[9] After peaking at the blastocyst stage, the average telomere lengths have been reported to shorten during the remainder of gestation in the placenta and fetal membranes in both human and mouse pregnancies.[10,11] In adult tissues, critical telomere shortening has been shown to produce apoptosis through p53-dependent and independent signaling events.[12,13]

Several published reports have confirmed that the detrimental effects of overall telomere shortening are produced by an increase in the amount of short telomeres. Telomerase is a ribonuclear protein that functions as a unique DNA polymerase that replaces and/or extends the telomere sequences at the ends of the chromosomes; however, its expression is mainly limited to rapidly dividing cells including stem cells, germ cells, and cancer.[14]

Homozygous telomerase-deficient mice demonstrate increased telomere shortening with each generation resulting in the progressive development of age-related diseases by the fourth generation, culminating in complete infertility and shortened life spans by the sixth generation.[15] Herrera et al[16] have reported that the age-related diseases occurring in telomerase-deficient mice were associated with a progressive shift of the telomere distribution curves to the left, resulting in an increase in the amount of short telomeres. Also using telomerase-deficient mice, Vera et al[17] have observed that mice with the highest rates of increase in short telomeres were the ones that had shorter lives, thereby confirming the key mechanistic role for short telomeres.

Using a combination of homozygous and heterozygous telomerase-deficient mice, Armanios et al[18] demonstrated that the increase in short telomeres was sufficient and necessary to cause the degenerative defects associated with aging. Hemann et al[19] utilized intergenerational crosses between homozygous and heterozygous telomerase-deficient mice to demonstrate that the shortest, not the average telomere, lengths are critical in regard to cell viability and chromosome stability. These investigators concluded that while most studies report changes in the average telomere lengths, it is not the average but rather the shortest telomeres that produce telomere dysfunction and limit cellular survival ultimately leading to aging.[19]

Based on the functional importance of short telomeres in regard to cellular senescence, apoptosis and aging, the studies described in this report sought to assess for the first time the relative increase in short telomeres and their modulation in gestational tissues during late pregnancy in the mouse. Specifically, quantitative fluorescence in situ hybridizations studies were performed to determine the distribution of telomere lengths in DNA samples extracted from mouse placentas and fetal membranes. In addition, quantitative studies were performed to determine the relative increase in representative short telomeres during the last 5 days of gestation in the mouse using a quantitative polymerase chain reaction (qPCR) modification of the classic telomere restriction fragment (TRF) assay technique.

Materials and Methods

For these studies, placental and fetal membrane tissues were harvested from timed-pregnant CD-1 mice on gestational days (GD) 14−18 (with normal parturition occurring on GD18.5−19) under an Institutional Animal Care and Use Committee−approved animal research protocol. Outbred CD-1 mice were utilized for these studies because of their average telomere lengths of 16−26 kb (which are similar to the 10−15 kb average telomere lengths in humans), in contrast to the much longer (ie, 50−150 kb telomeres found in inbred mouse strains [eg, C57BL/6 mice]).[20-22]

For the studies described in the following text, mice were euthanized under isoflurane anesthesia after the collection of placentas, fetal membranes, maternal liver, and other tissues. The harvested tissues were rinsed in phosphate-buffered saline and then snap froze in liquid nitrogen; the frozen tissues were then stored at −80°C until utilized for the studies described in the following text.

To determine the relative size distribution of telomere segments on CD-1 mouse chromosomes, 30 placental and fetal membrane samples (ie, 5 each from GD14, 16, and 18 for both tissues) were shipped frozen to Life Length Technologies (Madrid, Spain) for analysis using their commercial high-throughput quantitative fluorescence in situ hybridization technique.

Specifically, the frozen tissue was thawed and enzymatically disaggregated into single-cell suspensions. The cells were then seeded into clear bottom, black-walled, 384-well plates, fixed with methanol/acetic acid, and treated with pepsin to digest cell walls and cytoplasm. These cells were then hybridized with a fluorescent peptide nucleic acid (PNA) probe that recognizes a triplicate telomere sequence repeat (ie, 3 TTAGGG sequence motifs); thus, the more telomere triplicate repeats, the more fluorescent probes bind to the telomere. The intensity of the fluorescent signal from the PNA probes was proportional to the length of the telomere segments in the interphase cells.

After completion of the PNA hybridizations, the cells were treated with 4′,6′-diamidino-2-phenylindole (DAPI) to stain total DNA. Subsequently, quantitative data acquisition and analysis were performed using a high-content screening opera system using Acapella software (Perkin Elmer, Waltham, MA). The telomere length distribution and median telomere lengths were calculated using proprietary Life Length algorithms. Of the original 30 samples, 28 produced data of sufficient consistency to undergo further analysis, whereas 2 of the GD18 fetal membrane samples did not.

Telomere length distribution histograms were provided for all of the tissues and gestational days analyzed; representative telomere distribution histograms from the fetal membranes are provided in this manuscript. The median telomere lengths were statistically analyzed using the Kruskal-Wallis analysis of variance on ranks and multiple comparisons tests (using the Dunn method) to determine significant differences in the median telomere lengths between the 3 gestational day groups (ie, where $P \leq .05$). Paired and unpaired *t* test analyses were utilized to determine significant ($P \leq .05$) differences between the median telomere lengths in the placentas vs fetal membranes.

To quantify the relative changes in short telomere segments, 30 placental, 29 fetal membrane, and 30 maternal liver samples were utilized for the following studies. Each sample is from an individual mouse resulting in the use of more than 30 pregnant CD-1 mice (ie, 4−7 tissue samples for each of the 5 gestational days analyzed (ie, GD 14−18).

The relative increase in representative short telomeres was determined using our qPCR modification of the classic TRF assay technique as follows. Total DNA was extracted from mouse placental, fetal membrane, and maternal liver tissues using High Pure PCR Template Prep kits (Roche Applied Science, Mannheim, Germany) according to the manufacturer's routine protocol. The concentration and the purity (based on the 260/280 nm ratio) of the isolated DNA was determined using a NanoDrop spectrophotometer (ThermoFisher Scientific, Carlsbad, CA), a well-established method for determining the DNA concentration and purity in microvolume aqueous samples.[23]

The classic telomere (or terminal) restriction fragment assay technique consists of restriction endonuclease (RE) digestion of total DNA resulting in the release of intact telomere fragments (ie, the RE digests the genomic DNA but not the telomere DNA), followed by performing a Southern blot and hybridization with labeled telomere probes (for examples of TRF[22,24]).

Using the TRF technique, the DNA extracted from the pregnant mouse tissues underwent restriction endonuclease treatment using *Hinf I* and *Rsa I* followed by resolution by size on a horizontal DNA gel using the E-Gel Power Snap Electrophoresis system (ThermoFisher Scientific). Instead of performing the Southern blots, representative short telomere fractions (ie, 3 kb and 5 kb DNA fractions; note that the average telomere lengths in CD-1 mice are reported to be 16−26 kb[20]) were collected from the gels. Subsequently, real-time qPCR was performed in triplicate using the telomere PCR primers reported by Gil and Coetzer[25] and the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad Laboratories), with the qPCR run using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories) and DNA (2.5 or 5 ng): 40 cycles at 95°C × 10 seconds and 55.7°C × 30 seconds.

Using the same 3 and 5 kb DNA sample fractions, separate real-time qPCRs were performed using specific primers for the mouse *GAPDH* gene. The GAPDH PCR template sequences were not cut during the RE digestion, thereby allowing the stable amplification of the GAPDH amplicons to serve as the single copy control gene (ie, stable GAPDH amplification was confirmed by the coefficient of variation for the quantification cycle value being ≤7% in each of the qPCR runs; see Table 1).

TABLE 1
Amplification data for GAPDH gene

Samples	Mean Cq ± SD	C.V.	n
3 kb DNA fractions			
Fetal membrane	24.18 ± 0.93	3.8%	29
Placenta	25.25 ± 1.30	5.1%	30
Maternal liver	25.11 ± 1.77	7.0%	30
5 kb DNA fractions			
Fetal membrane	26.94 ± 1.05	3.9%	29
Placenta	28.58 ± 1.59	5.6%	30
Maternal liver	28.38 ± 1.99	7.0%	30

PCR Cq data for GAPDH qPCR runs are presented as the mean ± SD. C.V. confirms ≤7% variation in amplification of the control gene (GAPDH) in each of the qPCR runs. *Cq*, quantification cycle; *C.V.*, coefficient of variation; *qPCR*, quantitative polymerase chain reaction.

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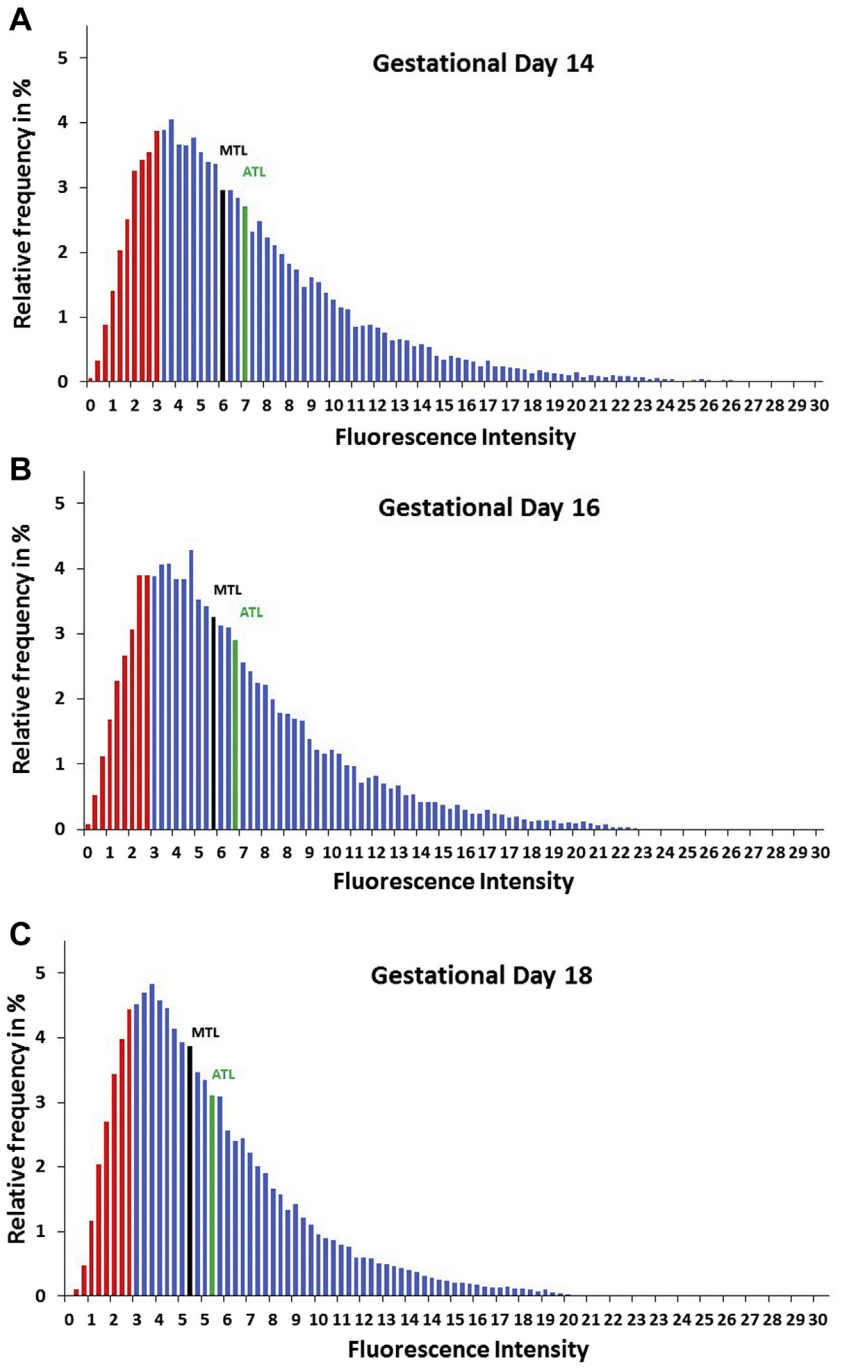
FIGURE
Distribution of telomere lengths in mouse fetal membranes



Representative histograms demonstrate the distribution of telomere lengths found on the chromosomes contained within interphase cells in mouse fetal membrane tissues. **A,** Histogram for GD14 fetal membrane telomeres. **B,** Histogram for GD16 fetal membrane telomeres. **C,** Histogram for GD18 fetal membrane telomeres. Telomere lengths (in fluorescence intensity) were determined using a commercial high-throughput quantitative fluorescence in situ hybridization technique; and the telomere length frequency is in relative frequency in percentages. These histograms demonstrate that the distribution skewing toward the left (ie, toward shorter telomere lengths) increased from GD14 to GD18.

ATL, average telomere length; *GD*, gestational day; *MTL*, median telomere length.

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The qPCR calculation method reported by Pfaffl[26] allows the determination of the relative fold increase or decrease in the qPCR amplicon levels of a target gene for a reference sample (in this case GD14) compared with the other samples (ie, GD15−18); therefore, the Pfaffl method was used to calculate the relative increase in telomere DNA in the representative short telomere fractions using GD14 as the reference value. The data were analyzed using the Kruskal-Wallis analysis of variance on ranks and multiple comparisons tests (using the Dunn method) with significance indicated at $P \leq .05$.

Results

Representative histograms for the distribution of telomere lengths in the mouse fetal membrane cells are shown in the Figure. As observed, the telomere lengths (in fluorescence intensity) compared with relative frequency (in percentages) generates a distribution curve skewed to the left (toward shorter telomere segment lengths).

Comparing the distribution curve for GD18 to curves for GD16 and GD14, there are fewer long telomere segments (ie, shorter tail to the right) and a higher frequency of short telomere segments (ie, a higher mode [peak] for the GD18 curve compared with the other 2 gestational days). There is also a shift to the left (toward smaller) for the average and median telomere lengths in the GD18 compared with the other 2 histograms.

Based on all of the fetal membrane telomere histogram data, the median telomere length averages were 18.7 kb for GD14, 17.9 kb for GD16, and 16.4 kb for GD18 (see Table 2). Histograms for the telomere lengths for the placental tissue generated similar distribution curves skewed toward the left (ie, shorter telomeres); however, the curve shifts between GD14 and GD18 were not as robust as observed with the fetal membranes (data not shown). Interestingly, the median telomere length averages for the placental tissues were significantly longer than the telomeres in fetal membranes (ie, 20.1 kb, 20.8 kb, and 20.7 kb, respectively; $P < .05$) as shown in Table 2.

The quantitative short telomere data generated using the TRF/DNA gel fractionation/real-time qPCR technique confirmed a significant increase in the relative quantity of short telomeres (ie, as indicated by the representative 3 kb and 5 kb telomere fragments) in DNA isolated from gestational tissues harvested from timed pregnant CD-1 mice during the last 5 days of gestation (see Table 3). For the fetal membranes, the 3 kb telomere fragments demonstrated a 3-fold increase by GD16, a 4-fold increase by GD17, and a >5-fold increase by GD18 compared with GD14 (all $P <$ .05). The 5 kb telomere fragments isolated from the fetal membranes demonstrated a similar progressive increase, which peaked at >9-fold by GD18 ($P <$ .05 compared with GD14).

For the placental tissues, the 3 kb telomere quantity remained relatively unchanged until it peaked at >9-fold on GD18 (ie, less than 1 day before the onset of parturition [$P <$ .05 for GD18 compared with GD14]). The same was true for the 5 kb telomere segments, which peaked at 7.8-fold on GD18 ($P <$ .05), except for a transient increase on GD15 (see Table 3).

Similar TRF/DNA gel fractionation/real-time qPCR studies were performed using adult liver tissue from pregnant and nonpregnant CD-1 mice. These studies demonstrated little variation between the amounts of representative short telomeres during GD14−18 and no significant difference between the samples from the nonpregnant and pregnant mice as observed in Table 4.

Comment

The studies described in this report have demonstrated for the first time a significant increase in short telomeres in gestational tissues of near-term pregnant CD-1 mice. Even by GD14, the distribution of telomeres on the chromosomes found in the gestational tissues were observed to be skewed toward the shorter lengths. This skewed distribution is consistent with telomeres found in aged mice and in the late generations of telomerase-deficient mice and not the more bell-shaped distribution of the telomere lengths found in young reproductive-age adult mice.[16,27]

Providing additional support to the increase in short telomeres in near-term gestational tissues, these studies have demonstrated a significant increase in representative short telomeres (ie, 3 kb and 5 kb telomere fragments) during the last third of gestation in the fetal membranes of the mouse, which peaks on the day before the onset of parturition (ie, GD18). These observations are consistent with the progressive aging and degradation of the fetal membranes that occurs during the same time period in the mouse. In contrast, the significant increase in short telomeres in the placental tissue did not occur until GD18, suggesting that significant aging and apoptosis of this physiologically important tissue appears to be delayed until shortly before the fetus is born (consistent with the essential role for a normally functional placenta until the end of pregnancy).

Although several investigators have reported telomere shortening in gestational tissues and have suggested a possible link to parturition, the novelty of the telomere gestational clock hypothesis being proposed here is that it provides a cohesive signaling pathway all the way from short telomeres to the onset of labor. Several lines of evidence by our laboratory and by other investigators provide support for the plausibility of this hypothesis. Bonney et al[11] have reported progressive telomere shortening throughout gestation in the mouse, which was associated with increased levels of activated p53 protein.

Although these investigators evaluated cellular senescence as the endpoint for their studies, other researchers have found short telomere-stimulated p53 leads to not only cellular senescence but also to apoptosis resulting in cell death.[28] Increasing levels of apoptosis, especially in the trophoblast cells, has been described for many years in gestational tissues including the placenta and fetal membranes in mice, humans, and other mammals peaking near the end of gestation.[29-32] Research performed to assess levels of cell-free fetal DNA

TABLE 2
Median telomere lengths

Gestation	Fetal Membranes	n	Placentas	n
GD14	18,705 ± 444	5	20,053 ± 297[a]	5
GD16	17,883 ± 562	5	20,834 ± 686[a]	5
GD18	16,364 ± 2663	3	20,679 ± 856[a]	5

Median telomere lengths in base pairs (mean ± SD). *GD*, gestational day.

[a] $P <$ .05 compared with respective fetal membrane telomere lengths.

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TABLE 3
Relative quantity of short telomeres in gestational tissues

	Fetal membranes				Placentas			
Gestation	3 kb telomeres	n	5 kb telomeres	n	3 kb telomeres	n	5 kb telomeres	n
GD14	1.06 ± 0.40	6	1.33 ± 0.84	6	1.10 ± 0.41	6	1.06 ± 0.45	6
GD15	1.07 ± 0.30	6	2.61 ± 0.79	6	2.95 ± 0.55	6	5.45 ± 1.45[a]	6
GD16	3.10 ± 1.48[a]	5	4.10 ± 3.29	5	1.22 ± 0.54	5	0.90 ± 0.37	4
GD17	4.09 ± 1.05[a]	5	3.61 ± 1.04	5	2.56 ± 0.67	6	1.20 ± 0.77	6
GD18	5.48 ± 1.90[a]	7	9.27 ± 5.11[a]	7	9.30 ± 5.66[a]	7	7.81 ± 2.39[a]	7

Data (mean ± SD) are reported as fold increase in short telomeres compared with GD14. *GD*, gestational day.

[a] $P <$ .05 compared with GD14.

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TABLE 4
Relative quantity of short telomeres in adult liver tissues

Gestation	3 kb telomeres	n	5 kb telomeres	n
GD14	1.01 ± 0.40	5	1.01 ± 0.19	5
GD15	1.08 ± 1.11	5	0.73 ± 1.24	5
GD16	2.05 ± 1.96	5	1.08 ± 1.23	5
GD17	1.33 ± 0.43	5	1.14 ± 0.54	5
GD18	3.80 ± 1.94	5	1.80 ± 0.60	5
Nonpregnant	1.83 ± 0.30	5	0.62 ± 0.13	5

Data (mean ± SD) are reported as fold increase in short telomeres compared with GD14. There were no significant difference between the nonpregnant and pregnant 3 kb or 5 kb telomere fractions. *GD*, gestational day.

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through gestation have confirmed a marked increase of 10- to 40-fold that also peaks at the end of gestation.[33,34]

Published reports from our laboratory and by other researchers have provided a link between apoptosis in gestational tissues and the release of cell-free DNA.[35-37] In addition, our laboratory has demonstrated that the cell-free DNA released by the placental and fetal membrane tissues produce a robust innate immune response mediated by DNA stimulation of TLR9.[35,37] Other investigators have performed in vivo studies using small DNA fragments (ie, oligodeoxynucleotides) to demonstrate the ability of DNA fragments to stimulate parturition in 100% of the treated pregnant mice.[38,39]

These studies confirmed that DNA-induced parturition is produced by an innate immune response mediated by TLR9, resulting in an increase in proinflammatory cytokines and the influx of inflammatory cells, including macrophages, into the uterine tissues (ie, phenomena similar to that described in human gestational tissues with the approach of parturition).[38,39] Similar studies have been performed using pregnant mice to show that DNA extracted from human fetal tissue is also able to stimulate preterm pregnancy loss (resorptions) mediated by TLR9 stimulation and associated with a proinflammatory cytokine response and the influx of inflammatory cells.[40] Thus, this series of in vitro and in vivo studies provide a line of experimental evidence supporting the feasibility of a telomere based gestational clock.

The significant contribution and novelty of the studies described in the current report is the observation that short telomeres increase severalfold in gestational tissue as the onset of parturition approaches; the importance being that short telomeres have been shown to be the molecular mediators that produce the telomere dysfunction effects and limit cellular viability resulting in apoptosis and tissue aging (as described in the introductory text).

Several recent reports regarding telomere shortening in human gestations support the potential translation of these pregnant mouse telomere observations into mechanisms that might be occurring in human pregnancies. For example, confirming a relationship between telomere lengths and gestational length, Smeets et al[41] observed significantly shorter telomeres in individuals who had been born preterm (<37 weeks) compared with those born at term (>37 weeks).

After correcting for prematurity, the gestational period for African-American women has been observed to be several days shorter than white women through mechanisms that are currently unknown.[42] However, in 2016 Jones et al[43] provided some potential insights into this issue by studies that reconfirmed this racial difference in gestational length and then demonstrated that the average telomere lengths in placental villi and chorion membrane tissue obtained from African-American women were significantly shorter than those found in these gestational tissues from white women.

Another unexplained gestational length observation is the fact that spontaneous premature deliveries (with or without preterm premature rupture of the membranes) occur more frequently in women carrying male fetuses than female fetuses throughout pregnancy.[44] Providing support for a possible relationship between this gender difference and short telomeres, Wilson et al[45] reported in 2016 that the progressive decrease in telomere lengths in placental tissue was more rapid in pregnancies carrying males compared with females, resulting in significantly shorter telomeres in males throughout gestation.

Preterm deliveries in the United States are associated with multiple risk factors including psychological stress, low socioeconomic status, poor-quality neighborhoods, cigarette smoking, low education levels, and African-American race.[46] Several of these risk factors produce metabolic effects that lead to oxidative stress, shorter telomeres, and lower telomerase activity.[47] Published reports have demonstrated that oxidative stress can accelerate telomere shortening through its ability to uniquely targets the guanine-rich telomere sequences, resulting in single- and double-strand breaks of the DNA within the telomere segments.[48]

Several of the risk factors for preterm birth have been reported to be associated with telomere shortening in the placenta and/or fetal blood. For example, 3 recent studies using validated psychological stress scales have confirmed a significant inverse relationship between increasing maternal stress and decreasing telomere lengths in fetal cord blood.[49-51] In regard to maternal education, 2 recent publications have demonstrated an inverse association between the attained level of maternal education and telomere lengths in cord blood.[52,53]

Poor-quality neighborhoods are characterized by several things including air pollution, lack of green space, and close proximity to major roads and vehicle emissions. A paper by Bijnens et al[54] reported that the maternal residential proximity to green spaces was associated with an average of 3.6% longer telomere lengths in the placenta.

These authors also reported that maternal residential proximity to major roads and vehicle emissions had a significant association with placental telomere lengths; specifically, they observed that doubling the distance to major roads was associated with a 5.3% increase in placental telomere lengths.[54]

In another recent study, Martens et al[55] observed that increased levels of maternal residential small particulate air pollution was associated with a 12−13% reduction in placental telomere lengths, especially during the second trimester. Cigarette smoking has been associated with preterm delivery in addition to growth restriction. The report by Salihu et al[56] demonstrated a significant association between decreased cord blood telomere lengths for active smokers greater than passive smokers, compared with pregnant women who were nonsmokers.

In the United States, African-American women have a 2- to 3-fold higher rate of preterm delivery compared with white women.[46] When considering the previously described risk factors for telomere shortening and preterm delivery, African-American women have multiple risk factors, all of which are distinct and potentially additive in regard to pathologic telomere shortening.

African-American woman have been reported to have significant levels of psychological stress, have attained lower education levels, and have higher neighborhood deprivation indices compared with white women.[46,57] Their neighborhoods often have significant levels of air and vehicle pollution and contain minimal (if any) safe or available green space. Thus, it could be theorized that the accumulated effects of these risk factors leading to significant telomere shortening provide a possible biologic explanation for the racial disparity in preterm birth in the United States.

Telomere shortening could also explain the nativity effect (ie, the phenomenon in which gestational length becomes shorter when minority immigrant women reside in the United States for a period of time).[58] The shortened telomere effect could also explain why highly educated, affluent African-American women continue to have an increased, albeit lower, risk of preterm delivery when compared with equally educated, affluent white women, an observation suggesting that improved socioeconomic status and education do not completely mitigate the telomere shortening effects of psychological stress produced by implicit bias and other racial issues.[59]

In summary, the mouse studies described in this report have provided support for a telomere gestation clock mechanism that could be operational in the mouse. Furthermore, these hypothesis generating studies provide a foundation for future research regarding the actual role for telomere shortening in human pregnancies.

The strength of this study is the ability to make seminal observations in a physiologically normal, unperturbed model system (ie, the pregnant mouse) with a gestational length short enough to clearly observe day-to-day changes. The weaknesses of this study relate to the fact that it was an observational study performed in pregnant mice. Future studies need to be performed to attempt to demonstrate a cause-and-effect relationship between the increase in short telomers and decrease in gestational length through direct modulation of telomere lengths in mouse gestational tissues. Likewise, future studies will need to be performed to assess the role of short telomeres in gestational tissue in regard to the regulation of the length of pregnancy and the onset of parturition in humans.

The telomere gestational clock is a novel hypothesis supported by several preliminary mouse studies and interesting associations in human pregnancies between maternal conditions (eg, stress, education, pollution, neighborhood quality, and race) and telomere lengths as discussed in this manuscript. However, robust investigation in the future is needed to confirm or refute an actual mechanistic role for short telomeres regarding the regulation of the length of pregnancy in humans and other mammals. ■

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