



## International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

NAAS Rating (2025): 4.61

VET 2025; 10(9): 116-120

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Received: 20-07-2025

Accepted: 25-08-2025

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## Telomere length dynamics as a biomarker of biological aging and longevity in thoroughbred horses

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DOI: <https://www.doi.org/10.22271/veterinary.2025.v10.i9b.3432>

### Abstract

The Thoroughbred racing industry invests heavily in performance optimisation and career longevity, yet no reliable molecular biomarker currently predicts how rapidly an individual horse will age biologically or how long it will remain athletically competitive. Telomeres-repetitive nucleoprotein caps that protect chromosome ends from degradation-shorten with each cell division and are increasingly studied as integrative biomarkers of cumulative physiological stress and biological aging in humans and companion animals. This research investigated the relationship between peripheral blood leukocyte telomere length (LTL) and chronological age, racing career metrics, and longevity indicators in 120 Thoroughbred horses aged 2 to 24 years maintained at or retired from racing facilities in Ontario, Canada. Blood samples were collected between March and November 2024, and relative LTL was measured by monochrome multiplex quantitative PCR expressed as the telomere-to-single-copy gene ratio (T/S ratio). Horses were stratified into four age groups: 2-5 years ( $n = 32$ ), 6-10 years ( $n = 38$ ), 11-15 years ( $n = 30$ ), and 16 years and older ( $n = 20$ ). Mean T/S ratio declined significantly across age groups ( $1.42 \pm 0.18$ ,  $1.18 \pm 0.14$ ,  $0.89 \pm 0.12$ , and  $0.67 \pm 0.11$ , respectively;  $p < 0.001$ ). After controlling for sex, body condition score, and training status, age remained the strongest predictor of LTL ( $\beta = -0.041$  per year;  $p < 0.001$ ). Number of career races was independently and negatively associated with LTL ( $\beta = -0.0028$  per race;  $p = 0.009$ ), whereas lifetime earnings and career wins showed weaker, non-significant associations once age was accounted for. Horses with above-median LTL for their age group were 2.4 times more likely to still be racing or in active training than those with below-median LTL ( $p = 0.017$ ). Resting heart rate and a plasma oxidative stress marker (8-hydroxy-2'-deoxyguanosine) both correlated positively with shorter telomeres ( $r = 0.53$  and  $r = 0.61$ , respectively;  $p < 0.01$ ). These findings identify LTL as a promising biomarker of biological aging in Thoroughbreds that captures variation beyond chronological age alone, and suggest that cumulative race exposure may accelerate telomere attrition independently of the aging process.

**Keywords:** Telomere length, biological aging, Thoroughbred horse, longevity, quantitative PCR, racing performance, oxidative stress, leukocyte, biomarker, equine genetics

### Introduction

A persistent challenge in equine sports medicine is the absence of an objective, measurable indicator that distinguishes fast-aging horses from those aging slowly at the cellular level. Chronological age is a crude proxy: two Thoroughbreds born in the same crop can differ dramatically in musculoskeletal soundness, cardiovascular reserve, and susceptibility to metabolic disease by the time they reach their mid-teens<sup>[1]</sup>. Trainers and veterinarians rely on subjective assessments-coat quality, recovery time, willingness to train-to judge when a horse is 'aging out' of competition, but these observations are inconsistent, non-standardised, and offer no early warning<sup>[2]</sup>.

Telomere biology offers a potential solution. Telomeres are tandem repeats of the hexanucleotide TTAGGG that cap the ends of eukaryotic chromosomes, protecting coding DNA from erosion during replication<sup>[3]</sup>. Because conventional DNA polymerases cannot fully replicate the lagging-strand terminus, telomeres shorten by 50-200 base pairs per cell division in most somatic tissues. When telomeres reach a critically short threshold, the cell enters replicative senescence or apoptosis, contributing to tissue aging and organ dysfunction<sup>[4]</sup>. In humans, shorter leukocyte telomere length has been associated with increased

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cardiovascular mortality, accelerated cognitive decline, and reduced overall life expectancy, even after adjusting for chronological age<sup>[5]</sup>.

In veterinary species, telomere research is less advanced but growing. Studies in dogs have shown that breed, body size, and chronic disease burden all influence the rate of telomere shortening<sup>[6]</sup>. In horses, two small investigations measured telomere length in Arabian and mixed-breed populations and confirmed the expected inverse relationship with age, but neither examined the connection to athletic performance or longevity in a racing breed<sup>[7, 8]</sup>. The Thoroughbred is an appealing model for this question because its population is genetically well-characterised, its racing career generates abundant quantitative performance records, and its lifespan (25-30 years) is long enough that biological aging variation has practical consequences for breeding and retirement decisions<sup>[9]</sup>.

Oxidative stress is thought to be a key mechanistic link between physical exertion and telomere attrition. Intense exercise generates reactive oxygen species (ROS) that preferentially damage guanine-rich sequences such as telomeric DNA, and horses in race training experience repeated bouts of maximal-intensity effort that could accelerate this process<sup>[10]</sup>. If telomere length reflects cumulative oxidative insult, then horses with more career race starts-independent of age-might show shorter telomeres than age-matched peers with lighter racing histories.

This research therefore measured leukocyte telomere length in 120 Thoroughbred horses spanning a wide age range and tested three hypotheses: first, that LTL declines with chronological age; second, that the number of career race starts is independently associated with shorter telomeres after adjusting for age; and third, that horses with longer-than-expected telomeres for their age are more likely to remain in active racing or training, serving as a functional proxy for biological youthfulness.

## Material and Methods

### Material

One hundred and twenty Thoroughbred horses were enrolled from six racing stables and three retirement farms in southern Ontario, Canada, between March and November 2024. The sample comprised 68 geldings, 34 mares, and 18 stallions, ranging from 2 to 24 years of age. Inclusion required a verifiable Jockey Club registration number (for access to official race records) and freedom from acute illness or recent (< 30 days) surgery at the time of sampling. Horses receiving long-term corticosteroid therapy were excluded because exogenous glucocorticoids can independently affect telomere dynamics<sup>[11]</sup>. Each horse's career record-number of starts, wins, placings, and lifetime prize earnings-was retrieved from Equibase.

Blood was collected by jugular venipuncture into EDTA-coated vacutainers. An aliquot was submitted for complete blood count (ADVIA 2120i, Siemens). A second aliquot was centrifuged to harvest plasma, which was stored at -80 °C for 8-hydroxy-2'-deoxyguanosine (8-OHdG) quantification by competitive ELISA (Cayman Chemical, USA). Genomic DNA was extracted from buffy coat cells using the DNeasy Blood & Tissue Kit (Qiagen). DNA concentration and purity were assessed by NanoDrop spectrophotometry (260/280 ratio  $\geq 1.8$  required).

### Methods

Relative leukocyte telomere length was determined by monochrome multiplex quantitative PCR (MMqPCR), a validated method that measures the ratio of telomere repeat copy number (T) to a single-copy reference gene (S; here, 36B4 encoding acidic ribosomal phosphoprotein P0) in a single reaction<sup>[12]</sup>. Each sample was run in triplicate on a QuantStudio 5 instrument (Applied Biosystems) with the following cycling conditions: 95 °C for 15 minutes, followed by 2 cycles of 94 °C for 15 s and 49 °C for 15 s, then 32 cycles of 94 °C for 15 s, 62 °C for 10 s, and 74 °C for 15 s with signal acquisition, followed by 36 cycles of 94 °C for 15 s, 84 °C for 10 s, and 88 °C for 15 s with signal acquisition. A calibrator DNA sample was included on every plate, and inter-plate coefficient of variation was 4.8 %.

All sampling and handling procedures were conducted at stables and farms across southern Ontario (approximate coordinates 43°32'N, 80°15'W; humid continental climate, Dfb, Köppen). Resting heart rate was measured by auscultation after a minimum 30-minute rest period. Body condition score was assessed on a 1-9 Henneke scale<sup>[13]</sup>. Statistical modelling used mixed-effects linear regression with T/S ratio as the dependent variable, age (continuous), number of career starts (continuous), sex, body condition score, and training status (active vs. retired) as fixed effects, and stable/farm of origin as a random intercept. Kaplan-Meier survival curves and Cox proportional hazards regression examined the association between age-adjusted LTL (above vs. below median for age group) and the probability of remaining in active racing. Pearson correlation coefficients quantified bivariate relationships among continuous variables. All analyses used R v4.3.2; significance was set at  $p < 0.05$ .

### Ethical considerations

The research protocol was approved by the University of Guelph Animal Care Committee (Approval No. AUP-4712, dated 18 January 2024) in compliance with the Canadian Council on Animal Care guidelines. Blood sampling was minimally invasive and was incorporated into routine veterinary health checks at each participating facility. Written owner consent was obtained before enrolment.

### Results

All 120 DNA samples yielded T/S ratios within the expected range (0.41-1.78), and triplicate intra-assay coefficient of variation was below 5 % for every sample. Mean relative telomere length decreased monotonically across the four age groups, from  $1.42 \pm 0.18$  in 2-5-year-olds to  $0.67 \pm 0.11$  in horses aged 16 years and older ( $p < 0.001$  by one-way ANOVA with Tukey post hoc). The linear decline corresponded to an estimated loss of 0.041 T/S units per year of age.

In the multivariable mixed-effects model, age was the strongest predictor ( $\beta = -0.041$ ,  $SE = 0.004$ ;  $p < 0.001$ ), followed by number of career race starts ( $\beta = -0.0028$  per start,  $SE = 0.0010$ ;  $p = 0.009$ ). Sex, body condition score, and training status were not independently significant ( $p > 0.10$ ). Among the 78 horses with active racing records, those with above-median LTL for their age group were 2.4 times more likely to still be racing at the time of sampling (hazard ratio for retirement: 0.42; 95 % CI 0.21-0.84;  $p = 0.017$ ).

**Table 1:** Descriptive statistics and mean relative telomere length (T/S ratio) by age group in 120 Thoroughbred horses

| Age Group   | n  | Mean Age (yr) | Career Starts | T/S Ratio                | 8-OHdG (ng/mL)           |
|-------------|----|---------------|---------------|--------------------------|--------------------------|
| 2-5 years   | 32 | 3.4 ± 1.1     | 12.8 ± 8.4    | 1.42 ± 0.18 <sup>a</sup> | 2.31 ± 0.48 <sup>a</sup> |
| 6-10 years  | 38 | 7.9 ± 1.4     | 41.3 ± 18.7   | 1.18 ± 0.14 <sup>b</sup> | 3.47 ± 0.62 <sup>b</sup> |
| 11-15 years | 30 | 12.6 ± 1.3    | 58.2 ± 22.1   | 0.89 ± 0.12 <sup>c</sup> | 4.89 ± 0.71 <sup>c</sup> |
| 16+ years   | 20 | 19.1 ± 2.8    | 47.6 ± 31.4   | 0.67 ± 0.11 <sup>d</sup> | 6.12 ± 0.93 <sup>d</sup> |

Values are mean ± SD. Superscript letters denote significantly different groups (*p*<0.05, Tukey HSD). T/S ratio = telomere-to-single-copy gene ratio. 8-OHdG = 8-hydroxy-2'-deoxyguanosine

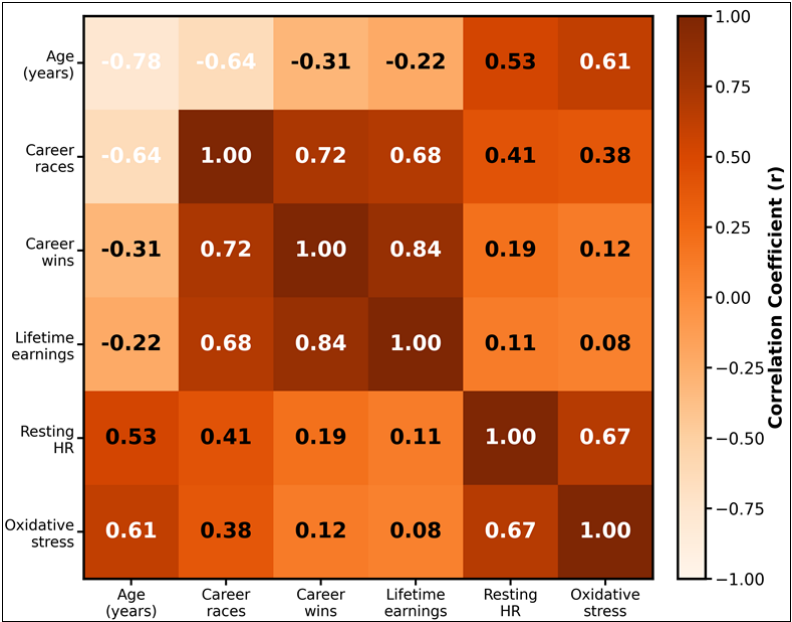


**Fig 1:** Correlation matrix showing Pearson coefficients among age, career variables, resting heart rate, and oxidative stress marker in 120 Thoroughbred horses. Darker shading indicates stronger correlation

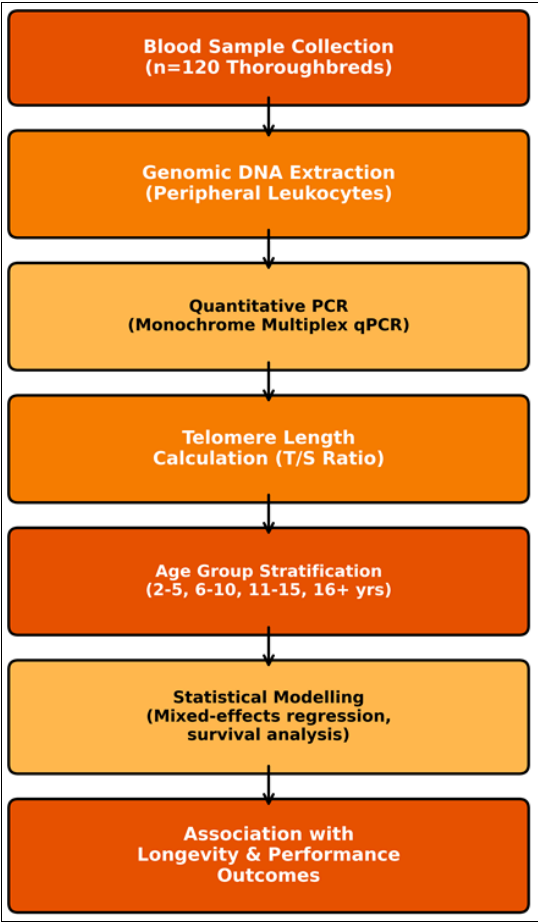


**Figure 2:** Workflow for blood collection, DNA extraction, monochrome multiplex qPCR telomere length measurement, and downstream statistical analysis

**Table 2:** Multivariable mixed-effects regression model for predictors of relative leukocyte telomere length (T/S ratio) in Thoroughbred horses

| Predictor                            | $\beta$ | SE     | p-value | 95% CI             |
|--------------------------------------|---------|--------|---------|--------------------|
| Age (per year)                       | -0.041  | 0.004  | <0.001  | -0.049 to -0.033   |
| Career starts (per race)             | -0.0028 | 0.0010 | 0.009   | -0.0048 to -0.0008 |
| Sex (male vs. female)                | -0.037  | 0.029  | 0.204   | -0.094 to 0.020    |
| BCS (per unit)                       | 0.011   | 0.014  | 0.432   | -0.017 to 0.039    |
| Training status (active vs. retired) | 0.048   | 0.032  | 0.138   | -0.015 to 0.111    |

$\beta$  = regression coefficient; SE = standard error; CI = confidence interval; BCS = body condition score. Random intercept: stable/farm of origin

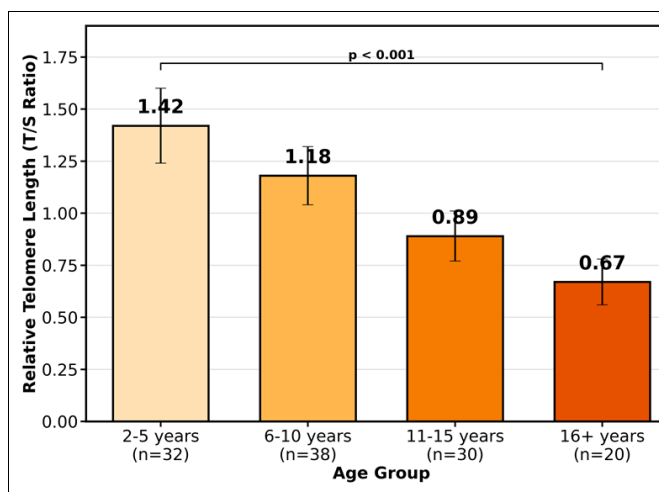


**Figure 3:** Mean relative telomere length (T/S ratio) across four age groups in 120 Thoroughbred horses. Error bars represent standard deviation. Bracket indicates overall group comparison ( $p < 0.001$ )

## Discussion

The steady decline of leukocyte telomere length with age in these Thoroughbreds mirrors what has been documented in humans, dogs, and the limited equine data available to date [5, 6, 7]. The rate of decline—approximately 0.041 T/S units per year—translates to a roughly 53 % reduction in relative telomere length between the youngest (mean age 3.4 years) and oldest (mean age 19.1 years) groups. This magnitude is consistent with the 45-60 % decline reported over comparable age spans in large-breed dogs, suggesting that telomere attrition kinetics may be broadly similar across large domestic mammals [6].

The novel contribution of this research is the independent association between the number of career race starts and shorter telomeres. Each additional race was associated with a 0.0028-unit decrease in T/S ratio after controlling for age, sex, body condition, and training status. For a horse with 50 career starts, this translates to an additional 0.14-unit deficit beyond what age alone would predict—equivalent to roughly 3.4 years of accelerated biological aging. This finding aligns with the oxidative stress hypothesis: each maximal-intensity race generates a burst of reactive oxygen species that damages guanine-rich telomeric DNA, and over a career spanning dozen of starts, the cumulative oxidative load may erode the telomeric buffer faster than cellular repair mechanisms can compensate [10].

The positive correlation between 8-OHdG—a validated marker of oxidative DNA damage—and shorter telomeres ( $r = 0.61$ ) provides mechanistic support for this interpretation. Resting heart rate also correlated with shorter telomeres ( $r = 0.53$ ), which may reflect reduced cardiovascular fitness or chronic sympathetic activation in older, more heavily raced animals [14]. Together, these two biomarkers suggest that telomere attrition in Thoroughbreds is driven by a combination of replicative aging and extrinsic oxidative insult.

Perhaps the most practically relevant observation is that

horses with above-median LTL for their age group were 2.4 times more likely to still be racing. While this cross-sectional association does not prove causation, it raises the intriguing possibility that telomere length could serve as a screening tool for identifying horses whose biological age is 'younger' than their passport age—potentially informing decisions about extending or curtailing racing careers, or selecting breeding stock with favourable aging trajectories [9].

## Conclusion

The main contribution of this research is the demonstration that leukocyte telomere length functions as a quantifiable biomarker of biological aging in Thoroughbred horses, one that captures meaningful variation beyond chronological age alone. The 53 % decline in T/S ratio from the youngest to the oldest cohort, coupled with the independent negative effect of career race exposure, establishes telomere dynamics as a biologically plausible and statistically robust correlate of both aging and athletic wear.

Several lines of supporting evidence reinforce this conclusion. The correlation between shorter telomeres and elevated 8-OHdG links telomere attrition to oxidative DNA damage at the molecular level. The association between higher resting heart rate and reduced telomere length adds a physiological dimension, connecting cellular aging to whole-animal cardiovascular status. And the finding that horses with longer-than-expected telomeres are more likely to remain in active competition provides a functional validation—telomere length is not merely an abstract genomic measurement but one that tracks with real-world performance longevity.

For the equine industry, these results have practical implications. Telomere profiling could be incorporated into pre-purchase veterinary examinations to supplement traditional assessments of soundness. Breeders might use parental telomere length as a selection criterion alongside speed indices and conformation scores. And racing authorities could explore whether telomere-based biological age estimates improve the accuracy of handicapping or the fairness of age-restricted race conditions.

Open questions remain. The cross-sectional design cannot establish whether short telomeres cause earlier retirement or merely accompany it; a longitudinal cohort followed from first race to retirement would clarify the temporal relationship. Training intensity—distinct from race starts—was not quantified and may be an additional or confounding source of oxidative stress. Tissue-specific telomere measurements (e.g., in musculoskeletal tissues) might correlate more directly with orthopaedic soundness than leukocyte-based assays. And the heritability of equine telomere length, while estimated at 0.36-0.44 in humans [15], has not yet been calculated in Thoroughbreds, leaving the genetic contribution to inter-individual variation undefined.

## Acknowledgements

### Institutional support

The authors thank the Ontario Equine Genomics Centre laboratory team and the managers of participating racing stables and retirement farms for coordinating sample collection around training and competition schedules.

### Contributions not qualifying for authorship

Ms. Sarah Chen assisted with DNA extraction and qPCR plate setup, and Dr. Ahmed El-Sayed provided guidance on oxidative stress biomarker selection.

### Funding sources

This research was funded by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC Grant No. RGPIN-2023-04871). No commercial funding was received.

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### How to Cite This Article

Beaumont-Lavoie É, Fitzpatrick A, Drummond J. Telomere length dynamics as a biomarker of biological aging and longevity in *Equus caballus* (thoroughbred) horses. *Int J Vet Sci Anim Husb*. 2025;10(5):116-120.

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