Telomere length in newborn, 'small-for-gestational-age' dairy calves

Telomeerlengte bij melkveekalveren met kleine geboortegrootte

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ABSTRACT

In human medicine, both telomere length (TL) and small birth size have been linked to an increased disease risk later in life, although there are conflicting data linking size and TL at birth. The aim of this study in neonatal dairy heifers was to explore whether birth size was associated with TL at birth, and, if so, whether this relationship was also associated with the animals' lifespan (the animals were followed up over a period of six years and nine months). In this study, there was no association between TL at birth and the birth size category. However, small-for-gestational-age dairy calves tended to have a reduced lifespan compared to their average, for-gestational-age counterparts. These findings suggest that fetal growth may not impact telomere biology directly. More research is warranted to understand early life TL dynamics and its effects on dairy cattle longevity.

SAMENVATTING

In de humane geneeskunde werd zowel telomeerlengte (TL) als een kleine geboortegrootte in verband gebracht met een verhoogd risico op ziekten op latere leeftijd. Er is echter sprake van tegenstrijdige resultaten met betrekking tot het verband tussen de grootte en TL bij de geboorte. Het doel van deze studie bij pasgeboren melkveevaarzen was om te onderzoeken of de geboortegrootte geassocieerd was met de TL bij geboorte en indien dit het geval was of deze relatie ook geassocieerd was met de levensduur van de onderzochte dieren (met een follow-upperiode van zes jaar en negen maanden). Er werd geen verband gevonden tussen TL bij de geboorte en de geboortegrootte-categorie, maar kalveren die klein waren voor de drachtduur hadden over het algemeen een kortere levensduur dan kalveren van gemiddelde grootte. Deze bevindingen suggereren dat foetale groei mogelijk geen directe invloed heeft op de telomeerbiologie. Meer onderzoek is nodig om de dynamiek van TL tijdens het vroege leven en de effecten daarvan op de levensduur bij melkvee beter te begrijpen.

INTRODUCTION

Telomeres are repetitive DNA nucleotide sequences interacting with proteins that form a cap at the ends of eukaryotic linear chromosomes and protect chromosomal integrity (Blackburn, 1991; Haussmann and Vleck, 2002). With every cell division, telomeres are not fully replicated leading to age-related telomere shortening (Slykerman et al., 2019). In humans, the fetal origins of adult disease, or the Barker hypothesis, led to research linking low birthweight or being born small for gestational age (SGA) with an increased risk of adult cardiovascular and metabolic disease (Calkins and Devaskar, 2011). A short telomere length (TL) has been negatively associated with an individual's lifespan and disease risk later in life (Steenstrup et al., 2017; Schneider et al., 2022). Thus, there might be a link between low birthweight and in-

creased adult disease risk, mediated by a shorter TL (Slykerman et al., 2019). While in human medicine, some studies reveal a positive association between size and telomere length at birth (de Zegher et al., 2017; Lee et al., 2017), others found no difference in TL according to birth size category (Akkad et al., 2006; Kajantie et al., 2012; Niu et al., 2019).

Similar to what has been described in humans, a cow's future health (Ilska-Warner et al., 2019) and productive lifespan (Brown et al., 2012; Seeker et al., 2018) have been associated with TL and telomere attrition rate in dairy cows (Seeker et al., 2018; Seeker et al., 2018b; Ilska-Warner et al., 2019). Moreover, it has recently been described that the gestational environment influences both telomere length and size at birth, with both maternal (age, parity, milk yield) and environmental (increased late gestation temperature-humidity index) influencing factors (Meesters et al., 2023; Meesters et al., 2024a). The authors also linked being born SGA to reduced fertility, productive performance and survival in dairy heifers (Meesters et al., 2024b). This raises the question whether the prenatal stress of impaired intrauterine growth could be linked to accelerated telomere attrition, hence, shortened telomeres at birth. Although, to the best of the authors' knowledge, no research has been performed linking TL at birth to birth size categories in cattle. The authors hypothesized that calves born small for gestational age might have a shorter telomere length at birth than those born average or large for gestational age (AGA or LGA, respectively), indicating a potential association between intrauterine fetal growth and telomere length at birth. Furthermore, the authors proposed that SGA calves may have a reduced lifespan compared to AGA and LGA calves, possibly explained by a shorter telomere length. Therefore, the aim of the present study was to examine whether calves born small for gestational have a shorter TL at birth than AGA and LGA calves, and to assess if birth size category is associated with differences in lifespan. These findings could reveal potential links between intrauterine growth patterns, neonatal TL, and lifespan in dairy cattle.

MATERIALS AND METHODS

This study was conducted on four dairy farms in Flanders (Belgium). Informed consent was obtained from the participating farmers. All purebred, singleton, and female Holstein-Friesian (HF) calves born at the four herds between August 2017 and November 2018 were enrolled in the study. Only calves born after a term gestation (265 to 295 days) were included in the study (N=210). Whole blood samples were collected by venipuncture (vena jugularis) in 10 mL Vacutainer® EDTA tubes (Becton Dickinson, Plymouth, United Kingdom), within the first ten days of life. Samples were analyzed at the Centre for Environmental Sciences, Hasselt University (Belgium) and leukocyte TL was measured following the protocol described by Meesters et al. (2023). In short, leukocyte DNA was extracted from the whole blood using the QIAamp DNA Mini Kit (Qiagen, Inc., Venlo, the Netherlands). Relative average leukocyte TL was measured and assessed in triplicate, as previously described by Martens et al. (2016), by the use of a modified quantitative real-time PCR (qPCR) protocol. Briefly, the telomeric region was amplified with the use of telomere specific primers (telg and telc), and one single-copy gene (beta-globulin) was amplified on a QS5 Fast Real-Time PCR System (Applied Biosystems, Hasselt, Belgium) in a 384-well format. Cycle thresholds after the amplification of the telomere specific region were normalized relative to the cycle thresholds after the amplification of the single-copy gene using the QBase+2 software (Biogazelle, Zwijnaarde, Belgium) (Martens et al., 2016). Relative average leukocyte telomere lengths were expressed as the ratio of telomere copy number to single-copy gene number (T/S), relative to the average T/S ratio of the entire sample set. All calves were categorized in terms of birth size, as small, average or large for gestational age (SGA, AGA, LGA, respectively) as described by Meesters et al. (2024a). Data was collected until the end of the study (31 May, 2024), at which point 150 animals were removed from the herd while 60 were still present on the farms. Reasons for culling (dead or sold) were noted by the farmers and were categorized as 'accident', 'claws and legs', 'disease', 'other', 'production', 'reproduction', and 'udder'. Lifespan was defined as the number of days from birth until herd exit (dead or sold). For sold animals (e.g. to other farms, fattening units, or slaughter), no further follow-up data were available, and their fate beyond herd exit could not be ascertained, thus being sold was classified as not surviving on the herds in this study.

Statistical analyses were conducted using R (version 2023.06.0). The telomere length was tested for normality using the Shapiro-Wilk test (P=0.001) and subsequently log-transformed. An ANOVA was used to analyze associations between birth weight, gestation length, age at first insemination (AFI), age at first calving (AFC), and TL and birth size category, followed by Tukey's post-hoc test to assess pairwise differences between categories where significant effects were found. A survival analysis was performed on all animals, using a censoring variable to differentiate animals that left the herd from those still alive at the end of the data collection. Survival data were analyzed by Kaplan-Meier survival analysis, using the Survival package in R (Therneau, 2023). Multivariable (with herd as a random effect), cox and frailty models with clustering (herd) were built to assess the effect of TL and birth size on lifespan (time to last observation). Statistical significance was set at $P<0.05$, with tendencies identified as $0.05<P\leq0.10$. Model residuals were assessed using a scatterplot of the studied residuals.

RESULTS AND DISCUSSION

To the best of the authors' knowledge, this is the first study to examine the association between birth size category and TL at birth, in term Holstein Friesian dairy calves.

The calves had a mean [± standard deviation (SD)] gestational age of 278 ± 4.7 days. Their mean age at sampling was 4 ± 2.2 days, the mean bodyweight at sampling was 40.5 ± 4.72 kg, and their mean age at first insemination and calving were 426 ± 57.2 days and 725 ± 73.5 days, respectively (Table 1). An ANOVA and Tukey analysis revealed a significant difference in birth weight between the SGA, AGA, and LGA calves (P<0.0001), as well as a significant difference between the gestation length of SGA versus AGA and LGA calves (P=0.028 and P=0.006, respectively). No significant differences were found in the AFI and AFC of SGA, AGA, and LGA calves. These results are consistent with previous observations, where there was no significant difference in AFI and AFC across the different birth size groups (Meesters et al., 2024b). The recorded AFC in the four herds was slightly lower than the 26 months average in Flemish dairy herds (CRV, 2020) (Table 1).

The average neonatal TL was 1.01 ± 0.173 (mean ± SD). The telomere length in SGA, AGA and LGA calves was 1.04 ± 0.210, 1.01 ± 0.164, and 1.00 ± 0.172, respectively. The ANOVA revealed no statistically significant differences in TL across the different birth size categories (P=0.81) (Figure 1). These results are consistent with previous reports in human medicine (Akkad et al., 2006; Kajantie et al., 2012; Niu et al., 2019). Conversely, in other human studies, significant positive associations between birth size and TL have been found (de Zegher et al., 2017; Lee et al., 2017). The reason for this disparity in results may lie in the selection criteria used in the different studies. In some, both term and preterm babies were included (Kajantie et al., 2012; Lee et al., 2017), in others, only birthweight was included, not corrected for gestational age (Kajantie et al., 2012), and some included mothers with a complicated pregnancy (e.g. hypertension, pre-eclampsia, etc.) (Kajantie et al., 2012; Tellechea et al., 2015; de Melo et al., 2017). In this study, only term pregnancies were included, and birth size was classified using birthweight adjusted for gestational age (Meesters et al., 2024a). A limitation of this study might be that the health status of the dam during gestation was unknown, as health status of the dam has been shown to influence a calf's TL at birth (Ilska-Warner et al., 2019). These results suggest that variations in fetal growth may not directly impact telomere length at birth, that other prenatal factors as well as genetics might play a more important role. It must be mentioned that the calves in the present study were up to ten days old at the moment of blood sampling. This implies that although sick animals were excluded from the study, some of the included calves could have suffered from health issues before they were measured and blood sampled. This limitation is of importance because leukocytes or peripheral white blood cells consist of different cell types (macrophages, granulocytes, lymphocytes) known to differ in TL (Lin et al., 2016). Leukocytes appear to be a reasonable proxy for other tissues in dairy cows

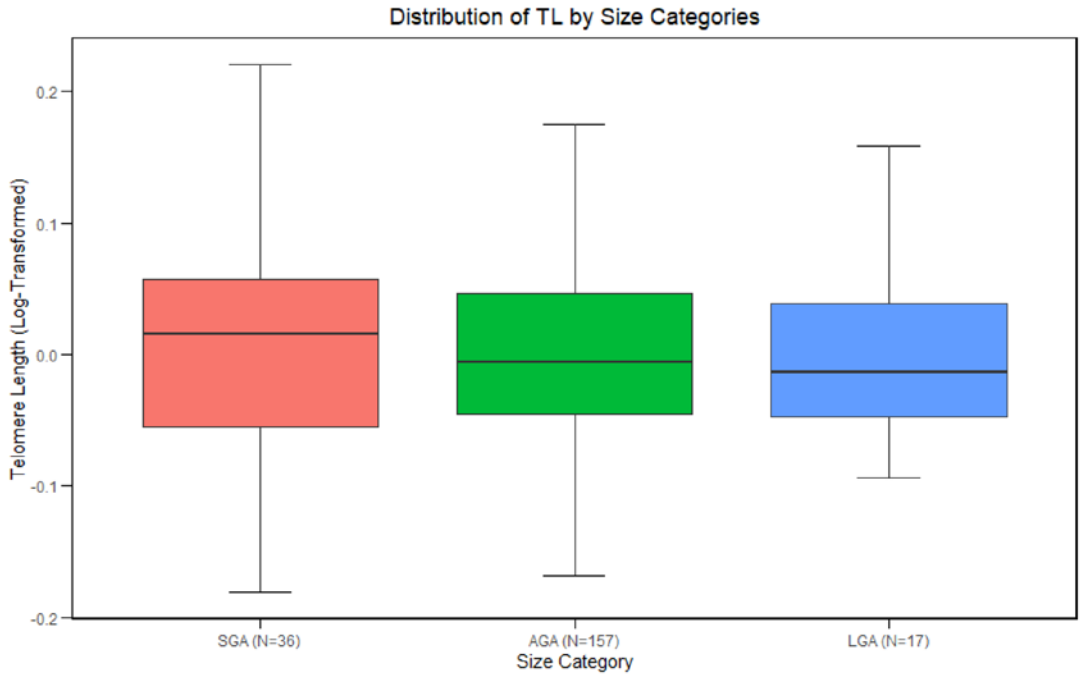

Figure 1. Boxplots showing the distribution of telomere length (TL) across different birth size categories: small, average or large for gestational age (SGA, AGA, LGA) (P=0.81; N=210).

Table 1. Distribution of mean ± SD age at measurement (Age, days), birth weight (kg), gestation length (days), age at first insemination (AFI, days), and age at first calving (AFC, days) according to birth size category: small, average, large for gestational age (SGA, AGA, LGA, respectively), and the population average (N=210).

Birth size category	N	Age	Birth weight	Gestation length	AFI	AFC
SGA	36	4 ± 2.4	34.4 ± 3.07[a]	280 ± 4.4[a]	423 ± 44.1[a]	739 ± 59.2[a]
AGA	157	4 ± 2.2	41.1 ± 3.53[b]	278 ± 4.6[ab]	428 ± 59.4[a]	725 ± 76.7[a]
LGA	17	4 ± 2.3	47.6 ± 3.11[c]	276 ± 4.4[a]	417 ± 59.7[a]	696 ± 61.3[a]
Population Average	210	4 ± 2.2	40.5 ± 4.72	278 ± 4.7	426 ± 57.2	725 ± 73.5

The superscripts (a, ab, c) indicate significant differences between groups.

Table 2. Distribution of reasons for culling (N=150) in small, average, and large for gestational age (SGA, AGA, LGA, respectively) dairy cows.

Birth size	Dead/Sold	Total	Accident	Claws and legs	Disease	Other	Pro-duction	Repro-duction	Udder
SGA	**Dead**	8	1	0	7	0	0	0	0
	Sold	18	0	3	1	2	1	11	0
AGA	**Dead**	29	2	1	22	0	0	3	1
	Sold	84	0	16	4	6	5	35	18
LGA	**Dead**	5	0	0	3	0	0	2	0
	Sold	6	0	2	0	0	1	1	2

when tissue biopsies are not available (Laubenthal et al., 2016). However, measurements of TL in blood leukocytes are always reflections of the cell pool used at that specific time, where leukocytes have high turnover rates, which could also be influenced by infections and chronic diseases (Hägg, 2018). The ratio of the different cell types may change in response to stress or disease, and might consequently alter leukocyte TL (Seeker, 2018). Therefore, the results of the present study may have been influenced by previous disease in calves that were older at sampling. Despite this complication, leukocytes remain the most frequently studied cell type in telomere studies (Seeker, 2018), probably due to their accessibility (Cawthon et al., 2003).

A second limitation of the different ages at blood sampling was the significant effect of calf age at sampling on TL, which the authors described in a previous study using the same animals of the present dataset. In that study, a significantly shorter TL was found in calves that were older at blood sampling (Meesters et al., 2023). This age-dependent change in TL could therefore bias a potential relationship between TL and weight. To bypass possible changes in leukocyte TL across different birth size groups, blood sampling at birth is the best option.

The average lifespan or last observation on the herd was 1370 ± 766.5 days for SGA calves, 1595 ± 661.3 days for AGA calves, and 1633 ± 595.5 days for LGA calves, which did not differ significantly between the different size categories (P=0.56) (Figure 2). The average lifespan and productive lifespan in Flemish herds in 2023 were 1905 days and 1001 days, respectively (CRV, 2025), which seems longer than the lifespan of the animals in the present study. However, no standard deviation nor range were provided by CRV, so comparison to the region's average was difficult. Although a wide range of reasons for culling was documented, the majority of animals across all birth size groups were culled due to disease or reproductive problems (Table 2). It must be noted that the animals that were sold, were considered 'non-survivors' within the context of this analysis, even though their life may have continued on another farm (e.g. fattening).

The multivariable models reveal that SGA calves have a tendency towards a reduced lifespan compared to AGA calves, with a difference of 219 days (P=0.08). These results are in line with recent findings, as calves born SGA showed significantly lower survival rates until a second calving and had higher risk of leaving the herd prematurely (Meesters et al., 2024b) (Figure 2).

While there was a numerical difference, there was no statistical difference between the average lifespan of SGA and LGA calves (P=0.20), which might be explained by the low number of animals in both the SGA and LGA categories. The cox model (P=0.7) and frailty model using clustering (herd) (P=0.7) were not significant. Neither TL, birth size categories, nor the inclusion of a frailty term for herd, appear to be significant predictors for lifespan in this study. Ideally,

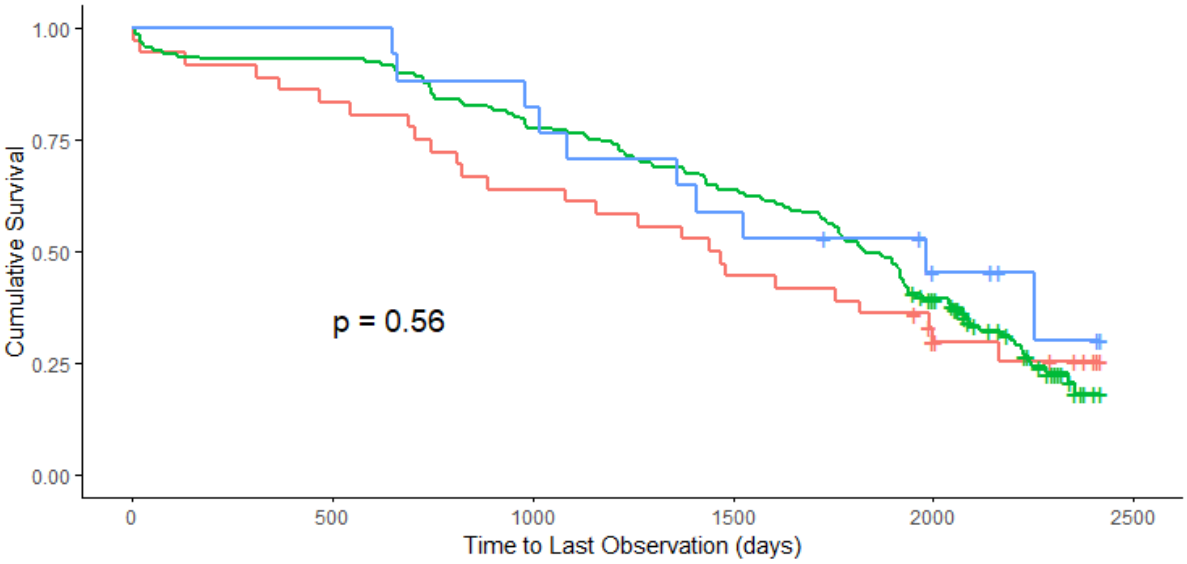

Figure 2. Kaplan-Meier survival analysis plot of survival for each calf size category. There is no significant difference between time to last observation on the herd between the different calf size categories: small, average or large for gestational age (SGA, AGA, LGA) (P=0.56; N=210).

follow-up TL measurements should have been done for each animal during their lifetime, to assess TL attrition rates for each birth size group. Different attrition rates might better explain differences in lifespan, as has already been described in cattle and other species (Whittemore et al., 2019; Seeker et al., 2021). However, in women born SGA, telomere attrition rate is not accelerated in their third decade of life compared to women born AGA (de Melo et al., 2017). Additionally, including (genomic) estimated breeding values for birth weight and longevity would have allowed to isolate the genetic predisposition for birth size and longevity from other prenatal influencing factors. More longitudinal studies with larger sample sizes are warranted and should include genetic factors and maternal health, in order to address other factors that might influence TL at birth as well as TL attrition rates during a cow's productive life.

CONCLUSION

Telomere length does not differ between SGA, AGA and LGA dairy calves. However, SGA calves tend to have a reduced lifespan compared to AGA calves. This suggests that variations in fetal growth may not directly impact telomere biology. Notwithstanding, interpretation of the results should be done with care, as there were limitations in the present study, such as age at sampling, the limited number of animals specifically in the SGA and LGA groups, and the inability to follow each animal to its actual end of life for true assessment of lifespan. Further research with larger study populations, and with blood sampling at birth is needed to better understand the relationship between birth size, telomere length and productive lifespan in dairy cattle.

REFERENCES

Akkad, A., Hastings, R., Konje, J., Bell, S., Thurston, H., Williams, B., (2006). Telomere length in small-for-gestational-age babies. *BJOG: An International Journal of Obstetrics & Gynaecology 113*, 318-323.

Blackburn, E.H., (1991). Structure and function of telomeres. *Nature 350*, 569-573.

Brown, D.E., Dechow, C.D., Liu, W.S., Harvatine, K.J., Ott, T.L., (2012). Hot topic: Association of telomere length with age, herd, and culling in lactating Holsteins. *Journal of Dairy Science 95*, 6384-6387.

Calkins, K., Devaskar, S.U., (2011). Fetal origins of adult disease. *Current Problems in Pediatric and Adolescent Health Care 41*, 158-176.

Cawthon, R.M., Smith, K.R., O'Brien, E., Sivatchenko, A., Kerber, R.A., (2003). Association between telomere length in blood and mortality in people aged 60 years or older. *The Lancet 361*, 393-395.

CRV, (2020). E-book: *CRV Beslissen van Kalf tot Koe*. Popkema, B. Kleiboer and A. Booij (editors). https://crv4all.be/be/service/beslissen-van-kalf-tot-koe

CRV, (2025). *Koeien en Bedrijven in Cijfers Vlaanderen*. [Accessed 15 May 2025], https://crv4all.be/be/service/koeien-en-bedrijven-in-cijfers-vlaanderen

de Melo, A.S., Reis, R.M.d., Calado, R.T., de Carvalho Cavalli, R., Bettiol, H., Cardoso, V.C., Ferriani, R.A., Barbieri, M.A., Vieira, C.S., (2017). The telomere attrition rate is not accelerated in women born small for gestational age: A birth cohort study. *Gene 600*, 16-20.

de Zegher, F., Díaz, M., Lopez-Bermejo, A., Ibáñez, L., (2017). Recognition of a sequence: more growth before birth, longer telomeres at birth, more lean mass after birth. *Pediatric Obesity 12*, 274-279.

Hägg, S., (2018). Telomere length dynamics and atherosclerotic disease. *Circulation Research 122*, 546-547.

Haussmann, M.F., Vleck, C.M., (2002). Telomere length provides a new technique for aging animals. *Oecologia 130*, 325-328.

Ilska-Warner, J.J., Psifidi, A., Seeker, L.A., Wilbourn, R.V.,

Underwood, S.L., Fairlie, J., Whitelaw, B., Nussey, D.H., Coffey, M.P., Banos, G., (2019). The genetic architecture of bovine telomere length in early life and association with animal fitness. *Frontiers in Genetics 10*, 1048.

Kajantie, E., Pietiläinen, K.H., Wehkalampi, K., Kananen, L., Räikkönen, K., Rissanen, A., Hovi, P., Kaprio, J., Andersson, S., Eriksson, J.G., Hovatta, I., (2012). No association between body size at birth and leucocyte telomere length in adult life—evidence from three cohort studies. *International Journal of Epidemiology 41*, 1400-1408.

Laubenthal, L., Hoelker, M., Frahm, J., Dänicke, S., Gerlach, K., Südekum, K.H., Sauerwein, H., Häussler, S., (2016). Short communication: Telomere lengths in different tissues of dairy cows during early and late lactation. *Journal of Dairy Science 99*, 4881-4885.

Lee, S.-P., Hande, P., Yeo, G.S.H., Tan, E.-C., (2017). Correlation of cord blood telomere length with birth weight. *BMC Research Notes 10*, 469.

Lin, J., Cheon, J., Brown, R., Coccia, M., Puterman, E., Aschbacher, K., Sinclair, E., Epel, E., Blackburn, E.H., (2016). Systematic and cell type-specific telomere length changes in subsets of lymphocytes. *Journal of Immunology Research 2016(1)*, 5371050.

Martens, D.S., Plusquin, M., Gyselaers, W., De Vivo, I., Nawrot, T.S., (2016). Maternal pre-pregnancy body mass index and newborn telomere length. BMC Medicine 14, 148.

Meesters, M., Van Eetvelde, M., Martens, D.S., Nawrot, T.S., Dewulf, M., Govaere, J., Opsomer, G., (2023). Prenatal environment impacts telomere length in newborn dairy heifers. *Scientific Reports 13*, 4672.

Meesters, M., Van Eetvelde, M., Verdru, K., Govaere, J., Opsomer, G., (2024a). Small for gestational age calves: part I - concept and definition, contributing prenatal factors and neonatal body morphometrics in Holstein Friesian calves. *Animals 14*, 2125.

Meesters, M., Van Eetvelde, M., Verdru, K., Govaere, J., Opsomer, G., (2024b). Small for gestational age calves: part II - reduced fertility, productive performance, and survival in Holstein Friesian heifers born small for their gestational age. *Animals 14*, 2157.

Niu, Z., Li, K., Xie, C., Wen, X., (2019). Adverse birth outcomes and birth telomere length: a systematic review and meta-analysis. *The Journal of Pediatrics 215*, 64-74.e66.

Schneider, C.V., Schneider, K.M., Teumer, A., Rudolph, K.L., Hartmann, D., Rader, D.J., Strnad, P., (2022). Association of telomere length with risk of disease and mortality. *JAMA Internal Medicine 182*, 291-300.

Seeker, L.A., (2018). Characterisation of telomere length dynamics in dairy cattle and association with productive lifespan. PhD thesis, Royal (Dick) School of Veterinary Sciences. https://era.ed.ac.uk/handle/1842/29626

Seeker, L.A., Ilska, J.J., Psifidi, A., Wilbourn, R.V., Underwood, S.L., Fairlie, J., Holland, R., Froy, H., Bagnall, A., Whitelaw, B., Coffey, M., Nussey, D.H., Banos, G., (2018b). Longitudinal changes in telomere length and associated genetic parameters in dairy cattle analysed using random regression models. *PLOS ONE 13*, e0192864.

Seeker, L.A., Ilska, J.J., Psifidi, A., Wilbourn, R.V., Underwood, S.L., Fairlie, J., Holland, R., Froy, H., Salvo-Chirnside, E., Bagnall, A., Whitelaw, B., Coffey, M.P., Nussey, D.H., Banos, G., (2018). Bovine telomere dynamics and the association between telomere length and productive lifespan. *Scientific Reports 8*, 12748.

Seeker, L.A., Underwood, S.L., Wilbourn, R.V., Dorrens, J., Froy, H., Holland, R., Ilska, J.J., Psifidi, A., Bagnall, A., Whitelaw, B., Coffey, M., Banos, G., Nussey, D.H., (2021). Telomere attrition rates are associated with weather conditions and predict productive lifespan in dairy cattle. *Scientific Reports 11*, 5589.

Slykerman, R.F., Joglekar, M.V., Hardikar, A.A., Satoor, S.N., Thompson, J.M.D., Jenkins, A., Mitchell, E.A., Murphy, R., (2019). Maternal stress during pregnancy and small for gestational age birthweight are not associated with telomere length at 11 years of age. *Gene 694*, 97-101.

Steenstrup, T., Kark, J.D., Verhulst, S., Thinggaard, M., Hjelmborg, J.V.B., Dalgård, C., Kyvik, K.O., Christiansen, L., Mangino, M., Spector, T.D., Petersen, I., Kimura, M., Benetos, A., Labat, C., Sinnreich, R., Hwang, S.-J., Levy, D., Hunt, S.C., Fitzpatrick, A.L., Chen, W., Berenson, G.S., Barbieri, M., Paolisso, G., Gadalla, S.M., Savage, S.A., Christensen, K., Yashin, A.I., Arbeev, K.G., Aviv, A., (2017). Telomeres and the natural lifespan limit in humans. *Aging (Albany NY) 9*, 1130-1142.

Tellechea, M., Gianotti, T.F., Alvariñas, J., González, C.D., Sookoian, S., Pirola, C.J., (2015). Telomere length in the two extremes of abnormal fetal growth and the programming effect of maternal arterial hypertension. *Scientific Reports 5*, 7869.

Therneau, T.M., (2023). A package for survival analysis in R. *R Package Version 2(7)*, 2014.

Whittemore, K., Vera, E., Martínez-Nevado, E., Sanpera, C., Blasco, M.A., 2019. Telomere shortening rate predicts species life span. In: *Proceedings of the National Academy of Sciences*, USA 116, 15122-15127.