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Mathematical modelling of aging acceleration of the human follicle due to oxidative stress and other factors

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Abstract

There is a gradual telomere shortening due to the inability of the replication machinery to copy the very ends of chromosomes. Additionally, other factors such as high levels of oxidation (free radicals or reactive oxygen species (ROS)), for example due to cumulated stress, inflammation or tobacco smoke, accelerate telomere shortening. In humans, the average telomere length is about 10-15kb at birth and telomeres shorten at a pace of 70 bp per year. However, when cells are exposed to reactive oxygen species, telomere attrition happens at a faster pace, generating a wide variety of telomere size distribution in different length percentiles, which are different to what is expected just by age. In this work, the generational age of a cell is associated with its telomere length (TL), from certain maximum to the minimal TL that allows replication. In order to study the accumulation of aged Granulosa cells (GCs) in human follicles, from preantral to preovulatory size, a mathematical model is proposed, regarding different degrees of accelerated telomere shortening, which reflect the action of reactive oxygen species in addition to the telomere shortening that happens after cell division. In cases of cells with TL shorter than cells with average TL, with low telomerase activity and accelerated telomere shortening, the mathematical model predicts an aged outcome in preovulatory follicles. The model provides a plausible explanation for what has been observed in oocytes from older women, which have been exposed to ROS for a longer period of time and have bad outcomes after in vitro fertilization.

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1. Introduction

Reproductive aging in women is a continuous process that begins before birth and extends through the transition to menopause. The main mechanism behind this process lies in the depletion of the ovarian follicles, remaining after menopause a pool of follicles which will never be activated, known as non growing follicles (NGFs) (Hansen et al., 2008). The female reproductive system ages in such a way that it fails at a relatively young age (51 ± 8 years on average) compared with the lifespan of women. Although menopause itself is an easily recognizable end point for reproductive life, dysfunctions due to reproductive aging begin years earlier. The peak of fertility in a woman's life occurs around the age of 25, after which a general decline in fertility takes place and increases severely from the age of 35 onwards (Menken et al., 1986). The wide age range at which natural menopause occurs indicates that there is great variation in the reproductive aging process in women. Clinically, it is proven that when a woman reaches perimenopause, her fertility is severely compromised (Santoro et al., 1996). At that point in the reproductive aging process, treatment options are limited due to ovarian resistance to exogenous gonadotropins and aneuploidy of most of the remaining oocytes (Treff et al., 2011).

The reasons of this biological variation of ovarian aging can be multiple, some of them are the initial number of follicles, different rates of follicular atresia, distinct TL in cumulus cells, oocytes and GCs, diverse rate of telomere shortening and varied levels telomerase activity. There are different mathematical models studying the impact of diverse factors on ovarian aging, some of them consider the relation between follicular decline with ageing and the role of hormones, for example (Thilagam, 2016), in which by means of simulations, the time evolution of the number of ovarian follicles influenced by hormone levels are examined. However, this work is not focused on the evolution of the number of ovarian follicles but on the GCs aging in relation to the telomerase activity and the degree of oxidation.

Telomeres are specialized chromatin loci, localized at chromosomes ends which safeguard the genome integrity. During chromosome replication, the end of the chromosome is shortened. Over time, due to each cell division,

the telomere ends become shorter giving rise to the end-replication-problem (Olovnikov, 1973) which was associated to the discovery of Hayflick and Moorhead (1961) who showed that human fibroblasts could only undergo a limited number of cell divisions upon in vitro expansion which leads to the phenomenon known as replicative senescence. In 1998, Hayflick introduced the idea that cells were mortal because their telomeres shortened (Hayflick, 1998), and nowadays the number of divisions that cells can undergo is denoted as the Hayflick limit. Telomeres are replenished by an enzyme, the telomerase reverse transcriptase, that counteracts the effects of telomere shortening by adding de novo telomeric repeats onto chromosome ends (Greider and Blackburn, 1985). When telomeres reach a critically short length, the DNA damage response pathways are activated, leading to cell senescence or apoptosis (Karlseder et al., 1999; Collado et al., 2007). The accumulation of senescent cells in tissues leads to aged organs (Munoz–Espin and Serrano, 2014).

The process of organ aging can be accelerated if cells are exposed to damaging agents which cause accelerated telomere attrition. Thus, after mitosis, one of the daughter cells could increase its age by two or more generations (Rubelj and Vondracek, 1999; Proctor and Kirkwood, 2002). Therefore, there is a gradual telomere shortening due to the inability of DNA polymerase to replicate the very ends of chromosomes, which can be incremented by the action of other agents. In some cases, there can be an abrupt telomere shortening, as for example in the sudden senescence syndrome which can drive a cell to senescence even with a single mitotic event (Jones et al, 1985). Abrupt telomere shortening was studied in (Rubeljz and Vondracek, 1999) with a model for stochastic nature of cellular aging. Oxidative stress has been shown to accelerate telomere shortening (T. von Zglinicki et al., 2000). In fact, many factors can influence telomere shortening (Starkweather et al., 2014; Razgonova et al., 2020): different diseases, including immune-associated diseases (Yudoh et al., 1999; Katayama and Kohriyama, 2001; Fujii et al., 2009); chronic stress (Epel et al., 2004; Georgin-Lavialle et al., 2010); mental and depresive disorders (Vakonaki et al., 2018); drug abuse (Pavanello et al., 2011) and genetic factors.

In the previous article (Portillo et al., 2019), the mathematical model assumed that when a cell divides it produces two daughter cells whose generational age increases by one, due to telomere shortening via the end-replication problem and mild oxidation. In this work, we modified the previous model to include higher level of oxidation that accelerate telomere shortening by as-

suming mitosis produces two cells whose generational age may be any greater than the cell mother one.

The paper is organized as follows. Different percentiles of TL according to age are estimated in Section 2. The mathematical model is introduced in Section 3. Numerical experiments are conducted in Section 5. Finally, the last section is devoted to the discussion.

2. Different percentiles of telomere length according to age

Human cells can undergo a limited number of cell divisions, since TL shortens during DNA replication and, at a critical threshold, cells reach an indivisible state called replicative senescence. This limit is reached when the telomere length is reduced to 3000 bases. In (Wagner et al., 2018), a study was carried out which showed a normal distribution of telomere length with age in lymphocytes. These data indicated that TL across different populations has reproducible upper and lower boundaries, establishing lymphocyte telomere length measured in kilobases versus age for different percentiles.

We called TL_y the telomere length measured in number of bases at y years. In order to calculate h, the number of times that a cell can be divided before reaching the senescent state (Hayflick limit), we must contemplate the number of bases the telomere loses in each division. Telomere length reductions are due not only to the end replication problem but also to other factors such as oxidative stress. According to (Wagner et al., 2018), the number of base pairs lost per cell division ranges between 50 and 200. We considered the mean value 125, including losses by the end replication problem and mild oxidation. Therefore, the value of h for each age would be approximately

$$\frac{TL_y - 3000}{125}, \tag{1}$$

rounding to the next integer number.

Using (1) we estimated the values of h for different percentiles and different ages, considering Figure 2A in (Wagner et al., 2018) which displays lymphocyte telomere length measured in kilobases versus age for different percentiles. Table 1 shows the value h for human lymphocytes for different percentiles at 25 and 40 years old.

The objective of our work is to study the aging of the human follicle. Therefore, we must apply a conversion to the values of the previous table,

Percentile	LTL_{25}	$h_{25}(L)$	LTL_{40}	$h_{40}(L)$
1%	5600	21	4600	13
10%	6800	30	5900	23
50%	8000	40	7400	35
90%	9400	51	8400	43
99%	10600	61	9900	55

Table 1: h for lymphocytes for different percentiles at 25 and 40 years old.

which represent TL in lymphocytes, to TL in GCs. For this purpose, following the formulas for the evolution of leukocytes telomeric length (LTL) and granulosa cells telomeric length (GTL) in (Xu et al., 2017),

$$\begin{aligned} LTL(year) &= -0.067 \times (year - 21) + 3.55, \quad year \in [21, 39], \\ GTL(year) &= -0.089 \times (year - 23) + 4.05, \quad year \in [23, 39], \end{aligned}$$

$$\begin{aligned} LTL(25) = 3.280, GTL(25) = 3.8720, \quad GTL(25)/LTL(25) = 1.1798, \\ LTL(39) = 2.3440, GTL(25) = 2.6260, \quad GTL(39)/LTL(39) = 1.1203, \end{aligned}$$

we estimated

$$\begin{aligned} GTL(25) &= 1.2\, LTL(25), \\ GTL(40) &= 1.1\, LTL(40). \end{aligned}$$

With this data, we updated LTL_{25} in Table 1 by $1.2\, LTL_{25}$ in Table 2 and the values of LTL_{40} in Table 1 by $1.1\, LTL_{40}$ in Table 2, then we used (1) again to estimate the values of h.

In the numerical experiments with our model we will consider the values of h 40, 50 and 60 to cover different percentiles at the ages of 25 and 40.

3. Mathematical model to classify a population in subpopulations according to the generational age

We assumed that a cell species expressed as C has a certain maximum and minimum telomeric length, being the Hayflick limit the minimal telomeric length that permits replication. Then following (Wesch et al., 2016), the

Percentil	GTL_{25}	$h_{25}(G)$	GTL_{40}	$h_{40}(G)$
1%	6720	30	5060	16
10%	8160	41	6490	28
50%	9600	**53**	8140	**41**
90%	11280	66	9240	50
99%	12720	78	10890	63

Table 2: h for granulosa cells for different percentiles at 25 and 40 years old.

generational age of a cell was associated with its telomere length, regardless of when it was formed. Considering h the number of times that a cell can be divided before reaching the senescent state, the generational age of a cell subpopulation was indicated by subscript i, for $i = 0, 1, \ldots, h-1, h$, thereby, cells with maximum telomeric length were considered as generational age of zero and were denoted by C_0, cells whose telomeric length was strictly between the maximum and the minimum value $C_1, \ldots, C_{h-1}$, which can undergo mitosis and replicate, and senescent cells C_h, which have reached the Hayflick limit. The populations of cells of each generational age at a given time t was denoted by $N_i(t)$, for $i = 0, 1, \ldots, h-1, h$.

3.1. Model 1: telomere shortening due to the end replication problem and mild oxidative stress

It is appropriate to remember the former model (Portillo et al., 2019), because several of the parameters that we will use are similar and to show the differences between both models. Let m be the rate of mitotic replication per cell per unit of time. A cell undergoes mitosis to produce two cells whose generational age increases by one; this can occur only when $i \neq h$. Let d be the rate of mortality events per cell per unit of time. Cells of any generation are susceptible to death. Let r be the rate of telomerase activity per cell per unit of time which acts rejuvenating the cell and moving back to the previous generational age. Only $i \neq 0$ cells can be acted by telomerase.

If all rates are taken constant, the average subpopulations $N_0, N_1, \ldots, N_h$ satisfy the following coupled linear ordinary differential equations

$$\begin{aligned} N_0'(t) &= -(m+d)N_0(t) + rN_1(t), \\ N_i'(t) &= 2mN_{i-1} - (m+d+r)N_i(t) + rN_{i+1}(t), \ i = 1, \ldots, h-1, \\ N_h'(t) &= 2mN_{h-1} - (d+r)N_h(t). \end{aligned} \quad (2)$$

Denoting by $\mathbf{N}(t) = [N_0, N_1, \ldots, N_h]^T$ the system of equations (2) can be rewritten as

$$\mathbf{N}'(t) = A\mathbf{N}(t), \tag{3}$$

where

$$A = \begin{pmatrix} -(m+d) & r & 0 & \cdots & 0 \\ 2m & -(m+d+r) & r & 0 & 0 \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ 0 & \cdots & 2m & -(m+d+r) & r \\ 0 & \cdots & 0 & 2m & -(d+r) \end{pmatrix} \tag{4}$$

is a tridiagonal matrix of dimension $(h+1) \times (h+1)$.

It is well known that the solution of (3) with initial condition $\mathbf{N}(t_0) = \mathbf{N}_0$ is $\mathbf{N}(t) = exp(A(t-t_0))\mathbf{N}_0$.

3.2. Model 2: increased telomere shortening due to higher level of oxidative stress and other factors

In order to consider increased telomere shortening due to oxidative stress and other circumstances, this model is similar to the previous one except that after mitosis cells may increase their generational age at any greater than the previous one. In fact, oxidation due to physical or emotional stress and other factors can cause a dramatic loss of telomeric repeats during mitosis and different parts of the population may pass to any subsequent generational age. Let suppose a ratio x_1 of cells jumps two generational ages, a ratio x_2 jumps three generational ages, a ratio x_3 jumps four generational ages, and in general a ratio x_i of cells jumps $i+1$ generational ages. Under these hypotheses, the average subpopulations $N_0, N_1, \ldots, N_h$ satisfy the following coupled linear ordinary differential equations

$$\mathbf{N}'(t) = A\mathbf{N}(t), \tag{5}$$

where $\mathbf{N}(t) = [N_0, N_1, \ldots, N_h]^T$ and the matrix A of dimension $(h+1)\times(h+1)$ is

$$\begin{pmatrix}
-(m+d) & r & \cdots & & & 0 & 0 \\
(2-\sum_{i=1}^{h-1} x_i)m & -(m+d+r) & r & \cdots & & 0 & 0 \\
x_1 m & (2-\sum_{i=1}^{h-2} x_i)m & & \ddots & & 0 & 0 \\
x_2 m & x_1 m & \cdots & -(m+d+r) & r & 0 & 0 \\
\vdots & \ddots & & \ddots & \ddots & \vdots & \vdots \\
x_{h-3}m & x_{h-4}m & \cdots & (2-\sum_{i=1}^{2} x_i)m & -(m+d+r) & r & 0 \\
x_{h-2}m & x_{h-3}m & \cdots & x_1 m & (2-x_1)m & -(m+d+r) & r \\
x_{h-1}m & x_{h-2}m & \cdots & x_2 m & x_1 m & 2m & -(d+r)
\end{pmatrix}. \tag{6}$$

We notice that in this model the matrix can be filled from the main diagonal downwards, unlike the previous model where except for the diagonal immediately below the main one, the rest were null. While matrix (4) is tridiagonal, the matrix (6) is an unreduced lower Hessenberg matrix.

Proposition 1. *The solutions of the systems with matrices (4) and (6,) corresponding to the models 1 and 2, preserve positivity, i.e. a nonnegative initial condition leads to a nonnegative solution over time.*

Proof. The solution of (3) with nonnegative initial condition $\mathbf{N}(t_0) = \mathbf{N}_0$ is $\mathbf{N}(t) = exp(A(t-t_0))\mathbf{N}_0$.

If the model 1 is considered, the matrix (4) can be written as $A = M - (m+d+r)I$ where M is the following matrix with nonnegative coefficients

$$M = \begin{pmatrix}
r & r & 0 & \cdots & 0 \\
2m & 0 & r & 0 & 0 \\
\vdots & \ddots & \ddots & \ddots & \vdots \\
0 & \cdots & 2m & 0 & r \\
0 & \cdots & 0 & 2m & m
\end{pmatrix}.$$

Then, $\mathbf{N}(t) = exp(-(m+d+r)(t-t_0))exp(M(t-t_0))\mathbf{N}_0$ and therefore the solution is nonnegative over time.

A similar result is deduced for matrix (6) considering as matrix M a matrix equal to (6) except the main diagonal which is $[r, 0, \ldots, 0, m]$. □

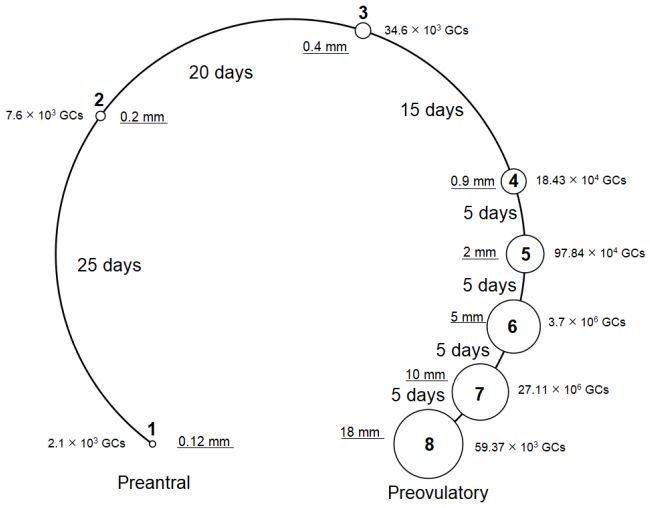

Figure 1: Classification of follicles in the human ovary based on data from (Gougeon, 1996).

3.3. *Simulation of the human follicular growth: from preantral to preovulatory follicle*

Human preantral follicle takes approximately 85 days to reach preovulatory size, going through eight classes, from class 1 for preantral follicle to class 8 for preovulatory class. Figure 1 displays classification of follicles in the human ovary according to (Gougeon, 1996). We focused the study on the evolution of the population of GCs which are the most important somatic cells for determining the size of follicles (Wang et al., 2014). A follicle enters a given class when its number of GCs attains the lower limit of this class and leaves this class when its number of GCs enters into the subsequent class (Gougeon, 1996). We assumed the population of GCs of each class and its respective generational age subpopulations satisfied an ordinary differential system similar to the one of Subsection 3.2. To go through the eight stages corresponding to the development of the follicle from preantral to preovulatory class seven ordinary differential systems concatenated in time were considered, with a vector $\mathbf{N}_0(T_1) = \tilde{\mathbf{N}}$ of initial values

$$\begin{aligned} &\mathbf{N}_j'(t) = A_j \mathbf{N}_j(t), \qquad t \in [T_j, T_{j+1}], \quad \text{for } j = 1, \ldots, 7, \\ &\mathbf{N}_j(T_j) = \mathbf{N}_{j-1}(T_j), \end{aligned} \tag{7}$$

where the matrices A_j had the same structure as (6) but with values m_j, d_j and r_j corresponding to the class j. This is a concatenation of seven linear

systems and thus the solution of (7) can be written as

$$\mathbf{N}_7(t) = exp(A_7(t - T_7))exp(A_6(T_7 - T_6)) \dots exp(A_1(T_2 - T_1))\tilde{\mathbf{N}}.$$

Then, according to the Proposition 1, the solution preserves positivity, i.e. a nonnegative initial condition leads to a nonnegative solution over time. The parameters involved in the systems were calculated in the same way as in (Portillo et al., 2019).

3.4. Aging ratio

The average total number of cells of all generational ages at a given time t is denoted by

$$n(t) = \sum_{i=0}^{h} N_i(t).$$

We divided the population into three sections, young cells in the first section, middle-aged cells in the second one and aged cells in the third one. But unlike what was done in the previous work, where the last third of h was considered aged cells, we wanted aged cells to follow the same criteria for different sizes of h. Then, regarding that the value of h of GCs for 50th percentil at 25 years old is around 50, we took as a reference the last third of $h = 50$ to define aged cells. So we considered aged cells the cells corresponding to the last 17 generations, and the population of aged cells was denoted by

$$na(t) = \sum_{i=h-16}^{h} N_i(t),$$

middle-aged cells that were in the previous 17 (generations from $h - 32$ to $h - 17$ if $h \geq 32$, other case from 0 to $h - 17$) and young cells in the remaining first generations. An indicator of aging is the accumulation of short telomeres. We defined the aging ratio ra as the number of aged cells divided by the total number of cells, that is

$$ra(t) = na(t)/n(t). \tag{8}$$

The values of ra may vary between 0 and 1. Values of ra close to 0 corresponded to populations of young cells, while the closer to 1 its value was, the older the population was.

4. Qualitative analysis

In this section a qualitative study of the simplest model according to its key parameters is carried out. The matrix in equation (4) is non symmetric, so it is not obvious whether the eigenvalues are purely real or complex.

Proposition 2. *The matrix (4) has real eigenvalues and $h+1$ linear independent eigenvectors.*

Proof. Let it be $\rho = \sqrt{2m/r}$ and the diagonal matrix $D = diag(1, \rho^{-1}, \rho^{-2}, \ldots, \rho^{-h})$. Then

$$DAD^{-1} = B = \begin{pmatrix} -(m+d) & \sqrt{2mr} & 0 & \cdots & 0 \\ \sqrt{2mr} & -(m+d+r) & \sqrt{2mr} & 0 & 0 \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ 0 & \cdots & \sqrt{2mr} & -(m+d+r) & \sqrt{2mr} \\ 0 & \cdots & 0 & \sqrt{2mr} & -(d+r) \end{pmatrix}$$

is a symmetric matrix, from where the result is deduced. □

Proposition 3. *If $0 < r < 2m$ in model 1, then an estimation of the aging ratio (8), for t big enough, is*

$$ra(t) \approx est(t) = 1 - \left(\frac{h-17}{h}\right)\left(\frac{r}{2m}\right)^{17/2}. \tag{9}$$

Proof. The solution of (3) with nonnegative initial condition $\mathbf{N}(t_0) = \mathbf{N}_0$ is $\mathbf{N}(t) = exp(A(t-t_0))\mathbf{N}_0$. Let be $n = h+1$, $\lambda_1, \ldots, \lambda_n$ the eigenvalues of A and $\mathbf{u}_1, \ldots \mathbf{u}_n$ a basis of eigenvectors. Let it be $\mathbf{C} = [C_1, \ldots, C_n]^T = P^{-1}\mathbf{N}_0$, being P a matrix whose columns are the eigenvectors. Then, $\mathbf{N}(t) = C_1 e^{\lambda_1(t-t_0)}\mathbf{u}_1 + \cdots + C_n e^{\lambda_n(t-t_0)}\mathbf{u}_n$.

The matrix (4) of model 1 is a tridiagonal Toeplitz matrix whose eigenvalues and eigenvectors can be found explicitly by using exact formulae, see (Yueh and Cheng, 2008). Let it be $\rho = \sqrt{2m/r}$. The dominant eigenvalue and the eigenvector associated $\mathbf{u}_1 = [u_{1,1}, \ldots, u_{1,n}]^T$, are

$$\begin{aligned} \lambda_1 &= -(m+d+r) + 2\rho r, \\ u_{1,j} &= \rho^{j-1}(2mj - r\rho(j-1)),\ j = 1, \ldots, n. \end{aligned}$$

The remaining eigenvalues λ_i and the corrresponding eigenvectors $\mathbf{u}_i = [u_{i,1}, \ldots, u_{i,n}]^T$, $i = 2, \ldots, n$ are

$$\begin{aligned} \lambda_i &= -(m + d + r) + 2\rho r \cos(\theta_i), \\ u_{i,j} &= \rho^{j-1}(2m \sin(j\theta_i) - r\rho \sin((j-1)\theta_i)),\ j = 1, \ldots, n, \end{aligned}$$

where θ_i satisfies the equation

$$2m \sin((n+1)\theta) - m \sin((n-1)\theta) - \rho(r+m) \sin(n\theta) = 0.$$

Taking into account that λ_1 is the dominant eigenvalue, for t big enough, $\mathbf{N}(t) \approx C_1 e^{\lambda_1 (t - t_0)} \mathbf{u}_1$. Consequently,

$$ra(t) = na(t)/n(t) \approx est(t) = \frac{\sum_{j=n-16}^{n} u_{1,j}}{\sum_{j=1}^{n} u_{1,j}} = 1 - \frac{\sum_{j=1}^{j=n-17} u_{1,j}}{\sum_{j=1}^{n} u_{1,j}}.$$

$$\sum_{j=1}^{n} u_{1,j} = 2m \sum_{j=1}^{n} j\rho^{j-1} - r \sum_{j=1}^{n} (j-1)\rho^j = 2mn\rho^{n-1} + (\frac{2m}{\rho} - r\rho) \sum_{j=1}^{n-1} j\rho^j + r\rho^2.$$

Applying Abel-summation by parts method and simplifying it is obtained

$$\sum_{j=1}^{n-1} j\rho^j = \frac{(n-1)\rho^{n+1} - n\rho^n + \rho}{(1-\rho)^2}.$$

Therefore,

$$\begin{aligned} \sum_{j=1}^{n} u_{1,j} &= \frac{2mn\rho^{n-1}(1-\rho)^2 + (\frac{2m}{\rho} - r\rho)((n-1)\rho^{n+1} - n\rho^n + \rho) + r\rho^2(1-\rho)^2}{(1-\rho)^2} \\ &\approx \frac{-r(n-1)\rho^{n+2}}{(1-\rho)^2}. \end{aligned}$$

$$\frac{\sum_{j=1}^{j=n-17} u_{1,j}}{\sum_{j=1}^{n} u_{1,j}} \approx \frac{n-18}{n-1} \rho^{-17} = \left(\frac{h-17}{h}\right) \left(\frac{r}{2m}\right)^{17/2}$$

and the estimation (9) is obtained. □

Remark 4.

$$\lim_{r\to 0} est(t) = 1.$$

The smaller r is, the greater the aging ratio.

Remark 5.

$$\lim_{r\to 2m} est(t) = \frac{17}{h},$$

(the estimation obtained taking into account that $\mathbf{u}_1 = [1,\ldots,1]^T$, when $r = 2m$, would be $17/(h+1)$).

Remark 6. *We observe, for example, that the values of $\frac{h-17}{h}$ for $h = 40$, 50 and 60 are 0.58, 0.66 and 0.72 respectively. Then, the smaller the h the greater it is the aging ratio.*

5. Numerical experiments

The following numerical experiments were devoted to check the model described in Subsections 3.2 and 3.3, where increased telomere shortening was considered, going through different scenarios. Certain parameter values were chosen, in later simulations. Ideally these would be measured. But the knowledge of the effects of oxidative stress and telomerase activity on TL is still limited and more studies are required. In the meantime we are supported by the qualitative analysis of the behaviour of the model in which, as we have seen, the smaller telomerase activity is, the greater the aging ratio and also the smaller the h index the greater the aging ratio.

First, we focus in $h = 50$, a value near the average among women aged 25. Supposing to pass from a certain generational age to a greater one, the further away these generational ages are, the less probability is, we considered two choices for the parameters x_i: the values $x_i = 10^{-i}$ for $i = 1,\ldots,16$ and the remaining values of x_i equal to zero, for the experiment $E1$ and the values $x_i = 2\times 10^{-i}$ for $i = 1,\ldots,16$ and the remaining values of x_i equal to zero, for the experiment $E2$. Figure 2 displays the evolution of cell populations for $r_1 = 0.2$, the value of telomerase rate at preantral stage. Green lines represent young cells, blue lines middle-aged cells and red lines aged cells. Dashed lines correspond to the experiment E1 data and solid line to the experiment E2 ones. Initially the number of young and middle-aged cells remain very similar in the two experiments. No aged cells appeared until after the first 25 days,

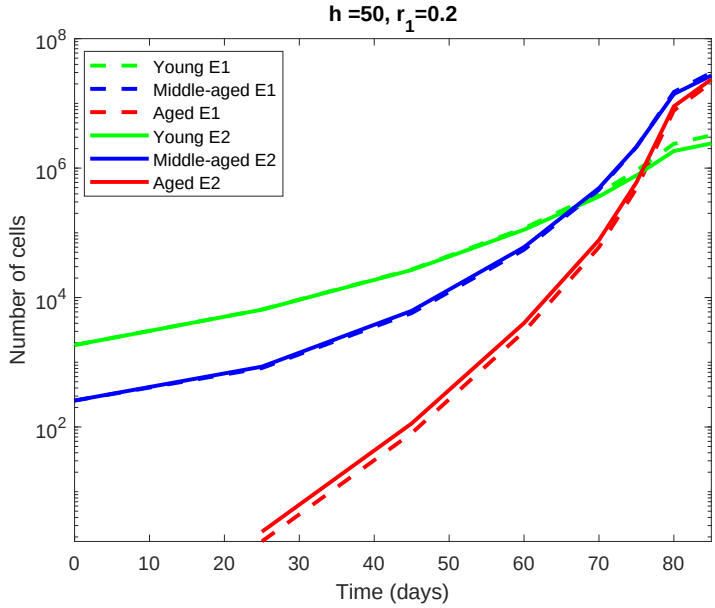

Figure 2: Populations of young cells (green), middle-aged cells (blue) and aged cells (red) from preantral follicle to preovulatory follicle versus time t (days) for $h = 50$ and telomerase rate $r_1 = 0.2$ for experiments E1 and E2.

but from that moment their growth was more pronounced than the middle-aged ones and this in turn is greater than that the young ones. On day 85, middle-aged cells predominated over the others and were higher in $E1$ than in $E2$. They were closely followed by the aged cells ones which were fewer in $E1$ than in $E2$. In addition, there were more young cells in $E1$ than in $E2$. Finally the aging ratio was 0.40 in $E1$ and 0.46 in $E2$. These results suggest that telomere losing by oxidizing agents promotes accelerated cell aging.

Next experiments were concerned about the aging ratio of GCs of preovulatory follicle at day 85, which are critical for the maturation of oocytes, since GCs, transformed in corpus luteum, must divide and increase their size (Alila and Hansel, 1984) to sustain early pregnancy (Csapo et al., 1972). The literature indicates that there is a link between stress and shorter TL, see for instance (Reichert and Stier, 2017; Mehrsafar et al., 2020) and the references in them. Indeed, a review investigating the connections between oxidative stress and telomere shortening in vivo was conducted in (Reichert and Stier, 2017) concluding that the information regarding the in vivo effects of oxidative stress on TL remains limited, because most studies performed so far have used an in vitro approach, this means that our understanding of this link still remains incomplete. For this reason, as an additional alternative, this work was aimed to carry out an in silico study of the effect of oxidative stress on TL, for which several values of the parameters x_i were used to simulate different degrees of accelerated telomere shortening (see Table 3, only the non zero values are indicated).

On one hand, we wanted to study how the aging ratio of the preovulatory follicle was affected by diverse degrees of oxidation and several rates of telomerase activity when the parameter h was fixed to 40, which corresponds to a low percentile of GCs telomere length at 25 years old and an intermediate percentile at 40 years old. On the other hand, under stress, telomerase activity decreases (Razgonova et al., 2020), so for somewhat reduced telomerase activity, we studied the evolution of the follicle preovulatory aging ratio for different telomeric lengths and different degrees of oxidation.

Aging ratio ra of preovulatory follicle versus telomerase rate r_1 for $h = 40$ and the values of the parameters x_i in Table 3 are displayed in Figure 3. The solid lines correpond to several values of x_1 and the remaining x_i equal to zero. The higher the value of x_1 the higher was the aging ratio. Dashed lines correpond to several values of x_1 and x_2 and the remaining zero and dotted lines correspond to several values of x_1, x_2 and x_3 and the remaining zero. For the same sum of the values x_i the aging ratio was higher in dashed lines

x_1	x_2	x_3	line and color
0			solid green
0.1			solid cyan
0.2			solid blue
0.15	0.05		dashed blue
0.1	0.07	0.03	dotted blue
0.3			solid magenta
0.2	0.1		dashed magenta
0.15	0.1	0.05	dotted magenta
0.4			solid red
0.3	0.1		dashed red
0.25	0.1	0.05	dotted red
0.5			solid black
0.4	0.1		dashed black
0.35	0.1	0.05	dotted black

Table 3: x_i values in the Figures 3, 4 and 5.

and dotted lines. That is, passing to more distant generations gave rise to a higher aging ratio.

For $h = 40$, even with mild oxidation, the aging ratio was high which was worsened by low values of telomerase activity and increased oxidation values. This could be one of the causes of low fertility for $h = 40$, which occurs in the average of women in their forties and in younger women in lower percentiles of telomere length.

A least squares approximation of the $a+br_1$ type has been made from the data in Figure 3. Table 4 shows the results for the different values of the x_i parameters and also the mean squared error (MSE). It can be seen that with increasing oxidation the value of a raises, i.e. for $r_1 = 0$ the aging ratio is higher. In addition, $|b|$ diminishes, i.e. the aging ratio decreases more slowly as the telomerase activity increases.

High levels of chronic stress reduce the ability to induce telomerase activity by 25% compared to moderate or low levels of chronic stress (Razgonova et al., 2020). Next we simulated a reduction in telomerase activity by taking $r_1 = 0.2$. In Figure 4 is showed the aging ratio ra of preovulatory follicle versus parameter h for $r_1 = 0.2$ and the same values of the parameters x_i than in the former experiment. Similar conclusion can be done respect to

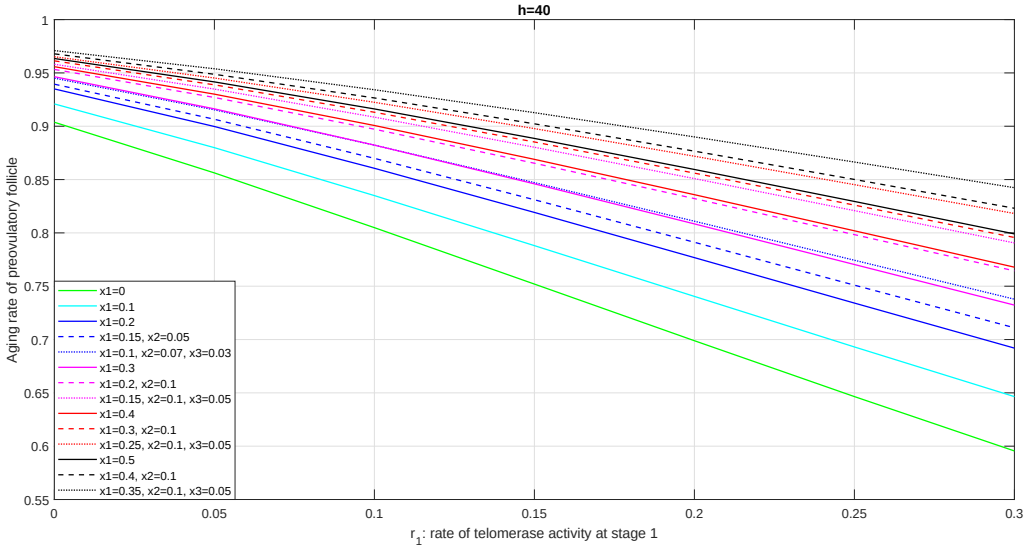

Figure 3: Aging ratio of preovulatory follicle (at day 85) versus telomerase rate r_1 for $h = 40$ and several values of x_i.

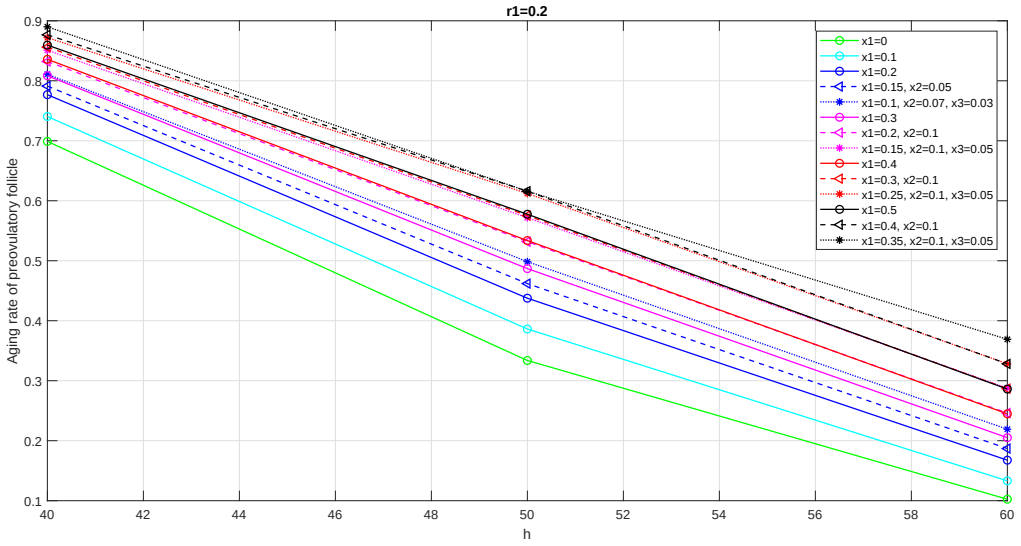

Figure 4: Aging ratio of preovulatory follicle (at day 85) versus h for $r_1 = 0.2$ and several values x_i.

x_1	x_2	x_3	$a + br_1$	MSE
0			$0.9065 - 1.0358r_1$	0.0015
0.1			$0.9246 - 0.9226r_1$	0.0020
0.2			$0.9393 - 0.8171r_1$	0.0024
0.15	0.05		$0.9438 - 0.7678r_1$	0.0025
0.1	0.07	0.03	$0.9492 - 0.6965r_1$	0.0024
0.3			$0.9512 - 0.7202r_1$	0.0027
0.2	0.1		$0.9578 - 0.6350r_1$	0.0026
0.15	0.1	0.05	$0.9622 - 0.5630r_1$	0.0024
0.4			$0.9607 - 0.6322r_1$	0.0029
0.3	0.1		$0.9658 - 0.5568r_1$	0.0027
0.25	0.1	0.05	$0.9692 - 0.4939r_1$	0.0024
0.5			$0.9684 - 0.5530r_1$	0.0029
0.4	0.1		$0.9723 - 0.4868r_1$	0.0027
0.35	0.1	0.05	$0.9749 - 0.4320r_1$	0.0024

Table 4: $a + br_1$ least squares approximation of the aging ratio at day 85 for $h = 40$ and several values of x_i.

the parameters x_i used which would reflect that the more oxidation the more aging. For $h = 50$, for the values of x_i dashed and dotted in black, even dotted in red, an aging ratio similar to $h = 40$ with $x_1 = 0$ or mild stress is reached. In other words, with levels 25% above mild stress, more than 10-year advance of aging occurs. Something similar is observed also for the value $h = 60$ (Figure 4).

In this case we have opted for a least squares approximation of the $a+b/h$ type, from the data in Figure 4, due to the similarity with the form of the estimation of the aging ratio of the qualitative study (9). The results for the different values of the x_i parameters are displayed in Table 5. As oxidation increases the values of $|a|$ and b decrease. On the other hand, the derivative $-b/h^2$ is negative, so the function is decreasing. Therefore when h increases the decrease of aging ratio is slower when there is more oxidation. This behavior can be noted in Figure 5 where the functions in Table 5 are depicted.

6. Discussion

The accumulation of short telomeres impacts negatively the function of tissues (Blasco, 2007; Donate and Blasco, 2011) and may cause degenerative

x_1	x_2	x_3	$a + b/h$	MSE
0			$-1.0953 + 71.6915/h$	0.0034
0.1			$-1.0749 + 72.7213/h$	0.0048
0.2			$-1.0337 + 72.6968/h$	0.0123
0.15	0.05		$-0.9998 + 71.9905/h$	0.0156
0.1	0.07	0.03	$-0.9365 + 70.3481/h$	0.0200
0.3			$-0.9751 + 71.7685/h$	0.0190
0.2	0.1		$-0.8926 + 69.5301/h$	0.0240
0.15	0.1	0.05	$-0.8004 + 66.6734/h$	0.0276
0.4			$-0.9027 + 70.0933/h$	0.0246
0.3	0.1		$-0.8119 + 67.3581/h$	0.0285
0.25	0.1	0.05	$-0.7154 + 64.1899/h$	0.0311
0.5			$-0.8199 + 67.8306/h$	0.0291
0.4	0.1		$-0.7239 + 64.7417/h$	0.0320
0.35	0.1	0.05	$-0.6477 + 61.9110/h$	0.0180

Table 5: $a + b/h$ least squares approximation of the aging ratio at day 85 for $r_1 = 0.2$ and several values x_i.

pathologies (Alder et al., 2008; Armanios et al., 2009; Martinez and Blasco, 2017) therefore the aim of the mathematical model was to simulate the effect of different factors which could accelerate GCs population aging, possibly compromising the viability of the human follicle.

The Hayflick limit is related to replicative senescence, restricting the number of divisions that a cell can undertake (Hayflick and Moorhead, 1961). Taking into account that the limit number of cell divisions depends on age and even within the same age, there is enough biological variety distributed into percentiles, different limit number of cell divisions were considered. The work focused on the age range of 25 years, the moment of maximum fertility up to 40 years, when fertility falls drastically.

The model exposed in this article attempts to describe the loss of bases in telomeres during cell division above the expected average, due to factors such as physical or emotional stress, mental and depression disorders or drug abuse and their influence on the acceleration of human follicle aging. For that purpose, we have considered that in addition to telomere attrition due to mitosis, cells may increase their generational age not only by one, as in the previous model (Portillo et al., 2019), but by two or more, that is, daughter

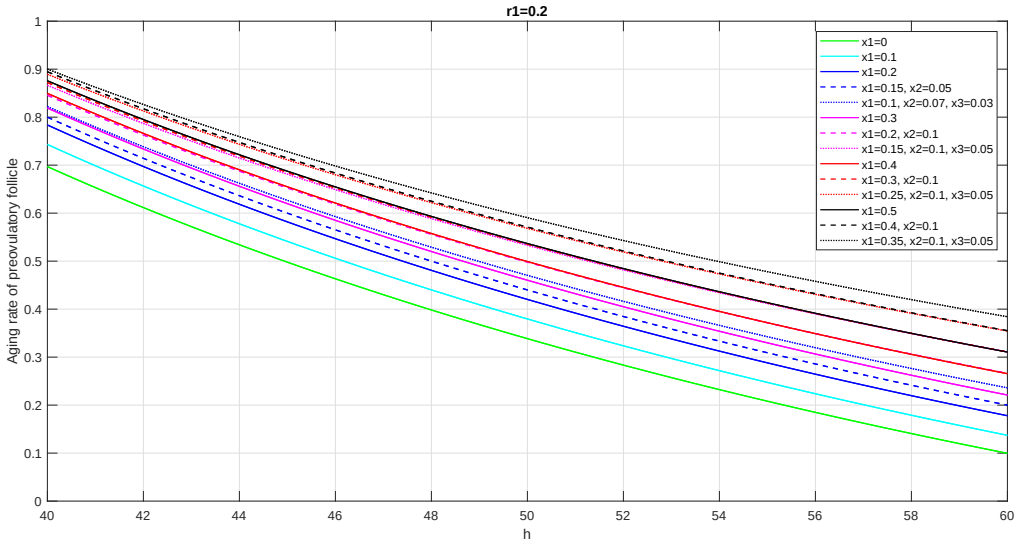

Figure 5: $a + b/h$ least squares approximation of aging ratio of preovulatory follicle (at day 85) versus h for $r_1 = 0.2$ and several values x_i.

cells may pass at any generational age older than the mother's generational age.

Specifically, we have considered the limit number of cell divisions $h = 50$ as the mean value in GCs for women aged 25 to see the effect of oxidative stress, and other reasons accelerating telomere shortening, on the accumulation of aged cells. The model indicated that after 85 days of the folliculogenesis process that goes from the preantral to the pre-ovulatory stage, the middle-aged cells dominated over the aged ones and these in turn, over the young ones. Comparing the E1 and E2 experiments, when the factors that produce telomere shortening were duplicated, it was observed that the middle aged cells decreased and in turn the aged cells increased. Consequently the aging ratio grew.

Different degrees of accelerated telomere shortening were studied for the limit number of cell divisions $h = 40$, corresponding to GCs of women either around the 50th percentile for aged 40 or in lower percentiles at younger ages. Our results show increased loss of telomere length, when daughter cells were simulated to advance to generations further away from that of their mothers. In this circumstances, the model showed accelerated follicular aging. Even

$h = 40$	$x_1 = 0$	$x_1 = 0.1$	$x_1 = 0.2$	$x_1 = 0.3$	$x_1 = 0.4$
$r_1 = 0.05$	0.86	0.88	0.90	0.92	**0.93**
$r_1 = 0.10$	0.80	0.83	0.86	0.88	0.90
$r_1 = 0.15$	0.75	0.79	0.82	0.85	0.87
$r_1 = 0.20$	**0.70**	0.74	0.78	0.81	0.84

Table 6: Aging ratio for the average value of h for women of 40 years old, for several values of telomerase activity and oxidation.

with mild oxidation power, the aging ratio was strikingly high and this was worsened by low values of telomerase activity and increased oxidation values.

Focusing on women in their 40s, for whom $h = 40$ is the average value in the GCs according to Table 2, some data extracted from Figure 3 are shown in Table 6, for the sake of clarity. In the most favourable case displayed, $x_1 = 0$ and $r_1 = 0.2$, the aging ratio is 0.7, which is considerably near to 1. When oxidation increases or telomerase activity decreases, the aging ratio worsens. This may be one of the reasons why fertility declines dramatically in women around 40.

Physical or emotional stress among other causes lead to decreasing telomerase activity (Razgonova et al., 2020). This has been simulated by decreasing the rate of telomerase activity at preantral stage. Several values of the limit number of cell divisions (the Hayflick limit) and different degrees of accelerated telomere shortening were considered. There was a clear relationship between the oxidation ratio (the higher the ratio, the greater the chance that cells move into higher generational states with each cell division) and the aging ratio. The mathematical model predicted more than 10-year advance of aging with levels 25% above mild stress, which is similar to the magnitude of accelerated cell aging observed in (Epel et al., 2004). Understanding this relationship and how these ratios are interconnected could be key to comprehend and quantify the effects of oxidative stress on human follicular aging and, similarly, on other cell groups.

One way for future research is to explore the continuous counterpart of the discrete models used in this work, modeling the evolution of the age density of a population via an age-structured PDE.

Summary

- The work was focused on the age range of 25 years, the moment of maximum fertility up to 40 years, when fertility falls drastically. The number of times that a cell can be divided before reaching the senescent state, for GCs at 25 and 40 years old, for different percentiles, was estimated.
- In the previous article (Portillo et al., 2019), the mathematical model assumed that when a cell divides it produces two daughter cells whose generational age increases by one, due to telomere shortening via the end-replication problem and mild oxidation. In the present work, higher level of oxidation that accelerate telomere shortening are included by assuming mitosis produces two cells which may pass at any generational age older than the mother's generational age.
- The qualitative behaviour of the simplest model has been studied through the key parameters m, r and h.
- The simulations suggest that excessive telomere losing by oxidizing agents promotes accelerated cell aging.
- In cases of cells with short TL, with low telomerase activity and accelerated telomere shortening, the mathematical model predicts an aged outcome in preovulatory follicles.
- It can be deduced from the least square approximations of the data obtained in the simulations that the improvement in the aging ratio, when the index h or the telomerase activity increases, is slower when there is more oxidation.
- The model provides a plausible explanation why fertility declines dramatically in women around 40.

Conflict of interest statement

Nothing declared.

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