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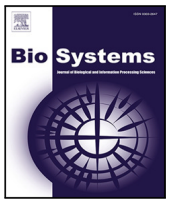

Influence of oxidative stress on women's fertility: A model with a generational age Caputo's fractional derivative

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ABSTRACT

Cellular aging associated with telomeric shortening plays an important role in female fertility. In addition to natural decline, due to the loss of telomeric repeats during cell division, other factors such oxidative stress (OS), accelerate telomere shortening by causing a dramatic loss of telomeric repeats. Thus, mathematical models to better understand the accelerated aging leading to infertility are lacking in the literature. An initial and boundary value problem (IBVP) with a diffusion-advection equation was considered to describe the evolution of a cell population undergoing a gradual decrease of the proliferation potential due to the end-replication problem (Olovnikov, 1973). In this paper we propose a continuum model that attempts to capture the random telomere shortening caused by OS, replacing the advection term with a Caputo's fractional derivative of order β, $0 < \beta < 1$, with respect to the generational age. The distance between the order of the Caputo derivative and 1 was considered the oxidation parameter. The mathematical model was applied to the human follicular growth from preantral to pre-ovulatory follicle, in young and older women to study the influence of oxidation and low telomerase activity on the aging rate of the pre-ovulatory follicle. We observed that as OS increases, the generational age of granulosa cells (GCs) increases as well, suggesting that telomeres of these GCs will be aged. Although middle-aged women treated with antioxidants could reduce the negative effects of OS on telomeres, antioxidants in combination with good levels of telomerase activity yield the best results regarding the reduction of generational aging of GCs.

1. Introduction

Ovarian aging happens early in women's lifespan, compared to other organs (Polonio et al., 2020; Varela et al., 2018; Faddy et al., 1992). This has several consequences such as infertility and an increased risk of aging-associated diseases (Babayev and Duncan, 2022; Polonio et al., 2020; Varela et al., 2018). Indeed, ovarian aging, characterized by the decrease in the number and the quality of oocytes starts at the age of 35 years, leading to a depletion of follicles by menopause (Córdova-Oriz et al., 2024; Chico-Sordo et al., 2021).

In some women, ovarian aging happens earlier in life leading to diminished ovarian reserve and premature ovarian insufficiency at young ages, forcing women to seek for assisted reproductive technologies (ART). One of the pathways involved in ovarian aging is telomere attrition (López-Otín et al., 2023). When telomeres reach a critically short length, the regenerative capacity of tissues is drastically reduced (Varela and Blasco, 2010; Hao et al., 2005), leading to organ failure (Martínez and Blasco, 2017), including reproductive organs (Lee et al., 1998).

Telomeres are nucleoprotein structures localized at chromosome ends which confer them identity, so that the cell does not confuse them with broken DNA which must be repaired (Martínez and Blasco, 2017). Their function is to protect chromosomes from degradation activities and fusions, safeguarding genome integrity (Córdova-Oriz et al., 2024). Telomeric DNA in mammals consist of tandem repeats of the sequence 5'-TTAGGG-3' ending in a single-stranded overhang rich in guanines that can invade the doble stranded DNA to form the telomeric loop (De Lange, 2015; Griffith et al., 1999). This loop is stabilized by a complex of proteins called shelterin, which also coats the telomeric

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DNA to protect it (de Lange, 2018; Palm and de Lange, 2008). The enzyme telomerase is a reverse transcriptase, (Greider and Blackburn, 1985) composed by a proteic part and a RNA component (Greider and Blackburn, 1987), which can add the novo repeats onto chromosome ends, preventing telomere attrition (Whittemore et al., 2019; Povedano et al., 2018). However, telomerase is not detectable in most tissues of the body, but remains active in the germ line (Polonio et al., 2023; Córdova-Oriz et al., 2022; Chico-Sordo et al., 2021). Indeed, granulosa cells of infertile patients (Xu et al., 2017; Butts et al., 2009) and mice with reproductive senescence (Polonio et al., 2023) show less telomerase activity than controls, linking the telomere pathway to infertility.

Another factor, that negatively affects aging, is OS due to mitochondrial dysfunction which generates reactive oxygen species (ROS) (López-Otín et al., 2023). ROS increase ovarian aging and affect telomere biology and fertility (Chakraborty et al., 2023). Interestingly, 80% of telomerase localizes to the nucleus of the cell and 20% to mitochondria where it exerts a function directed to lowering OS, DNA damage and apoptosis (Miwa et al., 2016; Jaiswal et al., 2013). At the organismal level, both telomere and mitochondrial dysfunction affect oocyte quality and the rate of aneuploidy (Cozzolino et al., 2024; Hao et al., 2023a; Treff et al., 2011). Indeed, long telomeres have been correlated with longer female fertility (Michaeli et al., 2022), better oocyte competence and embryo quality (Cheng et al., 2013). ROS have also been reported to alter oocyte quality (Rajani et al., 2012).

Because the current society is delaying the decision to have children due to socioeconomic changes, the aging of the ovary becomes an issue for ART. Thus, different strategies are under investigation to reactivate ovarian function (Polonio et al., 2020). One of them is the use of antioxidants as resveratrol, which comprises polyphenols present in grapes and other fruits (Burns et al., 2002). It has a protective effect on several diseases (Meng et al., 2020), improves telomere length (TL) and telomerase activity (TA) in mice (Liu et al., 2013). Another strategy is the use of sexual steroids, which reactivate telomerase (Córdova-Oriz et al., 2023; Guo et al., 2003) through the estrogen responsive element present in the promoter of telomerase gene (Calado et al., 2009). Telomerase reactivation results in improved health, tissue regeneration, fertility and increased lifespan in mice (Bär et al., 2016; Bernardes de Jesus et al., 2012; Jaskelioff et al., 2011). It would be expected that, since telomerase and telomere biology are quite similar in mice and humans, the benefits of telomerase reactivation on health, tissue regeneration, fertility, and lifespan observed in mice could also apply to women.

Numerous mathematical models focus on the dynamics of cell populations, such as those examining proliferation in clonal cancer (Ortega-Sabater et al., 2023). However, we are interested in mathematical models related to telomere length and replicative senescence, as they may offer valuable frameworks to explore the intricate biological processes of aging, for example incorporating stochastic processes (Rat et al., 2023). Models applied to the study of granulosa cell aging in the human follicle were proposed in (Portillo and Peláez, 2021) and (Portillo et al., 2019) with systems of ordinary differential equations and in (Portillo et al., 2023) using partial differential equation with zero-flux boundary conditions. The generational and temporal evolution of a stem cell population were studied in (Portillo et al., 2024), with time-dependent parameters.

There are different types of mathematical models involving redox-related mechanisms depending on the type of biological pathways considered (Allboani et al., 2024) and also depending on whether ordinary, partial differential or stochastic differential equations are used (Guimera et al., 2019). Although there are some discrete models (Portillo and Peláez, 2021) for the study of the influence of oxidation on telomere shortening, to the best of our knowledge there are no continuous models with spatial fractional derivatives that mathematically simulate the effect of oxidation. Thus, the Caputo's fractional derivative model is introduced to fill this gap. This spatial fractional model is effective in describing accelerating telomere shortening due not only to the end-replication problem but also due to different degrees of oxidation.

Fractional derivatives are increasingly being recognized as powerful tools to model biological systems because their ability to capture complex behaviors that traditional integer-order derivatives may overlook (Burrage et al., 2024). For instance, biological systems often exhibit memory-dependent processes, where the current state is influenced not only by immediate conditions but also by past states (Caputo and Cametti, 2016; Magin, 2010). Fractional derivatives inherently account for this memory effect, enabling more accurate representations of phenomena (Caputo and Cametti, 2021; Area et al., 2015). Spatial fractional derivatives provide a better fit for real-world biological phenomena exhibiting heterogeneous behaviors, where the interaction rates vary across space.

Several questions can be explored within this modeling framework, such as whether the variable representing OS (through β) would influence telomere-related dynamics in a similar way at younger or older ages, or how hypothetical interventions, such as modulating the variable associated with telomerase activity to reflect the potential effects of antioxidants, might impact these dynamics. While this model does not intend to make direct biological predictions, our results suggest that could be useful for the generation of hypotheses regarding biological processes. For instance, one possible implication could be that in women in their 40s with fertility problems, follicle development could be improved by reducing OS through a healthy lifestyle and the use of antioxidants, as well as with Danazol treatment to reactivate endogenous telomerase.

Fig. 1 illustrates, on the left, a healthy preovulatory follicle depicted in pink, while on the right, an aged preovulatory follicle is shown in brown with small black spots, representing the effects of OS associated with follicular aging. Aged follicles have shorter telomeres, and the lipids in cellular membranes are oxidized.

2. Mathematical model incorporating oxidation impact

To model telomere shortening, we first considered when it happens gradually, due to the end-replication problem (Olovnikov, 1973, 1971). This is the result of the inability of the replication machinery to copy the very ends of chromosomes (Olovnikov, 1973, 1971). To reflect this, we assumed that the average telomere length of a cell shortens by a constant factor during each cellular division. The cell has a certain maximum and minimum mean telomeric length, being the Hayflick limit the minimal telomeric length that does not permit replication (Hayflick and Moorhead, 1961) leading to cell senescence or apoptosis (Martínez and Blasco, 2017). We denoted by x_H the maximum proliferation potential of a cell, i.e. the maximum number of times that a cell can divide before reaching the senescent state. We referred to the generational age of a cell as its proliferation potential, which is associated with its telomere length, regardless of when the cell was formed. Let x be the variable representing the generational age, $x \in [0, x_H]$, and $N(x,t)$ the population density of a type of cells at generational age x and time t.

In this model it was assumed that cells divide symmetrically denoting by m the rate of mitotic replication per cell per unit of time, d, the rate of mortality events per cell per unit of time and r, the rate of telomerase activity per cell per unit of time which acts rejuvenating the cell, as it incorporates telomeric repeats.

Reconsidering the continuum model of our previous work (Portillo et al., 2023), the following initial and boundary value problem is proposed to describe the evolution of the cell population when gradual decrease of the proliferation potential is due to the end-replication problem

$$N_t(x,t) = DN_{xx}(x,t) - vN_x(x,t) + \rho N(x,t), \quad 0 < x < x_H,\ t > 0, \tag{1}$$

$$DN_x(0,t) - vN(0,t) = 0, \quad t > 0, \tag{2}$$

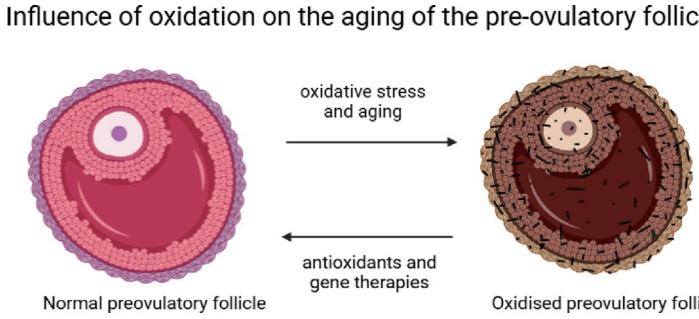


Fig. 1. Influence of oxidation on growing follicles. The scheme shows a healthy fully developed antral follicle, which will be ovulated, on the left panel. On the right panel, an aged follicle is depicted. This stage can be achieved due to natural aging, but it could also happen in the presence of reduced oxygen species. The use of antioxidants, which can reduce ROS and telomerase reactivation through sexual steroids could prevent follicular aging from happening. The figure has been created with Biorender.

$$DN_x(x_H,t) - vN(x_H,t) = 0, \quad t > 0, \tag{3}$$

$$N(x,0) = f(x), \quad 0 < x < x_H, \tag{4}$$

where $D = (2m+r)/2$ is the diffusion constant, $v = 2m-r$ the advection coefficient, and $\rho = m-d$ can be interpreted as an effective proliferation rate. The diffusion-advection equation (1) is supplemented with zero-flux Robin boundary conditions (2)–(3) and the initial distribution of the population (4).

Besides telomere shortening during chromosome replication, due to the end-replication problem (Olovnikov, 1973, 1971), our model considers telomere shortening due to other factors such as high level of oxidation, which accelerate telomere shortening. Most endogenous reactive oxygen species (ROS) are produced in the mitochondria. The ATP that is required to drive cellular processes is created in the mitochondria in a process that consumes oxygen and carbohydrate substrates. However, some electrons leak to react with molecular oxygen and form ROS. When levels of ROS exceed those necessary for cellular signaling and overwhelm the detoxification capacity of a biological system, they can cause oxidative damage to telomeres (Metcalfe and Olsson, 2022). In addition to being generated during cellular metabolism in mitochondria, ROS can be produced in response to different environmental stimuli such as growth factors, inflammatory cytokines, ionizing radiation, UV, chemical oxidants, chemotherapeutics hyperoxia, toxins, and transition metals (Cui et al., 2012). Cigarette smoke has also been confirmed to associate with increased OS in the ovary (Li et al., 2020; Kim et al., 2018; Mai et al., 2014; Sobinoff et al., 2013). Because ROS can attack and shorten telomeres, it will cause for daughter cells to increase their generational age even to the point of senescence.

The advection term is mainly responsible for the population moving to the right in the x-range, i.e. decreasing the proliferation potential and increasing aging population. Telomeres lose 50−100 DNA bases per cell division due to the end-replication problem (Hodes, 1999). This would cause the daughter cells to be older than the mother but close in generational age. The derivative D_x is local and serves to model the telomeric shortening caused by the end-replication problem. However, OS may cause accelerated aging due to the loss of an indetermined number of DNA bases (even thousands), as oxidation of telomeric DNA may happen at any guanine residue present in the telomere sequence. That is, any cell, because of OS, could pass into any subsequent generational age. This means that the generational age variation affects the whole previous interval. For this reason we proposed a generational age Caputo's fractional derivative, to simulate the effect of oxidation, due to its integral character, which means that it involves all previous generational ages.

For any positive constant β, $0 < \beta < 1$, the Caputo fractional derivative of order β is defined by (see, for example, (Diethelm, 2010))

$$D^{\beta}_{C,x}N(x,t) = \int_0^x \frac{(x-s)^{-\beta}N_x(s,t)}{\Gamma(1-\beta)}\,ds. \tag{5}$$

We consider the following IBVP for regular enough solutions

$$N_t(x,t) = DN_{xx}(x,t) - vD^{\beta}_{C,x}N(x,t) + \rho N(x,t), \quad 0 < x < x_H,\ t > 0, \tag{6}$$

$$DN_x(0,t) - vN(0,t) = 0, \quad t > 0, \tag{7}$$

$$DN_x(x_H,t) - vN(x_H,t) = 0, \quad t > 0, \tag{8}$$

$$N(x,0) = f(x), \quad 0 < x < x_H. \tag{9}$$

The Robin boundary diffusion-subadvection model (6)–(9) takes advantage of its diffusion-advection counterpart (1)–(4) describing generational age global dependence.

The initial and boundary value problem with fractional differential equation (6)–(9) was solved numerically by the method of lines. First, for the case where the solution respect the variable x lies in $C^1[0,x_H]\bigcap C^{p+1}(0,x_H]$ for some positive integer p, the problem was discretized in the variable x by approximating the derivatives by finite differences. We used a uniform mesh on $[0,x_H]$. Let M be a positive integer and $h = x_H/M$ the space step. Let $x_j = jh$ for $j = 0,1,\ldots,M$ be the nodes. We denoted $N_j(t) = N(x_j,t)$.

We employed $D^{\beta}_{C,x,L1}N_j$, the $L1$ discretization of the Caputo fractional derivative $D^{\beta}_{C,x}N_j$ as in (Gracia et al., 2020)

$$D^{\beta}_{C,x,L1}N_j = \frac{1}{h^{\beta}\Gamma(2-\beta)}\left(-d_jN_0 + \sum_{k=1}^{j-1}(d_{j-k+1}-d_{j-k})N_k + d_1N_j\right), \tag{10}$$

where

$$d_k = (1-\beta)\int_{k-1}^{k} s^{-\beta}ds, \quad \text{for } k = 1,2,\ldots. \tag{11}$$

We used second-order centered finite differences for the discretization of $N_{xx}(x_j,t)$ for $j = 1,\ldots,M-1$. Finally, we implemented the following finite differences of second order to approximate the boundary conditions

$$\frac{D}{h}\left(-\frac{3}{2}N_0 + 2N_1 - \frac{1}{2}N_2\right) = vN_0,$$

$$\frac{D}{h}\left(\frac{1}{2}N_{M-2} - 2N_{M-1} + \frac{3}{2}N_M\right) = vN_M,$$

then

$$N_0 = \frac{1}{\frac{3}{2}+\frac{vh}{D}}\left(2N_1 - \frac{1}{2}N_2\right), \tag{12}$$

$$N_M = \frac{1}{-\frac{3}{2}+\frac{\upsilon h}{D}}\left(\frac{1}{2}N_{M-2}-2N_{M-1}\right). \tag{13}$$

After the discretization in the variable x, we arrived at a system of ordinary differential equations in the variable t for the vector $\vec{N}(t) = [N_1(t), \ldots, N_{M-1}(t)]^T$,

$$\vec{N}'(t) = DA_{2,h}\vec{N}(t) - \upsilon A_{\beta,h}\vec{N}(t) + \rho\vec{N}(t) + \frac{D}{h^2}\begin{pmatrix} \frac{(2N_1-\frac{1}{2}N_2)}{\frac{3}{2}+\frac{\upsilon h}{D}} \\ 0 \\ \vdots \\ 0 \\ \frac{(\frac{1}{2}N_{M-2}-2N_{M-1})}{-\frac{3}{2}+\frac{\upsilon h}{D}} \end{pmatrix}$$

$$- \frac{\upsilon(2N_1-\frac{1}{2}N_2)}{h^\beta\Gamma(2-\beta)(\frac{3}{2}+\frac{\upsilon h}{D})}\begin{pmatrix} -d_1 \\ -d_2 \\ \vdots \\ -d_{M-2} \\ -d_{M-1} \end{pmatrix}, \tag{14}$$

where

$$A_{2,h} = \frac{1}{h^2}\begin{pmatrix} -2 & 1 & 0 & \cdots & 0 \\ 1 & -2 & 1 & \cdots & 0 \\ \vdots & \vdots & \ddots & & \vdots \\ 0 & \cdots & 1 & -2 & 1 \\ 0 & \cdots & 0 & 1 & -2 \end{pmatrix}, \tag{15}$$

$$A_{\beta,h} = \frac{1}{h^\beta\Gamma(2-\beta)}\begin{pmatrix} d_1 & 0 & 0 & \cdots & 0 \\ d_2-d_1 & d_1 & & \cdots & 0 \\ \vdots & \ddots & \ddots & \cdots & \vdots \\ d_{M-2}-d_{M-3} & \cdots & d_2-d_1 & d_1 & 0 \\ d_{M-1}-d_{M-2} & \cdots & d_3-d_2 & d_2-d_1 & d_1 \end{pmatrix}. \tag{16}$$

The association between β and OS becomes clearer when examining the discretization of the fractional derivative. In this formulation, the resulting matrix is a lower triangular matrix with all elements below the main diagonal being non-zero, indicating that older generational ages can be reached from any prior generational age. The absolute value of the coefficients of the matrix $A_{\beta,h}$ decreases as we move away from the main diagonal, which reflects that the probability of drastic generational aging decreases with generational distance. On the other hand, the smaller the β, the larger the absolute value of the coefficients of the matrix $A_{\beta,h}$ further away from the main diagonal, meaning that there are higher probability of drastically aging. This behavior is analogous to the effects of OS, which introduces long-range influence across generational states, breaking the local character of interactions. Therefore, β would serve as a natural parameter to represent OS and the size of β would be associated with the degree of oxidation. The greater the distance between β and 1, the higher the oxidation. As an example, we write the matrices $A_{0.9,1}$ and $A_{0.99,1}$ for $M-1=8$ and $h=1$ (see the equation in Box I).

In summary, the model based on the first-order derivative is local, meaning it affects generational ages close to a given one, as in the case of the end-replication problem. In contrast, the model employing Caputo's fractional derivative is global, as it influences the entire range of preceding generational ages, similar to what occurs in oxidative processes. Moreover, the smaller the order of the fractional derivative β, the further the likelihood that generational ages farther from a given generational age will reach it, which corresponds to the process observed as oxidation increases.

3. Numerical experiments

3.1. Aging rate and model parameters

Definition 1. Fixed a number q, we considered the aging rate at a given time t, the number of cells of generational age between $x_H - q$ and x_H divided by the total number of cells

$$ra(t) = \frac{\int_{x_H-q}^{x_H} N(x,t)\,dx}{\int_0^{x_H} N(x,t)\,dx}.$$

That is, the aging rate (ra) is the proportion of cells whose proliferation potential is less than or equal to q. 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 the value is, the older the population becomes. As in the previous article (Portillo and Peláez, 2021) we fixed $q = 17$.

The model is applied to the human follicular growth from preantral to preovulatory follicle, a process that takes approximately 85 days and involves eight classes, according to (Gougeon, 1996). The study focused on the evolution of the population of GCs which are the most important somatic cells to determine the size of follicles, with N representing their population density from preantral to preovulatory stages.

We used the same mitosis rate and mortality rate for each follicle class as in the previous article (Portillo and Peláez, 2021). The parameter s was entered as the proportionality factor between r and m, i.e. $r = s\,m$, so that the coefficient of advection was $\upsilon = m(2-s)$. Small s values represent low telomerase activity, in particular $s = 0$ means no telomerase activity.

We employed initial density function of Gaussian (normal) type concentrated around 1,

$$N(x,0) = \frac{2100}{\sqrt{2\pi\sigma^2}}e^{-(x-\nu)^2/2\sigma^2},$$

with $\nu = 1$ and $\sigma = 0.1$. As was seen in (Portillo et al., 2023), $x_H = 50$ happens in the average of women aged 25, while $x_H = 40$ occurs in the average of women in their forties and in younger women in lower percentiles, whose biological age is older than their chronological age. Regarding the spatial discretization we took as step size $h = 0.2$. The system of ODEs (14) achieved after spatial discretization was solved numerically by means of the Matlab function ode15s.

3.2. Effects of oxidative stress

An imbalance between free radicals and antioxidants leads to OS, which contributes to aging, while both internal and external sources of ROS further accelerate the aging process (Tan et al., 2018). OS contributes to infertility in women through multiple mechanisms. An excessive accumulation of ROS within the follicle may surpass the antioxidant capacity of the follicular fluid, leading to direct damage to the oocytes (Adeoye et al., 2018). Thus, we studied the influence of OS on the aging of the pre-ovulatory follicle, through β parameter in women in their forties, when fertility declines (Voros et al., 2025). The total GCs for different values of β are displayed in Fig. 2. Black solid lines correspond to total granulosa cell values in healthy populations, according to (Gougeon, 1996). The total GCs for $x_H = 50$ (average of women aged 25) in Fig. 2(a) and for $x_H = 40$ (average of women aged 40) in Fig. 2(b) for different degrees of oxidation. In all cases considered, the total number of granulosa cells are within Gougeon's limits which is a guarantee of the model's parameters.

Next we set to understand how different degrees of OS may affect the density function of GCs at the end of folliculogenesis (day 85) of pre-ovulatory follicle development (Wang et al., 2021). We considered a moderate telomerase activity ($s = 0.3$) for $x_H = 40$ and varied β parameter. We considered a Gaussian initial density concentrated aroud 1, for four values of β. In Fig. 3(a) $\beta = 1$, meaning no damage due to OS. Results of the simulation show that the center of mass of the population end follicular development at a generational age of 20. The generational age is shifted towards older generational ages as the β parameter is decreased. In addition, the population of aged cells is increased, observed at the right end of the interval, where the

$$A_{0.9,1} = \begin{pmatrix} 1.0511 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ -0.9757 & 1.0511 & 0 & 0 & 0 & 0 & 0 & 0 \\ -0.0288 & -0.9757 & 1.0511 & 0 & 0 & 0 & 0 & 0 \\ -0.0124 & -0.0288 & -0.9757 & 1.0511 & 0 & 0 & 0 & 0 \\ -0.0070 & -0.0124 & -0.0288 & -0.9757 & 1.0511 & 0 & 0 & 0 \\ -0.0045 & -0.0070 & -0.0124 & -0.0288 & -0.9757 & 1.0511 & 0 & 0 \\ -0.0032 & -0.0045 & -0.0070 & -0.0124 & -0.0288 & -0.9757 & 1.0511 & 0 \\ -0.0024 & -0.0032 & -0.0045 & -0.0070 & -0.0124 & -0.0288 & -0.9757 & 1.0511 \end{pmatrix},$$

$$A_{0.99,1} = \begin{pmatrix} 1.0057 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ -0.9987 & 1.0057 & 0 & 0 & 0 & 0 & 0 & 0 \\ -0.0029 & -0.9987 & 1.0057 & 0 & 0 & 0 & 0 & 0 \\ -0.0012 & -0.0029 & -0.9987 & 1.0057 & 0 & 0 & 0 & 0 \\ -0.0007 & -0.0012 & -0.0029 & -0.9987 & 1.0057 & 0 & 0 & 0 \\ -0.0004 & -0.0007 & -0.0012 & -0.0029 & -0.9987 & 1.0057 & 0 & 0 \\ -0.0003 & -0.0004 & -0.0007 & -0.0012 & -0.0029 & -0.9987 & 1.0057 & 0 \\ -0.0002 & -0.0003 & -0.0004 & -0.0007 & -0.0012 & -0.0029 & -0.9987 & 1.0057 \end{pmatrix}.$$

Box I.

population is concentrated (Fig. 3(b), $\beta = 0.99$; 3(c), $\beta = 0.98$ and 3(d), $\beta = 0.97$). The comparison of the distributions for these four values of the parameter β is in Fig. 3(e).

In order to study the population aging in more detail, we analyzed the aging rate for the above four β values (1, 0.99, 0.98 and 0.97) and various values of the parameter s related to telomerase activity (TA) (Péntek et al., 2023). Fig. 4(a) shows the aging rate versus telomerase activity ranging from low values 0.2 to higher values 0.8. Our results indicate that the aging rate decreases as telomerase values increase, even when oxidative stress in higher ($\beta = 0.97$). Comparing the different lines (on solid blue line $\beta = 1$, on dashed blue line $\beta = 0.99$, on the blue dashed dotted line $\beta = 0.98$ and on the blue dotted line $\beta = 0.97$), the model shows that the lower the β the higher the aging rate. In addition, for low values of TA factor, there can be a 10% to 15% increase in aging rate for these small changes in β. Besides, Fig. 4(b) depicts aging rate versus degree of oxidation: on red line $s = 0.7$, on red line with plus $s = 0.6$, red line with circles $s = 0.5$, red line with triangle $s = 0.4$, red line with square $s = 0.3$, red line with diamond $s = 0.2$. The lower the s TA factor, the higher the aging rate. For instance, for $\beta = 0.97$, there would be almost a 30% difference between the different telomerase activity values considered.

For $1 - \beta = 0 : 0.005 : 0.03$ and $s = 0.2 : 0.1 : 0.8$ the values of the aging rate are calculated. Table 1 shows the aging rate at 85 days of pre-ovulatory follicle development, as a function of maximum telomere length (x_H), telomerase activity factor (s) and oxidation factor ($1-\beta$). In the case of maximum telomeric length $x_H = 50$, which corresponds to GCs of women in the 50th percentile at age 25, low telomerase activity or oxidation did not greatly affect the aging rate of GCs belonging to pre-ovulatory follicles. However, for the maximum telomeric length $x_H = 40$, which corresponds to GCs of women in the 50th percentile at age 40, low telomerase activity or oxidation substantially influenced the aging rate of GCs from preovulatory follicles. This is more noticeable if the two circumstances are combined (higher oxidation and lower telomerase activity). Then we used the Linear model Poly22 of Matlab to fit to aging rate data by second degree two-variable polynomial

$$g(1-\beta, s) = p00 + p10\,(1-\beta) + p01\,s + p20\,(1-\beta)^2 + p11\,(1-\beta)\,s + p02\,s^2,$$

whose coefficients are shown in Table 2.

Exploring potential pathways from OS to ovarian aging, we generated the surface data for $x_H = 50$, Fig. 5(a), while Fig. 5(b) is the fit surface to data for $x_H = 40$. As a general trend, the lower the factor of telomerase activity s and the higher the oxidation degree $1 - \beta$, the higher the aging rate. This tendency is more pronounced in populations in their 40s ($x_H = 40$) than in their 25s ($x_H = 50$). For example, for the maximum degree of oxidation considered, $1 - \beta = 0.2$, with no telomerase activity at $x_H = 50$, the aging rate is 0.4 and at $x_H = 40$, the aging rate is 35% higher. However, if the telomerase activity factor is $s = 0.8$ the aging rate for $x_H = 50$ is 0.2 and for $x_H = 40$ it is 20% higher.

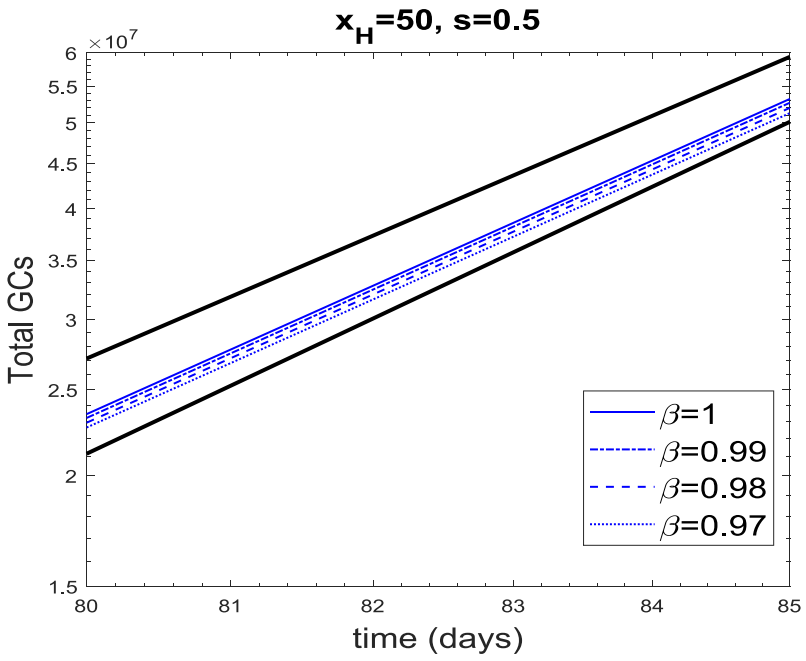
Table 1
Aging rate at 85 days of pre-ovulatory follicle development, as a function of maximum telomere length (x_H), telomerase activity factor (s) and oxidation factor ($1 - \beta$). Value $x_H = 50$ corresponds to GCs of women in the 50th percentile at age 25: low telomerase activity or oxidation did not greatly affect the aging rate. Value $x_H = 40$ corresponds to GCs of women in the 50th percentile at age 40: low telomerase activity or oxidation substantially influenced the aging rate.

	$x_H = 50$	$1-\beta$						
		0	0.005	0.01	0.015	0.02	0.025	0.03
	0.8	0.0004	0.0050	0.0091	0.0129	0.0166	0.0202	0.0240
	0.7	0.0006	0.0060	0.0107	0.0149	0.0191	0.0232	0.0275
	0.6	0.0011	0.0076	0.0129	0.0178	0.0225	0.0272	0.0319
s	0.5	0.0017	0.0101	0.0164	0.0219	0.0272	0.0324	0.0379
	0.4	0.0029	0.0147	0.0223	0.0284	0.0341	0.0399	0.0459
	0.3	0.0046	0.0246	0.0339	0.0401	0.0456	0.0514	0.0576
	0.2	0.0074	0.0540	0.0651	0.0675	0.0692	0.0723	0.0769
	$x_H = 40$							
	0.8	0.0520	0.0637	0.0745	0.0848	0.0949	0.1049	0.1148
	0.7	0.0724	0.0862	0.0986	0.1105	0.1220	0.1333	0.1444
	0.6	0.0994	0.1156	0.1299	0.1432	0.1561	0.1687	0.1811
s	0.5	0.1340	0.1534	0.1696	0.1845	0.1986	0.2124	0.2259
	0.4	0.1774	0.2016	0.2199	0.2359	0.2508	0.2653	0.2795
	0.3	0.2304	0.2641	0.2846	0.3006	0.3151	0.3293	0.3433
	0.2	0.2933	0.3545	0.3764	0.3881	0.3981	0.4086	0.4199

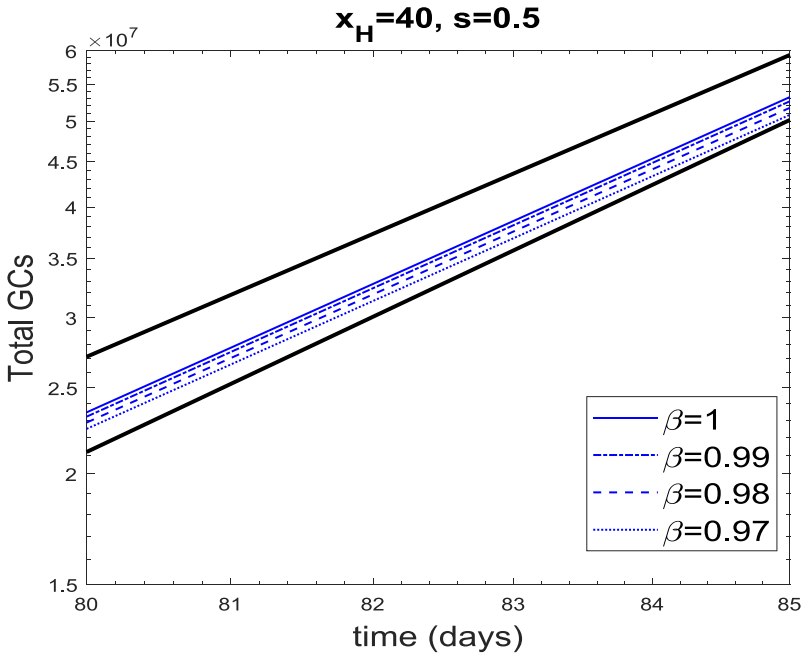
3.3. Effects of antioxidants and sexual hormones

One important question in the field of reproductive medicine is to what extent aged ovaries can recover their function and be able to develop follicles (Sadeghi, 2024). To this end, we modeled the action of antioxidants and sexual hormones, which can contribute to maintain telomeres, either by telomerase reactivation (Calado et al., 2009) or OS amelioration (Haendeler et al., 2004; Badás et al., 2015).

If we consider the aging rate at the end of follicular development (85 days), the worst-case scenario of the simulation, occurs when $x_H = 40$, $s = 0.2$ and $1 - \beta = 0.03$, where $ra = 0.42$ (lower right corner in Table 1 and Fig. 6(a)), for which it would be desirable to apply some treatment



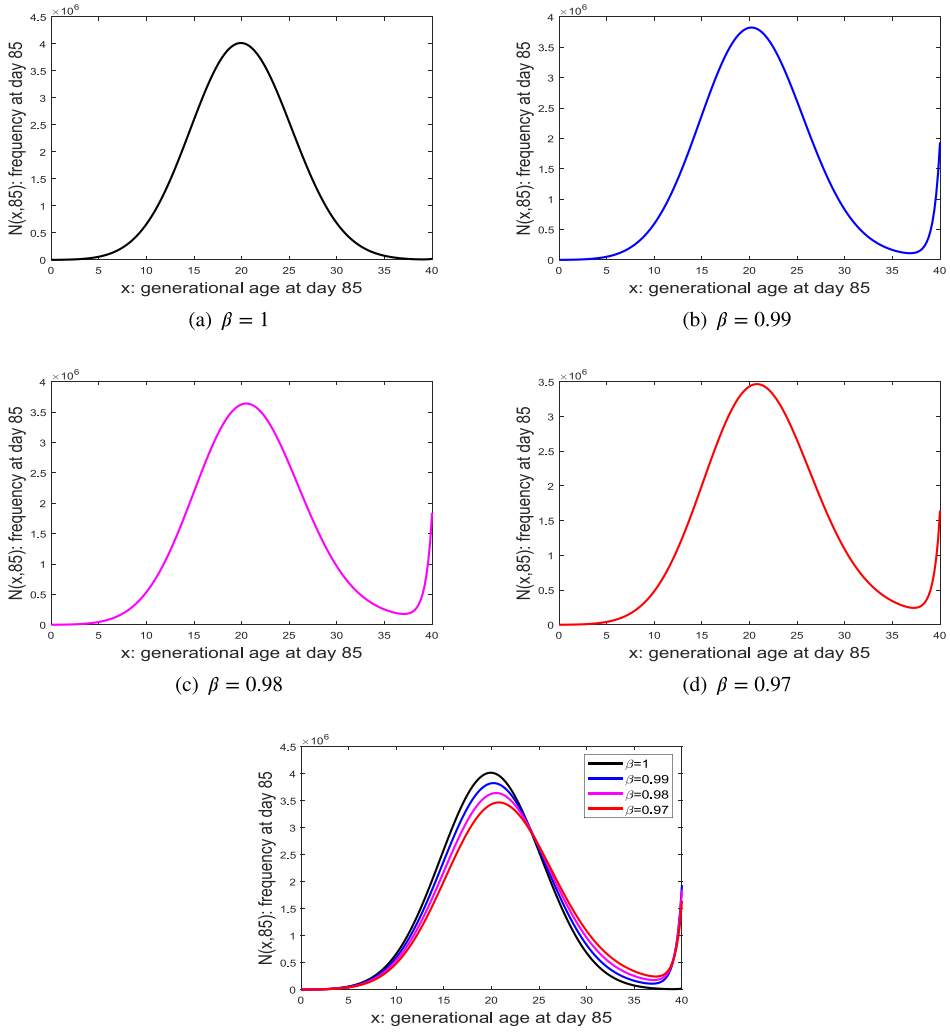
(a) $x_H = 50$ (average of women aged 25)



(b) $x_H = 40$ (average of women aged 40)

Fig. 2. Effect of oxidation on the number of GCs at the end of folliculogenesis. The graphs show the total GCs for $x_H = 50$ (average of women aged 25; (a)) and $x_H = 40$ (average of women aged 40; (b)) and different values of β (degree of oxidation). Black solid lines correspond to total granulosa cell values in healthy populations, according to Gougeon (1996). In all cases considered, the total number of GCs are within Gougeon's limits. Note that for the lowest value of β, the corresponding blue line approximates the lower black line, and this effect becomes more pronounced in the case of $x_H = 40$.

to improve its aging rate. Danazol, a sexual steroid hormone, could be applied to reactivate the expression of the endogenous telomerase gene (Guo et al., 2003). In addition, effective natural antioxidants could provide the means to delay or reverse ovarian aging. For instance, resveratrol protects against infertility and positively affects telomere length (Liu et al., 2013). Treatment with antioxidants over a population with $x_H = 40$, $s = 0.2$ (low telomerase activity) and $\beta = 1$ (effective antioxidant), based on the results of (Liu et al., 2013), leads to the $ra = 0.29$, i.e. a reduction of 13% (Fig. 6(b)). Treatment with Danazol, in the presence of oxidation $\beta = 0.97$, and a complete reactivation of telomerase, simulated by increasing the s parameter, from $s = 0.2$ to $s = 0.8$ would produce a $ra = 0.11$, i.e. a 30% reduction is achieved (Fig. 6(c)), suggesting that complete telomerase reactivation is more effective than antioxidants alone. Next, we studied both agents in combination (Table 3 and Fig. 6(d)), moving along the diagonal of Table 3 from $s = 0.2$ and $1 - \beta = 0.03$ to $s = 0.8$ and $1 - \beta = 0$, and found that with effective antioxidant ($\beta = 1$) and complete telomerase reactivation ($s = 0.8$) the $ra = 0.05$, representing a 37% reduction

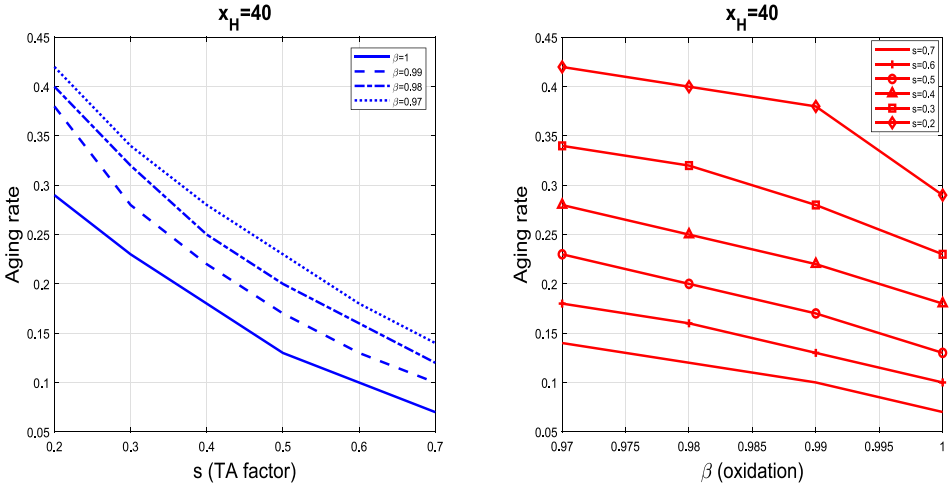


(a) $\beta = 1$ (b) $\beta = 0.99$

(c) $\beta = 0.98$ (d) $\beta = 0.97$

(e) Comparison of $\beta = 1$, $\beta = 0.99$, $\beta = 0.98$, $\beta = 0.97$

Fig. 3. Evolution of the density function of GCs on day 85 of pre-ovulatory follicle development. The graphs GCs density for $x_H = 40$ and $s = 0.3$, with no OS ($\beta = 1$) in (a); with $\beta = 0.99$ in (b); with $\beta = 0.98$ in (c); with $\beta = 0.97$ in (d) and the merge of all of them in (e). Note that when β decreases, the oxidation parameter $(1 - \beta)$ increases and the population concentrated at the right end of the interval is also increased, meaning that the population was more aged.

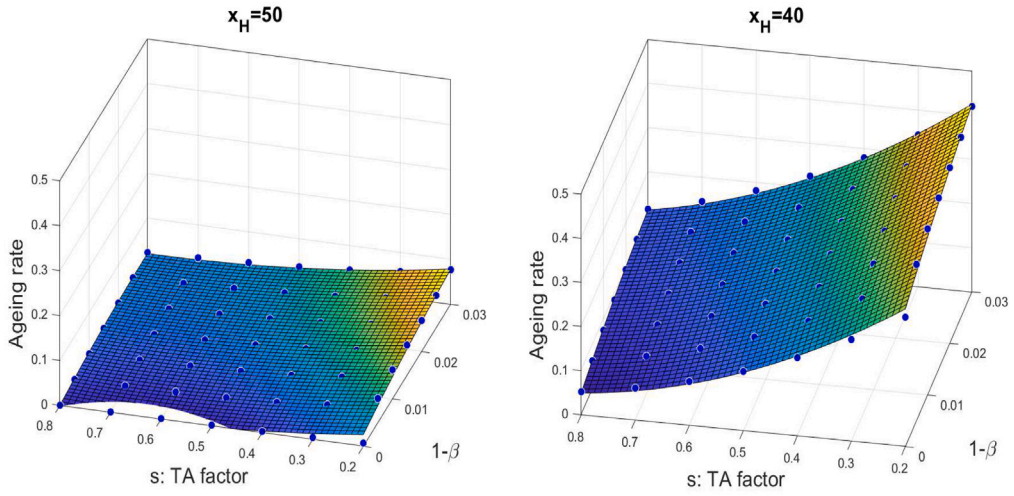


(a) Aging rate versus telomerase activity. (b) Aging rate versus degree of oxidation.

Fig. 4. Aging rate of the population in the presence of OS and telomerase. (a) Aging rate versus telomerase activity for several values of β. Note that when β decreases, the oxidation parameter $(1 - \beta)$ increases and the aging rate went up. (b) Aging rate versus degree of oxidation for several values of s (TA factor): the lower the TA factor, the higher the aging rate.

Table 2
Fitting of aging rate data by second degree two-variable polynomial $g(1-\beta, s) = p00 + p10\,(1-\beta) + p01\,s + p20\,(1-\beta)^2 + p11\,(1-\beta)\,s + p02\,s^2$ obtained using linear model Poly22 of Matlab to fit to aging rate data for $1-\beta = 0 : 0.005 : 0.03$ and $s = 0.2 : 0.1 : 0.8$.

Coefficients (with 95% confidence bounds)	$x_H = 50$		$x_H = 40$	
p00	0.06163	(0.04901, 0.07426)	0.4853	(0.4709, 0.4997)
p10	2.955	(2.253, 3.656)	5.591	(4.792, 6.391)
p01	−0.2107	(−0.259, −0.1624)	−0.9744	(−1.029, −0.9193)
p20	−28.98	(−47.44, −10.53)	−42.09	(−63.12, −21.05)
p11	−1.717	(−2.516, −0.9179)	−2.741	(−3.652, −1.83)
p02	0.1675	(0.1214, 0.2136)	0.5367	(0.4841, 0.5893)



(a) $x_H = 50$, average of women aged 25 (b) $x_H = 40$, average of women aged 40

Fig. 5. Analysis of the combined effects of telomerase activity and OS in the aging rate. The tridimensional graphs show the variations in the aging rate according to the levels of telomerase activity (0.2 to 0.8) and the degree of OS ($1-\beta$), in younger women ($x_H = 50$; (a)) or older women ($x_H = 40$; (b)). Note that in younger women, the low telomerase activity and high degree of oxidation did not greatly affect the aging rate, while in older women, whose cells had lower division potential, low telomerase activity or high levels of oxidation did influence the aging rate.

Table 3
Aging rate at 85 days of pre-ovulatory follicle development for $x_H = 40$ (average of women aged 40) with either antioxidant treatment, sexual hormones treatment or both. The aging rate was reduced in all three treatments, with the combined treatment having the best results.

$x_H = 40$		$1-\beta \rightarrow$ Antioxidant treatment			
		0.03	0.02	0.01	0
s	0.2	**0.4199**	0.3981	0.3764	**0.2933**
↓	0.4	0.2795	0.2508	0.2199	0.1774
Sexual	0.6	0.1811	0.1561	0.1299	0.0994
Hormones	0.8	**0.1148**	0.0949	0.0745	**0.0520**

in aging rate. Thus, both agents in combination were more effective than each one separately. Lastly, all these results can be observed in Fig. 6(e), where the black line is the starting condition and, upon the different treatments, the lines moved towards the left part of the graph, meaning a reduction in generational age.

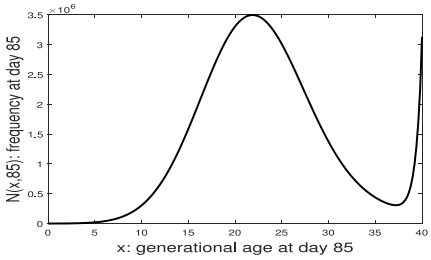
Finally, we addressed the possible improvement of the aging rate for women in their 40s who have fertility problems by combining treatments that activate telomerase activity with treatments that have an antioxidant effect (Fig. 7). The model shows that in cases where telomerase activity is low ($s = 0.2$) and the antioxidant can effectively remove all OS, the slope is more pronounced, although the *ra* remains higher compared to the situation in which telomerase activity is high ($s = 0.8$) with no antioxidants. In this case the slope (blue line) is not so steep, but *ra* is much lower.

4. Discussion

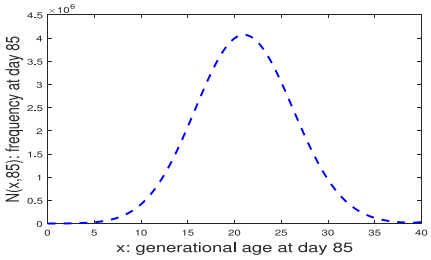
During the process of chromosome replication, telomeres lose length, primarily due to the end-replication problem (Olovnikov, 1973, 1971). This shortening can be exacerbated by OS, which accelerates the degradation of telomeric DNA, potentially resulting in cellular senescence (López-Otín et al., 2023). To capture the influence of OS on telomere dynamics, we proposed a model with a Caputo's spatial fractional derivative. The adverse effect of oxidation could make any cell pass into any subsequent generational age. Spatial fractional derivatives are able to describe non-local effects (as OS on the generational age of the cell) better than classical integer-order derivatives because their integral formulation (Prodanov and Delbeke, 2016). The model allows OS to be graduated by the order of the fractional derivative. So the higher the oxidation, the greater the distance of the order of the fractional derivative to one. We have used the aging rate to capture in a single number the population aging. Values of the aging rate close to 0 corresponded to populations of young cells, while values near 1 indicate old populations.

4.1. Oxidative stress and telomerase in the aging rate

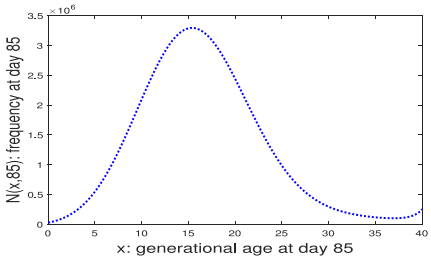
Here we have studied the effects of OS on telomeres of GCs during folliculogenesis. GCs localize around the oocyte, to nurture it during folliculogenesis. They produce estrogens, secrete factors and nutrients required for oocyte growth and maturation, ovulation and fertilization (Liu et al., 2023). Thus, these cells reflect what happens in the oocyte and are a good, non-invasive way to assess oocyte quality (Uyar et al., 2013) and fertility results (Kordus and LaVoie, 2017).



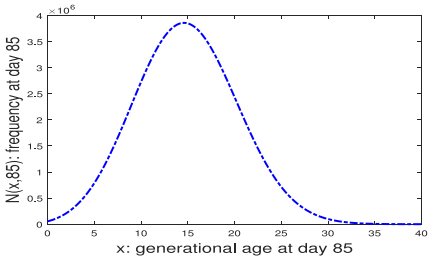
(a) Aged follicle: $\beta = 0.97, s = 0.2, ra = 0.42$



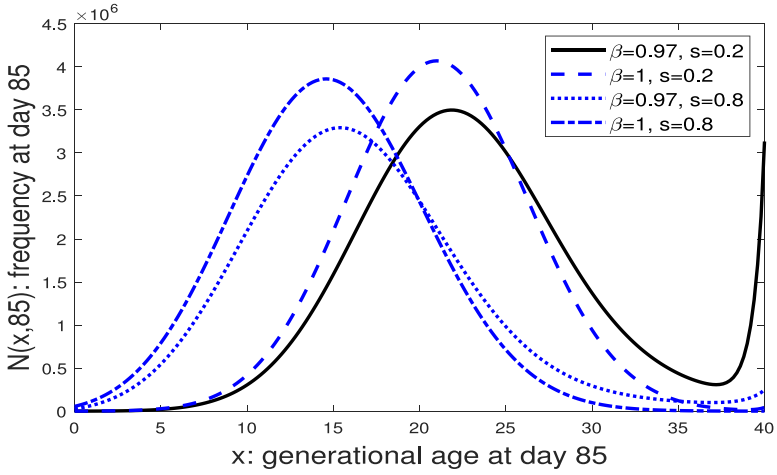
(b) Antioxidant treatment: $\beta = 1, s = 0.2, ra = 0.29$



(c) Sexual hormones treatment: $\beta = 0.97, s = 0.8, ra = 0.11$



(d) Joint treatment: $\beta = 1, s = 0.8, ra = 0.05$



(e) Comparison of the simulation of various treatments

Fig. 6. Dynamics of the density function after antioxidants and sexual-hormone treatments. Evolution of the density function for $x_H = 40$ (average of women aged 40) and Gaussian initial density concentrated around 1 for three possible treatments: antioxidant, sexual hormones and both treatments jointly. (a) Aged follicle without treatment: $\beta = 0.97, s = 0.2, ra = 0.42$. (b) Antioxidant treatment: $\beta = 1, s = 0.2, ra = 0.29$. (c) Sexual hormones treatment: $\beta = 0.97, s = 0.8, ra = 0.11$. (d) Joint treatment: $\beta = 1, s = 0.8, ra = 0.05$. (e) Comparison of the previous simulations. Note that with the three proposed treatments the population shifted to the left of the interval, which means that the aging rate decreased, with the best result being obtained with the combined treatment.

We have found that the number of GCs is reduced when OS is stronger, both in young and middle-aged women, although in our conditions this number did not exceed the lower limits found by (Gougeon, 1996). In fact, OS play an important role in granulosa cell apoptosis (Péntek et al., 2023; Devine et al., 2012) and telomere damage (López-Otín et al., 2023). Telomeric DNA is vulnerable to oxidative damage because these repeats are rich in guanine bases, whose redox potential is low, making them susceptible to oxidation (Liu et al., 2022). Because telomeres have a complex closed structure their repair may be impaired (Coluzzi et al., 2014), leading to persistent DNA-damage signals what may result in cells undergoing senescence or apoptosis (Lagnado et al., 2021; Collado et al., 2007). In addition, the oxidation of guanine bases may difficult replication, causing unscheduled telomere shortening (Richter and von Zglinicki, 2007), preventing TRF1 and TRF2 shelterins to bind the DNA (Coluzzi et al., 2014), leading to telomere dysfunction (Lin and Epel, 2022).

Granulosa cell division is key to sustain follicle development (Chico-Sordo et al., 2021) and the telomerase activity found in these cells (Hao et al., 2023b), which is an uncommon event in most somatic cells (Martínez and Blasco, 2017), may serve to sustain follicular growth (Córdova-Oriz et al., 2024; Chico-Sordo et al., 2022; Iwata, 2017). We have also found that the generational mean age of the population of GCs at day 85 of follicle development is increased as OS increases. These facts are in agreement with other studies in blood cells (Borghini et al., 2024). In addition, the decrease of telomerase activity leads to an increment of oxidative stress in yeast (Zeinoun et al., 2024) and mammals (Péntek et al., 2023; Pérez-Rivero et al., 2008).

In addition, the decrease of telomerase activity leads to an increment of OS in yeast (Zeinoun et al., 2024) and mammals (Pérez-Rivero et al., 2008). Interestingly, in the case of older women there is an abrupt increment in the aging rate of the population, compared with younger women. These results suggest that OS and low telomerase activity are more detrimental at older ages. Thus, aged GCs may not

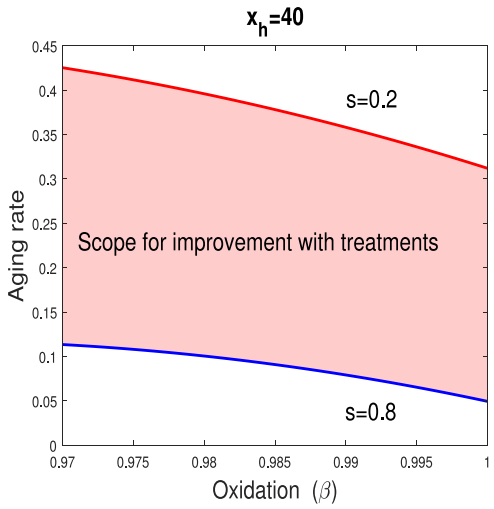


Fig. 7. Analysis of the improvement margin in older women. Margin of improvement of the aging rate when treatments to increase telomerase activity and to reduce oxidation are combined for women aged 40. The red line indicates the decrease in the aging rate by reducing oxidation when telomerase activity is low ($s = 0.2$), while the blue line does so when telomerase activity is high ($s = 0.8$). The red area indicates the margin of improvement when treatments that increase telomerase activity and reduce oxidation are combined. Note that when telomerase activity is low it is better to combine antioxidant and sexual hormones treatments.

fulfill their functions in oocyte nurturing and may decrease oocyte quality and competence for fertilization. These results are congruent with women attending fertility clinics, of about 40 years old, which produce less oocytes after ovarian stimulation and many times these oocytes do not have good quality (Carosso et al., 2022; Ferrario et al., 2015). Curiously, our results also show that at younger ages, the effects produced by OS do not cause so much aging on GCs not even in the presence of low telomerase activity. This may be due to the action of natural antioxidants which are more abundant at younger ages (Lim and Luderer, 2011).

4.2. Therapies to improve oxidative stress and telomerase

OS excess results from the imbalance between free radicals and the mechanisms to remove OS that cells employ to avoid toxicity in their DNA, proteins and lipids (Chaudhary et al., 2023). At the molecular level, hydroxyl radical ($^{\cdot}OH$) and superoxide anion ($O2^{\cdot-}$) are very reactive and harmful forms of oxygen (Halliwell, 1989). Indeed, ovaries have natural defenses against OS (Wang et al., 2017), such as superoxide dismutase which converts the superoxide anion into oxygen and hydrogen peroxide (Tamate et al., 1995) and catalase, an enzyme that converts hydrogen peroxide into H2O and oxygen (Harvey et al., 1995). However, their levels decay with age in GCs (Tatone et al., 2006). Thus, a strategy to solve the OS is the treatment with antioxidants to ameliorate the effects of OS (Ramírez-Martín et al., 2025). Another strategy to solve the accelerated telomere shortening due to OS, is telomerase reactivation (Córdova-Oriz et al., 2023), with sexual hormones (Calado et al., 2009; Guo et al., 2003), which can maintain telomere homeostasis (Martínez and Blasco, 2017). These strategies can be used separately or in combination.

For the study of antioxidants, we focused on women of older ages as they are the most frequent patients in fertility clinics. They have the disadvantage that natural antioxidants present in GCs (Adeldust et al., 2015) decrease with age (Díaz-Casado et al., 2019; Tatone et al., 2006). Thus, the detrimental effects caused by OS on telomeres (López-Otín et al., 2023), could not be so efficiently counteracted as in women of younger ages.

Based on what has been reported in mouse models (Liu et al., 2013), middle-aged women treated with antioxidants, such as resveratrol, could reduce the negative effects of OS on telomeres with the highest doses employed (this work). Another possibility to counteract cell aging (telomere shortening) would be with high telomerase activity, which also decreases with age (Flores et al., 2008). In the case of women with good endogenous levels of telomerase activity, then, the effects of OS would be milder (Péntek et al., 2023). However, low telomerase activity has been found in patients with ovarian insufficiency (Xu et al., 2017; Butts et al., 2009), indicating that this scenario will not probably be very frequent in older patients. Or if these women had good endogenous levels of telomerase activity, then, the effects of OS would be milder.

According to the mathematical model, the best-case scenario would be for women with good levels of telomerase activity or a combined treatment of antioxidants and sexual hormones. Indeed, these results are promising for middle-aged women, who could theoretically produce good quality oocytes, but in order to prove the results of the mathematical model, more experimental work is needed.

Finally, nonlinear models offer a nuanced representation of complex systems and represent an important line of future research to be explored.

CRediT authorship contribution statement

A.M. Portillo: Writing – review & editing, Writing – original draft, Visualization, Software, Investigation, Funding acquisition, Formal analysis, Conceptualization. **J.A. García-Velasco:** Writing – review & editing, Funding acquisition. **E. Varela:** Writing – review & editing, Writing – original draft, Resources, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

None Declared

Data availability

Data will be made available on request.

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