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Modeling the extension of ovarian function after therapeutic targeting of the primordial follicle reserve

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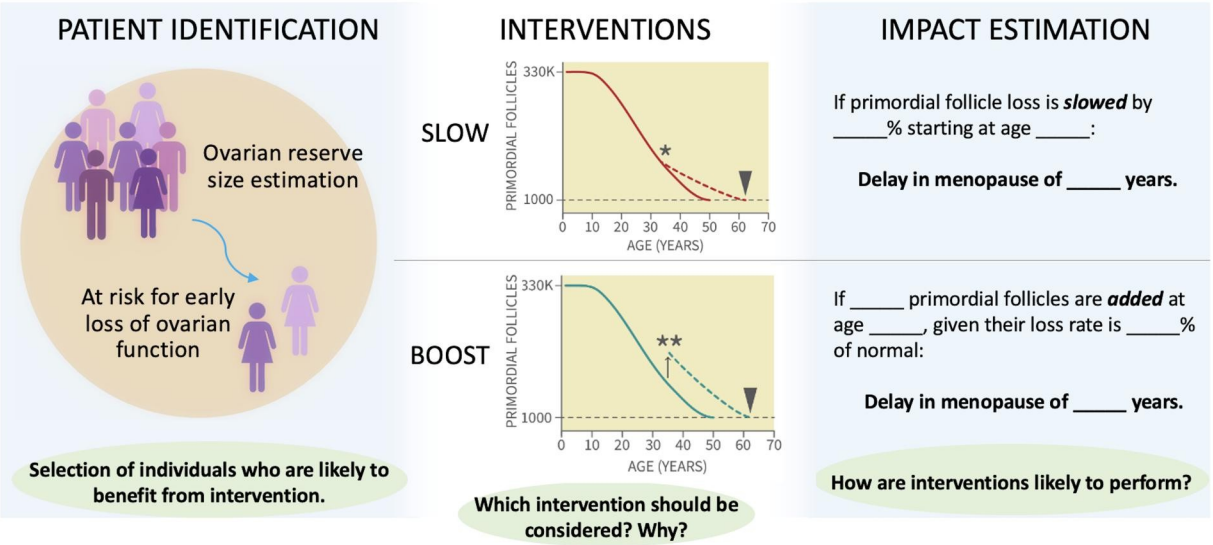
GRAPHICAL ABSTRACT



Interventions being developed to delay menopause: mathematically model strategies that might slow the loss of the ovarian reserve or boost primordial follicle numbers.

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ABSTRACT

BACKGROUND: Women are increasingly choosing to delay childbirth, and those with low ovarian reserves indicative of primary ovarian insufficiency are at risk for sub- and infertility and also the early onset of menopause. Experimental strategies that promise to extend the duration of ovarian function in women are currently being developed. One strategy is to slow the rate of loss of existing primordial follicles (PFs), and a second is to increase, or 'boost', the number of autologous PFs in the human ovary. In both cases, the duration of ovarian function would be expected to be lengthened, and menopause would be delayed. This might be accompanied by an extended production of mature oocytes of sufficient quality to extend the fertile lifespan.

OBJECTIVE AND RATIONALE: In this work, we consider how slowing physiological ovarian aging might improve the health and well-being of patients, and summarize the current state-of-the-art of approaches being developed. We then use mathematical modeling to determine how interventions are likely to influence the duration of ovarian function quantitatively. Finally, we consider efficacy benchmarks that should be achieved so that individuals will benefit, and propose criteria that could be used to monitor ongoing efficacy in different patients as these strategies are being validated.

SEARCH METHODS: Current methods to estimate the size of the ovarian reserve and its relationship to the timing of the menopausal transition and menopause were compiled, and publications establishing methods designed to slow loss of the ovarian reserve or to deliver additional ovarian PFs to patients were identified.

OUTCOMES: We review our current understanding of the consequences of reproductive aging in women, and compare different approaches that may extend ovarian function in women at risk for POI. We also provide modeling of primordial reserve decay in the presence of therapies that slow PF loss or boost PF numbers. An interactive online tool is provided that estimates how different interventions would impact the duration of ovarian function across the natural population. Modeling output shows that treatments that slow PF loss would need to be applied as early as possible and for many years to achieve significant delay of menopause. In contrast, treatments that add additional PFs should occur as late as possible relative to the onset of menopause. Combined approaches slowing ovarian reserve loss while also boosting numbers of (new) PFs would likely offer some additional benefits in delaying menopause.

WIDER IMPLICATIONS: Extending ovarian function, and perhaps the fertile lifespan, is on the horizon for at least some patients. Modeling ovarian aging with and without such interventions complements and helps guide the clinical approaches that will achieve this goal.

REGISTRATION NUMBER: Not applicable.

Keywords: aging / follicle reserve / oocyte / ovary / menopause / primordial follicles

Introduction

The age at natural menopause (ANM) is reached when loss of ovarian function results in 12 months passing in the absence of a menstrual period (Santoro and Johnson, 2019). The ANM time distribution is centered upon a median age of 51, with an upper limit around age 62. At the lower end, approximately 3.5% of women reach menopause before age 40, and one in 250 women do so before age 35 (Persani *et al.*, 2010; Golezar *et al.*, 2019; Li *et al.*, 2023). Menopause can be accompanied by many well-known symptoms and physiological changes, including compromised cardiovascular health, bone density, body fat/muscle composition, hot flushes, and others. The timing of the ANM may also affect the type and severity of menopausal symptoms. Those that reach menopause before 40 are considered to be affected by the condition 'premature ovarian insufficiency' (POI; Nelson, 2009; De Vos *et al.*, 2010; European Society for Human Reproduction and Embryology (ESHRE) Guideline Group on POI *et al.*, 2016). Reaching menopause at these earlier ages means that the symptoms of menopause and the earlier menopausal transition (below) can be experienced for half, or even more than half, of women's lives.

Before menstrual cycles cease, they become increasingly irregular during a phase termed the menopausal transition (Hoyt and Falconi, 2015; Paramsothy *et al.*, 2017; Santoro *et al.*, 2021). The time-of-onset distribution for the menopausal transition ranges from ages 42 to 54, and lasts between 2 and 10 years for most women (Paramsothy *et al.*, 2017). Menstrual cycle irregularity experienced during the menopausal transition can include 'unpredictable menstrual cycle endocrine patterns' including anovulatory (Burger, 2011) cycles interspersed by months of amenorrhea (Harlow *et al.*, 2008). Some of the symptoms and consequences of reproductive aging can begin during the menopausal transition.

These significant consequences of the menopausal transition and the onset of menopause have led to the practical consideration of how the health span, and even lifespan, could be impacted by intervening and extending the duration of ovarian function. This is distinct from the current practice of replacement of ovarian hormones estrogen and progesterone, as in HRT. HRT provides relief of many symptoms of reproductive aging (Davis *et al.*, 2023), but cannot support the 'baseline' health status of the reproductive years for all individuals (Mukherjee and Davis, 2025). Reports of experimental strategies that may one day extend the duration of ovarian function in women, and thus delay the onset of the landmark menopausal transition and ANM events are therefore becoming more commonplace (reviewed in Llarena and Hine, 2021; Cavalcante *et al.*, 2023; Liu and Gao, 2023; see also Johnson *et al.*, 2024). In the case of women with elevated risk for POI as determined by clinical measures, a successful intervention would delay her loss of ovarian function such that she instead experiences menopause and its consequences years later than she would otherwise. A delay of menopause should also delay the menopausal transition (Hoyt and Falconi, 2015; Paramsothy *et al.*, 2017; Santoro *et al.*, 2021; Lawley *et al.*, 2024) and its accompanying symptoms. In the shorter term, interventions in some women diagnosed as having diminished ovarian reserve might also extend their window of fertility. Here, counteracting the loss of immature ovarian follicles might increase the numbers of retrievable (and ideally, high quality) oocytes in assisted reproductive treatments. Because socioeconomic disadvantage has been identified as a potential risk factor for early ovarian demise (Bleil *et al.*, 2018; Pan *et al.*, 2024), interventions to extend ovarian function may help correct a significant health disparity.

To benefit patients in these ways, two important questions need to be answered. First, how can we identify the individuals who are at the highest future risk of early loss of ovarian function and would thus be the most likely to benefit from these interventions? Herein, we consider current methods used to estimate the size of the ovarian reserve and thereby, the risk of early loss of ovarian function. Second, can we estimate how different

interventions under development are likely to perform, and use this to help identify which patients would benefit most from strategies that slow ovarian aging? Fortuitously, recent progress has been made in modeling how loss of the ovarian reserve of primordial follicles (PFs) occurs over time in individuals and across the population (Johnson *et al.*, 2022; Lawley *et al.*, 2024).

We have developed a mathematical model of PF behavior over time that produces accurate PF loss patterns seen in individuals and also the natural patterns of the menopausal transition and the ANM (Johnson *et al.*, 2022; Lawley *et al.*, 2024) across populations of women. This model has been used to address the question of why girls have large numbers of PFs at birth (Lawley and Johnson, 2023) and to probe the relationship between the timing of the menopausal transition and ANM in different women (Lawley *et al.*, 2024). Because of increasing interest and activity in developing interventions to extend ovarian function, we now consider how the model might be modified in order to simulate the performance of these strategies.

Strategies for extending ovarian function can be placed into two general categories (see Fig. 1 for graphical summaries). In the first category, the goal is slowing the rate of loss of existing PFs in the 'ovarian reserve', usually by the application of a pharmacological or biological agent (Zhang *et al.*, 2019; Valtetsiotis *et al.*, 2023), and thereby delaying menopause onset. Because the initial ovarian reserve at birth (a woman's 'starting supply' of PFs; Johnson *et al.*, 2022) has been associated with the eventual timing of menopause (Depmann *et al.*, 2015; McLaughlin *et al.*, 2015), an individual's PF loss rate may be normal, but early menopause will occur due to a low starting supply. Alternatively, known conditions appear to accelerate the loss of the PF reserve, meaning that even if born with a 'normal' number of PFs, an individual might exhaust her reserve earlier than others. Slowing PF loss could delay the onset of symptoms that accompany the menopausal transition and menopause, and could thereby have a significant impact upon the health and well-being of affected women for extended time periods. In such cases, significant success could be defined by delaying menopause to the median age of 51, as seen in the general population of women (Gold, 2011).

The second strategy to slow ovarian aging promises to increase or 'boost' the number of autologous PFs in the human ovary. In 'boost' strategy manifestations, the delivery of stem cells that can give rise to new follicles, the production of engineered ovarian tissue that contains new autologous follicles, and treatment with pharmacologic agents that appear to stimulate the development of new follicles can be considered. In each case, newly added PFs need to function normally in terms of their survival and growth pattern, and possible production of mature, fertilizable oocytes.

One practical strategy that we have considered in detail previously falls into the 'boost' category: ovarian tissue cryopreservation (OTC) in healthy women. Mathematical modeling suggests that OTC early in life, and tissue replacement just before menopause could significantly delay the loss of ovarian function (Johnson *et al.*, 2024). While PFs are not produced de novo, the use of OTC in this way 'boosts' PF numbers by delaying the loss of PFs over time that would occur if the tissue was left in place. Importantly, loss of PFs following tissue replacement, probably due to the tissue being avascular (Baird *et al.*, 1999), can be significant, and thus progress needs to be made in maximizing follicle survival within the cortex that undergoes OTC. Furthermore, this strategy requires two surgical procedures at minimum (and perhaps more if tissue is returned sequentially, in a 'fractionated' fashion; Johnson *et al.*, 2024). Some patients and providers may still find that the benefits of the procedures, i.e. the extension of ovarian function and its consequences, are worthwhile despite the invasive nature of OTC.

Pathological human conditions that accelerate the loss of primordial reserve (above) could also be treated by 'boosting' PF numbers in this way, but in those cases, if the underlying condition that leads to accelerated loss is not addressed, more autologous PFs may not result in extended ovarian function. Here again, we consider the early ovarian demise seen in classic galactosemia (CG; Gibson, 1995; Frederick *et al.*, 2018; Hagen-Lillevik *et al.*, 2021) and fragile X premutation (FXPM; Pastore and Johnson, 2014; Fink *et al.*, 2018). Most reports suggest that in these cases, girls are born with PFs in the normal range. In these cases and others involving idiopathic POI, if accelerated loss is not treated, any autologous newly delivered PFs might themselves undergo accelerated loss, and the treatment could have minimal or even no net benefit (Fig. 1). To prevent this, it may be necessary to correct the genetic defect associated with accelerated PF growth activation (PFGA; Kallen *et al.*, 2018) in the newly delivered, stem cell-derived follicles.

There are also patients for whom strategies that promise to deliver additional PFs are needed and are perhaps their only option. These patients have conditions that result in zero or very low numbers of PFs early in life. For example, very early in postnatal life, girls with Turner syndrome (Reindollar, 2011; Fukami, 2023) or triple X syndrome (Goswami *et al.*, 2003; Davis *et al.*, 2020) can have very few immature follicles or streak ovaries, which are devoid of follicles. For these individuals, only the addition of (autologous) PFs can extend or, in the case of streak ovaries, reinitiate ovarian function.

The conceptual and technical achievements necessary to advance these strategies to this point have been remarkable. It is important now to consider the likely efficacy of such approaches given their application to patients. When efficacy is defined as the duration that ovarian function is extended in the presence versus the absence of treatment, we need to be able to make predictions about the duration of ovarian function extension. If ovarian function is extended only for a short duration after a particular therapy that might include surgery, the intervention may be difficult to justify. We can consider specific patient conditions where 'slow' or 'boost' options or a combination of available approaches will be favored. It is also critical to consider whether and for how long the window of fertility might be affected by successful intervention(s).

After brief consideration of the symptoms and health consequences of the menopausal transition and menopause, we consider the current methods used to estimate the remaining ovarian reserve. In this way, we can begin to determine the patients for whom slowing ovarian reserve loss, or rescuing ovarian function, might be appropriate. Afterwards, we apply existing mathematical modeling approaches to evaluate how 'slow' or 'boost' interventions would impact ovarian aging. Results in comparison to the absence of treatment can be generated using an interactive tool so that variables can be explored and treatment outcomes can be estimated under different circumstances. Formal assessment of 'slow' and 'boost' strategies in this way can lead to general guidelines about the timing of their application to their eventual target population(s). We conclude by establishing potential criteria that could be used to monitor ongoing efficacy in different patients.

Methods

Literature review methodology

Literature searches were performed using PubMed to identify literature in three areas; both primary literature and review articles

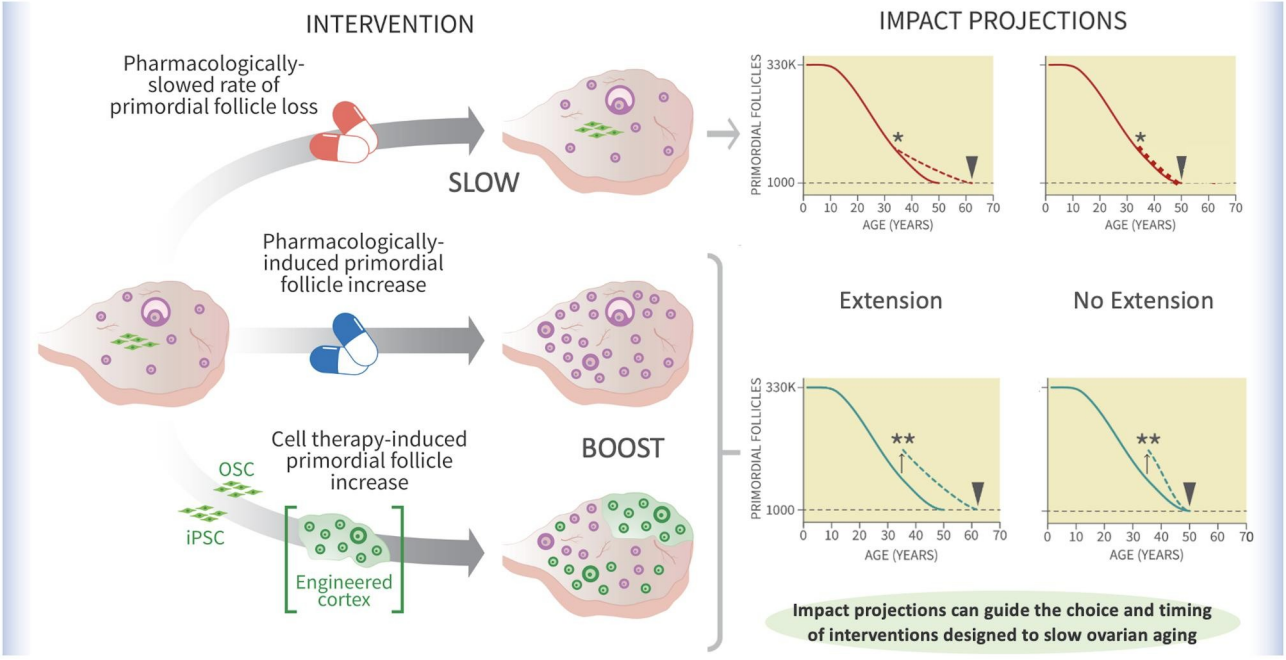

Figure 1. Intervention strategies and their desired impact(s). Two intervention strategies to extend ovarian function are considered, along with mockup projections of their impact upon ovarian aging. At the top, an intervention that 'slows' the rate of primordial follicle loss is shown. This may be done pharmacologically, using agents targeted either to mechanisms that drive follicle loss broadly or to specific disease conditions that accelerate loss. The desired impact of slowing the loss rate is indicated by and in the example plot at left, an intervention beginning at age 35 slows ovarian aging such that crossing the menopausal threshold (arrowhead and horizontal black dashed line) is delayed (red dashed line and arrowhead). The counter example where an unsuccessful treatment does not slow ovarian reserve loss is shown on the right. Below, two 'boost' strategies to increase primordial follicle numbers are shown. First, pharmacological agents could be applied that stimulate the production of new primordial follicles. Second, at the bottom, cell therapy strategies could be employed to produce new primordial follicles. The latter strategy could make use of induced pluripotent stem cells (iPSC) or native oogonial stem cells (OSC) to produce primordial oocytes and pregranulosa cells of primordial follicles. This strategy could optionally involve the production of an engineered ovarian cortex substrate (green parentheses) for follicle aggregation and delivery. Potential impacts of the generation of new follicles are shown in the bottom plots, with the 'boost' in follicle numbers indicated by the up arrow and In the left-hand example, the subsequent trajectory results in delayed menopausal threshold crossing (Extension), while the right example does not (No Extension, compare dashed lines and arrowheads). The No Extension outcome could occur if the rate of loss of newly engineered primordial follicles is abnormally fast, as might be seen in conditions that accelerate ovarian reserve loss; such a defect would need to be corrected in newly provided follicles.

were included due to the extensive and historical nature of the topics, and all non-peer-reviewed sources were excluded. First, publications detailing (i) methods to estimate the size of the ovarian reserve, (ii) established models of ovarian reserve decline over time, or (iii) the relationship between the ovarian reserve and the timing of menopause, were selected using the search terms, *ovarian reserve*, *primordial follicles*, *menopause*, and *aging*, where no limits were placed on publication year. Next, publications investigating pre-clinical methods to extend the duration of ovarian function were selected using the search terms, *human*, *menopause*, *delay*, *intervention*, *extension*, and *follicle*. Again, searches were performed where no date range was specified, with and without the term 'Review article' selected.

Model application to extension of ovarian function

Current mathematical approaches (Johnson *et al.*, 2022; Lawley *et al.*, 2024) were repurposed for predictive analysis of how methods that slow loss of the ovarian reserve or deliver additional primordial ovarian follicles to patients' ovaries are likely to perform. We consider 'slow' therapies and 'boost' therapies in terms of their anticipated impact upon the timing of menopause (Fig. 1). Specifically, if T_{therapy} and $T_{\text{no therapy}}$ denote the respective menopause ages for a woman with and without a given therapy, then the menopause delay is

$$D = T_{\text{therapy}} - T_{\text{no therapy}}. \quad (1)$$

In Supplementary Data Files S1, S2, and S3, we use mathematical modeling and analysis to derive explicit formulas and computational methods for predicting the menopause delay. An interactive online tool that allows users to explore how different therapies delay menopause given different parameters is available at: https://seanlawley.shinyapps.io/slowboost/

Results

Estimating the size of the ovarian reserve

We consider how the size of the ovarian reserve might be estimated. At minimum, each of the approaches discussed should provide guidance about the risk of early loss of ovarian function so that individuals and providers can assess whether intervening is warranted.

There is some evidence that antral follicle count (AFC) can be used to identify those at risk of early ovarian demise. In 2013, Wellons *et al.* reported that AFC as determined by transvaginal ultrasound can be used to determine a threshold AFC (≤ 4 vs >4), where AFC ≤ 4 significantly raised the risk of menopause in the next 8 years. This corresponded to a hazard ratio of menopause within those 8 years of 1.89 (95% confidence interval: 1.19, 3.02; *P*-value of 0.008), and in that time approximately 35% of women under or at the threshold reached menopause, while approximately 15% above the threshold reached menopause. AFC

measurement, and also the related measure of the number of oocytes retrieved after ovarian stimulation (Christensen *et al.*, 2020), can thus provide actionable information about the remaining ovarian reserve.

Anti-Müllerian hormone (AMH) levels (sometimes in combination with other measures) can also be used to estimate the size of the ovarian reserve and therefore the 'status' of ovarian aging (Ruth *et al.*, 2019; Moolhuijsen and Visser 2020; de Kat *et al.*, 2021; Zhang *et al.*, 2021; Chatziandreou *et al.*, 2023). AMH levels below the 10th percentile may be used as a threshold for the identification of a low functional ovarian reserve in an individual woman (Tehrani *et al.*, 2022). More recently, Chatziandreou *et al.* (2023) showed that a cutoff of 0.012 ng/dL AMH discriminates between women who will reach their last menstrual period an average of 20 months later (below threshold) versus an average of 60 months later (above threshold). This is consistent with the conclusion of a recent systematic review that prediction of the ANM remains imprecise when it is not imminent, but a very low AMH in young women can indicate increased risk of developing POI (Nelson *et al.*, 2023).

Finally, in a systematic review and meta-analysis, Younis *et al.* (2020) evaluated menstrual cycle length data and probed how it relates to the expected ovarian reserve size during the reproductive years. Because menstrual cycle length changes are well characterized during the reproductive years and across the menopausal transition (Paramsothy *et al.*, 2017; Lawley *et al.*, 2024), identification of women with low ovarian reserve may one day be achievable using specific cycle length parameters. The advent and spread of electronic tools (including wearable devices) that can track menstrual cycles can bring such tracking to large numbers of women. We next consider how ovarian aging might one day be extended in patients identified to have low ovarian reserve.

Delaying menopause by extending the duration of ovarian function

Currently, different approaches to prolong the duration of ovarian function are under experimental evaluation. These are provided in Table 1, which is organized by the different 'slow' and 'boost' modalities targeting follicles, as well as those that are 'non-follicle targeting'.

Menopause delay from slowing therapies

The first modeling exploration here evaluates the impact of slowing the rate of PFGA (see Fig. 1; see also Table 1). This approach would be relevant to two cases. First, it might be desirable to slow the rate of loss of the remaining ovarian reserve to extend ovarian function in patients who expect to reach menopause before the median age of 51. These patients might have low or even normal-size ovarian reserves, and the goal would be to extend fertility or to delay symptoms of reproductive aging. Second, in certain disease states the initial endowment of follicles around birth is thought to be normal, but loss of the ovarian reserve occurs in an accelerated fashion that can result in early menopause, including before the age of 40. For example, early ovarian demise occurs in CG (Gibson, 1995; Frederick *et al.*, 2018; Hagen-Lillevik *et al.*, 2021) or FXPM (Pastore and Johnson, 2014; Fink *et al.*, 2018), and in each of these cases, the ovarian reserve is thought to be normal around birth. A strategy where an agent(s) is delivered to slow the rate of PFGA would be highly desirable here as well. Intervening in these patients so that PFGA rates are slowed and 'normalized' holds the promise of extending ovarian function into the normal range.

Prior work by one of our groups tested a therapeutic strategy using a mouse model of CG, *GalT* knockout mice. The mouse model phenocopies the accelerated PF loss that leads to early cessation of ovarian function in girls and young women with CG (Balakrishnan *et al.*, 2019; Hagen-Lillevik *et al.*, 2021), and we showed that a 'slowing' treatment was able to normalize PF loss over time. This pre-clinical evidence showed that targeting specific stress and damage pathways in the mouse model can not only normalize PFGA rates (Balakrishnan *et al.*, 2017, 2019), but animals kept on the agent Salubrinal (which is an Integrated Stress Response pathway agonist (Boyce *et al.*, 2005; see Table 1)) from birth demonstrated normalized numbers of growing follicles present during adult life. Furthermore, litters were produced in an ongoing fashion in treated animals that would otherwise be sterile. These results, where follicle behavior after PFGA through ovulation and the production of mature oocytes were normalized by the treatment, are promising when considering the challenge in human patients at risk for early ovarian demise due to accelerated PF loss.

The goal of the above example was to impact follicle behavior (e.g. slow their loss and support normal development) directly. However, it should be noted that targeting ovarian tissue(s) outside of follicles (referred to here as 'non-follicle targeting') may slow PF loss indirectly. A body of evidence is available that shows that the ovarian stroma that exists between and around ovarian follicles may influence follicle growth and survival over time (Briley *et al.*, 2016; McCloskey *et al.*, 2020; Henning *et al.*, 2021; Machlin *et al.*, 2021; Landry *et al.*, 2022; Grosbois *et al.*, 2023; Amargant *et al.*, 2024). Because markers of tissue fibrosis in the ovarian stroma increase with aging, anti-fibrotic treatments are being evaluated for their action upon follicle behavior and loss. There is also evidence that cellular senescence occurs and increases in non-follicle (and follicular) cell types of the ovary with aging (Maruyama *et al.*, 2023; Shen *et al.*, 2023; Telfer *et al.*, 2023). In another example, targeting ovarian endothelial cells and the vasculature that develops around follicles has been shown to slow the rate of loss of the ovarian reserve (Xu *et al.*, 2022). It is possible that reducing either fibrosis or cellular senescence within 'non-follicle' tissues of the ovary or targeting ovarian vasculature could lead to a slowed rate of follicle loss in patients. Because systemic interventions may impact both follicles and surrounding cells in the ovary, there may be functional overlap between follicle and non-follicle targeting. With all of these potential approaches in mind, we turn to the mathematical modeling of approaches designed to 'slow' ovarian aging.

If a woman receives the therapy continuously from age t_0 until menopause at age T_{therapy}, we show in Supplementary Data File S1 that the resulting menopause delay in Equation (1) is

$$D_{\text{slow}} = \frac{1-a}{a}\left(T_{\text{no therapy}} - t_0\right), \tag{2}$$

where the parameter a quantifies how the therapy slows ovarian aging. Specifically, if ovarian aging (PF loss) occurs at rate r without the therapy, then ovarian aging occurs at rate ar during therapy, where $0 < a < 1$. For example, consider a woman who, without therapy, would have early menopause at age $T_{\text{no therapy}} = 43$ years, and suppose she receives therapy from age $t_0 = 31$ years until menopause, which slows PF loss by 40%. In this case, $a = 0.6$, and Equation (2) predicts that this therapy would delay menopause by $D_{\text{slow}} = \frac{0.4}{0.6}(43 - 31)$ years $= 8$ years, thereby bringing her menopause age to the population median of 51 years.

Table 1. Summary of interventions considered for the use of slowing ovarian aging in women.

Category	General approach	Specific examples	Mechanistic background	Caveats/challenges	References
		AMH	Negative regulator of the rate of PF growth activation.	Large-scale (recombinant) protein production.	Meinsohn *et al.* (2021), Pankhurst (2017), P [illegible]pin *et al.* (2018)
		CoQ10	Mitochondrial bioenergetics; antioxidant action. Slows the rate of PF loss in an aged mouse model.	Incompletely characterized in humans.	Ben-Meir *et al.* (2015)
Follicle targeting [illegible]	Medicinal/biological	NMN	NAD_b precursor, key cofactor for mitochondrial bioenergetic (electron transport chain, TCA cycle), and DNA repair enzymes.	Incompletely characterized in humans. Currently more associated with oocyte quality.	Arslan *et al.* (2024), Bertoldo *et al.* (2020), Huang *et al.* (2022)
		PSPC; myo-inositol	Integrated stress response enhancement and antioxidant action correspond to a slowed rate of PF loss in a galactosemic mouse model.	Incompletely characterized in humans. Non-galactosemic applications?	Hagen-Lillevik *et al.* (2022)
		Rapamycin	Cell cycle regulation via mTOR targeting: reduced mTOR activity corresponds to a slowed rate of PF loss in mice.	Incompletely characterized in humans, potential dose-dependent and off-target effects.	Garcia *et al.* (2019)
		Salubrinal	Integrated stress response pathway enhancement slows PF loss in a galactosemic mouse model.	Humans? Non-galactosemic applications?	Boyce *et al.* (2005), Balakrishnan *et al.* (2017, 2019)
SLOW	Diet/lifestyle	Caloric restriction	Bioenergetics/metabolism, reduced DNA damage, and oxidative stress.	Onerous/compliance difficulty over long time scales.	Isola *et al.* (2022), Li *et al.* (2015), Nelson *et al.* (1995), Shi *et al.* (2013), Flanagan *et al.* (2020)
		Diet composition	Specific conditions of caloric or protein restriction (but not branched-chain amino acid restriction alone) slow the rate of loss of the ovarian reserve in mice.	Incompletely characterized in humans.	Veiga *et al.* (2024)
	Ovarian stroma fibrosis	Metformin	Reduction of aging-associated fibrosis in ovarian stroma; targeting of mitochondrial complex I.	Reduces fibrosis; benefits for humans are hypothetical.	Landry *et al.* (2022), McCloskey *et al.* (2020), Reczek *et al.* (2024)
Non-follicle targeting [illegible]		Pirfenidone	Reduction of aging-associated fibrosis in ovarian stroma via TGF-$_\beta$ signaling downregulation and cytokine modulation.	Reduces fibrosis; benefits for humans are hypothetical.	Amargant *et al.* (2024)
	Senescent cells	Senolytics	Elimination of senescent cells has been shown to improve performance of other tissues and offset cell and tissue consequences of aging.	Benefits are predicted/hypothetical.	Maruyama *et al.* (2023), Shen *et al.* (2023)
	Angiogenesis	Anti-angiogenic treatment	Limiting blood vessel development corresponds to a slower rate of loss of the ovarian reserve.	Incompletely characterized in humans.	Xu *et al.* (2022)
	Pharmacological	Combination adriamycin, bleomycin, vincristine, doxorubicin	The 'paradoxical' increase in non-growing follicles in patients treated with this chemotherapeutic regime requires further mechanistic study.	Chemotherapy/off-target effects. Need for 'new' PFs to function normally.	McLaughlin *et al.* (2017)

(continued)

Table 1. Continued

Category	General approach	Specific examples	Mechanistic background	Caveats/challenges	References
		iPSC	Production of autologous PF cells (oocyte and somatic pregranulosa cells) via iPSC reprogramming; PF assembly and delivery to ovary.	Complexity, need for production and surgical delivery of large numbers of autologous PFs, need for delivered PFs to function normally, cost.	Kobayashi *et al.* (2022), Saitou and Hayashi (2021), Sosa *et al.* (2023)
BOOST	Cell therapy	Oogonial/germline stem cells (OSC)	Isolation of putative OSC from ovary, assembly of PFs after mixing with somatic pregranulosa cells, and delivery to ovary.		Alberico *et al.* (2022), MacDonald *et al.* (2023)
Increase PF number		Other stem cell approaches, 'artificial ovary'	Production of extra-follicular cells and/or extracellular matrix scaffolds, likely to be used in combination with stem cell production and assembly of new PFs. Scaffold containing follicles delivered to ovary.		Amorim and Shikanov (2016)
	Cortex cryopreservation	Tissue freezing, return	Cryopreservation is expected to result in net reduction of PF loss over time, resulting in extended ovarian function.	Multiple surgeries, need for consistent PF survival during procedure, need for PFs to function normally after transplantation.	Johnson et al. (2024)

• Systemic interventions intended to target primordial follicles directly may impact extrafollicular tissues of the ovary, and vice versa.
AMH: anti-Müllerian hormone; CoQ10: coenzyme Q10; iPSC: induced pluripotent stem cell; mTOR: mechanistic target of rapamycin; NAD_{+}: nicotinamide adenine dinucleotide; NMN: nicotinamide mononucleotide; OSC: oogonial stem cell; PF: primordial follicle; PSPC: purple sweet potato color; TCA: tricarboxylic acid cycle; TGF-β: transforming growth factor beta.

If the slow therapy does not continue past menopause (i.e. the therapy ceases before age T_{therapy}), we show in Supplementary Data File S1 that the resulting menopause delay in Equation (1) is

$$D_{\text{slow}} = (1-a)\,t_{\text{dur}}, \quad (3)$$

where t_{dur} is the total duration of time that the woman receives the slow therapy. For example, if a woman receives therapy from age 30 to age 40 years which slows the rate of PF loss by 30%, then $t_{\text{dur}} = 10$ years, $a = 0.7$, and Equation (3) predicts that the resulting menopause delay is $D_{\text{slow}} = 0.3 \times 10 \text{ years} = 3$ years. We note that Equations (2) and (3) agree in the case that therapy ceases at age T_{therapy}.

We stress here that the results in Equations (2) and (3) are model-independent (see details in Supplementary Data File S1 and Figure S1). That is, these results do not depend on any specific mathematical or statistical model of PF loss. Rather, these results only depend on assuming that menopause coincides with the primordial reserve dipping below a threshold number of remaining PFs.

In Fig. 2, we plot the delay in menopause in Equation (2) as a function of the age at start of therapy, t_0, for therapies which slow the rate of PF loss by 20% (blue, $a = 0.8$) and 40% (red, $a = 0.6$). The dark blue and red lines are for a woman who would have menopause at the population median of 51 years in the absence of therapy, and the blue and red shaded regions depict women who would have menopause between ages 47 and 55 years, which constitutes the bulk of the population (Weinstein *et al.*, 2003).

Figure 2 illustrates that the extension of ovarian function is increased by starting the therapy earlier in life, assuming that the therapy is well tolerated and continues until the eventual (delayed) menopause age. This result is intuitive, since the therapy can offer relatively little benefit if the vast majority of PFs have already been lost by the time a woman starts the treatment. Fig. 2 also illustrates how the menopause delay depends non-linearly on how the therapy slows PF loss. Indeed, assuming the therapy begins at age $t_0 = 40$ years and continues until menopause, slowing the rate of PF loss by 40% rather than 20% increases the menopause delay from 2.8 years to 7.5 years for the median woman. This non-linear dependence can be understood by noting that further decreasing the rate of PF loss allows the therapy to continue longer. Another salient implication of this analysis is that for a given therapy start age t_0, women who would have earlier menopause ages without the therapy (i.e. the women for whom the therapy would perhaps be most desirable) are the women who will receive the smallest menopause delay. The impact of slowing therapies can be explored by using the interactive tool to produce ranges of expected menopause delay in the presence of specified intervention(s).

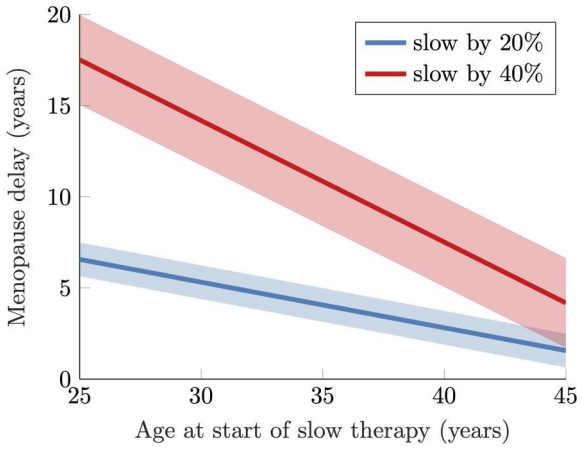

Figure 2. Modeling interventions that slow the rate of primordial follicle (PF) loss in women. The menopause delay is plotted as a function of the age at the start of therapy, t_0, for therapies that slow the rate of PF loss by 20% (blue, $a = 0.8$) and 40% (red, $a = 0.6$). The dark blue and red lines are for a woman who would have menopause at the population median of 51 years in the absence of therapy, and the blue and red shaded regions depict women who would have menopause between ages 47 and 55 years in the absence of therapy.

Menopause delay from boost therapies

The second exploration here models how 'boosting' PF numbers by varying the numbers of new follicles added to the ovaries would likely influence subsequent ovarian aging (mockup in Fig. 1; see also Table 1). The production of PFs de novo and engineered ovarian tissue that contains these newly produced follicles is underway (Kobayashi *et al.*, 2019, 2022; Pierson Smela *et al.*, 2023; Sosa *et al.*, 2023). It is anticipated that such tissue can be delivered to the host ovary surgically and that the autologous PFs within will develop normally and support ongoing ovarian function.

Induced pluripotent stem (iPS) cells have been used to produce the component oocytes and granulosa cells of ovarian follicles, and stem cells that can produce the theca cell lineage (Vlieghe *et al.*, 2024) have recently been reported. Boosting follicle numbers has been achieved pre-clinically via the delivery of stem cells that can give rise to new follicles in the mouse (Johnson *et al.*, 2004; Hayashi *et al.*, 2012; Hayashi and Saitou, 2013; Hikabe *et al.*, 2016; Yoshino *et al.*, 2021), and more recently this work has been extended to human cells (White *et al.*, 2012; Woods *et al.*, 2013; Alberico *et al.*, 2022) used in a clinical strategy to improve oocyte quality (Morimoto *et al.*, 2023).

Different methods using these stem cells are in development and promise to produce a complete 'artificial ovarian cortex' (Amorim and Shikanov, 2016; Sosa *et al.*, 2023). Extremely rapid progress is being made using stem cells and tissue engineering approaches, and the major remaining hurdle seems to be ensuring that follicles produced in this way behave normally in terms of their survival and development over time.

As mentioned, some patients' ovaries have either very low numbers of PFs, and in some cases can be entirely devoid of PFs. In this case, 'boosting' the ovary with additional PFs may be the only option, as too few native follicles are present to respond meaningfully to any 'slowing' therapy. Here, it is critical to consider the defect behind accelerated ovarian demise, as the delivery of additional autologous PFs may result in a similarly rapid loss of any 'boosted' ovarian reserve (see also Discussion). Gene therapy approaches may be required to repair the genetic lesions that led to the primary loss of the ovarian reserve so that ongoing ovarian function can be achieved.

Last here, treatment with specific chemotherapeutic agents has been shown to correspond to significantly elevated PF numbers in treated patients, suggesting that the development of new follicles can be induced (McLaughlin *et al.*, 2017). In part due to the surprising outcome of a treatment that might instead have been expected to damage the ovarian reserve, further work in this area is needed to identify mechanisms that might be exploited to induce this effect. Given that somewhat precise numbers of autologous PFs can one day be produced for patients, and given that these PFs will perform normally in terms of survival and development, we now explore how such 'boost' approaches would likely impact the duration of ovarian function.

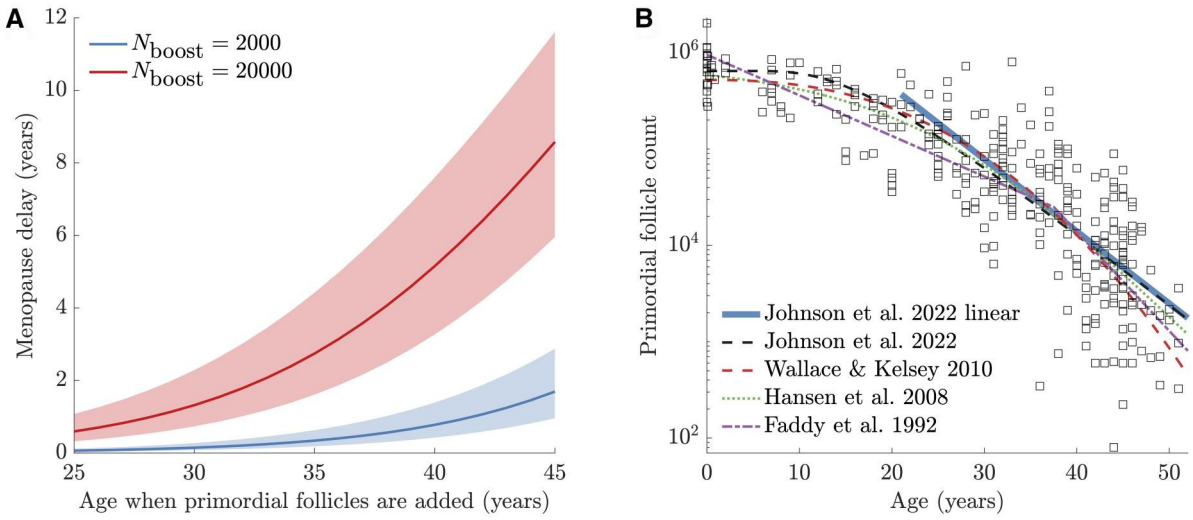

Figure 3. Modeling interventions that add or 'boost' primordial follicle (PF) numbers in women. (**A**) The impact of adding either 2000 (blue) or 20 000 (red) PFs to a woman's existing PF reserve is simulated. (**B**) Comparison of prior PF decay models against PF data reported in Wallace and Kelsey (2010).

In Fig. 3A, we plot the delay in menopause in Equation (1) for either 2000 PFs added (blue) or 20 000 PFs added (red) as a function of the age when the PFs are added. As in Fig. 2, the solid curves correspond to a woman who would have menopause at age 51 years in the absence of therapy, and the shaded regions represent women who would have menopause between ages 47 and 55 years in the absence of therapy.

Unlike Fig. 2, the results in Fig. 3A depend on the specific model of PF loss, and we use the model proposed in Johnson *et al.* (2022). However, we show in Fig. 3B that the menopause delays predicted by several prior models of PF loss are all very similar (see also Supplementary Fig. S2 and Supplementary Data File S2 for examples of menopause delay given specific numbers of boosted PFs). One benefit of this model is that it allows us to obtain an explicit formula for the menopause delay in Equation (1). Specifically, for such a 'boost' therapy in which N_{boost} PFs are added at age t_0, we derive the following formula in Supplementary Data File S1:

$$D_{boost} = \frac{1}{\lambda} \ln\left(1 + \frac{N_{boost}}{F(t_0)}\right), \quad (4)$$

where $\lambda > 0$ is the rate of PF loss, $F(t_0)$ is the number of PFs across both ovaries just before the new PFs are added, and ln( ) denotes the natural logarithm. The result in Equation (4) is plotted in Fig. 3 for F and λ given by the model proposed in Johnson *et al.* (2022; Equations S16 and S17 in Supplementary Data File S2).

We now highlight several important implications of this analysis. First, in contrast to the slow therapies, the menopause delay is increased by applying the boost therapy later in life (but not after menopause). Second, and also in contrast to slow therapies, it is evident from Equation (4) that women with smaller PF reserves (who thus would reach menopause earlier without an intervention) stand to gain the greatest extension of ovarian function for a given age at treatment, t_0.

Finally, and perhaps most strikingly, this analysis predicts that such a boost therapy would yield relatively minor extensions of ovarian function, even for the currently optimistic assumption that $N_{boost} = 2000$ PFs can be added. Indeed, Fig. 3 predicts that adding 2000 PFs to a median woman at age 45 would delay her menopause by less than 2 years. Importantly, we note that the result in Equation (4) (which is plotted in Fig. 3) assumes that the added PFs age at the same rate as the natural PFs. If the added PFs have slower rates of PFGA, then the therapy would offer a greater benefit. Conversely, if the PFs are lost more quickly than natural PFs, then the benefit of the therapy would be even more modest (see Fig. 1, 'No Extension'). We note that the online tool mentioned in the Methods section also allows users to interrogate the effects of changing the aging rate of added PFs.

The analysis above also allows us to obtain an upper bound on the menopause delay induced by a boost therapy. As mentioned above, the delay is maximized by adding the PFs just before menopause. Estimates of the number of PFs across both ovaries at menopause range from 1000 to 2000 (Richardson *et al.*, 1987; Faddy *et al.*, 1992; Wallace and Kelsey, 2010). Estimates of the PF decay rate λ later in a woman's life range from $\lambda = 0.17$/year (Johnson *et al.*, 2022), to $\lambda = 0.24$/year (Faddy *et al.*, 1992), to $\lambda = 0.3$/year (Faddy and Gosden, 1995). Therefore, assuming that (i) menopause does not occur until the reserve dwindles to only 1000 PFs, (ii) the boost is applied at this exact time, and (iii) taking $\lambda = 0.17$/year thus gives the following rather generous upper bound on the possible menopause delay from adding N_{boost} PFs,

$$D_{boost} \leq \frac{1}{0.17} \ln\left(1 + \frac{N_{boost}}{1000}\right) \text{ years}. \quad (5)$$

This upper bound is plotted in Fig. 4. This plot shows that even in the optimistic scenario that PFs are added just before menopause, at least hundreds of additional PFs are needed to extend ovarian function for more than about 1 year.

Combining slow and boost therapies

We have shown that slow therapies may offer little in terms of ovarian function extension unless an intervention with significant slowing action (in the model, this is provided as a percentage of the endogenous rate of PF loss) is begun early in reproductive life, potentially as early as a woman's early twenties. If the intervention does not slow the PFGA rate enough, and it is not applied early enough, slowing PFGA may have little impact.

In contrast, boost therapies are likely to offer little ovarian function extension unless (i) the number of additional PFs is at least several hundred (and more likely, several thousand) and (ii) the therapy is applied shortly before menopause. It is

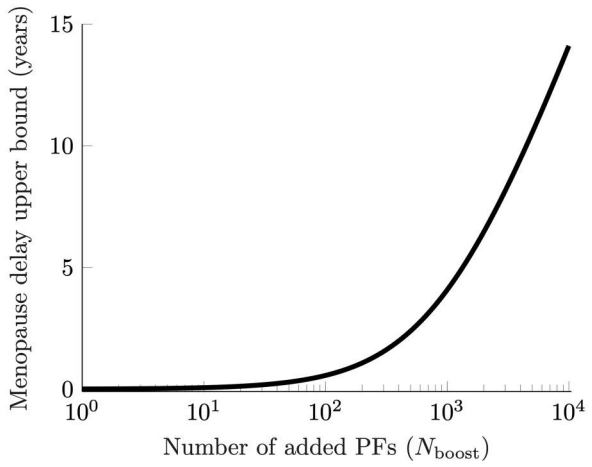

Figure 4. Upper bound for the menopause delay from a 'boost' therapy. The curve plots the upper bound for the menopause delay from a boost therapy. The curve depicts the near-optimal scenario that the primordial follicles (PFs) are added when only a total of 1000 natural PFs remain across both ovaries.

therefore natural to ask whether combining slow and boost therapies can result in greater extension of ovarian function than either therapy can achieve separately. Because combination therapies could be applied in many different ways, we consider only two specific 'simplified' examples as follows.

Providing combined 'slow' and 'boost' therapies at a young age, say 25, will only slightly extend ovarian function beyond that of only providing 'slow' therapy at age 25. This is because the impact of adding additional PFs at this early time has minimal impact unless many thousands of PFs are added. However, adding a slowing agent along with a boost later in life (e.g. at age 45) would certainly delay menopause longer than boost therapy alone, and perhaps very significantly so depending upon the slowing rate as well as the number of new PFs delivered. The interactive tools in their current, separate, forms allow the extrapolation of the potential effects of combined therapies along these lines.

Discussion

Different clinical measurements can provide some information about patients threatened with early ovarian demise and thus loss of fertility and the symptoms of the menopausal transition and of menopause. As summarized, AFC and AMH determination, as might occur during a reproductive endocrine evaluation, and also newer consumer-facing tools can all provide broad information about the size of the ovarian reserve. Thus, not only can patients and providers discuss strategies to slow ovarian aging from the standpoint of who may benefit from intervention, but they can also discuss who is unlikely to benefit from intervention. For example, a woman whose testing indicates a large remaining ovarian reserve consistent with menopause around or later than the median age of 51 might decide that extending ovarian function for health and well-being purposes is unnecessary. If the intervention is compatible with patient goals and is desired, as in a case of likely POI, however, existing mathematical tools can be seen to be useful as outcomes of 'slow' and 'boost' strategies are compared and contrasted.

Application of predictive modeling to different strategies designed to treat accelerated ovarian aging at the level of the PF reserve provides insights about which strategies are likely to have the most impact, and how slowing the rate of PF loss and/or boosting the number of PFs acutely should perform over time. As mentioned, we assume in the first case that PF loss can be slowed in such a way that does not disrupt ovarian physiology after PFGA occurs, and in the second case that newly delivered PFs will behave like the pre-existing PFs present from the time of birth (or can be corrected so that their growth and survival rates are 'normalized'). Under these conditions, general expected outcomes for the two strategies can be predicted. As can be seen in the provided summary figures and by use of the interactive online tools, the earlier that therapies that slow PF loss are applied, the greater their extension of ovarian function. Conversely, the later that the ovarian reserve is 'boosted' by additional PFs, the greater the extension of ovarian function. These initial performance estimates of such techniques are useful in terms of establishing quantitative boundaries for slowing interventions (e.g. to be efficacious, loss of the primordial reserve must be slowed by *this* amount for a patient with a particular profile with treatment started by *this* age) and for boosting interventions (to be efficacious, *this* many follicles need to be added at *this time* relative to the menopausal transition and ANM).

Advantages

The first advantage of our approach is that the model can be easily tuned to explore the impact of different rates of PF loss over different time windows or the consequences of new PFs behaving differently than those that were pre-existing. Another advantage of this approach is that it is consistent with prior work. While our underlying random walk model accurately recapitulates ovarian aging landmarks (Johnson *et al.*, 2022), the simulation of outcomes of slow and boost strategies is consistent with output from different PF decay models (Supplemental Fig. S2). We show that our specific modeling method can also be modified to simulate the impact of experimental strategies that promise to offset follicle loss; this includes modifiable variables that reflect real-world intervention timing and follicle behavior.

That the model can reveal what many would consider non-intuitive outcomes is an additional advantage. It is intuitive that the earlier an intervention that slows the rate of PF loss is applied, the greater the delay in the loss of ovarian function. However, the quantitative influence of the size of a woman's ovarian reserve upon therapy-induced extension of function may be more difficult to predict, and visualization over time helps solve this challenge (Figs 2 and 3). Separately, it is intuitive that acutely adding additional PFs to a woman's ovarian reserve will extend the duration of ovarian function, but the quantitative influence of the number and timing of new PFs delivered is easier to appreciate given summary model output (Fig. 3). As mentioned, we can say generally that for slowing PF loss, 'the earlier the intervention, the better', and for boosting PF numbers, 'the later the intervention, the better'. Considered specifically, this approach can help inform which patients would benefit from which approach(es), so that individualized treatment plans can be developed; it can further inform how patients are monitored to test whether treatments are working (see discussion of intervention validation below).

Validating the efficacy of interventions

The main limitation of our modeling approach is the need to validate how well predictive output matches treatment outcomes for different interventions. Validation would ideally be performed prospectively. We envision that validation will include monitoring of ovarian biomarkers such as AMH, menstrual cycle length, and the detection of cycle variability and symptoms that indicate

menopausal transition onset, as well as the timing of the final menstrual period. These parameters can be compared in a blinded fashion between women treated with 'slow' or 'boost' methods versus no-intervention controls. No matter the actual intervention tested, ovarian aging occurs over years to decades, and tracking menopausal transition and menopause onset timing across different groups will take long periods of time. We note also that careful direct validation could include direct evaluation of the behavior of preantral ovarian follicles. Live imaging techniques that could accomplish this are beyond our capabilities currently, but technological progress should eventually lead to the ability to monitor ovaries in this way. Last, the model as presented currently only accounts for single interventions where follicles behave in a stable manner after intervention. Thus the impact of acute exposures is not accounted for when follicle loss trajectories are generated. The interactive tool could be easily modified so that follicle loss can be simulated in response to short-term exposures if needed. We appreciate that progress in this area may occur very rapidly, and that we may be underestimating how soon evidence-based interventions can lead to significantly extended ovarian function.

Final considerations

Our modeling and simulation approaches have been applied to general features of follicle loss during ovarian aging (Johnson *et al.*, 2022; Lawley *et al.*, 2024), and its application to therapeutic strategies previously (Johnson *et al.*, 2024) and here may also prove to be useful to the field. As mentioned in the Introduction section, there are patients who experience accelerated PF loss due to a particular disease state. In the near future, the modeling approach can be modified in order to account for specific amounts of PF loss acceleration in order to establish boundaries for how an intervention might normalize ovarian aging in a personalized way. The impact of accelerated or slowed PFGA can be appreciated in terms of the behavior of new 'boosted' PFs in the interactive tool, and this also can be used to help validate methods that produce new, autologous patient ovarian tissue and follicles in vitro. For now, mathematical modeling appears to be a useful tool for exploration of how different strategies to extend the duration of ovarian function are likely to perform. Increasing attention to the problems faced by the many women who will experience early ovarian demise can lead to high impact, potentially geroprotective interventions that appear poised to extend their health span.

Supplementary data

Supplementary data are available at *Human Reproduction Update* online.

Data availability

All mathematical details of this work are included either in the manuscript itself or in the supplementary data files. The code used to produce the interactive tool (https://seanlawley.shinyapps.io/slowboost/) is available in a publicly accessible repository: https://doi.org/10.6084/m9.figshare.28279349. Research data underlying this article are available in a separate publicly accessible repository: https://doi.org/10.6084/m9.figshare.24454732.v1.

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Authors' roles

J.J.: conceptualization of study, manuscript drafting, and preparation of final manuscript. J.W.E.: manuscript drafting and preparation of final manuscript. A.S.: general literature review and manuscript drafting. K.M.: general literature review and manuscript drafting. E.E.T.: summary of translational fertility preservation concepts and manuscript drafting. R.A.A.: summary of clinical concepts and manuscript drafting. S.D.L.: study concept, manuscript drafting, and preparation of final manuscript.

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Conflict of interest

There are no conflicts of interest to disclose.

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