nature aging

Perspective

https://doi.org/10.1038/s43587-023-00509-8

# Female aging: when translational models don't translate

Received: 9 May 2023

Accepted: 25 September 2023

Published online: 5 December 2023

Check for updates

Gabrielle Gilmer[1,2,3,4,14], Zachary R. Hettinger[1,2,5,6,14], Yetsa Tuakli-Wosornu[7,8], Elizabeth Skidmore[9], Julie K. Silver[2,5,10,11], Rebecca C. Thurston[12], Dawn A. Lowe[13] & Fabrisia Ambrosio[1,2,5]

For many pathologies associated with aging, female patients present with higher morbidity and more frequent adverse events from treatments compared to male patients. While preclinical models are the foundation of our mechanistic understanding of age-related diseases, the most common models fail to recapitulate archetypical female aging trajectories. For example, while over 70% of the top age-related diseases are influenced by the systemic effects of reproductive senescence, we found that preclinical studies that include menopausal phenotypes modeling those seen in humans make up <1% of published aging biology research. The long-term impacts of pregnancy, birthing and breastfeeding are also typically omitted from preclinical work. In this Perspective, we summarize limitations in the most commonly used aging models, and we provide recommendations for better incorporating menopause, pregnancy and other considerations of sex in vivo and in vitro. Lastly, we outline action items for aging biology researchers, journals, funding agencies and animal providers to address this gap.

In the 1949 book *The Second Sex*, French existentialist philosopher Simone de Beauvoir contemplated the origins of perceived female inferiority, outlined inequities that exist between men and women, and offered a profound argument for the dismantlement of this partisanship[1]. Over 70 years later, inequities related to sex and gender persist on multiple scales and across diverse domains. Here, we consider the basic biology of aging. Specifically, we comment on limitations in the most commonly utilized preclinical models in aging biology research and the resulting obstacles encountered in our mechanistic investigation of female aging.

Our understanding of the basic biology of aging has flourished in recent decades, owing in large part to the use of model organisms including *Drosophila melanogaster* (fruit flies), *Caenorhabditis elegans* (roundworms) and Rodentia (rodents)[2]. Such model systems have allowed for stratification of the diverse impacts of aging on organismal health into a smaller set of underlying features (that is, the hallmarks of aging)[3]. Information gained from preclinical studies has demonstrated that the effects of time's arrow can be manipulated by interventions such as caloric restriction[4], exposure to youthful circulatory factors[5] and removal of senescent cells[6]. The animal models used have provided

[1]Discovery Center for Musculoskeletal Recovery, Schoen Adams Research Institute at Spaulding Rehabilitation, Boston, MA, USA. [2]Department of Physical Medicine and Rehabilitation, Spaulding Rehabilitation Hospital, Charlestown, MA, USA. [3]Medical Scientist Training Program, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA. [4]Cellular and Molecular Pathology Graduate Program, University of Pittsburgh, Pittsburgh, PA, USA. [5]Department of Physical Medicine & Rehabilitation, Harvard Medical School, Boston, MA, USA. [6]Department of Geriatric Medicine, University of Pittsburgh, Pittsburgh, PA, USA. [7]Department of Social and Behavioral Sciences, Yale School of Public Health, Yale University, New Haven, CT, USA. [8]Department of Physical Medicine and Rehabilitation, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. [9]Department of Occupational Therapy, School of Health and Rehabilitation Sciences, University of Pittsburgh, Pittsburgh, PA, USA. [10]Department of Physical Medicine and Rehabilitation, Massachusetts General Hospital, Boston, MA, USA. [11]Department of Physical Medicine and Rehabilitation, Brigham and Women's Hospital, Boston, MA, USA. [12]Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. [13]Divisions of Rehabilitation Science and Physical Therapy, Department of Rehabilitation Medicine, University of Minnesota, Minneapolis, MN, USA. [14]These authors contributed equally: Gabrielle Gilmer, Zachary R. Hettinger. e-mail: fambrosio@mgh.harvard.edu

and continue to provide unique opportunities to monitor, measure and manipulate aging phenotypes over a condensed lifespan, offering novel insights into human physiology and pathophysiology.

A central tenet of animal models is that the systems used share essential physiological characteristics with humans. Unfortunately, this all too often is not the case when it comes to female aging. For example, female patients present with osteoporosis four times more frequently than male patients, resulting in a higher incidence of hip fractures[7]. Yet, aging female rodents do not naturally present with a decline in bone mass[8]. Female individuals also present with sarcopenia earlier in their lifespan than male indviduals[9], whereas sex differences in rodents are minimal[10]. The incidence of Alzheimer's disease (AD) and non-AD dementia is higher in female individuals when compared to age-matched male humans[11,12]. Yet, recapitulating this difference has proved challenging in animal models, and several studies have demonstrated that female rodents better retain memory-related functions over time in comparison to age-matched male rodents[13,14]. Female individuals are twice as likely to present with knee and hand osteoarthritis in the clinic[15], but in our recent study, we found that male mice presented with more severe cartilage degeneration than female mice[16]. Although these are just some reported examples of the discrepancies between clinical and preclinical observations, many more disconnects likely exist but have not yet been identified due to the predominant use of male models in aging biology studies[17].

This gap in our understanding of how aging affects the onset and progression of diseases in female patients has probably contributed to worsened health outcomes. Meta-analyses on disability status over the past 20 years from France, Spain and the USA have consistently demonstrated that people who are female live with higher morbidity than age-matched male counterparts[18–20]. Female patients are 50% more likely to have a heart attack misdiagnosis[21] and 33% more likely to have a stroke misdiagnosed than male patients[22]. Between 1997 and 2000, 80% of drugs removed from the market were due to adverse events caused in female consumers[23], with most of these drugs intended to treat age-related diseases. Even as of 2020, female patients still reported more side effects due to prescription drugs for age-related diseases when compared to male patients[24,25]. Although psychological, social and economic factors clearly contribute to these disparities, our lagging understanding of aging female physiology represents a major barrier in our ability to prevent, diagnose and treat diseases in older female individuals.

In an attempt to better identify, understand and ultimately resolve these preclinical and clinical discrepancies, the purpose of this Perspective is to (1) outline current issues confounding the study of sex as a biological variable (SABV) within aging biology research, (2) identify advantages and disadvantages of current models used to study female aging and (3) define action items to increase the translatability of preclinical aging studies for older female people.

At the outset, it is important to note the distinction between sex and gender and to define a priori the terms used throughout this Perspective. Sex is "a multi-dimensional biological construct based on anatomy, physiology, genetics, and hormones", while gender is "a multi-dimensional construct that encompasses gender identity and expression, as well as social and cultural expectations about status, characteristics, and behavior as they are associated with certain sex traits"[26]. Although gender is clearly an important contributor to the disparities observed within our population, in this Perspective we focus on differences as they pertain to sex. For simplicity and consistent language, throughout this Perspective, we use the term 'female' to refer to people or animals sexed as female at birth, as typically defined according to appearance of the genitalia.

## Shortcomings in the study of female aging at the bench

Female aging in humans is inextricably intertwined with reproductive senescence and menopause, leading to systemic endocrine alterations that affect tissues and organs throughout the body[27]. Other features commonly associated with female aging include the impacts of pregnancy and breastfeeding on health later in life[28,29]. The ubiquity of these features highlights their critical importance in understanding female aging trajectories.

### Female aging after menopause

With current lifespan estimates, female individuals spend on average over one-third of their lives after menopause, with an increased disease incidence corresponding with this phase[30]. Clinically, menopause onset is defined as the time when menstrual cycles have ceased for at least 12 months with a corresponding loss of ovarian follicular function (that is, ovarian senescence)[31]. Equally important are the substantial changes in circulating sex hormone profiles[32].

While the field of reproductive aging has long recognized the impacts of menopause-related disruption to the hypothalamic–pituitary–gonadal axis[30], the role of reproductive senescence in the etiology of many other age-related diseases has been largely overlooked in preclinical studies. Probably representing a major contributor to this gap, the most used models to study aging do not exhibit or maintain human menopausal phenotypes. Neither fruit flies nor roundworms demonstrate menstrual cycling (shedding of the uterine lining resulting from changes in sex hormone circulation) or estrous cycling (cyclical changes in sex hormones that do not result in uterine lining shedding). The most commonly used rodent models have an estrous cycle, but not a menstrual cycle, and the estrous cycle shows sex hormone fluctuations similar to those observed in female humans[33,34]. At 9–12 months of age (the equivalent of ~30–38 years in humans[35]), the estrous cycle of rodents becomes irregular and the estrus period prolonged, a phase referred to as 'estropause' or, in mice, 'mouseopause'[36]. However, a majority of aged rodents spontaneously rejuvenate their ovarian follicles in middle age and reestablish the circulation of sex hormones comparable to that of a premenopause state, despite being unable to reproduce[27,36–39]. The remaining rodents that fail to rejuvenate their ovarian follicles transition to an anestrous state of low ovarian sex hormone levels, akin to a perimenopause state in humans (that is, the transition from regular menstrual cyclicity to irregularity that eventually leads to cessation of menstrual cycles)[27,36,37]. Therefore, a majority of the most widely used aging female rodents reflects a premenopause state and a minority reflects a perimenopause state[27,37,40,41]. Moreover, while the only known organisms to definitively exhibit features of human menopause are toothed whales, some chimpanzees[42], spiny mice[43], and humans, there is evidence of late life reproductive deficiencies across many mammalian species[44]. However, the extent to which these alterations recapitulate the systemic effects seen in post-menopausal humans, such as alterations in sex hormone profiles, is unclear[45].

To better understand the potential impact of the lack of menopausal phenotypes among commonly used model organisms, we performed a literature search to determine the number of clinical trials, mammalian studies and mammalian studies that considered menopause in aging research (see Supplementary Note for detailed search information). We focused on the top 22 most prevalent diseases in older adults (aged 50 years or older), as outlined in a recent systematic analysis of the Global Burden of Disease study[46]. We then classified these top diseases into five categories: cardiovascular, metabolic, orthopedic, cancer and cognitive/neurological (Fig. 1a and Supplementary Table 1). Among adults aged 50–74 years old, male individuals displayed a higher incidence of cardiovascular diseases, such as stroke and heart disease, while female individuals displayed a higher incidence of musculoskeletal disorders, such as low back pain and osteoarthritis. Sensory deficits such as loss of hearing and vision were also more prevalent in female individuals at this age range. In adults 75 years and older, female individuals continued to display an increased incidence of musculoskeletal disorders and sensory deficits, in addition to an increased incidence of stroke,

https://doi.org/10.1038/s43587-023-00509-8

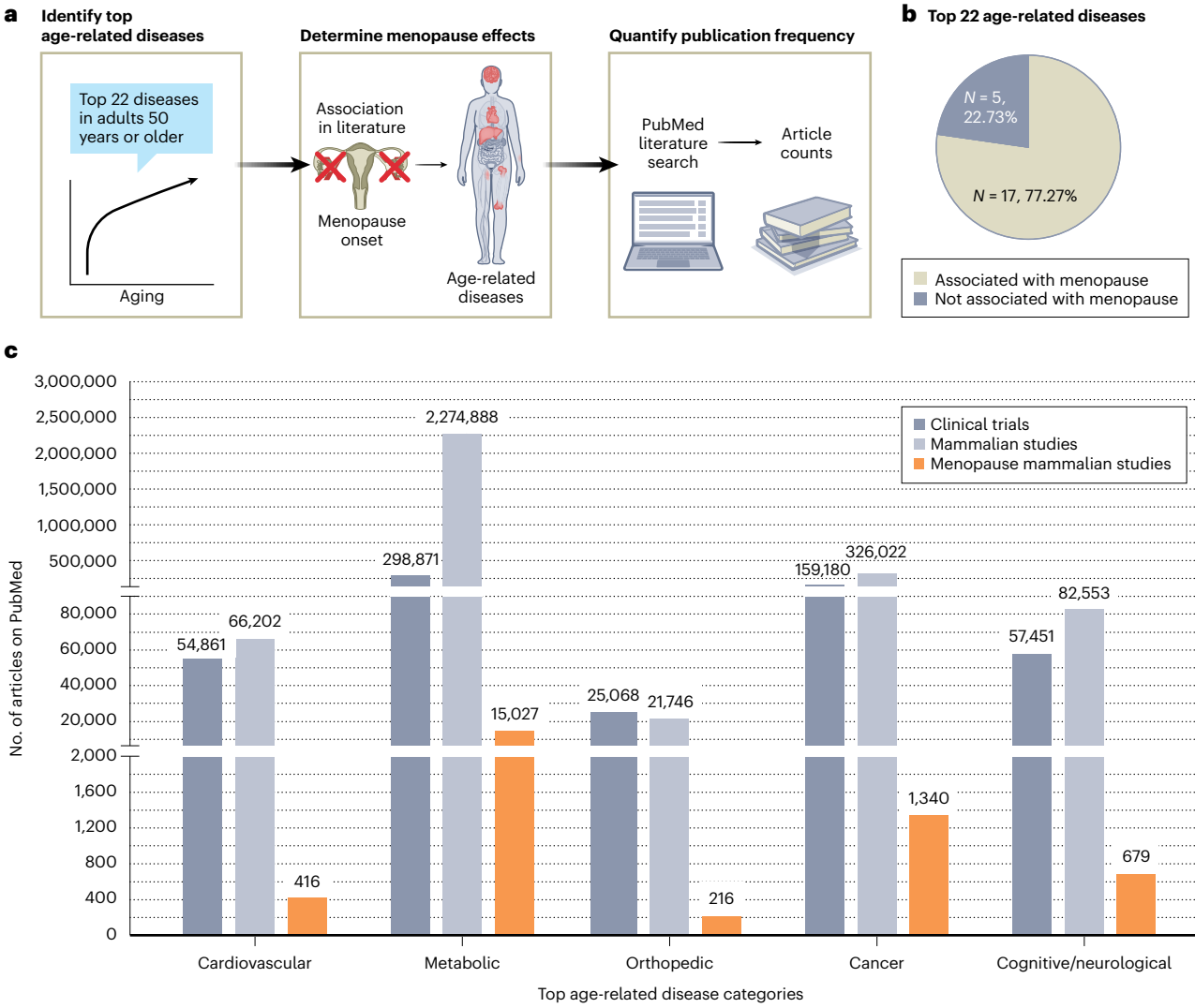


**Fig. 1 | Menopause is associated with the majority of age-related diseases, but few preclinical studies incorporated a menopausal phenotype. a**, The top 22 age-related diseases[46] were grouped into five disease categories. On 4 March 2023, we performed a PubMed literature search for known menopause-related effects in humans when considering these top 22 age-related diseases (Box 1 and Supplementary Table 1). On 4 July 2023, another PubMed literature search was performed to determine the number of clinical trials (**a**), mammalian animal studies (**b**) and menopause-inclusive mammalian animal studies that included a menopausal phenotype (**c**) on each disease category (Box 1). A summary of disease categories and specific search terms is available in Supplementary Table 1. **b**, The percentage of the top age-related diseases that are known to be associated with onset of menopause. References used to determine the association between each disease and menopause are listed in Supplementary Table 1. **c**, The number of articles on PubMed for clinical trials, mammalian studies and menopause-inclusive mammalian studies that included a menopausal phenotype.

heart disease and diabetes when compared to male counterparts (Supplementary Table 2).

When we considered articles that supported or refuted an association between menopause and age-related pathologies, we found over 70% of the most prevalent age-related diseases are associated with and/or potentially impacted by menopause[47] (Fig. 1b and Supplementary Table 1). Despite this, few studies have factored menopause into basic biology of aging studies. As of 4 July 2023, we identified 595,251 published clinical trials and 2,771,411 published preclinical mammalian studies relating to these five disease categories (Fig. 1c). Less than 1% (17,678) of the preclinical studies relating to these diseases considered menopause (Fig. 1c).

### Pregnancy, birthing and breastfeeding

Other critical features typically missing from the most utilized mammalian models of female aging relate to pregnancy, birthing and breastfeeding. In the USA, 86% of female individuals give birth at some point in their lifetime[48], and epidemiological studies have revealed that childbirth can affect both immediate health as well as long-term health of the childbearer[28]. People with a history of complications during pregnancy have an increased risk of cardiovascular and metabolic diseases later in life[28]. Breast cancer risk is lower in people who were younger at the time of their first full-term pregnancy[49], had a higher number of childbirths[29], had a history of preeclampsia[50] or who breastfed for longer after giving birth[51]. Breastfeeding is also associated with decreased risk of ovarian cancer[52], type II diabetes[53] and high blood pressure later in life[54]. Not considering these prevalent biological factors in aging biology research can impede the ability of preclinical studies to most effectively elucidate phenotypes and outcomes in older female individuals.

## Opportunities for more representative models of female aging

In 2013, a now widely referenced work reported that mice fail to mimic the responses of inflammatory diseases seen in humans, thereby

## BOX 1

# Closing the gap in our understanding of female aging

This Box provides specific and actionable recommendations to researchers, peer-reviewed journals, providers of animals for aging research and funding agencies to close the gap in our understanding of female aging

**Researchers**

- Increase the use of rigorous menopause models in middle-aged animals for aging and age-related drug testing studies, such as in the NIH-sponsored intervention testing program.
- In addition to reporting chronological age, report the state of ovarian senescence in rodent preclinical studies via levels of circulating sex hormones or other surrogate measures.
- Expand the science that evaluates the effect of pregnancy, birthing and breastfeeding in preclinical aging studies.
- Consider both the age and sex of the cell donor as well as sex-specific circulatory factors used in cell culture experiments (for example, using charcoal-stripped FBS and phenol-red-free media).
- Increase the number of aging studies that disentangle chromosomal versus hormonal sex differences by using the four core genotype model and analogous cell culture design.
- Further validate and characterize the spiny mouse as a potential natural menopause model in rodents.
- Advocate and encourage incorporation of the above listed recommendations among peers, funding agencies and journals.

**Peer-reviewed journals**

- Increase attention to reporting of sex in publications of animal research and the inclusion of sex-based statistical analyses, unless otherwise justified.
- Appropriately distinguish between 'sex' and 'gender' in the description of participants and animals.
- Encourage acknowledgment of limitations in models of female aging.
- Commission special issues that focus on the systemic effects of female aging.

**Providers of animals for aging research**

- Make a cohort of animals with simulated menopause available to the scientific community.
- Make female breeders readily available for studies into the effects of reproduction on aging trajectories.
- Track and publicize animal requests and availability by age, sex, strain, sexual status (for example, nulliparous) and whether menopause was induced. This can serve as a form of accountability for the field and toward identification of female aging research barriers.

**Funding agencies**

- Monitor adherence and implementation of SABV (for example, in progress reports), with an emphasis on the interactions between sex and age.
- Release funding announcements for studying sex-based differences and diseases that disproportionately affect older female individuals.
- Support research efforts specifically intended to distinguish chromosomal versus hormonal sex differences.
- Consider the use of relevant and translatable female aging models as illustrative of innovation and scientific rigor in grant proposals.

highlighting the translational inadequacy of the models most commonly used[55]. This work led to increased adoption of 'dirty' (that is, pet store) or 'humanized' (that is, engineered with human genes or engrafted with human organs) mice, both of which better recapitulate the intricacies of immune responses in humans[56]. The aging biology community can similarly benefit from reevaluation of the animal models used to study female aging with the long-term goal of making preclinical studies more translatable.

### Ovariectomy model of menopause

Of available menopause models, the simplest is arguably ovariectomy (OVX; Table 1). OVX surgically excises ovaries from the animal, representing a quick and reproducible procedure that recapitulates the loss of sex hormones that is typical of human menopause. Given that OVX eliminates all ovarian sex hormones, it also provides a unique opportunity to study signaling cascades and phenotypes resulting from individual sex hormones. For example, OVX followed by estradiol versus progesterone treatment can help to disentangle the distinct role of each on downstream phenotypes[27]. However, OVX as a model of menopause has several limitations. First and most obviously, natural menopause retains ovaries intact, while OVX does not. As a result, OVX creates an abrupt cessation in ovarian function, which contrasts the progressive loss of ovarian function observed with natural menopause over time[27]. This gradual decline in function is of interest given that many menopause-related symptoms first present during this transition period (that is, perimenopause)[57]. To address this limitation, some researchers have excised the ovaries at the conclusion of estropause, thereby allowing for a pseudo-perimenopause phase followed by a postmenopausal phase[27]. Another limitation is that OVX also results in depletion of all ovarian-produced sex hormones, including those that do not change with menopause, such as testosterone[27].

From an implementation perspective, most studies incorporating OVX models to date have used young animals, even though menopause in humans manifests in the setting of aging cells and tissues[27]. Age is probably an important consideration, as the systemic effects of menopause in a young organism can vary greatly when compared to the effects in aged counterparts[58]. For example, young female patients who undergo oophorectomy have clinically distinct presentations compared to older female patients undergoing natural menopause[59,60]. While the reasons for this difference are unclear, the manifestation of age-related disease following natural menopause presumably occurs through a combination of cell-intrinsic (for example, genetic vulnerability of aged cells) and cell-extrinsic (for example, a change in the circulating sex hormone profile) factors, rather than a single cause alone. As such, OVX performed in young animals probably misses key mechanistic insights into diseases that manifest in older female humans.

### VCD model of menopause

In contrast to surgical ablation, the 4-vinylcyclohexene diepoxide (VCD) model of menopause allows for a slow and progressive loss of ovarian-based sex hormones while preserving the ovaries intact (Table 1). VCD is an ovarian-specific toxin that causes primordial and primary ovarian follicle apoptosis[37,61]. This chemically induced model of menopause requires daily intraperitoneal injections of VCD in an oil-based vehicle to deplete follicles, allowing for evaluation of both perimenopause and menopause phases[37].

**Table 1 | Strengths and limitations of current rodent models of menopause for aging biology research**

| Model | Strengths | Limitations |
|---|---|---|
| OVX | • Short time to onset of menopause<br>• Cost effective and highly reproducible<br>• Can be used to systematically determine the influence of single sex hormone replacement following OVX | • Does not retain ovaries intact, as is the case with natural menopause<br>• Abrupt menopause transition with no perimenopause phase<br>• Depletes all sex hormones, including those that are not affected by menopause |
| VCD | • Slow and progressive menopause onset<br>• Retains ovaries intact<br>• Recapitulates perimenopause and menopause stages | • Time and labor intensive<br>• Potential for investigator toxicity (requires additional safety precautions) |
| Spiny mouse | • Naturally occurring menopause<br>• Non-invasive and least harmful to the animal | • Absence of age- and sex-matched non-menopausal mice<br>• Display a unique regenerative capacity; how this affects the presentation of menopause-related phenotypes is unclear<br>• Least characterized of murine menopause models<br>• Long lifespan relative to other commonly used rodents, and a long time to menopause onset<br>• Not commercially available |

While the VCD model may better recapitulate the menopause transition and is less invasive than OVX, it is a more time-, labor- and cost-intensive model. When considering possible toxic effects to animals receiving VCD, no secondary toxicity to organs beyond the ovaries in young[62] or middle-aged female mice[63] has been reported. In rats, while the model was minimally toxic in prepubescent animals, 100% of sexually mature rats suffered from peritonitis following VCD injections[64]. To our knowledge, the VCD model has not yet been used or validated in middle-aged or aged rats. One alternative to mitigate the presentation of peritonitis is oral administration of VCD and triptolide (an herb from a woody vine natively found in China), which induces infertility in rodents[65]. While we have not found evidence of this dual treatment approach as a model of menopause, it has the potential to attenuate some of the toxic side effects of intraperitoneal VCD administration. Another limitation of this model is that, like OVX, VCD has typically been performed in young animals, thereby potentially masking systemic cell and tissue responses that are age dependent.

Despite limitations, the potential impact of the OVX and VCD approaches for understanding menopause mechanisms is considerable. To illustrate this point, we revisit two of the aforementioned examples of the disconnect between preclinical and clinical findings, osteoarthritis and osteoporosis. As noted above, the incidence of knee osteoarthritis is higher in age-matched female patients than in male patients over the age of 50 years[15], with female patients presenting with more severe cartilage degeneration at the time of joint replacement than male patients[66]. Yet, male mice have more severe age-related cartilage degeneration than non-menopausal female mice[16]. Menopause induction by either OVX or VCD more closely recapitulates the clinical presentation, as evidenced by an accelerated and more severe cartilage degeneration compared to non-menopausal mice[63,67]. Considering osteoporosis, female patients demonstrate a precipitous drop in bone mass[7], a phenotype that is not recapitulated by mice undergoing natural aging[8]. By contrast, the onset of menopause in mice following OVX or injection with VCD results in notable loss of bone mass[62]. These examples illustrate how use of a menopause model can increase the translational relevance of preclinical findings.

## Spiny mice as a naturally occurring menopause model

*Acomys cahirinus*, the African spiny mouse, was recently reported to be the first mouse species known to both menstruate and undergo a slow and gradual menopausal transition similar to humans[43,68]. Specifically, spiny mice demonstrate a menopausal phenotype at 36 months, as evidenced by a progressive decline in primordial ovarian follicles. Ovarian cyclicity became more irregular between years one and three (akin to a perimenopause state), dropping precipitously in the fourth and final year of life (menopause onset). Importantly, the authors observed a substantial drop in estradiol between years 1 and 2 with no changes in circulating testosterone, similar to natural menopause seen in humans. Although initial research interest in spiny mice focused on elucidating mechanisms underlying their remarkable regenerative capacity[69], this model could be another promising research tool for incorporating menopausal phenotypes into aging biology studies. Future studies are needed to both validate this model and evaluate whether the menopause phenotype in these mice tracks with the menopause-induced systemic changes that are observed in humans (Table 1).

## Determination of menopause status in aging rodents

It is challenging to know which rodents exhibit a premenopause, perimenopause, or menopause state due to the relatively low quantity of collectable serum from rodents, the relatively low levels of circulating hormones and the insensitivity of commercially available assays. Mass spectrometry (either gas or liquid chromatography) is the gold standard for quantifying sex hormones in humans, and recent studies have mapped the sex hormone profiles seen in mice and rats across the estrous cycle using this approach[70]. However, mass spectrometry is expensive and requires a relatively large sample (~500 µl of serum per sex hormone). With the goal of increasing the translation and feasibility of using natural aging models, the development of more reproducible, reliable and cost-efficient assays that quantify female sex hormones in rodents is much needed. Until then, as a proxy for circulating sex hormone levels, vaginal lavage and cytology can be used to track the estrous cycle and estropause status[36,71]. Postmortem, histological evaluation of ovarian follicle numbers can also be used to determine estropause status[72]. Uterine weights also correlate with estrogen levels, offering a rapid screening method to estimate the hormone status of naturally aging and/or menopause models[73].

## Pregnancy, birthing and breastfeeding

Despite numerous clinical studies showing that pregnancy, childbirth and breastfeeding impact longevity and healthspan[28,49], these variables remain largely absent in preclinical work that does not focus on reproductive aging. Female breeders (that is, rodents used to generate rodent colonies) are typically excluded from research studies. As such, unless specifically requested, female rodents ordered through animal vendors are nulliparous (that is, have never produced a litter) and have never breastfed. The use of breeders offers an opportunity for researchers to develop mechanistic understandings of how physiological processes related to birthing may influence age-related diseases. Indeed, recent preclinical studies have identified pregnancy-related changes in immunity, microbiota composition, neural microstructure and muscle regeneration[74–77]. Another study demonstrated that breastfeeding alters the circadian rhythms of maternal mice[78]. These changes may translate to meaningful alterations in aging trajectories and age-related diseases, although further studies are needed. Consideration of variables such as number of pregnancies, litter sizes and complications (for example, stillbirth) will provide valuable insight into how childbearing features affect female aging trajectories.

## Cell culture considerations

Cell culture systems have served as an indispensable reductionist approach to evaluate aging mechanisms, but consideration of sex in these systems has generally been limited. The importance of SABV

in vitro and in vivo has been highlighted by the National Institutes of Health (NIH)[79]. Yet, in the 4 years following implementation of SABV requirements, 50% of studies still did not report the sex of cells used, and of those that did, only 22% used female cells[80]. Cells retain a memory of their origins; this is true of cells isolated from older animals and humans as well as cells isolated from male and female organisms[81,82]. Therefore, reporting of cell source represents an important study design consideration.

In addition, cell culture conditions have the potential to contribute confounding effects and may preclude identification of sex-dependent changes associated with aging. Cell culture studies typically use fetal bovine serum (FBS) or serum from other animals, which may mask the effects of endogenous hormone alterations in the context of aging[83]. Phenol red, commonly used in media as a pH indicator, has estrogenic activity[84], thereby similarly contributing potential confounding effects, especially when attempting to isolate downstream cellular responses to a menopause-induced loss of sex hormones.

Several steps can be taken to overcome these limitations in standard cell culture conditions. Cells can be cultured in phenol red-free media and in the presence of serum isolated from age-matched male or female organisms to better maintain phenotypic sex differences in vitro. Moreover, for studies aimed at investigating the role of the menstrual/estrous cycle or menopause in age-related phenotypes, media can be designed to mimic the circulatory environment across these life stages. For example, before the onset of estropause, female mice have circulating estradiol levels of $2.7 \pm 1.0$ pg ml$^{-1}$ and circulating progesterone levels of $31{,}323 \pm 6{,}108$ pg ml$^{-1}$ (ref. 70). Conversely, with OVX, female mice have undetectable levels of estradiol (<0.3 pg ml$^{-1}$) and low levels of progesterone ($3{,}940 \pm 2{,}135$ pg ml$^{-1}$)[70]. Media composed of charcoal-stripped FBS (that is, FBS with native sex hormones removed[85,86]) and supplemented with sex hormones at levels that recapitulate in vivo aging microenvironments can be valuable for better understanding the impact of menopause on female cellular aging. Similar practices have already been utilized in the literature for modeling menstrual cycles[87,88], illustrating the feasibility of this approach.

### Disentangling chromosomal versus hormonal sex differences

An interesting and important question in our understanding of the impact of aging on female cellular and tissue declines is whether the observed changes have predominantly genetic or hormonal origins. The 'four core genotypes' model includes mice engineered to have XX chromosomes with male or female gonads or mice with XY chromosomes with male or female gonads[89]. These models offer unique opportunities to disaggregate chromosomal from hormonal sex differences in the development of age-related diseases[89]. For example, the four core genotypes model revealed that the presence of XX chromosomes increases lifespan, independent of gonad[90]. As noted by the authors, an important limitation is that while this study accounted for chromosomal differences, it did not account for menopause[90]. It would be interesting to repeat these studies in menopause-induced mice to evaluate whether chromosomal effects on longevity persist. In other applications of the four core genotypes model, the XX genotype was shown to increase resistance to AD-related pathology, regardless of the type of gonad[91]. The primary limitation of the four core genotypes model is that these mice are not commercially available and, thus, need to be bred in house and aged. Additionally, this model does not consider the impact of other baseline X escapee genes, epigenetic regulation of autosomal genes by sex chromosomes[92], reactivation of a silent X chromosome, or secondary phenotypic effects (for example, the influence of sex chromosome gene expression on the hypothalamic–pituitary–gonadal regulation of sex hormones)[93,94]. Despite these limitations, the four core genotypes model has the potential to provide valuable insights into sex differences associated with age-related pathologies.

Parallel approaches can be used in cell culture experiments to disentangle hormonal versus chromosomal sex differences. For example, XY or XX cells from wild-type animals or humans can be cultured in media that contain male or female circulating factors. Using these approaches in vitro allows for detailed interrogation into the molecular mechanisms dictating chromosomal versus hormonal sex differences in a manner easily accessible to researchers.

## Closing the gap

Closing the gap in our understanding of sex-based differences in biological aging will require the engagement of stake holders at all levels of aging biology research, including researchers, peer-reviewed journals, funding agencies and animal providers. In Box 1, we present a list of recommendations, some of which echo those made by leaders in women's health[95–98].

## Conclusion

To date, few aging biology studies outside the field of reproductive biology have used models that recapitulate key features of aging female physiology, such as menopause. The result is a healthcare system that lacks mechanistic data on how to treat age-related diseases in female patients. Herein, we mapped progress and highlighted opportunities for advancing female aging biology research. We outlined the need for preclinical aging studies to incorporate more representative models of female aging, including menopause, pregnancy and breastfeeding. We also presented considerations of sex-based circulatory factors in vitro as well as investigations that disentangle chromosomal versus hormonal sex differences in aging biology. Finally, we reiterated recommendations made by women's health leaders and added suggestions for biomedical researchers, journals, animal providers and funders. Features unique to female aging are relevant to all aging biologists, not just those studying reproductive senescence or specializing in sex hormone signaling. These topics represent valuable opportunities for the field to tackle fundamental aging biology questions to the benefit of women's health.

## References

1. Beauvoir, S. D. *The Second Sex* (A. A. Knopf, 1968).
2. Mitchell, S. J., Scheibye-Knudsen, M., Longo, D. L. & de Cabo, R. Animal models of aging research: implications for human aging and age-related diseases. *Annu. Rev. Anim. Biosci.* **3**, 283–303 (2015).
3. Lopez-Otin, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. Hallmarks of aging: an expanding universe. *Cell* **186**, 243–278 (2023).
4. Green, C.L., Lamming, D.W. & Fontana, L. Molecular mechanisms of dietary restriction promoting health and longevity. *Nat. Rev. Mol. Cell Biol.* **23**, 56–73 (2022).
5. Conboy, M. J., Conboy, I. M. & Rando, T. A. Heterochronic parabiosis: historical perspective and methodological considerations for studies of aging and longevity. *Aging Cell* **12**, 525–530 (2013).
6. Chaib, S., Tchkonia, T. & Kirkland, J. L. Cellular senescence and senolytics: the path to the clinic. *Nat. Med.* **28**, 1556–1568 (2022).
7. Alpantaki, K. et al. Gender and age differences in hip fracture types among elderly: a retrospective cohort study. *Maedica* **15**, 185–190 (2020).
8. Sophocleous, A. & Idris, A. I. Rodent models of osteoporosis. *Bonekey Rep.* **3**, 614 (2014).
9. Kurose, S. et al. Prevalence and risk factors of sarcopenia in community-dwelling older adults visiting regional medical institutions from the Kadoma Sarcopenia Study. *Sci. Rep.* **10**, 19129 (2020).
10. Clemens, Z. et al. The biphasic and age-dependent impact of klotho on hallmarks of aging and skeletal muscle function. *Elife* https://doi.org/10.7554/eLife.61138 (2021).

11. Fisher, D. W., Bennett, D. A. & Dong, H. Sexual dimorphism in predisposition to Alzheimer's disease. *Neurobiol. Aging* **70**, 308–324 (2018).
12. Beam, C. R. et al. Differences between women and men in incidence rates of dementia and Alzheimer's disease. *J. Alzheimers Dis.* **64**, 1077–1083 (2018).
13. Bao, J. et al. Sex differences in the cognitive and hippocampal effects of streptozotocin in an animal model of sporadic AD. *Front. Aging Neurosci.* **9**, 347 (2017).
14. Mifflin, M. A. et al. Sex differences in the IntelliCage and the Morris water maze in the APP/PS1 mouse model of amyloidosis. *Neurobiol. Aging* **101**, 130–140 (2021).
15. Tschon, M., Contartese, D., Pagani, S., Borsari, V. & Fini, M. Gender and sex are key determinants in osteoarthritis not only confounding variables. a systematic review of clinical data. *J. Clin. Med.* https://doi.org/10.3390/jcm10143178 (2021).
16. Iijima, H. et al. Age-related matrix stiffening epigenetically regulates alpha-Klotho expression and compromises chondrocyte integrity. *Nat. Commun.* **14**, 18 (2023).
17. Carmody, C., Duesing, C. G., Kane, A. E. & Mitchell, S. J. Is sex as a biological variable still being ignored in preclinical aging research? *J. Gerontol. A Biol. Sci. Med. Sci.* **77**, 2177–2180 (2022).
18. Boerma, T., Hosseinpoor, A. R., Verdes, E. & Chatterji, S. A global assessment of the gender gap in self-reported health with survey data from 59 countries. *BMC Public Health* **16**, 675 (2016).
19. Crimmins, E. M., Kim, J. K. & Sole-Auro, A. Gender differences in health: results from SHARE, ELSA and HRS. *Eur. J. Public Health* **21**, 81–91 (2011).
20. Singh-Manoux, A. et al. Gender differences in the association between morbidity and mortality among middle-aged men and women. *Am. J. Public Health* **98**, 2251–2257 (2008).
21. Wong, C. W. et al. Misdiagnosis of heart failure: a systematic review of the literature. *J. Card. Fail.* **27**, 925–933 (2021).
22. Newman-Toker, D. E., Moy, E., Valente, E., Coffey, R. & Hines, A. L. Missed diagnosis of stroke in the emergency department: a cross-sectional analysis of a large population-based sample. *Diagnosis* **1**, 155–166 (2014).
23. Tom Harkin, O. J. S., Barbara A. M., Henry A. Waxman. *Drug safety: most drugs withdrawn in recent years had greater health risks for women* https://www.gao.gov/new.items/d01286r.pdf (2001; accessed 23 February 2023).
24. Zucker, I. & Prendergast, B. J. Sex differences in pharmacokinetics predict adverse drug reactions in women. *Biol. Sex. Differ.* **11**, 32 (2020).
25. Franconi, F., Brunelleschi, S., Steardo, L. & Cuomo, V. Gender differences in drug responses. *Pharmacol. Res.* **55**, 81–95 (2007).
26. Bates, N. et al. *Measuring Sex, Gender Identity, and Sexual Orientation* (The National Academies Press, 2022).
27. Diaz Brinton, R. Minireview: translational animal models of human menopause: challenges and emerging opportunities. *Endocrinology* **153**, 3571–3578 (2012).
28. Neiger, R. Long-term effects of pregnancy complications on maternal health: a review. *J. Clin. Med.* https://doi.org/10.3390/jcm6080076 (2017).
29. Lambe, M. et al. Parity, age at first and last birth, and risk of breast cancer: a population-based study in Sweden. *Breast Cancer Res. Treat.* **38**, 305–311 (1996).
30. Lobo, R. A. & Gompel, A. Management of menopause: a view towards prevention. *Lancet Diabetes Endocrinol.* **10**, 457–470 (2022).
31. Harlow, S. D. et al. Executive summary of the Stages of Reproductive Aging Workshop + 10: addressing the unfinished agenda of staging reproductive aging. *Menopause* **19**, 387–395 (2012).
32. Davis, S. R., Pinkerton, J., Santoro, N. & Simoncini, T. Menopause—biology, consequences, supportive care, and therapeutic options. *Cell* **19**, 4038–4058 (2023).
33. Sato, J., Nasu, M. & Tsuchitani, M. Comparative histopathology of the estrous or menstrual cycle in laboratory animals. *J. Toxicol. Pathol.* **29**, 155–162 (2016).
34. Ajayi, A. F. & Akhigbe, R. E. Staging of the estrous cycle and induction of estrus in experimental rodents: an update. *Fertil. Res. Pract.* **6**, 5 (2020).
35. Dutta, S. & Sengupta, P. Men and mice: relating their ages. *Life Sci.* **152**, 244–248 (2016).
36. Koebele, S. V. & Bimonte-Nelson, H. A. Modeling menopause: the utility of rodents in translational behavioral endocrinology research. *Maturitas* **87**, 5–17 (2016).
37. Brooks, H. L., Pollow, D. P. & Hoyer, P. B. The VCD mouse model of menopause and perimenopause for the study of sex differences in cardiovascular disease and the metabolic syndrome. *Physiology* **31**, 250–257 (2016).
38. Felicio, L. S., Nelson, J. F. & Finch, C. E. Longitudinal studies of estrous cyclicity in aging C57BL/6J mice: II. Cessation of cyclicity and the duration of persistent vaginal cornification. *Biol Reprod.* **31**, 446–53 (1984).
39. Nelson, J. F., Felicio, L.S., Randall, P. K., Sims, C., & Finch, C. E. A longitudinal study of estrous cyclicity in aging C57BL/6J mice: II. Cessation of cyclicity and the duration of persistent vaginal cornification. *Biol Reprod.* **27**, 327–39 (1982).
40. Walker, M. L. & Herndon, J. G. Menopause in nonhuman primates? *Biol. Reprod.* **79**, 398–406 (2008).
41. Herndon, J. G. et al. Menopause occurs late in life in the captive chimpanzee (*Pan troglodytes*). *Age* **34**, 1145–1156 (2012).
42. Wood, B. M. et al. Demographic and hormonal evidence for menopause in wild chimpanzees. *Science* **382**, 368–369 (2023).
43. Bellofiore, N., George, E., Vollenhoven, B. & Temple-Smith, P. Reproductive aging and menopause-like transition in the menstruating spiny mouse (Acomys cahirinus). *Hum. Reprod.* **36**, 3083–3094 (2021).
44. Winkler, I. & Goncalves, A. Do mammals have menopause? *Cell* **186**, 4729–4733 (2023).
45. Johnstone, R. A. & Cant, M. A. The evolution of menopause in cetaceans and humans: the role of demography. *Proc. Biol. Sci.* **277**, 3765–3771 (2010).
46. Diseases, G. B. D. & Injuries, C. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease study 2019. *Lancet* **396**, 1204–1222 (2020).
47. The Hormone Therapy Position Statement of The North American Menopause Society' Advisory, Panel. The 2022 hormone therapy position statement of The North American Menopause Society. *Menopause* **29**, 767–794, (2022).
48. Livingston, G. They're waiting longer, but US women today more likely to have children than a decade ago. *Pew Research Center* http://www.pewsocialtrends.org/2018/01/18/theyre-waiting-longer-but-u-s-women-today-more-likely-to-have-children-than-a-decade-ago/ (2018; accessed 19 April 2023).
49. Lord, S. J. et al. Breast cancer risk and hormone receptor status in older women by parity, age of first birth, and breastfeeding: a case–control study. *Cancer Epidemiol. Biomark. Prev.* **17**, 1723–1730 (2008).
50. Nechuta, S., Paneth, N. & Velie, E. M. Pregnancy characteristics and maternal breast cancer risk: a review of the epidemiologic literature. *Cancer Causes Control* **21**, 967–989 (2010).

51. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. *Lancet* **360**, 187–195 (2002).
52. Babic, A. et al. Association between breastfeeding and ovarian cancer risk. *JAMA Oncol.* **6**, e200421 (2020).
53. Morris, A. Risk factors: breastfeeding reduces risk of type 2 diabetes mellitus. *Nat. Rev. Endocrinol.* **14**, 128 (2018).
54. Park, S. & Choi, N.-K. Breastfeeding and maternal hypertension, *Am. J. Hypertension* **31**, 615–621 (2018).
55. Seok, J. et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc. Natl Acad. Sci. USA* **110**, 3507–3512 (2013).
56. Masopust, D., Sivula, C. P. & Jameson, S. C. Of mice, dirty mice, and men: using mice to understand human immunology. *J. Immunol.* **199**, 383–388 (2017).
57. Santoro, N. Perimenopause: from research to practice. *J. Womens Health* **25**, 332–339 (2016).
58. Lelovas, P. & Dontas, I. Concerns on modeling postmenopausal osteoporosis on young female rats. *J. Orthop. Surg. Res* **14**, 450 (2019).
59. The NAMS 2017 Hormone Therapy Position Statement Advisory Panel. The 2017 hormone therapy position statement of The North American Menopause Society. *Menopause* **24**, 728–753, (2017).
60. Hendrix, S. L. Bilateral oophorectomy and premature menopause. *Am. J. Med.* **118**, 131–135 (2005).
61. Kappeler, C. J. & Hoyer, P. B. 4-vinylcyclohexene diepoxide: a model chemical for ovotoxicity. *Syst. Biol. Reprod. Med.* **58**, 57–62 (2012).
62. Wright, L. E. et al. Comparison of skeletal effects of ovariectomy versus chemically induced ovarian failure in mice. *J. Bone Miner. Res.* **23**, 1296–1303 (2008).
63. Gilmer, G. et al. A network medicine approach to elucidate mechanisms underlying menopause-induced knee osteoarthritis. Preprint at *bioRxiv* https://doi.org/10.1101/2023.03.02.530756 (2023).
64. Muhammad, F. S. et al. Effects of 4-vinylcyclohexene diepoxide on peripubertal and adult Sprague–Dawley rats: ovarian, clinical, and pathologic outcomes. *Comp. Med.* **59**, 46–59 (2009).
65. Witmer, G. W. et al. Compromised fertility in free feeding of wild-caught norway rats (*Rattus norvegicus*) with a liquid bait containing 4-vinylcyclohexene diepoxide and triptolide. *J. Zoo. Wildl. Med.* **48**, 80–90 (2017).
66. Patel, J., Chen, S., Katzmeyer, T., Pei, Y. A. & Pei, M. Sex-dependent variation in cartilage adaptation: from degeneration to regeneration. *Biol. Sex. Differ.* **14**, 17 (2023).
67. Gilmer, G. et al. Uncovering the 'riddle of femininity' in osteoarthritis: a systematic review and meta-analysis of menopausal animal models and mathematical modeling of estrogen treatment. *Osteoarthr. Cartil.* **31**, 447–457 (2023).
68. Bellofiore, N. et al. First evidence of a menstruating rodent: the spiny mouse (*Acomys cahirinus*). *Am. J. Obstet. Gynecol.* **216**, 40.e1–40.e11 (2017).
69. Seifert, A. W. et al. Skin shedding and tissue regeneration in African spiny mice (*Acomys*). *Nature* **489**, 561–565 (2012).
70. Nilsson, M. E. et al. Measurement of a comprehensive sex steroid profile in rodent serum by high-sensitive gas chromatography-tandem mass spectrometry. *Endocrinology* **156**, 2492–2502 (2015).
71. McLean, A. C., Valenzuela, N., Fai, S. & Bennett, S. A. Performing vaginal lavage, crystal violet staining, and vaginal cytological evaluation for mouse estrous cycle staging identification. *J. Vis. Exp.* https://doi.org/10.3791/4389 (2012).
72. Acosta, J. I. et al. Transitional versus surgical menopause in a rodent model: etiology of ovarian hormone loss impacts memory and the acetylcholine system. *Endocrinology* **150**, 4248–4259 (2009).
73. Evans, J. S., Varney, R. F. & Koch, F. C. The mouse uterine weight method for the assay of estrogens. *Endocrinology* **28**, 747–752 (1941).
74. Falick Michaeli, T. et al. The rejuvenating effect of pregnancy on muscle regeneration. *Aging Cell* **14**, 698–700 (2015).
75. Lesteberg, K. E., Fader, D. S. & Beckham, J. Pregnancy alters innate and adaptive immune responses to Zika virus infection in the reproductive tract. *J. Immunol.* **205**, 3107–3121 (2020).
76. Hoekzema, E., van Steenbergen, H. & Straathof, M. et al. Mapping the effects of pregnancy on resting state brain activity, white matter microstructure, neural metabolite concentrations and grey matter architecture. *Nat. Commun.* **13**, 6931 (2022).
77. Faas, M. M. et al. Microbiota induced changes in the immune response in pregnant mice. *Front. Immunol.* **10**, 2976 (2019).
78. Lippert, R. N. et al. Maternal high-fat diet during lactation reprograms the dopaminergic circuitry in mice. *J. Clin. Invest.* **130**, 3761–3776 (2020).
79. Arnegard, M. E., Whitten, L. A., Hunter, C. & Clayton, J. A. Sex as a biological variable: a 5-year progress report and call to action. *J. Women's Health* **29**, 858–864 (2020).
80. Kim, J. Y., Min, K., Paik, H. Y. & Lee, S. K. Sex omission and male bias are still widespread in cell experiments. *Am. J. Physiol. Cell Physiol.* **320**, C742–C749 (2021).
81. Royall, L. N. & Jessberger, S. How stem cells remember their past. *Curr. Opin. Cell Biol.* **69**, 17–22 (2021).
82. Shah, K., McCormack, C. E. & Bradbury, N. A. Do you know the sex of your cells? *Am. J. Physiol. Cell Physiol.* **306**, C3–C18 (2014).
83. Arodin Selenius, L., Wallenberg Lundgren, M., Jawad, R., Danielsson, O. & Bjornstedt, M. The cell culture medium affects growth, phenotype expression and the response to selenium cytotoxicity in A549 and HepG2 cells. *Antioxidants* https://doi.org/10.3390/antiox8050130 (2019).
84. Berthois, Y., Katzenellenbogen, J. A. & Katzenellenbogen, B. S. Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. *Proc. Natl Acad. Sci. USA* **83**, 2496–2500 (1986).
85. Milo, G. E., Malarkey, W. B., Powell, J. E., Blakeslee, J. R. & Yohn, D. S. Effects of steroid hormones in fetal bovine serum on plating and cloning of human cells in vitro. *In Vitro* **12**, 23–30 (1976).
86. Liang, Z. R., Qu, L. H. & Ma, L. M. Differential impacts of charcoal-stripped fetal bovine serum on c-Myc among distinct subtypes of breast cancer cell lines. *Biochem. Biophys. Res. Commun.* **526**, 267–272 (2020).
87. Reus, T. L., Brohem, C. A., Schuck, D. C. & Lorencini, M. Revisiting the effects of menopause on the skin: functional changes, clinical studies, in vitro models and therapeutic alternatives. *Mech. Ageing Dev.* **185**, 111193 (2020).
88. Remoue, N. et al. Development of an in vitro model of menopause using primary human dermal fibroblasts. *Int. J. Cosmet. Sci.* **35**, 546–554 (2013).
89. Arnold, A. P. & Chen, X. What does the 'four core genotypes' mouse model tell us about sex differences in the brain and other tissues? *Front. Neuroendocrinol.* **30**, 1–9 (2009).
90. Davis, E. J., Lobach, I. & Dubal, D. B. Female XX sex chromosomes increase survival and extend lifespan in aging mice. *Aging Cell* **18**, e12871 (2019).
91. Davis, E. J. et al. A second X chromosome contributes to resilience in a mouse model of Alzheimer's disease. *Sci. Transl. Med.* https://doi.org/10.1126/scitranslmed.aaz5677 (2020).
92. Wijchers, P. J. & Festenstein, R. J. Epigenetic regulation of autosomal gene expression by sex chromosomes. *Trends Genet.* **27**, 132–140 (2011).

93. Blencowe, M. et al. Relative contributions of sex hormones, sex chromosomes, and gonads to sex differences in tissue gene regulation. *Genome Res.* **32**, 807–824 (2022).
94. Majdic, G. & Tobet, S. Cooperation of sex chromosomal genes and endocrine influences for hypothalamic sexual differentiation. *Front. Neuroendocrinol.* **32**, 137–145 (2011).
95. Miller, L. R. et al. Considering sex as a biological variable in preclinical research. *FASEB J.* **31**, 29–34 (2017).
96. Mazure, C. M. & Jones, D. P. Twenty years and still counting: including women as participants and studying sex and gender in biomedical research. *BMC Women's Health* **15**, 94 (2015).
97. Sandberg, K., Umans, J. G. & Georgetown Consensus Conference Work Group. Recommendations concerning the new U.S. National Institutes of Health initiative to balance the sex of cells and animals in preclinical research. *FASEB J.* **29**, 1646–1652, (2015).
98. Heidari, S., Babor, T. F., De Castro, P., Tort, S. & Curno, M. Sex and Gender Equity in Research: rationale for the SAGER guidelines and recommended use. *Res. Integr. Peer Rev.* **1**, 2 (2016).

## Acknowledgements

The authors thank J. Bergmann for assistance in the literature search as well as the members of the laboratory of F.A. for reviewing and editing this Perspective. The authors also gratefully acknowledge the funding sources that supported this work including the National Institute on Aging (NIA; R01 AG061005, to F.A.), NIA R01 AG052978 (to F.A.), NIA R01 AG066198 (to F.A.), NIH T32AG021885-19 (to G.G.), NIH T32GM008208 (to G.G.) and NIH T32AG021885-19 (to Z.R.H.).

## Author contributions

All authors made substantial contributions in the following areas: (1) conception and design of the study, acquisition of data, analysis and interpretation of data, drafting of the article; (2) final approval of the article version to be submitted; and (3) agreement to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy are appropriately investigated, resolved and the resolution documented in the literature. The specific contributions of the authors are as follows: Conceptualization: G.G., Z.R.H. and F.A. Writing—original draft: G.G., Z.R.H. and F.A. Writing—review and editing: G.G., Z.R.H., Y.T.-W., E.S., J.K.S., R.C.T., D.A.L. and F.A.

## Competing interests

R.C.T. is a consultant and advisor for Astellas Pharma, a consultant for Bayer and on the medical advisory board at Hello Therapeutics. The remaining authors declare no competing interests.

## Additional information

**Supplementary information** The online version contains supplementary material available at https://doi.org/10.1038/s43587-023-00509-8.

**Correspondence** should be addressed to Fabrisia Ambrosio.

**Peer review information** *Nature Aging* thanks Bérénice Benayoun and the other anonymous reviewer(s) for their contribution to the peer review of this work.