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Institute of Genetics and Animal Biotechnology
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Animal models in ovarian aging research – an updated review

Kaja Królikowska, Małgorzata Grzesiak*

Department of Endocrinology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University in Krakow, Gronostajowa 9, 30-387 Krakow, Poland

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Ovarian aging represents a multifactorial biological process characterized by progressive depletion of the follicular reserve, endocrine dysregulation, mitochondrial dysfunction, genomic instability, stromal remodeling, and chronic inflammation. While clinical studies in women provide essential insights, mechanistic dissection of ovarian senescence depends largely on animal models. This review presents a comparative and evolutionary synthesis of ovarian aging across invertebrate and vertebrate species, from *Caenorhabditis elegans* and *Drosophila melanogaster* to rodents, livestock, equine, and non-human primate models. We integrate conserved and divergent mechanisms, including telomere dynamics, oxidative stress, DNA damage repair, fibrosis, metabolic decline, immune activation, and endocrine transitions. We critically evaluate translational relevance, genetic manipulability, physiological similarity to humans, and suitability for longitudinal and interventional studies. Finally, we discuss emerging alternatives such as ovarian organoids, ovary-on-chip systems, and cross-species single-cell omics approaches. This integrative framework aims to guide rational model selection for mechanistic, translational, and therapeutic research in ovarian aging.

KEY WORDS: ovarian aging / invertebrates / vertebrates / animal models

In modern life, many women delay family planning because of improved social status, educational opportunities, and other novel determinants [Kramer *et al.* 2021]. Therefore, there is a need for more strategies to improve fertility and retard reproductive aging. Unfortunately, reproductive senescence is strongly correlated

*Corresponding author: m.e.grzesiak@uj.edu.pl

with chronological aging [Shen *et al.* 2023]. The ovarian reserve significantly declines after the age of 35 years, meaning that the quality and quantity of oocytes is becoming lower, thus the ability to achieve pregnancy declines.

The ovary is a heterogeneous organ composed of oocytes, granulosa cells, theca cells, stromal cells, vascular cells, and immune populations [Baerwald *et al.* 2012]. They support folliculogenesis, cyclic hormone production, and undergo specific age-related molecular changes, namely DNA damage, telomere shortening, mitochondrial dysfunction [Marchante *et al.* 2023, Wang *et al.* 2023], altered steroidogenesis [Danilovich and Ram Sariam 2006], and fibrosis [Shen *et al.* 2023]. These processes are predominantly conserved due to the universal nature of cellular senescence, but they can diverge among models due to species-specific reproductive strategies, lifespan differences, and structural variations in the ovary [Xue *et al.* 2026]. The analysis of orthologous genes expression showed similar patterns across species within comparable ovarian cell types [Wang *et al.* 2020, Zhou *et al.* 2024, Gaylord *et al.* 2025, Xue *et al.* 2026]. However, some divergences are quantified as species-specific gene expression changes, differences in cell-type proportions, or unique regulatory interactions [Xue *et al.* 2026].

There are many animal models for studying ovarian aging, beginning with invertebrates and ending with vertebrates. Although ovarian aging manifests differently across taxa, several mechanistic hallmarks appear evolutionarily conserved. These include mitochondrial dysfunction, accumulation of DNA damage, oxidative stress imbalance, apoptosis of granulosa cells, and decline in oocyte quality [Gaylord *et al.* 2025]. The most popular models are mice, rats, the nematode *Caenorhabditis elegans*, and the fly *Drosophila melanogaster*. Invertebrates have been used because of their high fertility, simple anatomy, short lifespan, and evolutionarily conserved genes. Vertebrates, on the other hand, have been used because of their physiological similarities to humans. They exhibit complex endocrine regulation, stromal fibrosis, immune infiltration, and, in primates, a distinct menopausal transition. Understanding which mechanisms are conserved *versus* species-specific is essential for rational model selection and translational interpretation [Lu *et al.* 2022]. It should be highlighted that using an alternative *in vitro* models, including follicle culture, ovarian organoids or ovary-on-chip, is emerging to reveal the molecular mechanisms and clinical translation in ovarian aging research. We herein present an overview of commonly used animal models in ovarian aging research, including their advantages and disadvantages.

The main hallmarks of ovarian aging

Ovarian aging represents a fundamental biological process that determines female reproductive capacity [Wang *et al.* 2023]. It is characterized by prominent endocrine changes, which correspond with reproductive senescence. The main hormonal markers used in diagnosis are the follicle-stimulating hormone (FSH), the anti-Müllerian hormone (AMH), 17β-estradiol (E2), and inhibin B. An increase in FSH

level accompanied by a decrease in E2 and inhibin B levels indicate the depletion of the follicle pool [Kelsey 2023]. AMH seems to be the best hormonal marker of ovarian reserve because it is produced by the granulosa cells of growing follicles, thus its level reflects follicle reserve and correlates inversely with the woman's age [Harris *et al.* 2023].

Shortening of telomeres leads to the aging of the genetic material, and this is accompanied by a decrease in the activity of the enzyme telomerase and other related proteins [Zhu *et al.* 2022]. The telomere features in the ovary are unique because of the complex process of folliculogenesis and the inherent interactions among oocytes, granulosa cells, and cumulus cells. A decreased estrogens level may inhibit telomerase activity, resulting in telomere shortening. This can disrupt the proliferation of granulosa cells and ultimately lead to ovarian dysfunction [Bayne *et al.* 2011]. Scientists discovered mitochondrial aberrations during ovarian aging manifested as lower number of mitochondrial DNA (mtDNA) copies and reduced production of ATP and NAD^+, which are needed to support oocyte development [Wang *et al.* 2023]. Another prominent feature of ovarian aging is stromal fibrosis, which impairs the course of folliculogenesis. Mice and humans exhibit age-related fibrosis in the ovaries. In aged ovaries, stromal fibrosis has been associated with disrupted ovulation and impaired tissue remodeling after ovulation, leading to ovarian dysfunction [Shen *et al.* 2023, Yang *et al.* 2024].

The marker of ovarian aging is also an oxidative stress. Reactive oxygen species (ROS) participate in the regulation of ovarian physiology [Qin *et al.* 2025]. As follicles grow, higher steroid levels promote the activity of aromatase, which in turn leads to the formation of ROS. Concurrently, the growing follicles release more E2, triggering the expression of catalase (CAT), thereby creating a dynamic equilibrium between ROS and antioxidants [Yan *et al.* 2022]. Proper control of meiotic arrest and the recovery of oocytes is crucial for female reproductive development. While ROS influence the progression of meiosis I, the advancement of meiosis II is primarily regulated by antioxidants present in the ovary [Wu *et al.* 2025]. The balance between ROS and antioxidants is disturbed within aging, marked by the excess of ROS that are not combated by antioxidant enzymes such as CAT, glutathione or superoxide dismutase 2 (SOD2), and leading to meiotic arrest dysfunction and granulosa cell apoptosis. Meanwhile, the apoptosis of granulosa cells causes a lack of nutrients for ovarian cells and triggers metabolic issues in the ovarian microenvironment, further worsening ovarian function [Yan *et al.* 2022]. Importantly, there are many genetic markers of ovarian aging, including *EXO1*, *HELQ*, *UIMC1*, and *MSH6* that are related to DNA repair, *IL11* and *NLRP11* that are responsible for immune functions, and genes related to hormonal regulation, such as *FSHB*, *STAR*, and *BCAR4* [Wu *et al.* 2025]. It is worth mentioning the non-coding RNA (ncRNA), which has been studied intensively in reproductive aging, and has been found in various mammalian species including pigs, mice, sheep, and cows, playing a functional role in follicular atresia [Wang *et al.* 2023].

Invertebrates

Caenorhabditis elegans (*C. elegans*)

This roundworm lives around 1-2 weeks and is easy to maintain in the laboratory. *C. elegans* has five pairs of autosomal chromosomes and one pair of sex chromosomes [Ellis and Wei 2010]. Its genome shares around 35% of protein-coding genes with the human genome. By the sex chromosomes, male (XO) and hermaphrodite (XX) individuals can be distinguished. The hermaphrodites initially generate male gametes, followed by internal fertilization after which fertilized eggs are deposited by allogamy [Luo and Murphy 2011]. Due to the organism's transparency, all organs can be easily observed. This model is useful for studying aging phenotype and senescence. It is also a good model for using genetic manipulation, creation of RNA libraries, and studying variation of mutagens [Scharf *et al.* 2021].

Due to the aforementioned characteristics, *C. elegans* has been utilized as a model organism for studying the initial decrease in reproductive ability [Lu *et al.* 2022]. It serves as a robust model for investigating the germ line and reproductive processes, encompassing germ line stem cell biology, germ cell apoptosis, the transition from mitosis to meiosis, oocyte maturation, and fertilization [Scharf *et al.* 2021]. Similar to humans, reproductive potential in *C. elegans* declines early and lasts for only about one-third of its lifespan [Luo and Murphy 2011]. The process of reproductive aging in *C. elegans* is genetically regulated, and it is constrained by a deterioration in oocyte quality as it is observed in humans [Athar and Templeman 2022]. Reproductive aging is typically linked to somatic aging yet remains a distinct process [Luo and Murphy 2011]. Research has shown that somatic tissues influence reproductive aging in *C. elegans*, including the transforming growth factor (TGF)-β and insulin/insulin-like growth factor (IGF) signaling pathways that implicate in maintaining oocyte and germline quality [Tatar 2010].

Drosophila melanogaster (*D. melanogaster*)

The lifespan of *D. melanogaster* is about 50 days at 25°C. Its developmental time depends on temperature and varies, taking about 8.5 days at 25°C, 7 days at 28°C, 11 days at 30°C, 19 days at 18°C, and more than 50 days at 12°C. *D. melanogaster* possesses four pairs of chromosomes, which include an X/Y pair and three pairs of autosomal chromosomes [Piper and Partridge 2018]. The genome has roughly 60% of genes conserved in humans, and 75% of known human disease genes is responsible for a range of disorders [Toivonen and Partridge 2009]. An advantage for research, *D. melanogaster* has tissues that show a greater resemblance to mammals than *C. elegans* [Tatar 2010]. It is also used for studying genetic and environmental factors on aging [Sun *et al.* 2013].

Female *D. melanogaster* can mate just eight hours after hatching, with peak fertility occurring in the first week of adulthood, during which a female can lay about 50-60 eggs daily. The reproductive system of female *D. melanogaster* consists of a pair

of ovaries, oviducts, and a uterus, with the ovaries resembling a budding lotus. The ovarian structure includes three types of stem cell populations: germline stem cells; somatic stem cells; and escort stem cells. These play a crucial role in understanding cell renewal and death [Piper and Partridge 2018]. This organism is linked with ovary aging thanks to the same processes that occur in humans, namely apoptosis, autophagy and necrosis [Sun *et al.* 2013]. In addition, research considering how nutrients affect reproductive health and lifespan was conducted on the *D. melanogaster* model [Ruchitha *et al.* 2024]. A study by Erwin and Blumenstiel [2019] investigated age-related gene expression changes in *D. melanogaster* and observed increased expression of piRNA pathway genes, suggesting enhanced genome maintenance efforts in aged ovaries. Additionally, there was a notable decrease in mitochondrial gene expression, indicating mitochondrial decline with age. Interestingly, despite these changes, there was no global depression of transposable elements, highlighting the robustness of germline genome integrity during aging [Erwin and Blumenstiel 2019]. Another findings revealed alterations in lipid profiles within the germ stem cell niche and developing follicles, indicating that lipid metabolism plays a role in ovarian aging in *D. melanogaster* [Li *et al.* 2022]. Furthermore, it was suggested that decreased germline stem cell activity and increased cell death in *D. melanogaster* might be correlated with aging of the whole organism [Zhao *et al.* 2008]. The fly was also used for studying the influence of diet on female fertility, showing that high-sugar food can lead to aberrations in folliculogenesis [Nunes and Drummond-Barbarosa 2023].

Overall, above studies underscore that invertebrate models have provided valuable information about the genetics and cellular biology involved in ovarian aging. However, both *C. elegans* and *D. melanogaster* belong to Ecdysozoa, which has undergone extensive gene loss since their divergence from their ancestor with humans, missing a large fraction of human orthologs [Murthy and Ram 2015]. Therefore, limitations of these organisms suggest the need for other model systems to facilitate deeper understanding of aging mechanisms in mammals, including humans.

Vertebrates

From fish to human, ovaries are responsible for oocytes and hormones production. However, ovarian structure, cellular composition, and regulatory pathways differ across species, reflecting diverse reproductive strategies and specific evolutionary trajectories [Nicol *et al.* 2022]. A key evolutionary transition in vertebrates is the shift from oviparity to viviparity. In oviparous animals, such as fish, amphibians and birds, the ovary is largely adapted for rapid oocyte growth and yolk deposition to support development outside the body. By contrast, viviparous mammals have evolved more complex follicular dynamics, tightly regulated ovulation [Xue *et al.* 2026]. This evolutionary differences are also crucial in the context of reproductive aging.

Fish, amphibians and birds

Fish models in ovarian aging research are limited due to differences with human reproductive physiology, however there are some studies, in which fish were used. For example, the impact of IGF signaling pathway during ovarian aging was determined in fugu (*Takifugu rubripes*) [Li *et al.* 2009], whereas medaka (*Oryzias latipes*) was employed to show changes in telomeres and telomerase activity during ovarian aging [Hatakeyama *et al.* 2008]. However, the most popular in ovarian aging research is zebrafish (*Dania rerio*) model, which elucidated mechanisms underlying oocyte quality decline and reproductive senescence [Zhao *et al.* 2008]. Faught *et al.* [2020] demonstrated that the absence of glucocorticoid receptor (GR) led to premature ovarian aging characterized by increased follicular atresia, yolk liquefaction, and inflammatory infiltrates in GR knockout (GRKO) zebrafish. Additionally, there was a decline in ovarian *tert* expression, indicating compromised telomere maintenance [Faught *et al.* 2020]. Recent study demonstrated the correlation between increased maternal age and decreased expression of mitochondria and telomere-related genes, such as *tomm40*, *mpc2*, *nbn*, and *tti1*, in zebrafish oocytes contributing to the decrease in the fertilization rate [Zhu *et al.* 2024]. Another study on zebrafish showed that exposure to environmental estrogens increased follicular atresia and changes in the oocyte volume, indicating potential acceleration in aging processes [Katti and Goundadkar 2022].

Similarly to fish, only a few studies have examined ovarian aging in amphibians, primarily due to their reproductive strategies strongly based on temperature and environmental cues [Lu *et al.* 2022]. However, some research has examined aspects of amphibian reproductive biology that may provide vital insights into ovarian aging. The dynamics of oocyte reserve across different age groups in frog (*Rana temporaria*) indicated a significant decline in the number of diplotene oocytes as frogs aged, suggesting a reduction in the ovarian reserve over time [Ogielska *et al.* 2013]. Thus, this observations indicate the reproductive senescence in frogs like in humans. The presence of ovarian aging processes in amphibians confirmed studies on the water frog (*Pelophylax esculentus*), showing germ cell degeneration influenced by age [Szydłowski *et al.* 2016]. Based on above data, amphibians could potentially serve as valuable models for studying ovarian aging, but further research in this area is required.

Birds show significant differences in the structure of their reproductive organs in comparison to humans. Their left oviduct and ovary degenerate in prenatal development as an adaptation to active flying [Johnson 2014]. What is thought provoking is the arrangement of yolk and follicles in birds follows a hierarchical structure akin to that of mammalian follicles [Johnson 2014]. Birds have similar age-related changes in hypothalamic responses, such as a greater reduction in luteinizing hormone (LH) and declining ovarian function, paralleling similar changes in women [Zhao *et al.* 2008]. Research indicates that reproductive aging pattern in female birds vary between species; laying hens exhibit relatively rapid reproductive aging,

making them suitable for laboratory studies, while budgerigars and wild seabirds show slower or negligible reproductive senescence, offering models for studying longevity and sustained fertility [Holmes *et al.* 2003]. Regarding domestic laying hens, their reproductive lifespan and decline in egg production with age (reduction in oocytes quantity and quality, increased granulosa cell apoptosis, and heightened oxidative stress in ovarian tissues) provide a practical framework for investigating ovarian senescence [Bai *et al.* 2024]. Not only ovarian aging mechanisms, but also the selection of anti-aging compounds have been extensively examined in hens. The D-galactose-induced ovarian senescence model [Liu *et al.* 2018] has been employed to examine the effect of natural compounds, such as nobiletin [Bai *et al.* 2024] and fisetin [Yang *et al.* 2024], on the oxidative stress and autophagy in ovarian tissues, revealing their capability to antioxidant defenses, reduction of reactive oxygen species accumulation, and promotion of autophagy. Another outcomes from hen models indicated that modulation of mitophagy and glycophagy pathways alleviated ovarian senescence, while FSH improved mitochondrial function and energy metabolism in aged hens [Dong *et al.* 2022, Yang *et al.* 2024]. To sum up, the use of avian models for studying ovarian aging provides both the physiological mechanisms involved and potential interventions.

Across abovementioned non-mammalian models, the most translational aspects to human ovarian aging include decline in oocyte quality and ovarian reserve, telomere shortening and impaired DNA maintenance, mitochondrial dysfunction, increased oxidative stress and inflammation. While physiological differences limit direct application, these models are valuable for examination of conserved molecular and cellular mechanisms underlying ovarian aging in humans.

Rodents

Rodents (mice and rats) are extensively used model species in studies on ovarian aging due to comparatively low cost of maintenance and low ethical concerns, short lifespan, well-characterized reproductive physiology, and availability of transgenic and knockout manipulations [Lu *et al.* 2022]. In contrast to women, rodents have shorter reproductive cycle without menstruation, and different hormonal profile. Furthermore, in rodents the reserve of primordial follicles is established postnatally, and depletion rates differ, potentially affecting aging timelines [Umeno *et al.* 2022].

Single-cell RNA sequencing (scRNA-seq) studies provide a comprehensive reference of ovarian aging across mouse and human populations, revealing conserved cellular specialization, involving sympathetic nerves and glia, alongside species-specific dynamics of follicle depletion, oocyte maturation, and stromal remodeling [Gaylord *et al.* 2025]. Furthermore, a doubling of immune cells in the aged murine ovary, especially lymphocytes, an age-related downregulation of collagenase pathways in stromal fibroblasts, which corresponds to ovarian fibrosis, was confirmed [Isola *et al.* 2024]. These results enable to understand the cell-type-specific mechanisms of ovarian aging in humans.

Numerous knockout mouse models were employed to decipher potential mechanisms of ovarian aging. Mice with FSH receptor (FSHR) knockout (FORKO) are sterile due to anovulation with E2 deficiency, and elevated androgens level, leading to several age-related issues such as obesity, skeletal deformities, cardiovascular problems, and neurological impairments that resemble those observed in aging women [Abel *et al.* 2000]. While the FORKO mice serve as a valuable model for studying the effects of aging and hormonal deficiency in ovaries, they have significant limitations, *i.e.* FORKO mice are infertile and acyclic that is akin to primary amenorrhea in women and does not reflect the normal reproductive aging process. Therefore, these mutants cannot accurately reflect conditions during peri- and menopause in women [Danilovich and Ram Sairam 2006]. It has been proposed that activation of the dioxin/aryl hydrocarbon receptor (AhR) pathway reflects the hypothalamic and ovarian aging processes, which may lead to the disruption of normal reproductive cycles in women [Valdez and Petroff 2004]. Research on transgenic mice that lack a functional AhR (AhRKO) supports this idea. AhRKO mice performed decrease in litter size, showed approximately 54% fewer preantral/antral follicles than wild-type mice, suggesting that AhR might influence the gathering of primordial follicles and regulate their further development. The AhRKO mice showed lower E2 concentration as a result of fewer antral follicles that might also impede normal follicular development [Danilovich and Ram Sairam 2006]. The interesting model to investigate distinct physiological ovarian phenotypes associated with aging is the use of immunodeficient mice, such as the NOD/SCID strain, which is an example of humanized mice [Marchante *et al.* 2023]. Older mice of this strain showed reduced ovarian reserve, follicular activation, and growth, deteriorated ovarian stroma, and decreased quantity and quality of harvested oocytes and embryos. Furthermore, the association between progressive ovarian aging and a reduced number of mitochondrial copies, oxidative damage, and apoptosis, and these age-related changes were established at the proteomic level [Marchante *et al.* 2023].

Beneficial studies on rodents model are those considering interventions aimed at delaying ovarian aging. Recent study examined therapeutic effect of saponins extracted from the Korean red ginseng mitigated aging-related damage in the ovary of C57BL/6 mice. Transcriptomic analysis revealed that the expression of genes encoding oxidative stress factors and NOD-like receptor protein 3 (NLRP3) inflammasome components were markedly reduced, demonstrating a possible therapeutic effect of saponins on the ovarian aging Chei *et al.* [2020]. Noteworthy, another investigation revealed therapeutic effect of mouse umbilical cord mesenchymal stem cells (mUCMSCs) on ovarian senescence. The transplantation of mUCMSCs into 18-month-old C57 mice ameliorated age-related ovarian decline marked by increased ovarian volume, restoration of follicular development, and elevated serum levels of E2, AMH, and inhibin B [Pan *et al.* 2023].

Aged rats exhibited a decline in ovarian function, characterized by reduced E2 levels and ovarian weight. Notably, there was a significant decrease in the expression

of autophagy-related genes (*Atg5*, *Atg12*, *Atg16L*, *Beclin1*, *Lc3B*), suggesting impaired autophagy. Furthermore, increased DNA methylation of these genes suggested epigenetic modifications in the aging process [Li *et al.* 2020]. The rat model of ovarian aging was also used to assessed the role of the sympathetic nervous system in regulating follicular development, revealing that the sympathetic nerve activity influenced the transition from the subfertile period to reproductive senescence. Understanding this neuronal regulation provides insights into the mechanisms underlying reproductive aging [Cruz *et al.* 2017]. Another research analyzed the therapeutic potential of resveratrol in aged rats, and showed increased level of AMH, enhanced antioxidant enzyme activities, and reduced oxidative stress marker. Additionally, resveratrol up-regulated sirtuin 1 expression and decreased caspase-3 abundance, indicating reduced ovarian apoptosis [Wu *et al.* 2022]. Interestingly, the research on rat model demonstrated that exposure to chronic hypoxia during gestation adversely affected the ovarian reserve of offspring in adulthood, namely it led to a reduction of primordial follicles pool and altered expression of genes associated with folliculogenesis, indicating accelerated ovarian aging in the next generation [Aiken *et al*. 2019].

Rodent models are valuable and cost-effective to study molecular and genetic aspects of ovarian aging. However, due to physiological and endocrine differences in comparison to humans, including menopause and the menstrual cycle, the caution is needed when translating findings to humans. Noteworthy, the useful tool in the study of ovarian aging represents a humanized mice model, ensuring more directly translatable results to human health compared to traditional rodent models.

Rabbits

The utility of the rabbit model in investigating various aspects of ovarian aging was very scarcely described. The study on the ovaries of aged rabbits revealed the accumulation of advanced glycation end-products (AGEs). Specifically, the AGE argpyrimidine was accumulated in granulosa cells, coinciding with changes in the expression of glyoxalase I (GLO1), an enzyme involved in detoxifying reactive metabolites that suggest a link between metabolic stress and impaired folliculogenesis in aging ovaries [de Nivelle *et al.* 2020]. Another research highlighted the rabbit model's relevance in diet-induced ovarian aging, indicating the decrease in follicular reserve and an accumulation of lipofuscin in the ovarian interstitial tissue, markers associated with cellular senescence. Additionally, increased expression of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) p65 was observed in the ovarian surface epithelium, suggesting an inflammatory response linked to oxidative stress [Díaz-Hernández *et al.* 2022]. Thus rabbits are particularly valuable for studying how diet, metabolism, and oxidative stress accelerate ovarian aging, offering a complementary model for understanding similar processes in humans.

Pigs

Pigs similarly to rodents, but unlike humans, are polyovulators [Soede *et al.* 2011]. Despite this, they have a reproductive cycle length, hormonal profile, and ovarian morphology, especially oocyte size, making them a highly translational model. The size of porcine ovaries facilitates surgical manipulation and sampling, allowing for detailed studies of ovarian structure and function [Lu *et al.* 2022]. Ovariectomized adult pigs are valuable model for studying menopause-related cardiovascular and bone disturbances [Bellino 2000]. Recent research determined porcine follicles treated with tert-butyl hydroperoxide (t-BHP) as a model of ovarian senescence, inducing several markers of aging, *i.e.* increased ROS level and decreased expression of antioxidant enzyme SOD2, increased expression of senescence-associated genes such as *p53*, *caspase 3*, and *Foxo1*, enhanced senescence-associated β-galactosidase (SA-β-Gal) staining [Shi *et al.* 2022, 2023]. High-throughput sequencing gene ontology analysis of ovaries from both young and older pigs identified genes related to histone and DNA methylation that play roles in ovarian aging processes, including apoptosis, embryonic development, reproduction, fertilization, ovarian cumulus expansion, and the ovulation cycle [Lu *et al.* 2022]. Noteworthy, recent study on porcine follicles provided valuable information about the mechanisms of ovarian aging and showed its new potential regulator, phospholipid phosphatase 3 (PLPP3), which aggravated oxidative stress, ferroptosis, and autophagy in granulosa cells, leading to an acceleration of cellular senescence [Quan *et al.* 2024]. Findings from porcine studies allow to conclude that it might be one of the most relevant non-primate models for human ovarian aging, particularly for studying evolutionary conserved processes such as oxidative stress, cell death pathways and epigenetic regulation of ovarian function. Importantly, pig ovaries are usually harvesting post-slaughter that allow to avoid ethical concerns.

Sheep and goats

Sheep and goats have been utilized in various ovarian studies, as their ovaries size, morphology, folliculogenesis duration and hormonal changes showed crucial similarities to humans [Montenegro *et al.* 2023]. The findings revealing the presence of multinucleated giant cells with lipofuscin accumulation – hallmarks of ovarian aging - in the ovarian stroma of mature ovine and caprine ovaries, supporting their use as models in ovarian senescence research [Montenegro *et al.* 2023]. The hormonal and ovarian changes during reproductive aging, including lower E2 and inhibin B levels, were observed in Sarda ewes. Furthermore, treatment with exogenous FSH improved follicular response, suggesting potential therapeutic avenues for age-related fertility decline [Berlinguer *et al.* 2012]. Recent findings also indicated that the signaling pathway initiating primordial follicle activation, specifically the phosphatidylinositol-3-kinase (PI3K)/Akt and the mammalian target of rapamycin (mTOR) pathway activated by Kit-ligand, was consistent between women and sheep [Montenegro *et al.* 2023]. The analysis of goat ovaries across different age groups (newborn, young, and aging) using scRNA-seq, identified age-related changes, particularly in granulosa

cells, *e.g.* down-regulation of antioxidant systems and oxidative phosphorylation pathways, up-regulation of apoptosis and inflammatory signaling pathways [Xu *et al.* 2023]. In goat oocytes, also typical age-dependent molecular changes were investigated, including increased apoptosis [Zhang *et al.* 2014]. Concluding, the ovine and caprine models are rarely used for ovarian research studies, however previous outcomes provided valuable insights into the general mechanisms of reproductive senescence and potential interventions, similarly to pigs.

Cows

Bovine model is utilized in ovarian aging research due to its physiological similarities to humans, including comparable ovarian size, estrous cycles, single ovulation and hormonal profile [Lu *et al.* 2022]. A study that compared older (13-14 years) with younger (1-4-years) cows has shown hormonal and follicular changes similar to those observed in women approaching menopause. In details, the elevated FSH and lower E2 concentration, reduced recruitment of 4-5 mm follicles, and smaller ovulatory follicles in two-wave cycles were observed [Malhi *et al.* 2006, Modina *et al.* 2014]. Likewise, another study showed that changes in plasma E2 and progesterone (P4) concentration in the luteal phase are similar to menopausal women [Cui *et al.* 2023]. It was also suggested that bovine model can be utilized to create quantitative and qualitative assessment of the ovarian follicular reserve and to devise strategies aimed to mitigate the impact of ovarian aging on fertility [Malhi *et al.* 2006]. Another findings indicated that oocytes from aged cows have reduced developmental competence, increased oxidative stress and mitochondrial dysfunction, highlighting the age-related decline in the oocyte quality [Malhi *et al.* 2007, Ferreira *et al.* 2024]. In aged cows, also granulosa cells showed signs of senescence, *i.e.* increased DNA damage, elevated ROS level, and higher expression of stress-related proteins [Iwata 2017, Nagata *et al.* 2020]. Interestingly, cows can naturally develop ovarian conditions analogous to human disorder, such as premature ovarian insufficiency, marked by reduced ovarian size, increased stroma content, lower levels of E2 and AMH or higher P4 concentration [Modina *et al.* 2014]. These findings suggest that cows could serve as a valuable model for studying cellular aging processes in oocytes and follicular cells, and are relevant for understanding reproductive senescence in humans. Although cows might be use for examination of conserved processes underlying ovarian senescence, such as oxidative stress, mitochondrial dysfunction and age-related decline in oocyte quality, such as porcine, caprine and ovine models, it might be more relevant for human studies due to fact that cows are monoovulators.

Horses

The mare has been recognized as a valuable natural model for studying ovarian aging due to its physiological similarities to human reproductive aging [Rizzo *et al.* 2020]. The mare's reproductive system is readily accessible for palpation and ultrasound, enabling frequent examinations or follicular interventions [Carnevale

2008]. Similarly to women, the horse is monoovular species with an extended follicular phase, which facilitates investigations into follicular development and regression [Nagy *et al.* 2000]. A comprehensive analysis of AMH level across the lifespan of mares revealed the AMH concentration peak between 5 to 15-year-old, and its decrease after 20-year-old, reflecting the decline in ovarian reserve observed in menopausal women [Carnevale 2008]. Previous research considered predominantly the effects of mare's age on the oocyte quality. It was found that oocytes from aged mares exhibited reduced centromeric cohesion, increasing the incidence of aneuploidy and chromosomal segregation errors [Rizzo *et al.* 2020]. In the study by Catandi *et al.* [2021], the reduced metabolic activity and altered lipids profile, leading to compromised developmental competence, was found in the oocytes from older mares. Furthermore, these oocytes as well as follicular cells showed impaired mitochondrial function, increased oxidative stress, higher abundance of glutamic acid and triglycerides, and a lower abundance of ceramides examined by metabolomic approach that could contribute to decreased oocyte quality and fertility in aged females [Catandi *et al.* 2023]. The mare model, belonging to monoovulators like cows, seems to be valuable for studies in oocyte and follicular development and the obtained results have the translational potential for human reproduction. However, other research on horses considering age-associated molecular processes in ovaries are limited.

Non-human primates

Non-human primates are most closely similar to humans regarding anatomy and physiology of the female reproductive tract [Holtze *et al.* 2021]. Many hormonal and morphological changes related to ovarian aging have been reported using this model [Black and Lane 2002]. Namely, rhesus monkey (*Macaca mulatta*) performed age-related increase in FSH and LH concentrations, depletion of primordial follicles and increased number of atretic follicles [Nichols *et al.* 2005]. Findings on vervet monkeys (*Chlorocebus aethiops sabaeus*) included a significant decline in primordial follicles and AMH level with age, as well as menstrual cycle irregularities and eventual cessation of menses, paralleling human menopausal transition [Atkins *et al.* 2014]. Furthermore, the ovaries of rhesus monkey and southern pig-tailed macaques (*Macaca nemestrina*) older females displayed cortical fibrosis, loss of follicles, and the absence of corpus luteum typical for ovarian senescence [Black and Lane 2002]. Recent study, utilizing scRNA-seq, provided insight into cell-type-specific mechanisms underlying ovarian aging in cynomolgus monkey (*Macaca fascicularis*). Transcriptional changes, particularly in oocytes and granulosa cells, were predominantly related to disturbances in antioxidant signaling pathways, indicating oxidative damage as a crucial factor in ovarian functional decline with age [Wang *et al.* 2020]. This model was also used for interventional study investigated the therapeutic potential of bone marrow mesenchymal stem cells (BM-MSCs) derived from juvenile macaques in reversing ovarian aging in elderly macaques, indicating that BM-MSC therapy could be a promising approach to mitigate age-related ovarian decline [Tian *et al.* 2021].

Non-human primates have menstrual cycle, endocrine profile, and reproductive lifespan similar to humans that makes this model valuable for studying age-related hormonal changes and follicular depletion [Lu *et al.* 2022]. However ethical concerns, expensive long-term maintenance of primates and challenging genetic manipulations are still crucial disadvantages of using them as research model.

Emerging models and future directions

Although animal models are predominantly used in ovarian aging studies, they often incompletely recapitulate human ovarian physiology and aging mechanisms. Recent technological advances, especially organoids, ovarian-on-chip systems, and integrated omics platforms, are transforming the study of ovarian aging. These approaches enable mechanistic dissection of aging pathways in human-relevant systems and facilitate cross-species comparisons to identify conserved molecular drivers [Benayoun *et al.* 2025, Wang *et al.* 2025].

Ovarian organoids are three-dimensional (3D) structures derived from primary ovarian tissue, stem cells, or induced pluripotent stem cells (iPSCs) that self-organize to mimic aspects of ovarian architecture and function. These systems recapitulate follicular niche interactions, extracellular matrix (ECM) dynamics, and hormone-responsive signaling better than traditional 2D culture. Importantly, organoids derived from young versus advanced-age ovarian tissue allow to compare intrinsic aging signatures independently on systemic aging [Zhang *et al.* 2025]. Microfluidic ovarian-on-chip platforms extend organoid systems by integrating mechanical forces, fluid flow, and endocrine signaling. This model enables examining age-associated stromal stiffness, testing inflammatory cytokine exposure or observing follicle activation dysregulation [Yan *et al.* 2023].

Ovarian aging is a multi-layered biological process, therefore multi-omics approaches provide the opportunity to make a deeper insight into ovarian heterogeneity within the oocyte, granulosa and theca cells, stromal fibroblasts or immune cells [Wei *et al.* 2024]. In aging ovaries, scRNA-seq has revealed increased inflammatory gene signatures in stromal cells, senescence-associated secretory phenotype (SASP) activation, reduced mitochondrial gene expression in oocytes and dysregulated DNA damage response pathways [Wei *et al.* 2022, Isola *et al.* 2024]. While scRNA-seq dissociates tissue architecture, spatial transcriptomic preserves positional information. This is particularly important in ovarian aging because fibrosis develops in spatially distinct cortical regions, follicular depletion occurs non-uniformly, and immune infiltration localizes to specific stromal niches. Therefore, integration of scRNA-seq with spatial mapping reconstructs the aging ovarian microenvironment at unprecedented resolution [Wu *et al.* 2024].

It should be also highlighted that omics tools allow to identify evolutionarily conserved mechanisms underlying ovarian aging across different species. In mice, that is a dominant model for ovarian aging, comparative analyses revealed conserved

pathways such as DNA damage, mTOR pathway activation, mitochondrial dysfunction, and inflammatory NF-κB signaling. However, species-specific differences include follicle pool size, reproductive lifespan scaling, and timing of primordial follicle activation [Gaylord *et al.* 2025]. In non-human primates, which are closely related to humans, transcriptomic analysis demonstrated conserved immune activation signatures, shared fibrosis-associated ECM remodeling, and parallel mitochondrial decline patterns [Wang *et al.* 2020].

Organoid and ovarian-on-chip technologies are redefining experimental modeling of ovarian aging by providing human-specific, dynamic, and controllable systems. When combined with single-cell, spatial, and integrative multi-omics approaches, they enable unprecedented resolution of molecular and cellular aging processes. Comparative omics across species further distinguishes fundamental aging mechanisms from species-specific adaptations. Together, these innovations establish a systems-level framework for understanding ovarian aging and pave the way for precision interventions aimed at extending reproductive health.

Conclusions

It is generally accepted that animal models are essential in ovarian aging research to overcome the limitations of human studies such as ethical issues, restricted access to ovarian tissue, and possibility of genetic manipulation. Based on evolutionary, mechanistic and translational aspect, all discussed models have advantages and disadvantages, thus the appropriate experimental approach should consider the research aim.

Invertebrate models provide valuable information about conserved genetic and cellular mechanisms underlying ovarian aging, similarly to oviparous animals, such as fish, amphibians and birds. Rodents are used most frequently for mechanistic and genetic studies, including genetic manipulation. Horses and cows (monoovular species) are excellent for natural aging studies, especially involving oocyte quality and follicular dynamic. Polyovulators (pig, goat and sheep) are useful for studying evolutionary conserved processes such as oxidative stress, cell death pathways and epigenetic regulation. Non-human primates are the most valuable for translational research, involving hormonal regulation, menopause, and long-term ovarian decline. Findings from this model have greater translational potential than rodents due to shared genetics, ovarian histology, and reproductive mechanisms. Noteworthy, complementing rodent studies (especially humanized mice model), non-human primates or *in vitro* models (organoids and ovarian-on-chip) seem to be the most appropriate research direction to enhance translational relevance of results (Tab. 1).

Table 1. Comparison of the most useful animal models for ovarian aging studies

Item	Non-human primates	Rodents (mice, rats)	Horses
Menstrual cycle	yes (monthly, similar to humans)	no (estrous cycle, not menstrual)	no (estrous cycle, seasonal breeders)
Ovarian aging similarity to humans	very high	moderate	high
Natural menopause	yes (*e.g.*, in macaques, vervets)	no (reproductive decline but not menopause)	some signs of ovarian senescence
Folliculogenesis	similar to humans (slow depletion)	fast follicle turnover	moderate; parallels to human follicle loss
Lifespan & study duration	long (10-30+ years)	short (1.5-3 years)	long (20-30 years)
Hormonal profile	very similar to humans	different levels, cycles shorter	similar trends (AMH decline, *etc.*)
Ethical considerations	high (strict oversight)	lower (relatively less regulated)	moderate (welfare important)
Cost & maintenance	very expensive	low cost	high cost
Genetic tools available	limited	extensive (CRISPR/KO models)	limited
Suitability for longitudinal studies	excellent	limited	good
Translatability to human health	excellent	moderate	high (especially metabolic and oocyte aging)

AMH – anti-Müllerian hormone; CRISPR – clustered regularly interspaced short palindromic repeats; KO – knockout.

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