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REVIEW ARTICLE

Effects of Aging on Mammalian Spermatogenesis From a Cellular Perspective

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ABSTRACT

Aging refers to the irreversible changes in the structure and function of organisms over time. Under the influence of social and economic factors, the apparent effect of aging on female fertility has been widely recognized; however, its effect on male fertility has not received sufficient attention. Spermatogenic stem cells can differentiate and produce gametes continuously for a long time in a man's life, but more significant cumulative effects often occur over time. These age-related effects mainly manifest as increased chromosomal abnormalities and DNA damage, lower levels of sex hormones, impaired testicular function, and reduced spermatozoa count and quality. In the past, more attention has been paid to some of the macroscopic changes associated with aging. In this review, we will focus on the cellular level, discussing the effects of aging on male germ cells and the changes in the spermatogenic microenvironment, which consists mainly of Sertoli and Leydig cells.

1 | Introduction

In modern society, the reproductive age of people is delayed year by year because of economic pressure as well as the shift in parenting ideologies (Secomandi et al. 2022). To date, more attention has been paid to the effect of age on female reproductive function, as women of advanced age are often accompanied by a distinct set of risk factors that affect offspring health, such as reduced ovarian reserve, increased probability of miscarriage, and increased risk for aneuploid offspring (Charalambous et al. 2023). In recent years, studies of aging on male reproductive capacity have been relatively underpowered and have generally focused on two aspects: (1) the effect of aging on the basic parameters of semen, such as the number of spermatozoa, motility, malformation rate, etc; (2) the effect of aging on fertility outcomes, such as an increase in spermatozoa DNA fragmentation that makes assisted reproductive technology (ART) success rate decline, and alterations in the epigenetic inheritance of spermatozoa affect the health of offspring, etc (Aitken 2024; Martins da Silva and Anderson 2022; Miyahara et al. 2023). However, studies of the effects of aging on spermatogenesis are relatively lacking. On the one hand, because the spermatogenic wave can be continuously generated over a long period of time in a man's lifetime, and the effects of aging on spermatogenesis are often time-dependent, such that the cumulative effects of aging are not immediately perceptible; On the other hand, the effect of aging on spermatogenesis, which is based on a series of alterations in the seminiferous microenvironment involving various stages of the spermatogenesis process, is difficult to sort out causality (Gunes et al. 2016; Perheentupa and Huhtaniemi 2009; Santiago et al. 2019). Meanwhile, in the case of humans, aging human populations are often accompanied by a range of metabolic or degenerative diseases, making it more difficult to study the

effect of increased age alone on spermatogenesis. In addition, due to the increasing diversity of experimental animal models of aging, there is often some variation in the clear definitions of aging as well as in the measures of associated trait measures, researchers also need to be aware of species distinctions during research. For example, in laboratory animal models such as jellyfish, worms, etc., due to their asexual reproduction characteristics, the impact of aging on their reproductive system is different from that of sexually reproducing animals (Carrageta et al. 2022). We have noted several recent publications for a review of testicular aging (S. Dong et al. 2022; Santiago et al. 2019). Researchers have addressed in detail the macroscopic characteristics of aging testes and the adverse effects of aging fathers on offspring. Therefore, in this review, we focus more on a range of cell level alterations in the simple aging (not accompanied by other systemic diseases) state, discussing the aging characteristics of germ cells and testicular somatic cells, their microscopic alterations, and the mechanisms that may influence spermatogenesis during aging.

2 | Overview of Mouse Spermatogenesis

Spermatogenesis is the process of developing from primordial germ cells (PGCs) into male gametes; The following cells are included: PGCs, spermatogonial stem cells (SSCs), spermatogonia, spermatocytes; spermatids as well as mature haploid spermatozoa (Griswold 2016). In mice, PGCs begin to appear in the ectoderm at embryonic Day 6.5 (E6.5), undergo a series of cell migrations, and eventually reach and infiltrate into the developing gonadal ridges at E11.5 (McLaren 1998; Saitou and Yamaji 2012). Once PGCs interact with embryonic Sertoli cells, myoid cells and Leydig cells at E12.5, sex determination is completed and the embryonic testis forms (Buganim et al. 2012; Griswold 2016). Subsequently, PGCs undergo a mitotic proliferation phase and, together with Sertoli cells, form spermatogenic cords and eventually become seminiferous tubules. Upon completion of seminiferous tubules deferens, the prospermatogonia will enter a nonproliferative phase until the birth of the mouse (Griswold 2016). After birth, spermatogonia migrate to the basement membrane within the third postnatal day and then acquire their proliferative potential as SSCs. Notably, after migration, only Neurogenin 3 (NGN3)-positive spermatogonia acquire proliferative potential, whereas NGN3-negative spermatogonia will directly transition into A1 cells, continue to differentiate and develop into haploid spermatozoa, which is "the first wave of spermatogenesis" (Nishimura and L'Hernault 2017; Yoshida et al. 2006) and is not covered in this review. A single spermatogonia (As) termed SSC. It is the starting point of subsequent spermatogenesis and is a germline stem cell (GSC) with self-renewal capacity (de Rooij and Griswold 2012). Under certain conditions, as cells can divide to form paired spermatogonia (Apr), after which the Apr continues to divide into a syncytium of 4, 8, and 16 cells (i.e., Aal4, Aal8, Aal16). These cells above, together with SSCs, are collectively referred to as "undifferentiated spermatogonia." These cell syncytia are collectively called progenitor spermatogonia with As cells (Krieger and Simons 2015). Subsequently, Aal cells undergo a stepwise transformation into A1 cells under the control of retinoic acid (RA) and multiple intrinsic factors. This marks the demarcation line of cell differentiation, after which irreversible and significant changes occur in the morphology and mitotic behavior of spermatogonia (Khanehzad et al. 2021; Lin et al. 2008; Raverdeau et al. 2012). Next, A1 cells transition through staged, synchronous mitoses into Types A2, A3, A4, Intermediate (In), and B spermatogonia. These cells are collectively referred to as "differentiated spermatogonia." Type B spermatogonia will continue to undergo mitosis, transitioning to preleptotene spermatocytes in the seminiferous epithelial tubules of Stages VII–VIII (de Rooij 2001, 2017). Afterward, they enter the progression of meiosis (Y. Chen et al. 2018; S. S. Chung et al. 2004). During meiosis, homologous chromosomes pair up and exchange genetic material through a process called recombination. This is followed by two rounds of cell division, resulting in the production of four haploid cells. In mouse spermatocytes, meiosis begins with the pairing of homologous chromosomes (Horisawa-Takada et al. 2021). During prophase I, several key events occur, including chromosome axis formation, homolog synapsis, and meiotic recombination. After prophase I, spermatocytes undergo two rounds of chromosome segregation, resulting in the production of haploid round spermatids. These round spermatids then undergo dramatic morphological and biochemical changes to form elongated mature spermatozoa (Biggs et al. 2020) (Figure 1).

3 | Effects of Aging on Male Germ Cells

In mice, aging leads to a decline in male fertility, primarily evidenced by a reduction in litter size, which is independent of mating frequency (Endo et al. 2024). Histological examination showed that the epithelium of the seminiferous tubules of 24-month-old mice was thinner than that of 2-month-old mice. In cross-sections of the testes of old mice, about 20% of the seminiferous tubules were obviously abnormal, including 8.4% of tubules showing germ cell depletion, 5.8% of tubules with delayed release of stage VIII spermatozoa, and 6.3% of tubules with disorganized seminiferous tubules with two-stage characteristics. Regarding the germ cell composition of the seminiferous epithelium, aged mice showed a significant decrease in the numbers of undifferentiated spermatogonia, pre-leptotene spermatocytes, pachytene spermatocytes, round spermatids, and spermatozoa. The remaining 80% also show a decrease in the number of germ cells (Endo et al. 2024). In addition, germ cells exhibit functional abnormalities. For example, the proliferation capacity of SSCs decreased, the apoptosis level of spermatocytes increased, and increased deformity rates (emergence of multinucleated germ cells) were related to factors such as oxidative stress, Inflammation, metabolite accumulation, and hormone levels (Aitken 2023; Feuz et al. 2024). The following sections will discuss these issues in detail, categorized by the stages of germ cell development.

3.1 | Effects of Aging on SSCs

In fact, undifferentiated spermatogonia may all have the potential to self-replicate, but they (As, Apr, and Aal spermatogonia) display different gene expression (Hara et al. 2014). GFRα1 and nanos2 are expressed in a large subset of As and Apr, which are capable of steady-state self-renewal, while many

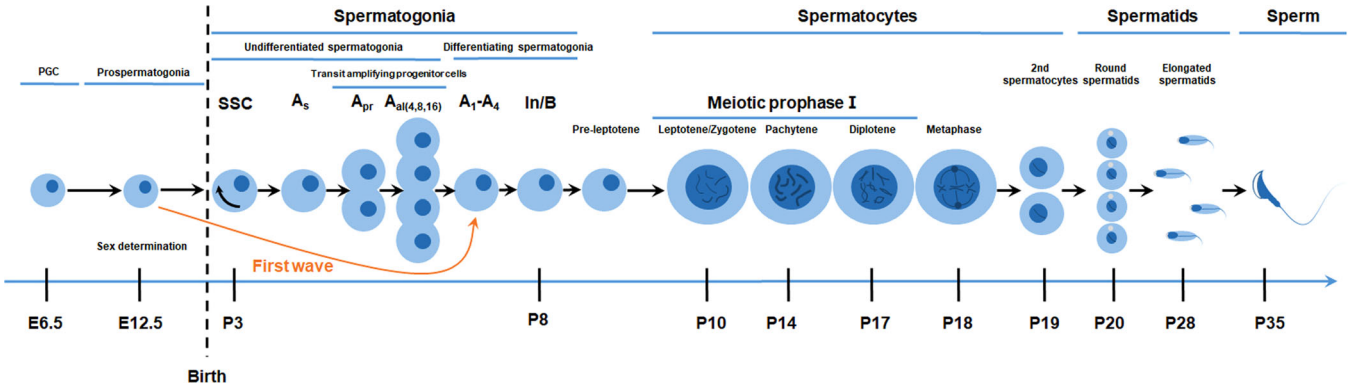

FIGURE 1 | Schematic diagram of mouse spermatogenesis process. PGC manifests at E6.5, sex differentiation happens at E12.5, and P3 establishes the pool of SSCs. The initial wave of spermatogenesis bypasses the undifferentiated spermatogonia stage. From P8 onwards, spermatogonia start to get ready to transform into spermatocytes. The first meiotic division between P10 and P18 was completed. Then starts to form round spermatids at P20, elongated spermatids at P28, and finally, at P35, the first wave of spermatogenesis concludes and mature sperm are generated. The above time points only occur in the first wave of spermatogenesis.

Aal express Ngn3 and tend to differentiate. However, Ngn3-expressing cells can also reexpress GFRα1 and assist in rebuilding the stem cell pool during steady-state spermatogenesis or following tissue injury (S. Sharma et al. 2019). Therefore, it is often challenging to discuss the impact of aging on SSCs in isolation, and consequently, many studies on the effects of aging on SSCs might include undifferentiated spermatogonia. When studying the effect of aging on SSCs, it is also important to distinguish between the results of in vivo and in vitro experiments. In vivo experiments may capture the effect of aging on the stem cell niche, while in vitro experiments cannot fully mimic the stable niche microenvironment. Additionally, SSC survival times in vitro may be longer than the normal lifespan of living bodies, so these results should be interpreted carefully (Ryu et al. 2006; Schmidt et al. 2011; X. Zhang et al. 2006).

The effect of aging on SSCs primarily focuses on its effects on the steady production of gametes, including the weakening of SSC self-renewal capacity, increased apoptosis of SSCs, impeded differentiation, and altered molecular expression levels (El-Domyati et al. 2009; Liao et al. 2021; Suzuki and Withers 1978). In a previous study, it was found that SSCs from young fertile male mice were unable to establish a colony of spermatogenesis when transplanted into the testes of older infertile male mice. This suggests that senescence may disrupt the SSC niche (Ryu et al. 2006; X. Zhang et al. 2006). Additionally, when SSCs from 2-year-old atrophic mouse testes were transplanted into young busulfan-treated recipients, there was a decline in the number of germ cell populations. This suggests that senescence may also impair SSC self-renewal capacity (X. Zhang et al. 2006). It is important to note that after SSCs are transplanted into new ecological niches, the community may remain stable for such a period of time that a significant decrease in SSC function may not be detected during the normal lifespan. This has also been demonstrated in xenograft experiments with mouse SSCs, suggesting that the intrinsic aging of SSCs may be slower than that of the niche (Ryu et al. 2006). A rat study based on spermatogonial transplantation demonstrates the effects of aging on SSCs. Researchers calculate the SSC activity from younger and older rat showed SSC activity from 18- and 21-month-old testes was less than 50% and also numbers of colony derived from aged donors were significantly lower than those from young donors (Paul et al. 2013).

In addition to macroscopic transplantation experiments, some mechanisms of SSC senescence have been uncovered. To continuously generate germ cells, SSCs must withstand exposure to metabolites that accumulate with age, such as reactive oxygen species (ROS), which have been shown to impair the normal physiological functions of multiple stem cells (Pervaiz et al. 2009; Takubo et al. 2013). However, the study of ROS in SSCs differs from that in other stem cells. Among SSCs, a certain level of ROS is essential for SSC self-renewal. In vitro studies based on GS cells showed that the ROS level of GS cells with EGFP transgene decreased significantly after 60 months of culture compared with 5 months, and transplanted into WBB6F1-W/W^v (W) mice cannot produce sperm (Kanatsu-Shinohara et al. 2019). SSC self-renewal is stalled in ROS-depleted SSCs, and increased ROS levels, achieved by the addition of hydrogen peroxide, enhance the self-renewal capacity of SSCs (Morimoto et al. 2013). NADPH oxidase 1 (Nox1) is thought to drive SSC self-renewal via the ROS-Bcl6b-Nox1 pathway by generating ROS in a feedforward manner, rather than relying on mitochondrially generated ROS (Morimoto et al. 2013; Morimoto et al. 2021). HIF1A plays a central role in regulating the self-renewal capacity of SSCs through its dual regulation by oxygen content and ROS levels (Movafagh et al. 2015). Knockout of HIF1A significantly inhibits SSC self-renewal, impairing the generation of secondary colonies after transplantation (Morimoto et al. 2021). In aging individuals, the testis experiences a decrease in blood supply may due to age-related changes in vasculature and thickening of the basement membrane (Brito et al. 2021). These changes result in a hypoxic seminiferous microenvironment and may inhibit mitochondrial oxidative phosphorylation, making SSCs more dependent on glycolysis as an energy source. This may promote the senescence of SSCs. Studies in Klotho-deficient senescent mouse models and 2-year-old rats have shown that intrinsic activation of c-Jun N-terminal kinase (JNK)-mediated glycolysis contributes to SSC senescence (Kanatsu-Shinohara et al. 2019). It remains unclear what is the causal relationship between oxygen levels, glycolysis levels and aging. SSCs are

known to depend on glucose for ATP production (Kanatsu-Shinohara et al. 2016; Kanatsu-Shinohara et al. 2019; Rato et al. 2012).

Senescence is accompanied by molecular changes in SSCs, including increased expression of *Brca1*, which may affect stem cell stability and promote stem cell pool attrition. The down-regulation of several genes involved in maintaining SSC stemness, including *Hmga2* (D. Huang et al. 2023; Nishino et al. 2008), *Tcf7l1*, *Tcf3*, *Dmrt1* (D. Huang et al. 2023; T. Zhang et al. 2016), *Gfrα*, *Itgβ1*, *csf1r*, *Itgα1*, *Runx2*, *Fgf3*, *Wnt7a*, and *Wnt5b*, suggests a loss of stemness in senescent SSCs (Nishino et al. 2008; Paul et al. 2013; T. Zhang et al. 2016). Additionally, transcription factors, adhesion molecules and receptors associated with SSC proliferative differentiation capacity, such as *Pparγ*, *Ncam1*, *Egfr*, *Fgfr2*, *Kdr*, and *Runx2* were also differentially expressed (Table 1). These results suggest a decline in SSC numbers in aging individuals (Aguirre et al. 2010; Kim et al. 2013; Levine et al. 2000; Paul et al. 2013; Sjöstrand et al. 2007; Wada et al. 2006). RIPK3 phosphorylation of MLKL is an activation marker of necroptosis and has been detected in SSCs of aged mice testes, suggesting higher levels of apoptosis in SSCs within aging individuals (Li et al. 2017). In summary, aging brings about a decline in the SSC population that is so great that it may not be able to stably maintain the level of its stem cell pool reserve.

Due to the phenomenon of "selfish spermatogonial selection," where SSCs harboring specific mutations have a significant growth advantage (Wood and Goriely 2022; Zhytnik et al. 2021), the prevalence of paternal age effect (PAE) diseases such as achondroplasia, Apert syndrome, and Costello syndrome increases in the offspring of aging individuals (J. Lim et al. 2012; Maher et al. 2014). This suggests that germ cells containing mutation sites can escape multiple checkpoints during spermatogenesis and transmit these sites to offspring, increasing the risk for older men to have affected offspring (Goriely and Wilkie 2012).

3.2 | Effects of Aging on Spermatogonia

SSCs develop into progenitor spermatogonia and differentiating spermatogonia, and the balance between these two germ cell populations is essential for homeostatic spermatogenesis. The effect of senescence on these populations varies due to their different biological characteristics. Recent studies suggest that the significant decrease in the number of undifferentiated spermatogonia maybe the primary cause of the reduced number of germ cells in subsequent stages (Endo et al. 2024). However, separate studies on progenitor spermatogonia are scarce due to limitations in cell isolation techniques and inadequate cell-specific markers. Given the relevant discussions on SSCs in the preceding text, it is prudent to consider that the effects of aging on undifferentiated spermatogonial features are often discussed in the context of SSCs.

A recent single-cell sequencing study showed that the ratio of undifferentiated to differentiated spermatogonia decreased in aging mouse testes relative to young mice. This suggests that aging disrupts the balance between maintenance and differentiation of spermatogonia, potentially leading to the exhaustion of spermatogonia associated with aging (W. Zhang et al. 2023). Similarly, another study found reduced spermatozoa numbers in seminiferous tubules with normal stages in aging testes, suggesting that this may be due to reduced levels of undifferentiated spermatogonia (Endo et al. 2024). Single-cell studies of human testes have shown variable results, with SSC abundances reduced in some samples while remaining unchanged in others. These findings suggest that the impact of aging on spermatogonia may vary between individuals and species, and may also be influenced by the health status of the older samples (Nie et al. 2022).

3.3 | Effects of Aging on Spermatocytes

Under the synergism of RA pulse and activin A, differentiating spermatogonia continue their developmental transition into preleptotene spermatocytes. At stage VIII of the seminiferous tubules, they cross over the blood-testis-barrier (BTB) into the inner side of the seminiferous tubules, where they become immune-privileged and enter meiosis (Gewiss et al. 2020; Khanehzad et al. 2021; Yan et al. 2008). During the first meiotic prophase, primary spermatocytes sequentially undergo leptotene, zygotene, pachytene, diplotene, and diakinesis substages. Three checkpoints exist during this phase: the DNA damage checkpoint, the synaptonemal checkpoint, and the spindle assembly checkpoint to ensure that meiosis proceeds normally (Xie et al. 2022).

It has been suggested that the age-related decrease in sperm production may result from a reduction in the number of germ cells preceding pachytene spermatocytes (Johnson et al. 1984). However, recent studies have found that aging also increases the level of apoptosis in spermatocytes. One piece of evidence is the increased number of degenerating spermatocytes and multinucleated spermatocytes found in aging testes, likely as a result of membrane fusion between adjacent spermatocytes (Johnson et al. 1990; Miething 1993; Paul et al. 2011). In addition to morphological alterations in spermatocytes, recent studies suggest that aging may confer higher meiotic error rates. Studies have found increases in the length of meiotic synaptonemal complexes and recombination associated with them in aging individuals, specifically as shown by a positive correlation between the number of MLH1 foci and age (Codina-Pascual et al. 2005; Vrooman et al. 2014). However, this does not affect normal gamete production as cells with errors in meiotic prophase I are effectively eliminated at the metaphase-anaphase transition of meiosis I. During the pachytene stage, senescence does not confer a significant change in the DSB repair marker Rad51. However, it does elevate the rate of sex chromosome pairing failure. This interferes with meiotic sex chromosome silencing (MSCI) and due to the abnormal chromosome pairing situation, cells will fail to pass the checkpoint in pachytene and will be induced to undergo apoptosis (Royo et al. 2010; Turner 2007; Vrooman et al. 2014). This may explain why there is an increase in apoptotic spermatocytes in aging testes. Abnormalities in meiosis are captured by checkpoints, initiating a death program. It is important to note that unlike in females, these changes may not significantly increase aneuploidy in male germ cells (Leonard and Leonard 1975), despite the fact that increased age is accompanied by a higher frequency of sex chromosome disomy or disease (Griffin et al. 1995). However, due to species distinctions, germ cell aneuploidy in humans may be slightly higher. Previous studies have found a slight elevation of aneuploidy in aging oligospermic

TABLE 1 | Aging-related changes in the levels of gene expression in germ cells.

Type of cell	Gene/protein	Species	Age comparison (aging versus youth)	Verification method	Function	Changes in aging animals (aging versus youth)	Refs.
SSCs	*Hmga2*	Cynomolgus monkeys (*Macaca fascicularis*)	18–21 years versus 8 years	snRNA-seq	Maintain stem cell stemness (Nishino et al. 2008)	Downregulated	D. Huang et al. (2023)
	Tcf7l1	Cynomolgus monkeys (*Macaca fascicularis*)	18–21 years versus 8 years	snRNA-seq	stem cell regulator	Downregulated	
	Tcf3	Cynomolgus monkeys (*Macaca fascicularis*)	18–21 years versus 8 years	snRNA-seq	TFs for SSC maintenance	Downregulated	
	Dmrt1	Cynomolgus monkeys (*Macaca fascicularis*)	18–21 years versus 8 years	snRNA-seq	TFs for SSC maintenance (T. Zhang et al. 2016)	Downregulated	
	Msh4, *Msh5*, *Brca1*, *Dmc1*, and *Rad52*	Rat	21 months versus 4 months	RNA-seq	DNA repair	Upregulated	Paul et al. (2013)
	Pparg	Rat	21 months versus 4 months	RNA-seq	Transcripts that were transcription factors or receptors (D. Huang et al. 2023)	Upregulated	
	Gfrα, *Itgβ1*, *csf1r*, Itgα1, *Runx2*, *Fgf3*, *Wnt7a* and *wnt5b*	Rat	21 months versus 4 months	RNA-seq	Involved in stem cell development, function, and maintenance either in SSCs or other types of stem cells.	Downregulated	
	Ncam1	Rat	21 months versus 4 months	RNA-seq, RT-PCR	Transcripts that were transcription factors or receptors (Sjöstrand et al. 2007)	Downregulated	
	Egfr	Rat	21 months versus 4 months	RNA-seq, RT-PCR	Transcripts that were transcription factors or receptors (Aguirre et al. 2010; Levine et al. 2000)	Downregulated	
	Fgfr2	Rat	21 months versus 4 months	RNA-seq	Transcripts that were transcription factors or receptors (Levine et al. 2000)	Downregulated	
	Kdr	Rat	21 months versus 4 months	RNA-seq	Transcripts that were transcription factors or receptors (Caires et al. 2009)	Downregulated	

(Continues)

TABLE 1 | (Continued)

Type of cell	Gene/protein	Species	Age comparison (aging versus youth)	Verification method	Function	Changes in aging animals (aging versus youth)	Refs.
	Runx2	Rat	21 months versus 4 months	RNA-seq	Transcripts that were transcription factors or receptors (Kim et al. 2013)	Downregulated	
	p-MLKL	Mice (C57BL/6)	18 months versus 4 months	IF	Activation marker of necroptosis	Upregulated	Li et al. (2017)
	Brca1	Cynomolgus monkeys (*Macaca fascicularis*)	18–21 years versus 7 years	snRNA-seq	Maintain stem cells in a quiescent state (Bai et al. 2013)	Upregulated	D. Huang et al. (2023)
	Hmgb1	Cynomolgus monkeys (*Macaca fascicularis*)	18–21 years versus 7 years	snRNA-seq	Via participation in modulation of TRIM30α expression to influence STING-mediated senescence (Lee et al. 2019)	Upregulated	
	Ttn, *Kif25*, *Kif2c*	Cynomolgus monkeys (*Macaca fascicularis*)	18–21 years versus 6 years	snRNA-seq	Meiosis-related genes	Downregulated	
Spermatocytes	*Brca2*, *Byn62*, *Tex15*	Cynomolgus monkeys (*Macaca fascicularis*)	18–21 years versus 6 years	snRNA-seq	Meiosis-related genes	Downregulated	D. Huang et al. (2023)
	Ogg1	Brown Norway rats	18 months versus 4 months	RNA Microarray, RT-PCR	Base excision repair (BER) pathway	Upregulated	Paul et al. (2011)
	Ercc1, *Xpa*, *Rpa1*, and *Ercc4* (Xpf)	Brown Norway rats	18 months versus 4 months	RNA Microarray, RT-PCR	Nucleotide excision repair (NER) pathway	Upregulated	
	Ape1, *Fen1*, *Xrcc1*	Brown Norway rats	18 months versus 4 months	RNA Microarray, RT-PCR, WB	Base excision repair (BER) pathway	Downregulated	
Spermatids	*Odf2*, *Pcsk4*, *Tcp11*	Cynomolgus monkeys (*Macaca fascicularis*)	18–21 years versus 6 years	snRNA-seq	Involved in spermatid differentiation	Downregulated	D. Huang et al. (2023)
	Odf2, Cfap100, Akap4	Cynomolgus monkeys (*Macaca fascicularis*)	18–21 years versus 7 years	snRNA-seq	Involved in cilium organization	Downregulated	
	Spata3, Spata6, Capza3	Cynomolgus monkeys (*Macaca fascicularis*)	18–21 years versus 8 years	snRNA-seq	Involved in perinuclear theca	Downregulated	

(Continues)

TABLE 1 | (Continued)

Type of cell	Gene/protein	Species	Age comparison (aging versus youth)	Verification method	Function	Changes in aging animals (aging versus youth)	Refs.
	Dnah1, Dnah10, Bicc1	Cynomolgus monkeys (*Macaca fascicularis*)	18–21 years versus 9 years	snRNA-seq	Involved in the regulation of sperm motility	Downregulated	

men (Dakouane et al. 2005). This may be a result of the combined effects of multiple internal and external environments and not solely derived from aging (Templado et al. 2013). In addition to the above, it has been found that senescence can cause spermatocytes at the pachytene stage to harbor a large number of differentially expressed genes (DEGs) related to oxidative stress and DNA damage responses (Paul et al. 2011). The base excision repair (BER) pathway, which repairs spontaneous base damage caused by oxidative stress, is an important mechanism involved in the aging process (Almeida and Sobol 2007; Demin et al. 2021; Yoon et al. 2022). Decreased expression of FEN1 and XRCC1, downstream players in the BER pathway, as well as APE1, an AP endonuclease that determines BER activity, was observed in aged animals (Paul et al. 2011). This may partially explain the increased DNA damage in spermatocytes at the pachytene stage of old male spermatocytes, suggesting that aging renders spermatocytes less competent for DNA damage repair.

3.4 | Effects of Aging on Spermatids and Spermatozoa

Secondary spermatocytes continue to develop and soon transform into round spermatids. At this point, the germ cells enter the spermiogenesis. During this phase, researchers have found multinucleated spermatids in aging testes, as well as some ultrastructural alterations of spermatids, which showed acrosome malformation, nuclear envelope redundancy, intranuclear inclusions, excessive cytoplasmic droplets, and irregular nuclei (Nistal et al. 1986; Paniagua et al. 1987). In addition, premature sloughing of immature spermatids was also found in the center of seminiferous tubules, which may be the result of premature spermatid release from senescent Sertoli cells (to be further elaborated on in the Sertoli cell section). Comparison of epididymal tissue from mice showed that spermatozoa from aged mice exhibited reduced spermatozoa counts, curved heads, missing or abnormally arranged mitochondria, reduced flagellar motility, and higher rates of DNA fragmentation (Endo et al. 2024). Further in vitro fertilization results indicated that the number of blastocysts produced by sperm from aged individuals decreased due to embryo degeneration or fragmentation (Endo et al. 2024). Altogether, these abnormalities in morphology and organelle structure are possible explanations for the declining number of spermatids with increased rates of malformations in aging individuals. Another noteworthy aspect is the epigenetic changes in spermatozoa (Ashapkin et al. 2023), particularly in the methylation modifications of spermatozoa histones and protamines. Spermatozoa from older men exhibit higher levels of 5-methylcytosine (5mC) methylation and trimethylation of histone 3 lysine 27 (H3K27me3), and lower levels of dimethylation and trimethylation of histone 3 lysine 9 (H3K9me2/3), as described in this review (Klutstein and Gonen 2023). Additionally, some studies have found that spermatozoa centromere methylation also accumulates with age (de Sena Brandine et al. 2023). Recent single-cell sequencing results (D. Huang et al. 2023) have identified several transcriptome-level alterations, for example, differential expression of *Odf2*, *Akap4*, *Ift74*, *Carma*, *Crisp2*, and *Capza3* in aging cynomolgus macaques, which are associated with spermatogenesis as well as spermatozoa flagellum formation (J. Bao et al. 2018; Fang et al. 2019; Ito et al. 2019; S. Lim et al. 2019; Shi

et al. 2019; Sosnik et al. 2010), and downregulation of genes *Spata3*, *Spata6*, *Dnah1*, *Dnah10*, and *Bicc1*, which are associated with acrosome function and normal spermatozoa structure formation (Ben Khelifa et al. 2014; Girault et al. 2021; Tu et al. 2021; Yuan et al. 2015) (Table 1).

4 | Effects of Aging on the Spermatogenic Microenvironment

The seminiferous microenvironment refers to the stable internal environment required for normal development of germ cells at all stages of spermatogenesis, with Sertoli and Leydig cells playing major roles (Cheng et al. 2010; Miyaso et al. 2022). Likewise, like other types of stem cells, SSCs rely on their niche to sustain their growth and initiate differentiation. The SSC niche refers to supporting cells and a growth factor environment that promotes SSC self-renewal (Oatley and Brinster 2012). These molecules are mainly produced by Sertoli cells, peritubular myoid (PTM) cells, Leydig cells, macrophages, endothelial cells, and the vasculature (Crisóstomo et al. 2018; Hofmann and McBeath 2022; W. Liu et al. 2024). Given the results of the transplantation experiment (Ryu et al. 2006; X. Zhang et al. 2006), aging of the spermatogenic microenvironment makes it difficult to maintain normal spermatogenesis Interestingly, a recent imaging study (Brito et al. 2021) in dogs revealed that aged dogs have a higher testicular pulsatility index compared to younger ones, indicating increased vascular resistance within the testes. This suggests that aging may reduce testicular blood supply; however, the cause of the increased vascular resistance remains unclear.

4.1 | Effects of Aging on the Functions of Sertoli Cells

Sertoli cells, the only somatic cells in the seminiferous epithelium, have close physical contact with germ cells and directly provide soluble growth factors and membrane-bound signals to germ cells while endocytosing apoptotic germ cells and remnants, maintaining the stability of the seminiferous microenvironment (Ni et al. 2019; O'Donnell et al. 2022). A single-cell sequencing study found that Sertoli cells may be the most sensitive cell type to aging in the testis, with the largest number of DEGs associated with aging (D. Huang et al. 2023). Systemic factors brought about by aging, as well as alterations in the local internal environment, decrease the number of Sertoli cells (Petersen et al. 2015; Wang et al. 1993) and a series of morphological changes such as amyloid-like inclusions, periodic loss of organelles, abnormal shape of the nucleus, loose endoplasmic reticulum, and enlarged lysosomes accompany aging (Bohl et al. 1991). This leads to a decline in Sertoli cell secretion and further decline in Sertoli function (Jiang et al. 2014).

4.1.1 | Disorders of Sertoli Cell Lipid Metabolism

A disturbance of lipid metabolism has been found in senescent Sertoli cells. Recent sequencing results (Nie et al. 2022) found a rise in the expression of genes related to sterol biosynthesis and transport, including *Hmgcr*, *Dhcr24*, *Msmo1*, *Sqle*, *Tspo*, *Fads1*, *Elovl5* in senescent Sertoli cells. This implicates an accumulation of lipid inclusions inside senescent Sertoli cells (Paniagua et al. 1991) (Table 2). Recently, a study based on a mouse model suggested a possible mechanism for the disorder of lipid metabolism in Sertoli cells. Sulfogalactosylglycerolipid (SGG), a sulfate glycolipid that is indispensable for spermatogenesis and sperm-egg binding, is first synthesized in primary spermatocytes (Tanphaichitr et al. 2018). Sertoli cells do not have the enzyme to synthesize SGG but express arylsulfatase A (ARSA) to degrade SGG from apoptotic germ cells and their remnants (Kongmanas et al. 2021). When Sertoli cells are unable to degrade SGG in a timely manner, they can show lysosomal swelling and lipid droplet accumulation, which is similar to the phenotype of senescent Sertoli cells. However, whether among senescent Sertoli cells it is because of decreased activity of ARSA leading to the accumulation of SGG accompanied by higher ROS and thus affecting spermatogenesis still needs further investigation.

4.1.2 | Decreased Secretory Capacity of Sertoli Cells

Sertoli cells secrete numerous signaling molecules, such as glial cell line-derived neurotrophic factor (GDNF) (Meng et al. 2000), fibroblast growth factor-2 (FGF-2) (Kubota et al. 2004; Mullaney and Skinner 1992), Wnt family proteins (Cui et al. 2020; Takase and Nusse 2016), Steel factor (SLF) (Rossi et al. 1993), neuregulin 1 (J. Zhang et al. 2011), activin A (de Winter et al. 1993), BMP4 (Pellegrini et al. 2003), EGF (Radhakrishnan et al. 1992), CXCL12 (Yang et al. 2013), SCF (Peng et al. 2023), and other factors to maintain the normal development of germ cells. However, current research is still focused on the normal physiological function of these molecules during spermatogenesis. In the context of aging, whether the secretory capacity of Sertoli cells for these molecules is diminishing, thereby affecting spermatogenesis, remains to be supported by clear evidence.

Among them, GDNF is one of the important derived factors of Sertoli cells. It is produced under the influence of follicle-stimulating hormone (FSH) from the pituitary and is regulated by Notch signaling (França et al. 2016) and Wnt/β-catenin signaling. By binding to the RET tyrosine kinase/GFRA1 receptor complex, it regulates self-renewal of SSCs (Hofmann 2008; Naughton et al. 2006; M. Sharma and Braun 2018). It has been previously shown that GDNF secretion levels decrease in a stepwise manner with increasing age (Ryu et al. 2006), perhaps representing an important factor in the decline of SSC populations within aging individuals. Periodic RA pulses are key regulators regulating four spermatogenesis characteristic events: spermatogonial differentiation, meiosis initiation, spermatozoa elongation, and spermatozoa release. Tsutomu Endo et al (Endo et al. 2017; Raverdeau et al. 2012) suggested that the RA pulses required before meiosis were mainly produced by Sertoli cells to maintain spermatogonial differentiation as well as meiotic entry. A recent single-cell sequencing study (D. Huang et al. 2023) revealed a significant downregulation of the RA receptor signaling pathway in senescent Sertoli cells, which may imply a diminished guidance of senescent Sertoli cells towards the progression of spermatogonial differentiation, resulting in a decreased rate of germ cells entering meiosis. WNT6, secreted by

TABLE 2 | Aging-related changes in the levels of gene expression in Sertoli and Leydig cells.

Type of cell	Gene/protein/pathway	Species	Age comparison (aging versus youth)	Verification method	Function	Changes in aging animals (aging versus youth)	Refs.
Sertoli cells	ZO-1, Claudin 11	Cynomolgus monkeys (*Macaca fascicularis*)	18–21 years versus 6 years	snRNA-seq, IF, WB	Involved in tight junction	Downregulated	D. Huang et al. (2023)
	Response to testosterone and the AR signal pathway	Cynomolgus monkeys (*Macaca fascicularis*)	18–21 years versus 6 years	snRNA-seq	Related to sex hormone signaling	Downregulated	
	Wnt signal pathway and the RA receptor signal pathway	Cynomolgus monkeys (*Macaca fascicularis*)	18–21 years versus 6 years	snRNA-seq	Growth factor-related	Downregulated	
	Wt1, *Gata4*, *Ar*, *Sox9*	Cynomolgus monkeys (*Macaca fascicularis*)	18–21 years versus 6 years	snRNA-seq	Transcriptional regulators	Downregulated	
	Timp1, *Il6ST*, *Ifitm2*/3	Human	62–76 years versus 17–22 years	Single-cell RNA-seq, IF	Secreted protein in inflammatory networks	Upregulated	Nie et al. (2022)
	Il1a、*Ifi27*、*Mif*、*Il6st*、*Ccl2*	Human	62–76 years versus 17–22 years	Single-cell RNA-seq	Inflammation-related	Upregulated	
	Hmgcr, *Dhcr24*, *Msmo1*, *Sqle*, *Tspo*, *FadS1*, and *Elovl5*	Human	62–76 years versus 17–22 years	Single-cell RNA-seq	Cholesterol biosynthesis	Upregulated	
	Hmgcs2, *Idh1*, *Fgfr1*, *Src*、*Aco2*, *Uqcrc1*, *Akr1b1* and *Pfkfb4*	Human	62–76 years versus 17–22 years	Single-cell RNA-seq	Generation of precursor metabolites and energy	Downregulated	
	Psat1	Human	62–76 years versus 17–22 years	Single-cell RNA-seq	Biosynthesis of amino acids	Downregulated	
	Ifit2, *Pcdh1*, *Cdh5*, *Sfrp1*, *Cgn11*, *Klf6*, *Anxa7* and *Ctgf*	Brown Norway rats	18–20 months to 4–6 months	mRNA sequencing	Sertoli cell and round spermatozoa binding-related genes	Upregulated	Fice and Robaire (2023)
	β-galactosidase	Mice (C57BL/6J)	23 months versus 2 months	IF	Biomarkers of aging	Upregulated	Endo et al. (2024)
Leydig cells	*Pten*, *Rhob* and *Rock1*/2	Human	62–76 years versus 17–22 years	Single-cell RNA-seq	Suppress cell survival and proliferation	Upregulated	Nie et al. (2022)
	Prdx6, *Sod2*, *Mt2a*, *Mt1x*, *Nampt*, and *Hif1a*	Human	62–76 years versus 17–22 years	Single-cell RNA-seq	ROS-related	Upregulated	

(Continues)

TABLE 2 | (Continued)

Type of cell	Gene/protein/pathway	Species	Age comparison (aging versus youth)	Verification method	Function	Changes in aging animals (aging versus youth)	Refs.
	Col1a1/2, *Col3a1* and *Col5a1/2*	Human	62–76 years versus 17–22 years	Single-cell RNA-seq	ECM organization	Downregulated	
	Vit, *Sfrp1*, *Igf2*, *Penk and Ltbp4*	Human	62–76 years versus 17–22 years	Single-cell RNA-seq	Encoding secreted proteins	Downregulated	
	Pdgfra, *Itm2a*, *Scara5*, *Spry1*, *Ror1*, *Fgfr1* and *Egfr*	Human	62–76 years versus 17–22 years	Single-cell RNA-seq	Membrane receptors, respond less well to growth factor	Downregulated	
	Ptch1/2 and *Hhip*	Human	62–76 years versus 17–22 years	Single-cell RNA-seq, IF	Key components of Hedgehog (HH) signaling pathway	Downregulated	

Sertoli cells as a Wnt family protein, activates Wnts by binding to receptors on the surface of undifferentiated spermatogonia/β-Catenin signaling, promoting their proliferation (Takase and Nusse 2016). On the other hand, WNT5A in Sertoli cells regulates actin dynamics through Ror2-mediated mTORC1 and mTORC2 to maintain the BTB structure and stabilize the apical ES, which is essential for the release of elongated cells as well as the maintenance of the blood-testis barrier (Fu et al. 2021). Similarly, in *Carf* knockout mice, Wnt signaling was significantly reduced in Sertoli cells, which reduced GDNF secretion and further impaired the SSC self-renewal and proliferation of undifferentiated spermatogonia (Cui et al. 2020). The recent finding that the Wnt signaling pathway is significantly downregulated in senescent Sertoli cells suggests a mechanism by which the functional degeneration of senescent Sertoli cells has a diminished effect on germ cell support (D. Huang et al. 2023).

Recent single-cell sequencing results (D. Huang et al. 2023) show that the expression of transcriptional regulators of senescent Sertoli cells, such as *Wt1*, *Gata4*, and *Ar* is significantly downregulated. This will affect the expression levels of *Fyn*, a gene closely associated with germ cell survival and differentiation (J. Luo et al. 2012; Maekawa et al. 2002). In addition to the above, senescent Sertoli cell bodies are accompanied by decreased expression of genes related to energy metabolism, including *Hmgcs2*, *Idh1*, *Fgfr1*, *Src*, *Aco2*, *Uqcrc1*, *Akr1b1*, and *Pfkfb4*. And upregulated expression of inflammation-related genes such as *Il1a*, *Ifi27*, *Mif*, *Il6st*, and *Ccl2* (Nie et al. 2022). The altered levels of these transcripts due to the trophic support effect of Sertoli cells on the germ cell lineage may suggest a reduction in the overall metabolic function of senescent Sertoli cells as well as an increased level of inflammation, thus compromising their ability to support germ cells (Table 2).

In the physiological state, FSH stimulates Sertoli cells to produce inhibin, which provides a negative feedback signal to the hypothalamus to inhibit further FSH production and secretion. Studies have found that senescent Sertoli cells have reduced production of inhibins, perhaps because of a reduction in the autocrine capacity of Sertoli cells (Haji et al. 1994; Tenover et al. 1988). This would perhaps affect the balance of hormones in the reproductive system. Because the hypothalamic-pituitary-gonadal (HPG) axis degenerates in aging individuals, it is unclear whether the altered levels of inhibin secretion are a result of degeneration of the upstream secretory glands or a consequence of aging supporting cells.

4.1.3 | Decreased Endocytic Capacity of Sertoli Cells

Sertoli cells have a prominent endocytic function in addition to their typical secretory function (Elliott et al. 2010; Hai et al. 2014; Nakanishi and Shiratsuchi 2004). On the one hand, at the end of spermatogenesis, the adherens junctions (AJs) between the germ cells and Sertoli cells allows the Sertoli cells to endocytose the cytoplasm of elongated spermatids and ultimately shape the spermatids morphologically (Yefimova et al. 2018). On the other hand, approximately 50% of the germ cells undergo stepwise apoptosis and are endocytotically cleared to maintain the appropriate ratio of germ cells to Sertoli cells and create a stable microenvironment for spermatogenesis (Y.

S. Dong et al. 2016; Nakanishi and Shiratsuchi 2004). In aging animals, researchers have also found that the seminiferous epithelium lumen harbors many residual bodies that should have been phagocytosed by Sertoli cells, as well as developmentally arrested round spermatids (Humphreys 1977). The accumulation of these materials would worsen the local spermatogenic environment and further impede subsequent spermatogenesis.

As early as 1986, it was discovered that Sertoli cells pass autophagy to digest meiotic spermatid remnants (Chemes 1986). Autophagy, which is essential for the normal functional maintenance of Sertoli cells, would affect the assembly of ectoplasmic specialization (ES) (Yamamuro et al. 2021). Specific knockout of autophagy-related proteins ATG5 or Atg7 in Sertoli cells will result in malformed spermatozoa heads as well as hypomotile spermatozoa (C. Liu et al. 2016). Through PDLIM1, autophagy affects the basal ES to regulate the BTB and also mediates the normal release of Apical ectoplasmic specialization (AEs) (at the Sertoli cell-spermatids interface) to control spermatids (C. Liu et al. 2016). Recent studies have found that the level of autophagy inside senescent Sertoli cells decreases, which may be related to their internal lipid metabolism as well as the disturbance of Tight-Junction (TJ) function (Ma et al. 2022).

4.1.4 | Altered Cell Junctions in Sertoli Cells

The intercellular BTB structure creates an immune-protective environment. During Stages VII–VIII of the seminiferous epithelial cycle, old TJ proteins are degraded and new TJ proteins are produced. This allows preleptotene/leptotene spermatocytes to reach the intraluminal compartment via the BTB, where they mature under physiological conditions and acquire immune-privileged immunity (Cheng and Mruk 2012; O'Donnell et al. 2022; S. Wu et al. 2020). In aging animals, it has been found that the intercellular spaces between adjacent Sertoli cells become larger, cellular communication weakens, and the typical BTB structure is interrupted or disappears (D. Huang et al. 2023; Levy et al. 1999). This may be due to the significant activation of the p-p38 MAPK pathway, which translocates Atf2 from the cytoplasm to the nucleus, enhances MMP9 transcription, further downregulates ZO-1, occludin, and claudin-11 expression to destabilize the TJ barrier (Ma et al. 2022). It may also be due to downregulation in the expression levels of genes encoding tight junction proteins (D. Huang et al. 2023) (Table 2).

4.1.5 | Impairment of Cell Communication Between Sertoli and Germ Cells

Sertoli cells have extensive cellular communication with other intra-testicular cells (Oatley and Brinster 2012), but in aging animals, this communication is globally dysregulated. For example, when Sertoli cells and germ cells from Brown Norway rats were co-cultured in vitro, senescent Sertoli cells significantly disrupted communication with germ cells by failing to induce von Ebner-like protein mRNA in germ cells (Syed 2001, 2002). The ErbB3-NRG1 interaction between Sertoli cells and germ cells is remarkably lost, and the CADM1-NECTIN3 pair cannot be observed between Sertoli cells and early SPC/late RS/elongating spermatids (D. Huang et al. 2023). On the one hand, senescence renders Sertoli cells deficient in guiding chemical signals to germ cells, leading to impaired spermatozoa development. On the other hand, cell junction genes between Sertoli cells and round spermatids are also obviously upregulated in senescent Sertoli cells, such as *Ifit2*, *Pcdh1*, *Cdh5*, *Sfrp1*, *cgn11*, *Klf6*, *Anxa7*, and *Ctgf* (Fice and Robaire 2023). This disorder can affect the physical grasping ability of Sertoli cells for germ cells, possibly leading to the premature release of spermatids. Regarding cellular communication between Sertoli and germ cells, there are also some very important molecules such as vimentin that affects the positioning of spermatids within germ cells and was upregulated in the testis of aged Sprague-Dawley male rats (Duan et al. 2007). In addition, the transcription factor AP-1 has been found to be involved in Sertoli cell-mediated control of germ cell apoptosis (Suomalainen et al. 2004). However, its role in the aging process is still unclear.

In short, senescence leads to a decline in Sertoli cell function. First, its ability to phagocytose dead cells in seminiferous tubules decreases, leading to two outcomes: (1) accumulation of residual bodies within the functional lumen, worsening the spermatogenic environment; and (2) if phagocytosis is weakened, recycling of material is blocked (e.g., vesicular transport secretions, phospholipid bilayer maintenance, etc.), causing Sertoli cells to become depleted of the material used for synthesis and tend to self-perpetuate (Humphreys 1977). Second, its metabolic secretory capacity is reduced and signaling between germ cells is weakened. This means that the signaling guidelines required for germ cell development cannot be maintained, causing a developmental block in germ cells and further deteriorating the local seminiferous microenvironment, forming a vicious cycle. However, the specific mechanisms still need further exploration by researchers.

4.2 | Effects of Aging on the Functions of Leydig Cells

Aging is often accompanied by a reduction in the frequency and amplitude of GnRH pulses, resulting in decreased downstream gonadotropin and androgen production (Greenhill 2022; Wang et al. 2002). Leydig cells, present between seminiferous tubules, are the primary source of androgens (Naamneh Elzenaty et al. 2022). Androgens bind to androgen receptors (AR) on PTM and Sertoli cells to indirectly participate in spermatogenesis (De Gendt et al. 2004). In addition, Leydig cells secrete a range of growth factors as well as steroids such as α-transforming growth factor β (TGF-β), insulin-like growth factor 1 (IGF1), insulin-like peptide 3 (INSL3), estrogen, and thyroid hormones among others to regulate spermatogenesis (Svechnikov et al. 2010). Androgens maintain spermatogenesis mainly through AR. When testicular androgen concentrations decrease, AR levels are also significantly reduced in Sertoli cells, further resulting in disturbed spermatogenesis (Smith and Walker 2014). Testosterone is one of the most important androgens and during spermatogenesis, it promotes meiotic progression, regulates the morphological transition of round spermatids and affects the ectoplasmic specialized structures of Sertoli cells

(Zhou et al. 2019). This includes facilitating the assembly of new BTB components during the passage of preleptotene spermatocytes through the BTB, facilitating the release of mature spermatozoa, and maintaining the connection of Sertoli cells to round spermatozoa (Cheng and Mruk 2010, 2012).

The net effect of increasing age is a reduction in testosterone levels. Two credible pieces of evidence come from human clinical trials. One study suggests that serum testosterone levels in men begin to decline from the age of 35 (Handelsman et al. 2015). Another, more detailed study reports that in men aged 40–70 years, total serum testosterone decreases at an annual rate of 0.4% (F. C. Wu et al. 2008), which may be due to aging of the hypothalamic-pituitary-testicular axis (Cannarella et al. 2022; Kaufman et al. 2019), with a decline in the number as well as a function of GnRH neurons leading to reduced GnRH secretion, reduced pituitary LH secretion levels with the caveat that pituitary gonadotropins are responsive to GnRH and remain normal (Iranmanesh et al. 2010), and reduced signals transmitted to Leydig cells (Greenhill 2022; Veldhuis et al. 2009). Meanwhile the deterioration of testicular microenvironment is also involved (Curley et al. 2019). On the one hand, stromal cell intrinsic factors, including the number of Leydig cells, secretory capacity, and responsiveness to LH also decrease with age (Anawalt and Matsumoto 2022; Mularoni et al. 2020; Nie et al. 2022). Some evidence comes from studies by *Zirkin* and *Papadopoulos* in Brown Norway rats (Chen et al. 2002; Grzywacz et al. 1998). Their results showed that aged rats had the same LH levels as young rats, but the reduced testosterone secretion in aged rats was due to a decrease in cellular responsiveness to LH. One of the mechanisms behind this is related to the low expression of mitochondrial translocator protein (18 kDa; TSPO) in aged Leydig cells. When the researchers knocked out TSPO in MA-10 cells, these cells showed disruption in mitochondrial function and membrane dynamics, and the ability to synthesize steroids decreased. The knockout phenotype was rescued by treatment with the mitochondrial fusion promoter M1 or by increasing mitochondrial fusion through optic atrophy 1 (OPA1) overexpression. Similarly, treatment of aged rats with M1 restored mitochondrial function in Leydig cells and increased testosterone synthesis. Impaired mitochondrial dynamics caused by TSPO may be an important mechanism for the reduced testosterone secretion capacity of aged Leydig cells (Garza et al. 2022). This is also likely because of defects in the LH cAMP signaling cascade in senescent Leydig cells, as well as their altered circadian rhythm of cAMP (Baburski et al. 2016). The cAMP/PKA signaling pathway is known to be critical for the expression of steroidogenic enzymes and the transport of cholesterol into mitochondria (Aghazadeh et al. 2015). In addition to this, decreased cholesterol import in aging Leydig cells, impaired function of mitochondria involved in cGMP signaling (Sokanovic et al. 2021), and abnormal activation of the MAPK pathway are important contributors (D. Luo et al. 2023; Sokanovic et al. 2014). In addition, STAR, TSPO, CYP11A1, and downstream steroidogenic enzymes were also reduced, which may indicate that steroidogenesis is impaired in aged Leydig cells (Culty et al. 2002; L. Luo et al. 2001, 2005).

On the other hand, elevated oxidative stress levels also play a significant role in the age-related decline in testosterone secretion (Beattie et al. 2013). Studies have shown that the aging of Leydig cells is accompanied by an increase in the oxidation of the intracellular environment (Cao et al. 2004; H. Chen et al. 2001; L. Luo et al. 2006). First, mitochondrial superoxide production was increased in Leydig cells of aged rats compared with young rats (H. Chen et al. 2001). Secondly, the antioxidant idants superoxide dismutase-1 and -2, glutathione peroxidase, and glutathione (GSH) decrease with age (Cao et al. 2004; L. Luo et al. 2006). Further studies by (Chen et al. 2015) revealed that SIRT1 and NRF2 are involved in maintaining the oxidant/antioxidant environment of Leydig cells, thereby sustaining steroid production. In Nrf2 knockout mice, a decrease in serum testosterone levels and Leydig cell testosterone occurs as early as middle age and becomes more severe with aging, and the decrease in testosterone is associated with an increased expression of protein nitrotyrosine residues (a marker of ROS) (H. Chen et al. 2015). SIRT1 has been shown to promote the entry of NRF2 into the nucleus and increase its transcriptional activity (Ding et al. 2016; K. Huang et al. 2013). The results of in vitro MA-10 cell studies showed that inhibition of *Sirt1* in MA-10 cells with nicotinamide (NAM), or Nrf2 with ML385, would reduce the production of progesterone. Consistently, the use of siRNA to inhibit the expression of these two genes also reduced progesterone and increased ROS. By treating Leydig cells formed by stem cells on the surface of seminiferous tubules with NAM, ROS increased, antioxidants GPx and CAT decreased, and testosterone production was also significantly reduced. After removing NAM, testosterone secretion was restored. In the presence of SIRT1 or NRF2 pharmacological activators magnolol or sulforaphane, oxidants could not cause Leydig cells to produce the same level of ROS as the control group, and testosterone production was higher than the control group due to the presence of these activators. These results suggest that *Sirt1* and *Nrf2* can maintain a normal oxidative/antioxidant intracellular environment in Leydig cells by inhibiting oxidative stress (J. Y. Chung et al. 2021). However, it remains unclear whether this mechanism is species conserved, and the mechanism by which changes in the intracellular oxidative/antioxidant environment affect testosterone production in Leydig cells is also uncertain.

Recent studies have provided additional insights into the mechanisms of senescence in stromal stem Leydig cells (SLCs), which contribute to the decline in testosterone production in aging individuals. Researchers have found that SLCs have a reduced capacity to differentiate during aging. A decrease in mitochondria-ER contacts (MERCs) among SLCs contributes to the dysfunction of SLC mitochondria and ER during aging. The intermediate filament protein nestin, which regulates MERC levels through a Keap1-nestin/Nrf2 competitive binding model, was found to decrease in expression. This affected the differentiation of SLCs. Melatonin supplementation can protect stem cells by reducing oxidation levels, restoring nestin expression, and maintaining mitochondrial homeostasis (Yao et al. 2022).

Recent advances in single-cell sequencing technologies have revealed molecular-level alterations in senescent Leydig cells. In aging individuals, these cells exhibit higher levels of transcriptome changes associated with ROS, such as *Prdx6*, *Sod2*, *Mt2a*, *Mt1x*, *Nampt*, and *Hif1a*. Additionally, there is decreased expression of genes associated with the extracellular matrix

(ECM), such as *Col1a1*/2, *Col3a1*, and *Col5a1*/2. Genes encoding cytokines, such as *Vit*, *Sfrp1*, *Igf2*, *Penk*, and *Ltbp4* are also downregulated. Researchers have also found that the responsiveness of Leydig cells to many cytokines decreases with age. This is due to the downregulation of several membrane receptors, including *Pdgfra*, *Itm2a*, *Scara5*, *Spry1*, *Ror1*, *Fgfr1*, and *Egfr* (Nie et al. 2022). Additionally, several transcription factors such as *Klf6*, *Jun6*, *Nr2f6*, *Hes1*, *Jun6*, and *Etv5* regulate DEGs related to senescence. This may further promote the senescence process in Sertoli cells. These results suggest that Leydig cells in aging individuals may experience increased levels of autoxidation and derangements in signaling (W. Zhang et al. 2023) (Table 2).

4.3 | Effects of Aging on Other Cells in the Spermatogenic Microenvironment

In addition to Sertoli cells and Leydig cells, the spermatogenic microenvironment also contains other types of cells, such as PTM cells, macrophages, endothelial cells, etc. Although there have been few studies specifically investigating the role of these cells in testicular aging, some results suggest some changes associated with aging.

Testicular endothelial cells are an essential component of the niche for SSCs. They express cytokines and growth factors such as GDNF, FGF-2, FGF-5, stromal cell-derived factor-1 (SDF-1), macrophage inflammatory protein 2 (MIP-2), and insulin-like growth factor binding protein 2 (IGFBP-2) to support the growth and maintenance of SSCs (Bhang et al. 2018; Kitadate et al. 2019). Recently, a study described changes in the function and characteristics of testicular endothelial cells in 2-year-old mice compared to 2-month-old mice. Senescence markers such as SA-β-gal and p21 were significantly upregulated in the testicular endothelial cells of 2-year-old mice. Co-culturing endothelial cells from mice of different ages with GSCs showed that endothelial cells from aged mice were unable to support the sustained proliferation of GSCs. Subsequently, the researchers used senolytics to perform rescue experiments, and aged endothelial cells treated with SRC/tyrosine kinase inhibitor dasatinib and natural flavonoid quercetin showed the same ability to support the proliferation of GSCs as young endothelial cells (Ozawa et al. 2023). The above results indicate that the ability of aged endothelial cells to maintain spermatogonia proliferation weakens with aging, which may be one of an important reason for the decline in testicular spermatogenesis in aged individuals.

PTM cells, the main cellular components of the wall of seminiferous tubules, and sertoli cells together delimit the niche at the base of the seminiferous epithelium (L. Y. Chen et al. 2016; Zhou et al. 2019). After Gdnf was specifically knocked out in PM cells, the undifferentiated spermatogonia of mice could not be replenished in time due to the lack of GDNF signal, which eventually led to infertility of mice (L. Y. Chen et al. 2016). Knocking out AR (also denoted NR3C4) specifically in PM cells rendered mice infertile with a reduction in all germ cell types, indicating that PM cells receive testosterone signals and are involved in regulating spermatogenesis (Welsh et al. 2009). In addition, according to in vitro research results, PM cells also express Colony stimulating factor-1 (CSF1), which enhances the self-renewal of SSCs (Oatley et al. 2009), secretes IGF1 and FGF-2, P-Mod-S (Skinner et al. 1989), Leukemia inhibitory factor (LIF) (Piquet-Pellorce et al. 2000) and stimulates supporting cells to produce TRF and ABP to regulate spermatogenesis (Hoeben et al. 1999).

Collagen I and laminin are important proteins that constitute the wall of seminiferous tubules and are secreted by PM cells. A study of human-aged male testes showed that collagen I expression increased at the base of seminiferous tubules, while laminin expression showed an irregular/unsmooth curve at the basement membrane (Nie et al. 2022). In addition, proteins involved in the maintenance and remodeling of basement membrane and ECM, such as COL3A1, LAMB1, and NID1, were downregulated (Nie et al. 2022). The above results indicate that the secretion of ECM of aged TPCs is reduced, and some proteins are deposited in the ECM, which may affect the elasticity of the ECM and thus impair the transport process of nutrients to affect SSCs. In addition, some proteins related to smooth muscle contraction, such as ACTA2, MYH11, MYLK, MYL9, and TPM2, were downregulated in aged TPCs, which may impair the ability of TPCs to assist sperm transport into the tubular lumen. In vitro experiments also demonstrated that the contractile capacity of aged TPCs was significantly lower than that of young TPCs (Nie et al. 2022). Another in vitro study from marmoset monkey PM cells (Proteomic Insights into Senescence of Testicular Peritubular Cells from a Nonhuman Primate Model) found that proteins associated with smooth muscle activity were also downregulated in aged cells (Stöckl et al. 2021) (Table 3). However, it is still unclear whether these in vitro findings reflect the changes in PM cells in vivo under aging conditions and need to be viewed critically.

5 | Summary and Future Perspectives

Advanced paternal age is becoming increasingly common due to socioeconomic and parenting perceptions. Aging affects cellular functions systemically, including the reproductive system. Although men can produce sperm throughout their lives, spermatozoa count and quality decline with age due to multiple factors involved in spermatogenesis. On one hand, aging may decrease blood supply to the testes, reducing oxygen and nutritional support (Brito et al. 2021). On the other hand, the accumulation of adverse environmental factors with age can lead to the buildup of metabolic waste and increased oxidative stress levels in the testicular intracellular environment (Santiago et al. 2019). These factors all worsen the seminiferous microenvironment. Sertoli cells, which are somatic cells that play an important role in aging, act as nurse cells for germ cells throughout spermatogenesis. They provide a physical and immunological barrier for germ cells and shape spermatids at later stages, and secrete key factors, including hormones, cytokines, and growth factors, which regulate germ cell proliferation, differentiation, and survival (O'Donnell et al. 2022; S. Wu et al. 2020). Senescent Sertoli cells exhibit a range of functional decline. First, insufficient promotion of stem cell self-renewal affects the maintenance of the stem cell pool. Second, degradation of their ectoplasmic specialized

TABLE 3 | Aging-related changes in the levels of gene expression in endothelial cells and peritubular myoid (PTM) cells.

Type of cell	Gene/protein	Species	Age comparison (aging versus youth)	Verification method	Function	Changes in aging animals (aging versus youth)	Refs.
Endothelial cells	*Hla-dra*, *Hla-drb1*, *Hla-dpa1*	Human	62–76 years versus 17–22 years	Single-cell	MHC Class II genes, antigen processing and presentation	Upregulated	Nie et al. (2022)
	Kdr, *Pdgfb*, *Tie1*	Human	63–76 years versus 17–22 years	Single-cell	"Vasculature development" and "regulation of angiogenesis"	Downregulated	
Peritubular myoid (PTM) cells	*Cdkn1a*, *Mdm2*, *Zmat3*, *Meg3*, and *Gadd45a*	Human	64–76 years versus 17–22 years	Single-cell	p53-Directed cell cycle arrest or cell death	Upregulated	
	p21 (encoded by *Cdkn1a*) and *Acta2*	Human	65–76 years versus 17–22 years	IF, Single-cell	Associated with cellular senescence	Upregulated	
	Sqstm1, *Bax*, and *Lamp2*	Human	66–76 years versus 17–22 years	Single-cell	Autophagy	Upregulated	
	Collagen I	Human	68–76 years versus 17–22 years	IF, Single-cell	Form the wall of seminiferous tubules	Upregulated	
	Laminin	Human	69–76 years versus 17–22 years	IF	Form the wall of seminiferous tubules	Abnormal morphology: irregular/unsmooth	
	Acta2, *Myh11*, *Mylk*, *Myl9*, and *Tpm2*	Human	70–76 years versus 17–22 years	Single-cell	Smooth muscle contraction	Downregulated	
	Col1a1/2, *Col3a1*, *Lama2*/4, and *Lama4*	Human	67–76 years versus 17–22 years	Single-cell	Core matrisome genes	Downregulated	
	CNN1	Common marmoset monkeys (*Callithrix jacchus*)	10–12 years versus 2–3 years	Immunohistochemistry	Regulates cell contraction	Upregulated	Stöckl et al. (2021)
	COL1A1	Common marmoset monkeys (*Callithrix jacchus*)	10–12 years versus 2–3 years	Immunohistochemistry	Participates in the formation of extracellular matrix	Upregulated	

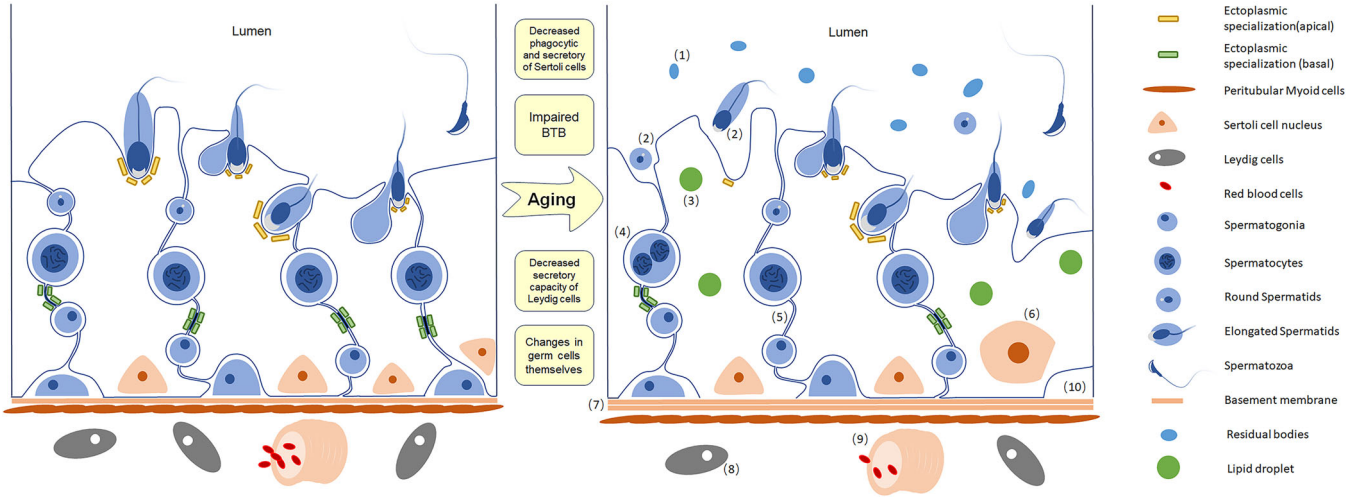

FIGURE 2 | Schematic diagram of the effects of aging on testicular spermatogenic epithelial cells. (1) Residual bodies that have not been phagocytosed by Sertoli cells in the lumen of seminiferous tubules. (2) Prematurely released round spermatids and elongated spermatids. (3) Dispersed lipid droplets are a sign of Sertoli cell lipid metabolism dysfunction. (4) One of the symptoms of spermatocyte abnormalities is multinucleated spermatocytes, which will eventually undergo apoptosis and be engulfed by Sertoli cells. (5) Disordered BTB structure. (6) Sertoli cells with abnormal nuclei. (7) Thickened basement membrane. (8) The number of Leydig cells has been reduced. (9) The amount of blood flowing to the testicles is comparatively reduced due to systemic variables related to age. (10) SSCs' capacity for differentiation and multiplication is reduced.

structure affects the stability of the BTB and deteriorates the seminiferous microenvironment. It also affects the ability to grasp spermatids at later stages, leading to the premature release of immature spermatids. Additionally, reduced secretion from Sertoli cells compromises their supportive role in spermatogenesis. Testosterone is primarily secreted by Leydig cells. Aging, accompanied by a decline in testosterone levels, affects not only the normal development of germ cells but also the normal function of Sertoli cells (Figure 2). The maintenance of spermatogenic processes is also influenced by other somatic cells, such as PTM cells, which construct the physical architecture of the stem cell microenvironment and have essential secretory capacity for the proliferation and differentiation of SSCs. In addition to alterations in the seminiferous microenvironment, senescent germ cells also exhibit characteristics of aging. These include downregulated expression of genes involved in SSC maintenance of stemness and hindrance of SSC self-renewal by low levels of ROS. There is also a decline in the level of differentiation of senescent spermatogonia, an elevated rate of meiotic errors in spermatocytes, and an increased rate of spermatid malformations (Figure 2). Recent advances in single-cell sequencing technology have led to the publication of many articles in the field of reproductive aging (Alfano et al. 2021; Hermann et al. 2018; D. Huang et al. 2023; Liao et al. 2021; Nie et al. 2022; W. Zhang et al. 2023). This has greatly aided our understanding of cellular transcript levels in the senescent state. However, it is important to consider the conservation of genes between different studied species. Recent reports (H. Bao et al. 2023) have also summarized the publication of senescence-related biomarkers, which is a great aid to the study of standardized senescence. Despite these advances, studying the effects of aging on spermatogenesis is difficult due to the overall level of alteration and the difficulty in controlling experimental variables. This presents limitations to both in vitro and in vivo findings. It is still unknown whether molecular-level alterations that accompany aging are a consequence or a cause of the aging phenotype. Determining causality will be essential to understanding the mechanisms underlying the effects of aging on spermatogenesis.

Author Contributions

T.L., W.X., L.Y., J.Z, and O.D. reviewed the literature. T.L. wrote the manuscript. S.Y. and Z.Z. conceived and revised the manuscript.

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Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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