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REVIEW ARTICLE

Autophagy in testicular physiology and male fertility: Mechanisms, dysregulation, and translational opportunities

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Abstract

Autophagy is a highly conserved intracellular degradation pathway that plays a central role in maintaining testicular homeostasis and male reproductive function. This review summarises recent advances in the understanding of autophagy in the testis, with particular emphasis on its roles in Sertoli cells, Leydig cells, and germ cells, as well as its regulatory mechanisms and implications for male infertility. Autophagy contributes to key processes, including spermatogenesis, spermiogenesis, blood–testis barrier integrity, and steroidogenesis, through coordinated organelle turnover and cellular remodelling. Core regulatory pathways such as mTOR, AMPK, PI3K/Akt, and oxidative stress–responsive signalling dynamically controls autophagic flux in response to metabolic and environmental cues. Emerging evidence highlights extensive molecular crosstalk between autophagy, apoptosis, ferroptosis, and ubiquitin-mediated proteostasis, with oxidative stress serving as a central upstream regulator. Dysregulation of autophagy, whether through impaired flux or excessive activation, is strongly associated with male infertility phenotypes, including azoospermia, oligozoospermia, and asthenozoospermia. These effects are driven by defects in germ cell development, mitochondrial function, and hormonal balance, and are further exacerbated by environmental toxicants, lifestyle factors, and ageing. Although autophagy-related proteins such as microtubule-associated protein 1 light-chain 3, p62, and Beclin-1 show promise as biomarkers, their clinical application remains limited by challenges in accurately measuring autophagic flux and context-dependent interpretation. Therapeutic modulation of autophagy using pharmacological agents and antioxidants represents a promising but complex strategy that requires precise, context-specific application. Overall, this review underscores the dual role of autophagy in male fertility and highlights the need for improved biomarkers and translational approaches.

Keywords: AMPK, autophagic flux, autophagy, Leydig cells, mTOR, spermatogenesis, Sertoli cells

INTRODUCTION

Autophagy is a highly conserved intracellular degradation pathway that regulates cellular homeostasis by transporting cytoplasmic components to double-membrane autophagosomes for lysosomal degradation. This process ensures nutrient recycling while also removing damaged organelles and protein aggregates under both basal and stress conditions.[1] A core set of proteins, including autophagy-related (ATG) proteins, Beclin-1, and microtubule-associated protein 1 light-chain 3 (LC3), orchestrate the autophagic process by regulating phagophore initiation, autophagosome formation, and lysosome fusion. In addition to bulk autophagy, selective pathways, such as mitophagy and aggrephagy, target the degradation of mitochondria and protein aggregates, respectively.[2,3]

Throughout spermatogenesis, Sertoli cells, Leydig cells and germ cells interact cooperatively to maintain testicular function. Sertoli cells form and maintain the blood-testes barrier (BTB), as well as provide metabolic and structural support to developing germ cells. Leydig cells produce testosterone, which is required for spermatogenesis. Germ cells undergo mitotic proliferation, meiosis, and extensive morphological remodelling during

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spermiogenesis.[1,4] These processes necessitate precise regulation of organelle turnover, cytoplasmic remodelling, and cellular quality control, all of which are heavily reliant on proteostatic mechanisms like autophagy.[1,5]

Recent research has directly linked autophagy to various aspects of testicular physiology, including stage-specific gene expression during spermatogenesis, regulation of spermatid elongation and spermiation, turnover of ectoplasmic specialisations and tubulobulbar complexes in Sertoli cells, and modulation of steroidogenic activity in Leydig cells.[4,6–8] Furthermore, environmental stressors, such as heavy metals, persistent organic pollutants, particulate matter, and engineered nanoparticles, interfere with autophagic flux in testicular cells, providing mechanistic insight into environmentally induced male infertility.[9–11]

This review will summarise recent advances in autophagy research in the testis, investigate molecular regulatory pathways, assess the impact of dysregulation on fertility, and identify translational opportunities for therapeutic intervention.

Autophagy regulation in specific testicular cells

Successful male reproduction is fundamentally dependent on the physiological integrity of the testes, which primarily comprise seminiferous tubules and the surrounding mesenchyme. Within the seminiferous tubules, the coordinated activity of Sertoli cells and various germ cell lineages drives spermatogenesis. Conversely, the interstitium or mesenchyme houses Leydig cells, which serve as the primary functional units for endocrine regulation. In recent years, an increasing body of evidence has established that autophagy is a critical mediator in numerous testicular cellular processes. Beyond its fundamental role in supporting spermatogenesis, autophagy serves as an essential regulatory mechanism for maintaining the integrity of ectoplasmic specialisations within Sertoli cells and the functionality of the BTB. Furthermore, autophagic activity within Leydig cells is indispensable for efficient steroidogenesis and the maintenance of systemic testosterone levels.[3]

Sertoli Cells (Support cells)

As essential somatic constituents of the seminiferous tubules, Sertoli cells provide the requisite nutritional and structural scaffolding for developing spermatozoa. Spermatogenesis proceeds in intimate association with the Sertoli cells' surface, where these cells exert rigorous regulatory control over the germinal epithelium.[12] Central to their endocrine function is the synthesis and secretion of androgen-binding protein (ABP), a testicular glycoprotein that facilitates the transport and localised concentration of testosterone and dihydrotestosterone.[13] Recent evidence suggests that testosterone acts as a molecular rheostat, selectively modulating the autophagic degradation of ABP within Sertoli cells to maintain androgen homeostasis.[12]

Beyond their secretory role, Sertoli cells are the primary components of the BTB, which is fundamental in determining germ cell capacity and maintaining the specialised microenvironment required for germ cell maturation.[14] The functional importance of autophagy in this context has been underscored by mouse models, in which Sertoli cell-specific disruption of autophagic pathways impairs ectoplasmic specialisation assembly and induces subsequent cytoskeletal disorganisation.[15] Mechanistically, autophagy preserves BTB integrity and actin-based ectoplasmic specialisation dynamics by mediating the targeted degradation of PDZ and LIM domain protein 1 (PDLIM1). The aberrant accumulation of this cytoskeletal-associated protein is known to compromise barrier stability.[15] Consequently, autophagic flux in Sertoli cells integrates structural maintenance with endocrine signalling, as testosterone-mediated inhibition of ABP degradation ensures the sustained protein half-life necessary for successful spermatogenesis.[12,13]

Leydig Cells (Steroid-producing cells)

In Leydig cells, autophagy, particularly lipophagy and mitophagy, is highly active and directly contributes to testosterone biosynthesis.[3] Autophagy facilitates the breakdown of intracellular lipid droplets and total cholesterol, releasing free cholesterol required for steroid hormone production.[14] Disruption of autophagy through genetic deletion of key autophagy genes, such as *Atg7* or *Atg5*, impairs cholesterol mobilisation and reduces testosterone synthesis,[16] highlighting the essential role of autophagy in endocrine function.

Germ Cells (spermatogenesis)

The development of male germ cells encompasses a precise sequence of spermatogenesis, maturation, and capacitation. Within these lineages, autophagy exerts a

multifaceted influence, modulating cell survival or programmed cell death depending on the specific physiological context. Autophagic flux is indispensable for successful spermatogenesis, particularly during the terminal phase of spermiogenesis. It facilitates acrosome biogenesis, flagellar assembly, and the systematic removal of redundant cytoplasm from developing spermatids.[3]

Consequently, the stringent regulation of autophagy is a prerequisite for the production of functionally competent spermatozoa. Beyond structural remodelling, autophagy is implicated in the induction of diploid germ cells [17] and plays a role in testosterone biosynthesis, potentially serving as a mechanism to supply the endogenous energy required for germ cell development.[18] Through these integrated pathways, autophagy ensures both the morphological integrity and the metabolic viability of the male gametes.

Key molecular regulatory pathway

The regulation of autophagy in testicular tissues is governed by a complex network of signalling pathways that respond to metabolic, oxidative, and environmental cues. The mechanistic target of rapamycin complex 1 (mTORC1) serves as the primary inhibitory regulator of this process. In testicular cells, physiological stressors, such as nutrient deprivation or energy deficiency, lead to the inactivation of mTORC1, thereby de-repressing the autophagic machinery.[19,20] This is complemented by the activity of AMP-activated protein kinase (AMPK), an intracellular energy sensor activated by low ATP/AMP ratios. Upon activation, AMPK induces autophagy through a dual mechanism: the indirect inhibition of mTORC1 and the direct phosphorylation of the Unc-51-like autophagy activating kinase 1 (ULK1) complex, which is essential for autophagy initiation.[21]

The phosphoinositide 3-kinase/protein kinase B/mechanistic target of rapamycin (PI3K/Akt/mTOR) signalling pathway represents another critical regulatory axis, acting predominantly as a negative regulator of autophagic flux. This pathway is particularly influential during testicular development, where it modulates the proliferation and differentiation of spermatogonia.[22] It exerts control by either suppressing autophagy, as evidenced in models of cryptorchidism, or by regulating the expression of key autophagy-associated proteins, such as LC3 and Beclin1.[23] Under conditions of oxidative stress, the accumulation of reactive oxygen species (ROS) leads to the suppression of the PI3K/Akt/mTOR axis, resulting in a compensatory increase in autophagic flux.[24] In parallel, hypoxia-inducible factor-1 alpha (HIF-1α) functions as a vital mediator under hypoxic or pathological conditions, such as diabetes. HIF-1α promotes mitophagy (selective degradation of mitochondria) via the activation and homodimerization of BNIP3, especially within Sertoli cells, to facilitate mitochondrial turnover.[25]

Recent investigations have identified further specialised regulatory mechanisms, including the Sox9–Srag pathway. The transcription factor Sox9 induces the expression of the *srag* gene, which enhances autophagy through its direct interaction with Beclin 1.[26] Furthermore, oxidative stress triggers the ROS–nuclear factor erythroid 2-related factor (NRF2)–sequestosome 1 (p62) signaling axis, promoting the degradation of damaged cellular components and reinforcing cellular defence mechanisms against proteotoxicity.[27]

The complex signalling pathways involved are summarised in FIG 1.

Molecular Crosstalk and Regulatory Networks in Testicular Autophagy

Molecular crosstalk among autophagy, apoptosis, ferroptosis, oxidative stress, and the ubiquitin system, further modulated by epitranscriptomic mechanisms, forms a highly integrated regulatory network in the testis that governs male fertility. This network primarily influences spermatogenesis and germ cell quality.[28,29] While autophagy generally maintains cellular homeostasis, its dysregulation, together with excessive activation of apoptosis and ferroptosis, contributes to impaired sperm production, including conditions such as oligozoospermia and asthenozoospermia.[30]

Molecular Crosstalk in Male Fertility

Autophagy and apoptosis are closely interconnected in testicular cells and share common regulatory proteins, including Bcl-2/Beclin-1 and p53, that determine whether damaged cells survive or undergo programmed cell death.[31] Under physiological conditions, autophagy acts as a protective mechanism by removing damaged organelles and maintaining cellular integrity. However, excessive oxidative stress, particularly through elevated ROS, can simultaneously activate apoptosis and autophagy in germ cells, ultimately leading to male

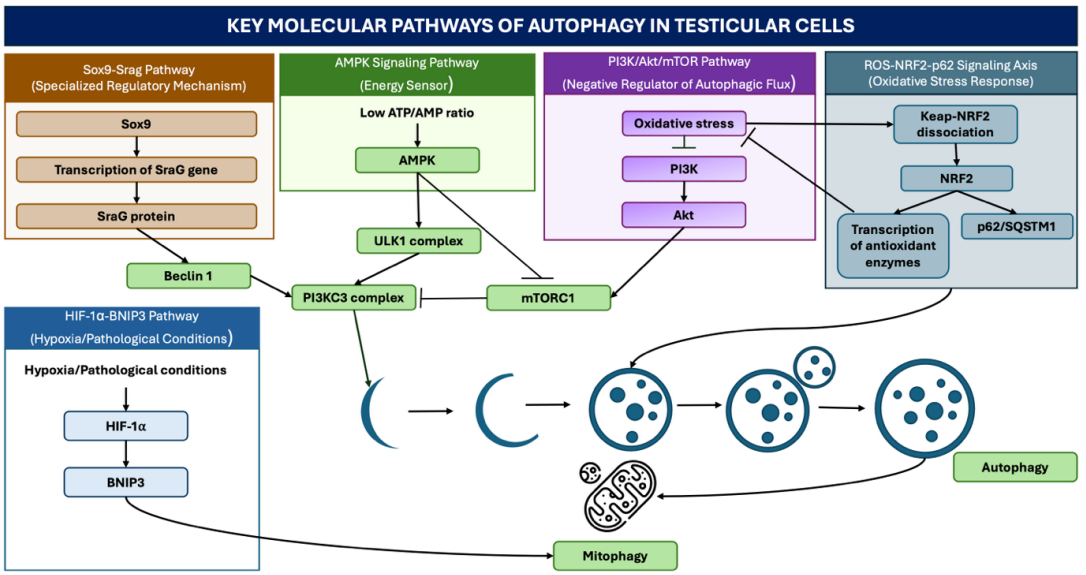


FIG. 1. Key molecular pathways of autophagy in testicular cells. Abbreviations: ⊥, inhibits/suppresses; AKT, protein kinase B; AMPK, AMP-activated protein kinase; ATP/AMP, adenosine triphosphate/adenosine monophosphate; BNIP3, Bcl-2/adenovirus E1B 19-kDa interacting protein 3; HIF-1a, hypoxia-inducible factor-1 alpha; Keap, Kelch-like ECH-associated protein; mTORC1, mechanistic target of rapamycin complex 1; NRF2, nuclear factor erythroid 2-related factor; p62/SQSTM1, sequestosome 1; PI3K, phosphoinositide 3-kinase; PI3KC3, phosphatidylinositol 3-kinase catalytic subunit type 3; ROS, reactive oxygen species; Sox9, SRY-Box Transcription Factor 9; SraG, small RNA G; and ULK1, Unc-51-like autophagy activating kinase 1.

infertility.[32]

Autophagy also exhibits significant crosstalk with ferroptosis, particularly through selective pathways such as mitophagy and ferritinophagy. Mitophagy facilitates the removal of damaged mitochondria[33], thereby reducing iron-dependent ROS production and limiting oxidative damage.[34,35] Conversely, ferritinophagy promotes ferritin degradation, increasing intracellular labile iron levels and enhancing susceptibility to ferroptosis.[36] This dual role highlights the context-dependent nature of autophagy, especially in spermatogonia, where iron metabolism and lipid peroxidation are tightly regulated.

Oxidative stress serves as a central driver linking these pathways. Elevated ROS levels induce lipid peroxidation, which damages sperm plasma membranes and contributes to ferroptotic cell death, while also causing DNA fragmentation that triggers apoptosis.[37–39] Thus, oxidative stress acts as a critical upstream regulator, coordinating the balance between survival and cell death pathways in the testis (FIG 2).

The inter-relationship between autophagy, apoptosis and ferroptosis is illustrated in FIG 2.

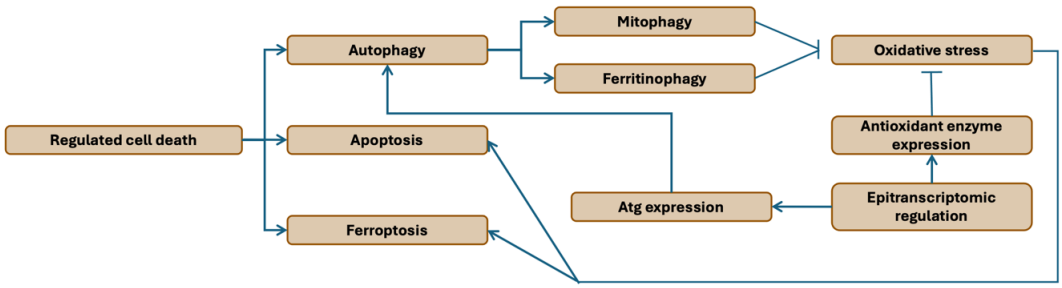
The Role of the Ubiquitin System

The ubiquitin–proteasome system (UPS) plays an essential role in protein quality control and is particularly important during spermiogenesis. It contributes to nuclear remodelling and cytoplasmic reduction by selectively degrading proteins required for spermatid maturation.[40]

The ubiquitin system also helps to degrade misfolded or unfolded proteins produced under stress conditions in the endoplasmic reticulum (ER).[41,42] In addition, ubiquitination of specific autophagy-related proteins, such as Atg12, regulates the balance between autophagy and apoptosis, thereby influencing cell fate decisions.[43]

Epitranscriptomic Control of Cell Fate

Epitranscriptomic regulation, particularly through N6-methyladenosine (m^6A) RNA methylation, represents an additional layer of control over testicular function. This modification regulates mRNA stability, translation efficiency, and degradation, thereby influencing gene expression programs essential for spermatogenesis.[44,45] Through modulation of



Mode of cell death	Sperm quality	Germ cell integrity	Blood-testis barrier integrity	Leydig cells' testosterone production
Autophagy	↑	↑	↑	↑
Apoptosis Ferroptosis	↓	↓	↓	↓

FIG. 2. Molecular crosstalk and regulatory networks in testicular autophagy. Abbreviations:; ↑, increase; ↓, decrease; ⊥, inhibits/suppresses; Atg, autophagy gene.

Atgs and apoptotic regulators, m^{6}A methylation directly affects the balance between cell survival and cell death.[46] In spermatogonial stem cells, these epitranscriptomic modifications play a crucial role in maintaining the balance between self-renewal and differentiation.[47] Furthermore, epitranscriptomic regulation influences cellular responses to oxidative stress by altering the stability of mRNAs encoding antioxidant defence proteins. This modulation affects the cellular capacity to counteract ROS accumulation and thereby influences susceptibility to ferroptosis.[48]

Impact on Male Fertility

Disruption of the coordinated network linking autophagy, apoptosis, ferroptosis, and the ubiquitin system has significant consequences for male fertility.[28] Dysregulation of these pathways contributes to spermatogenic failure, including non-obstructive azoospermia, which is associated with decreased expression of ferroptosis-inhibiting proteins, such as glutathione peroxidase 4 and increased intracellular iron levels.[49]

The interplay between these pathways also governs sperm maturation and quality. Proper regulation of autophagy ensures efficient removal of defective organelles and cytoplasmic components, while an imbalance in autophagy–apoptosis–ferroptosis signalling leads to impaired spermatogenesis and compromised BTB integrity.[28]

In Leydig cells, autophagy supports testosterone production by regulating lipid metabolism and mitochondrial function.[3] However, excessive ferroptosis, often associated with metabolic disorders, such as obesity or exposure to environmental toxicants, disrupts this process and contributes to endocrine dysfunction.[28]

From a therapeutic perspective, targeting these interconnected pathways offers promising strategies for the treatment of male infertility. Inhibition of ferroptosis using agents such as ferrostatin-1 or modulation of autophagy pathways has shown potential for mitigating oxidative stress–induced reproductive damage.[50,51] These approaches may provide a foundation for future interventions aimed at restoring testicular function and improving male fertility outcomes.

Autophagy Dysregulation and Male Infertility

Autophagy, a lysosome-dependent cellular recycling process, is essential for maintaining spermatogenesis, sperm maturation, and testicular structural integrity. It operates as a key quality-control mechanism by removing damaged organelles and proteins, thereby supporting germ cell development and endocrine function.[3,52] Dysregulation of autophagy, whether through impairment or excessive activation, has emerged as a major, yet often underappreciated, contributor to male infertility. Such dysregulation leads to decreased sperm quality, structural abnormalities, and impaired

hormonal regulation.[53]

Genetic and Clinical Consequences

Autophagy dysregulation is strongly associated with severe sperm abnormalities and clinical infertility phenotypes, including azoospermia, oligozoospermia, and teratozoospermia.[30] Genetic studies have provided direct evidence for this association. Whole-exome sequencing in patients with non-obstructive azoospermia (NOA) has identified pathogenic variants in key autophagy-related genes, including *ATG4A*, *ATG4B*, and *ATG4D*.[54] In parallel, reduced expression of core autophagy markers, such as LC3, Beclin1, ATG5 and ATG7, correlates with decreased sperm count[55] and increased DNA fragmentation index.[56] This observation indicates impaired autophagic activity in infertile individuals.

Functional studies further demonstrate that disruption of autophagy machinery has profound effects on spermiogenesis. Knocking out *ATG5* or *ATG7* in germ cells impairs cytoplasmic clearance and acrosome formation, resulting in round-headed sperm (globozoospermia) and infertility.[15,57] In Sertoli cells, silencing of autophagy-related genes leads to the accumulation of PDLIM1, causing cytoskeletal disorganisation, disruption of the BTB, and failure to support germ cell development.[15] In Leydig cells, autophagy is essential for maintaining cellular homeostasis and steroidogenesis. Its dysregulation reduces testosterone production, contributing to endocrine imbalance and impaired spermatogenesis.[30,55]

Environmental and Lifestyle Factors Affecting Autophagy

Environmental exposures and lifestyle factors represent major modulators of testicular autophagy and frequently act as stressors that induce abnormal autophagic responses. Obesity and high-fat diet (HFD) are associated with increased autophagic activity, as evidenced by elevated LC3-II levels in semen and experimental models. Notably, this overactivation can be detrimental, and pharmacological inhibition of autophagy using agents such as chloroquine has been shown to improve spermatogenesis in HFD-induced infertility models.[58]

Environmental pollutants further disrupt autophagy through distinct but converging mechanisms. Heavy metals such as cadmium induce autophagy by increasing LC3 and Atg5 expression, leading to BTB damage and testicular dysfunction.[59] Mycotoxins, including zearalenone (ZEA) and aflatoxin B1 (AFB1), inhibit the PI3K/Akt/mTOR signalling pathway, resulting in uncontrolled autophagy and increased death of Sertoli and germ cells.[60,61] Plasticisers, such as di-(2-ethylhexyl) phthalate (DEHP), induce mitophagy in spermatogonia via endoplasmic reticulum stress pathways, further impairing cellular function.[62,63]

Lifestyle-related stressors, including smoking, chronic alcohol consumption, and heat exposure, also disrupt the balance between autophagy and inflammatory responses. These factors contribute to elevated oxidative stress and increased sperm DNA fragmentation, further exacerbating reproductive dysfunction.[64–66]

Functional Outcomes and Clinical Implications of Autophagy in Male Fertility

The consequences of impaired autophagic flux in the male reproductive system are multifaceted and directly impact sperm quality, testicular function, and overall fertility. Defective autophagy disrupts the removal of excess cytoplasm and damaged organelles during spermiogenesis, leading to reduced sperm motility (asthenozoospermia) due to improper flagellar development and decreased tail flexibility.[30]

Autophagy has been reported to be essential for ROS clearance and mitochondrial maintenance[67], suggesting that defective autophagy may result in sperm cell damage.[32] Autophagy also plays a critical role in the function of testicular somatic cells. In Sertoli cells, impaired autophagy disrupts the BTB and actin cytoskeleton (ectoplasmic specialisation), compromising the microenvironment required for germ cell development.[15] In Leydig cells, dysregulated autophagy reduces the availability of cholesterol required for testosterone synthesis, leading to endocrine dysfunction.[16] Collectively, these alterations contribute to a broad spectrum of male infertility phenotypes, including azoospermia, oligozoospermia, asthenozoospermia, and teratozoospermia.

Age-related decline in autophagic capacity further exacerbates these effects. Reduced autophagy in ageing testes is associated with increased ROS accumulation, cellular senescence, and progressive deterioration of spermatogenic function. Environmental stressors such as heavy metals, heat, and toxicants may initially induce protective autophagy but ultimately promote apoptosis and tissue damage when exposure is prolonged or excessive.[68,69]

Therapeutic Implications and Intervention

Targeting autophagy represents a promising strategy for treating male infertility. However, therapeutic approaches must account for its dual role in promoting both cell survival and cell death. Pharmacological activation of autophagy has shown beneficial effects in conditions characterised by impaired autophagic flux. Agents such as rapamycin and cordycepin inhibit mTORC1 signalling, thereby enhancing autophagy.[70–72] In experimental models, cordycepin has been shown to improve sperm count and motility in aged rats by increasing antioxidant enzyme activity and restoring autophagic balance.[70] Similarly, rapamycin has been reported to restore the expression of cell junction proteins in BTB disruption models.[73]

Conversely, inhibition of autophagy may be advantageous in conditions characterised by excessive or dysregulated autophagic activity. Agents such as chloroquine and 3-methyladenine inhibit late stages of autophagy[72,74], and this effect has been associated with spermatogenesis in HFD-induced infertility models.[58] Data from these studies suggested that excessive autophagy contributes to pathology in these contexts.

Adjunctive antioxidant therapy also plays an important role in modulating autophagy-related damage. Antioxidants such as vitamin C have been shown to mitigate heavy metal–induced oxidative stress, thereby reducing excessive autophagy and apoptosis in testicular cells.[75–77] In addition, lifestyle modifications may help restore autophagic balance, as sedentary behaviour and high-fat diets are known to disrupt testicular homeostasis.[78]

CONCLUSION

Autophagy is a central regulator of testicular physiology, integrating metabolic, structural, and signalling pathways that are essential for spermatogenesis and endocrine function. Evidence from cellular, molecular, and experimental studies consistently demonstrates that tightly controlled autophagic flux is required to preserve germ cell quality, maintain Sertoli cell barrier integrity, and support Leydig cell steroidogenesis. However, autophagy operates in a highly context-dependent manner, and both insufficient and excessive activity are associated with testicular dysfunction and male infertility.

The interplay between autophagy and related processes, including apoptosis, ferroptosis, oxidative stress, and ubiquitin-mediated proteostasis, forms a complex regulatory network that governs cellular fate and reproductive outcomes. This network is particularly sensitive to disruption by environmental exposures, metabolic disorders, and ageing, all of which contribute to dysregulation of autophagy and the development of clinically relevant infertility phenotypes.

Despite substantial advances in mechanistic understanding, important translational challenges remain. Current biomarkers lack the specificity and sensitivity required to accurately reflect dynamic autophagic flux, thereby limiting their clinical applicability. In parallel, therapeutic strategies targeting autophagy must be carefully tailored to account for its dual protective and deleterious roles across different pathological contexts.

Future research should focus on developing reliable, non-invasive biomarkers, improving methodologies for in vivo assessment of autophagic flux, and conducting rigorously designed clinical studies to evaluate autophagy-targeted interventions. A more comprehensive understanding of cell-specific and context-dependent regulation of autophagy will be critical for translating these insights into effective diagnostic and therapeutic strategies to improve male reproductive health.

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