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PERSPECTIVE

FROZEN AND BREATHLESS: HYPOXIA'S ROLE IN SPERM CRYORESISTANCE

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Abstract

Hypoxia is a critical factor influencing sperm viability by compromising their structural integrity, functional activity, and fertilizing capacity. In reproductive biology and cryobiology, both hypoxia and cryopreservation are known to independently induce cellular stress; however, their combined effects on sperm remain inadequately explored. Here we systematize current knowledge on the effects of hypoxia on sperm cryoresistance, elucidate the molecular mechanisms underlying these effects and evaluate modern strategies for mitigating the combined damage caused by hypoxia and cryopreservation on sperm structure and function. We conducted a comprehensive literature review by analyzing peer-reviewed studies focused on the molecular and cellular responses of mammalian spermatozoa to hypoxia and cryopreservation. Key mechanisms investigated included oxidative stress pathways, mitochondrial function, membrane homeostasis, ion transport and genetic and epigenetic changes. The review also considered current experimental approaches and therapeutic interventions targeting these mechanisms. The analysis revealed that hypoxia disrupts cellular energy metabolism and enhances oxidative stress, leading to reduced sperm survival following cryopreservation. Additionally, cryopreservation itself causes further damage through membrane destabilization, osmotic imbalance and impaired intracellular signaling. These cumulative effects intensify structural and functional deterioration in sperm cells. Emerging mitigation strategies, including antioxidant supplementation, ion regulation, alterations to cryopreservation protocols and the use of hypoxic adaptogens demonstrate potential for improving post-thaw sperm viability. The combined impact of hypoxia and cryopreservation significantly impairs sperm integrity and function, primarily through oxidative and metabolic stress mechanisms. Targeted interventions aimed at counteracting these effects hold promise for enhancing sperm cryoresistance. Finally, this work highlights important avenues for further research and practical applications in reproductive medicine, particularly in fertility preservation and assisted reproductive technologies.

Keywords: cryopreservation; DNA integrity; epigenetic changes; hypoxia; oxidative stress; sperm.

INTRODUCTION

Hypoxia is a significant physiological stressor that adversely affects the human body, particularly by impairing male reproductive function. It disrupts key processes such as testicular steroidogenesis, spermatogenesis and sexual or erectile function – all of which are essential for maintaining male fertility (1, 2, 3, 4). Prolonged exposure to hypoxic conditions induces morphological abnormalities in sperm and significantly reduces their motility, viability and fertilization potential (5, 6). The use of such compromised spermatozoa in assisted reproductive technologies (ART) may negatively influence fertilization outcomes and early embryonic development (3).

Given that cryopreservation has become a fundamental component of ART for preserving male fertility, concerns have been raised regarding its impact on sperm already affected by hypoxia (7, 8). Emerging evidence suggests that sperm from individuals exposed to chronic hypoxia exhibit reduced cryoresistance, making them more susceptible to damage during freezing and thawing. This dual burden of hypoxic injury followed by cryoinjury may further compromise sperm function and reduce the success rate of ART procedures.

As chronic hypoxia becomes increasingly recognized as a factor limiting the effectiveness of sperm cryopreservation, there is a pressing need to elucidate the underlying biological mechanisms and to identify potential interventions. Understanding how hypoxia alters sperm physiology at the molecular level will aid in the development of novel strategies to enhance sperm survival and function during cryopreservation.

The aim of this study was to consolidate and analyze current knowledge on the effects of various forms of hypoxia on sperm cryoresistance, to investigate the molecular and cellular pathways involved in these responses and to evaluate the efficacy of modern approaches aimed at mitigating the detrimental effects of combined hypoxic and cryogenic stress on male gametes.

GENERAL HYPOXIA AND CRYOPRESERVATION EFFECTS ON THE MALE REPRODUCTIVE FUNCTION

General hypoxia effects

Hypoxia is broadly defined as a physiological condition in which the supply of oxygen (O_2) to tissues is inadequate to maintain normal cellular homeostasis. Based on its underlying cause, hypoxia can be categorized into four main types: hypoxic, anemic, stagnant, and histotoxic hypoxia (9). Hypoxic hypoxia arises from a decreased partial pressure of oxygen in the inspired air, as typically occurs at high altitudes. In contrast, anemic hypoxia results from a diminished oxygen-carrying capacity of the blood, often due to conditions such as anemia or hemorrhage. Stagnant hypoxia is caused by inadequate blood flow to tissues, while histotoxic hypoxia occurs when cells are unable to utilize oxygen effectively, often due to toxins or metabolic disturbances.

Hypoxic hypoxia most commonly develops in response to environmental stressors such as high-altitude exposure, air pollution, and climatic changes. Other forms of hypoxia are frequently associated with pathological conditions including cardiovascular diseases, varicocele, sleep apnea, and chronic anemia.

Hypoxia can present in various temporal and severity-related forms. Acute hypoxia is characterized by brief episodes of low oxygen availability (lasting from minutes to hours), typically due to transient vascular insufficiency. Cyclic hypoxia involves recurrent fluctuations between hypoxic and normoxic states, often resulting from intermittent blood flow restrictions. In contrast, chronic hypoxia refers to sustained low oxygen levels over extended periods (usually exceeding 24 h), frequently associated with persistent vascular or diffusion-related limitations (10).

The biological response to hypoxia is highly dependent on both the duration and intensity of exposure. While short-term or mild hypoxia may activate adaptive mechanisms such as angiogenesis, erythropoiesis, and cell proliferation, prolonged or severe hypoxia can induce cellular stress, leading to apoptosis, reduced proliferation, and tissue degeneration (11, 12, 13, 14). The susceptibility to hypoxia varies widely across different cell types. For instance, brain cells are highly vulnerable and can incur irreversible damage within minutes, whereas bladder smooth muscle cells can

survive under low oxygen conditions for several days (1).

Cellular adaptation to hypoxia involves complex transcriptional responses, prominently mediated by hypoxia-inducible factors (HIFs) such as HIF-1α. The extent of HIF-1α activation and the downstream gene expression profiles differ significantly among cell types, including renal tubular epithelial cells, breast epithelial cells, and smooth muscle cells. Notably, in oncological contexts, hypoxia can drive the selection of apoptosis-resistant cell populations, promoting not only resistance to hypoxic stress but also cross-resistance to chemotherapeutic and oxidative damage (15, 16).

In the male reproductive system, oxygen availability plays a critical role in multiple stages of reproduction, including spermatogenesis, fertilization, implantation, placentation, and fetal development (17). Hypoxia exerts its effects at several levels of spermatogenesis, from early spermatogonial stages to the final maturation of spermatozoa. Experimental models of acute hypoxia have demonstrated a reversible decline in the number of spermatogenic epithelial cells, as well as supportive Sertoli and Leydig cells within testicular tissue (5). However, chronic hypoxia may result in more permanent damage, including a significant decrease in testosterone levels and impaired Leydig cell function, ultimately disrupting the hormonal regulation of spermatogenesis.

Furthermore, hypoxia negatively affects sperm maturation, reducing motility, viability, and morphological integrity of mature spermatozoa (6). Many of these findings are derived from studies involving men exposed to high-altitude environments, which serve as natural models of chronic hypoxia. It has been consistently reported that such exposure leads to terato- and asthenozoospermia, a substantial increase in the proportion of morphologically abnormal sperm, and a marked reduction in sperm motility (1, 18).

General cryopreservation effects

In contrast to hypoxia, most research on the effects of cryopreservation has focused primarily on mature spermatozoa. The outcome of cryopreservation is closely influenced by the state of spermatogenesis, as evidenced in men with oligoasthenoteratozoospermia (19, 20). Additionally, the response to cryopreservation can vary depending on individual or species-specific characteristics (21, 22), as well as seasonal factors, particularly in species with defined breeding seasons (23, 24). Consequently, the initial quality and physiological status of spermatozoa play a critical role in determining their resilience to cryogenic stress.

In most cases, the adverse effects of cryopreservation are observed as reduced sperm motility, along with impaired fertilization capacity and a diminished ability to support embryo development. These functional declines pose significant challenges in the context of ART.

Cryopreservation of testicular tissue, rather than mature sperm, is most often employed as a fertility preservation strategy for prepubertal cancer patients who are not yet producing sperm (25, 26). Although this approach is still considered experimental, with no documented cases of live births resulting from transplanted cryopreserved tissue, it remains the only viable option for fertility preservation in this patient population.

Beyond its clinical potential in humans, testicular tissue cryopreservation also holds considerable promise for animal reproduction, particularly in the conservation of rare or endangered mammalian genotypes and for the improvement of breeding programs (27, 28). However, the procedure is not without drawbacks. Cryopreservation of testicular tissue often leads to a significant reduction in spermatogonial cell numbers, primarily due to the cytotoxic effects of cryoprotectants and ischemic injury incurred during the freezing and thawing process (29).

MOLECULAR MECHANISMS OF MALE REPRODUCTION IMPAIRMENT CAUSED BY HYPOXIA AND CRYOPRESERVATION

Oxidative stress

Both hypoxia and cryopreservation negatively affect sperm function by promoting oxidative stress which produces reactive oxygen species (ROS) as metabolic byproducts (30). These include superoxide anions, hydrogen peroxide, peroxyl radicals and hydroxyl radicals (31). Under hypoxic conditions, dysfunction of the mitochondrial electron transport chain (ETC) leads to the accumulation of NADH, which in turn enhances the production of superoxide

anions. Additionally, NADH oxidases are activated in response to low oxygen availability, further amplifying ROS generation. The excessive accumulation of ROS can damage mitochondrial DNA (mtDNA) and initiate the mitochondria-mediated apoptotic pathway (30). High ROS levels have several detrimental effects on sperm mitochondria, including a reduction in mitochondrial membrane potential ($\Delta\Psi m$), disruption of ATP synthesis and consequent impairment of sperm motility (30, 32).

Hypoxia-induced mitochondrial dysfunction in sperm leads to decreased $\Delta\Psi m$, reduced ATP output and elevated ROS levels (33). These changes exacerbate mitochondrial stress, damaging mtDNA and further impairing mitochondrial performance. Moreover, disruption of the ETC under hypoxic conditions contributes to a vicious cycle of declining ATP production and increasing ROS accumulation, severely compromising sperm motility and viability (34).

Similarly, during cryopreservation, rapid temperature shifts and osmotic stress during the freezing and thawing cycle enhance ROS production. This oxidative surge can reduce sperm survival, impair the acrosome reaction, decrease motility and compromise fertilization potential.

A deeper understanding of the molecular mechanisms underlying oxidative stress is essential for the optimization of cryopreservation protocols. Targeting ROS generation and reinforcing antioxidant defenses may help minimize cellular damage caused by both oxygen deprivation and cryogenic stress, ultimately improving sperm quality and reproductive outcomes (35).

Under hypoxic conditions, HIF-1α upregulates enzymes such as lactate dehydrogenase A and glucose transporter 1, promoting metabolic shifts that help restore cellular homeostasis by enhancing ATP production and reducing ROS generation. Thus, HIF-1α may play a protective role in mitigating oxidative stress (36, 37).

However, despite this adaptive response, studies have shown that in the testes of hypoxic mice, the activity of key antioxidant enzymes such as glutathione peroxidase 1 and superoxide dismutase 1 is significantly diminished, resulting in increased oxidative damage to germ cells (38).

HIF-1α also influences sperm physiology through its involvement in autophagy pathways. Hypoxia-induced activation of HIF-1 can elevate autophagy levels in sperm cells and reduce their vitality via both the HIF-1 signaling and glycolysis/gluconeogenesis pathways. Proteins such as histone H4, cathepsin L, glutathione synthetase and ENO1 have been identified as potential biomarkers of autophagy and sperm vitality, particularly in the context of asthenozoospermia (37).

To counteract oxidative stress during cryopreservation, supplementation with exogenous antioxidants has been shown to enhance the structural and functional integrity of spermatozoa and improve fertilization outcomes (30). A promising therapeutic strategy involves the use of extracellular vesicles derived from antioxidant-rich sources, such as mesenchymal stem cells. These vesicles have shown potential in protecting sperm cells from both cryopreservation-associated damage and hypoxia-induced oxidative injury (39).

Plasma membrane

The plasma membranes of spermatogenic cells and mature spermatozoa are rich in polyunsaturated fatty acids (PUFAs), which contribute to membrane flexibility — a critical feature for maintaining sperm motility. However, this high PUFA content also makes the membranes highly susceptible to lipid peroxidation, a process in which ROS attack and degrade membrane lipids and phospholipids. One of the toxic byproducts of this reaction is malondialdehyde (MDA), which disrupts membrane structure, rendering sperm and spermatogenic cells more vulnerable to damage. MDA also compromises cell functionality by interfering with DNA and protein integrity, impairing cellular processes such as division, differentiation, and development. Excessive ROS production and oxidative stress are particularly detrimental during the late stages of spermatogenesis, when spermatids transform into mature spermatozoa, as antioxidant defense systems are significantly weakened at this point (40).

Hypoxia further exacerbates these effects by reducing membrane fluidity and compromising the structural integrity of sperm cells. This is largely due to the increased production of ROS, which overwhelms antioxidant defenses and damages both the lipid and protein components of the sperm membrane.

ROS-induced lipid peroxidation alters the membrane's composition, reducing its fluidity and impairing sperm functionality (41, 42, 43). Cryopreservation similarly disrupts membrane fluidity. The freeze-thaw cycle can alter the lipid composition of the plasma membrane, often leading to increased rigidity (44). The degree to which sperm tolerate cryoinjury is partially dependent on their initial membrane lipid profile, which varies by species. For instance, feline spermatozoa exhibit low PUFA levels and a low unsaturated-to-saturated fatty acid ratio, potentially contributing to reduced oxidative damage and to better resilience during cryopreservation (45). Furthermore, hypoxia can impair ATP-dependent ion pumps, resulting in membrane depolarization and the influx of sodium and calcium ions, which further destabilize membrane integrity (46). Cryopreservation also interferes with calcium homeostasis in sperm cells. Some studies report reduced responsiveness to calcium signaling, which is essential for motility and fertilization, while others indicate elevated intracellular calcium levels post-thaw. This calcium overload may trigger mitochondrial permeability transition, compromising mitochondrial function and further harming the sperm (47, 48).

Cytoplasmic proteins

Oxidative damage to proteins in spermatogenic cells can significantly impair their function and development. ROS attack protein molecules by interacting with aromatic and heterocyclic amino acid residues such as tryptophan, tyrosine, and histidine, leading to alterations in protein structure and function (49). These oxidative modifications can include amino acid residue alteration, peptide bond cleavage, changes in the protein's three-dimensional conformation, and the formation of cross-linked protein aggregates. Such structural changes may disrupt the synthesis and enzymatic activity of proteins critical for meiosis and cell division during spermatogenesis. The resulting protein dysfunction can compromise cellular regulation, differentiation, and the overall progression of sperm development (50).

Hypoxia contributes further to protein alteration through multiple mechanisms, including enhanced oxidative stress, disrupted cellular signaling, and increased autophagic activity. These stress responses can collectively disturb the proteostasis essential for normal sperm function (43).

Additionally, cryopreservation introduces its own set of protein-related challenges. While cryoprotectants such as dimethyl sulfoxide (Me_2SO) and methanol (MeOH) are essential for minimizing ice crystal formation during freezing, they can also impose osmotic and chemical stress on cells (51). This stress may adversely affect the structural integrity and functional stability of sperm proteins, potentially impairing fertility.

DNA integrity

Oxidative DNA damage in cryopreserved spermatozoa remains a major challenge due to the sperm cells' limited capacity for DNA repair (19). Unlike somatic cells, sperm possess only one active enzyme for repairing oxidative base damage 8-oxoguanine DNA glycosylase (50). This enzyme is responsible for recognizing and excising 8-oxoguanine lesions, a common marker of oxidative DNA damage. However, the downstream components of the base excision repair pathway are largely absent or inactive in mature sperm, leaving the genome particularly vulnerable.

Hypoxia can also induce the production of ROS, highly reactive molecules capable of damaging DNA and compromising male fertility (52). This mechanism is strikingly similar to the oxidative damage observed after cryopreservation, where ROS lead to sperm DNA fragmentation (53).

DNA fragmentation arises not only from oxidative stress but also from defects in DNA repair pathways and in the activation of apoptosis (54). The nature of DNA fragmentation caused by hypoxia varies depending on whether the hypoxia is acute or chronic. In acute hypoxia, the reoxygenation phase rapidly generates ROS, leading to the formation of 8-oxoguanine (55). In contrast, chronic hypoxia contributes to DNA fragmentation, both through persistent oxidative stress and through the suppression of key DNA repair genes, further impairing the cell's ability to maintain genomic integrity (56).

Cryopreservation similarly compromises sperm DNA integrity by inducing oxidative stress (22, 57). One of the main consequences of ROS exposure during the freeze-thaw process is the formation of DNA strand breaks. ROS can attack the deoxyribose sugar and phosphodiester backbone of DNA, resulting in single-strand

breaks (SSBs) and, under more severe conditions, double-strand breaks (DSBs) (19). While SSBs can often be repaired by the base excision repair pathway in somatic cells, DSBs pose a greater risk to genomic stability. Without effective repair via homologous recombination or non-homologous end joining mechanisms, that are largely inactive in sperm, these breaks may lead to mutations, chromosomal rearrangements, or programmed cell death.

Moreover, cryoprotective agents such as ME_2SO can exert genotoxic effects under certain conditions. ME_2SO increases membrane permeability, potentially allowing ROS and other reactive molecules greater access to intracellular components, including DNA. It has also been shown to cause chromatin decondensation, which can leave DNA more exposed to oxidative attack. At high concentrations or with prolonged exposure, ME_2SO itself may generate secondary reactive species, compounding the extent of DNA damage (58).

Gene expression and epigenetic changes

Hypoxia is well known to alter the expression of genes involved in the regulation of spermatogenesis, negatively impacting the development of male germ cells. Disruption of oxygen supply shifts the metabolic balance between glycolysis and oxidative phosphorylation, potentially reducing the size of spermatogonial colonies and downregulating key spermatogonial stem cell (SSC) markers such as Plzf, Id4, Gfrα1, Etv5, and Sall4. These changes suggest that hypoxia may impair spermatogonial differentiation (59).

Experimental studies in mouse models have demonstrated that hypobaric hypoxia significantly decreases the population of undifferentiated spermatogonia (60). Transcriptomic analysis of testicular tissue from hypoxia-exposed animals compared to controls revealed the upregulation of 766 genes and downregulation of 965 genes. Notably, the offspring of hypoxia-exposed mice exhibited disrupted spermatogenesis, suggesting that epigenetic modifications induced by hypoxia may be heritable.

While cryopreservation has also been associated with the induction of epigenetic changes, including transgenerational effects, it is considered less likely to cause heritable alterations compared to hypoxia. This discrepancy may be attributed to the fact that cryopreservation primarily affects mature male germ cells (spermatozoa), whereas hypoxia impacts early-stage spermatogonia where epigenetic changes are more likely being occurred.

The effects of oxidative stress on histone structure and chromatin remodeling depend on the specific histone types involved and the extent of DNA packaging within sperm nuclei (22). Both hypoxia and cryopreservation are linked to epigenetic modifications such as DNA methylation and histone alterations, which may compromise sperm quality and reduce fertility (61, 62, 63).

Combined effect of hypoxia and cryopreservation on sperm

There is increasing evidence that deoxygenation may exert differential effects on sperm cryoresistance under in vivo and in vitro conditions. Controlled hypoxia applied to sperm prior to cryopreservation has shown promise in enhancing post-thaw survival. For example, Amin et al. (64) reported that reducing oxygen tension significantly improved sperm survival rates following cryopreservation. Treated sperm also exhibited enhanced motility and acrosome integrity.

In another study, reducing oxygen concentration to approximately 3% using oxidase treatment restored motility in 50% of cryopreserved mouse sperm, compared to only 31% in untreated controls. This finding suggests a strong association between lower oxygen levels and improved sperm function after thawing (65). Similarly, partial deoxygenation using nitrogen gas flushing demonstrated a beneficial effect on cryosurvival rate (66).

However, although partial deoxygenation improved structural and functional parameters, it did not completely prevent the overall decline in sperm quality after thawing. This underscores the notion that, while hypoxia can alleviate certain cryoinjuries, it cannot fully eliminate the detrimental impacts of cryopreservation (67). The effects of hypoxia and cryopreservation on male reproductive cells, as described above, are summarized in Table 1.

CONCLUSION

Hypoxia, as a systemic stress factor, affects spermatogenesis by disrupting energy metabolism, inducing oxidative stress, and

Table 1. Effects of hypoxia and cryopreservation on mammalian spermatozoa.

Type of damage/ effect on	Hypoxia outcomes	Cryopreservation outcomes
Oxidative stress	Excessive production of ROS. Most of the effects are at early stages of spermatogenesis (30, 33, 35, 72).	Excessive production of ROS. Most of the effects are on fully differentiated sperm cells (30, 76).
Mitochondrial activity	mtDNA damage; decreased ΔΨm; reduced ATP output (30, 32, 33).	mtDNA damage; decreased ΔΨm; reduced ATP output (70, 76).
Membrane stability	Reduced membrane fluidity; increased membrane depolarization (41, 42, 43, 74)	Altered membrane composition; increased membrane rigidity and depolarization; triggered mitochondrial permeability (44, 45, 47, 48, 75, 76).
Calcium homeostasis	Calcium disbalance (46).	Calcium disbalance (47, 48).
Cytoplasmic proteins	Disturbed proteostasis by oxidative stress (43, 45).	Disturbed proteostasis by oxidative stress and interactions with cryoprotectants (51)
DNA integrity	DNA fragmentation; apoptosis; suppression of key DNA repair genes (43, 52, 55, 56, 79).	DNA fragmentation; formation of DNA strand breaks; chromosomal rearrangements; apoptosis (19, 22, 53, 58).
Epigenetic changes	Epigenetic changes; transgenerational epigenetic modifications (60, 63, 71, 77, 78, 80, 81, 83).	Epigenetic changes; low transgenerational effect (61, 62, 73, 82).

altering membrane structures, ultimately influencing the cryoresistance of spermatozoa. On the other hand, cryopreservation targets already mature sperm cells, in which oxidative damage is the key mechanism of injury. Similar to hypoxia, cryopreservation compromises the integrity and lipid composition of the plasma membrane, reduces its fluidity, induces DNA fragmentation, alters cytoskeletal proteins, impairs mitochondrial function, increases lipid peroxidation and triggers premature acrosome exocytosis (68, 69). Given that oxidative stress is a central factor in both hypoxia- and cryopreservation-induced damage, it is possible that these stressors exert a synergistic detrimental effect on sperm function and viability. However, moderate hypoxia applied prior to freezing may shift sperm metabolism in a way that increases their resistance to subsequent cryoinjury. With growing understanding of the effects of hypoxia and cryopreservation on sperm, future research and clinical strategies in reproductive medicine should aim at a comprehensive evaluation of these conditions. The optimization of sperm preservation technologies should involve the regulation of oxygen levels, the application of antioxidant-based approaches and the use of innovative biotechnological solutions such as extracellular vesicle therapy. Therefore, future studies should focus on the development of effective methods to reduce oxidative stress caused by hypoxia and cryopreservation, which would minimize sperm damage and improve the success of germplasm preservation technologies in both human and animal reproductive systems.

REFERENCES

1. Luo Z, Tian M, Yang G, Tan Q, Chen Y, Li G, Zhang Q, Li Y, Wan P& Wu J (2022) *Signal Transduct Target Ther.* **7**, 218 https://doi.org/10.1038/s41392-022-01080-1
2. Oyedokun PA, Akhigbe RE, Ajayi LO & Ajayi AF (2023) *Mol. Cell Biochem.* **478(4)**, 875-885. https://doi.org/10.1007/s11010-022-04559-1
3. Tao H-P, Jia G-X, Zhang X-N, Wang Y-J, Li B-Y & Yang Q-E (2022) *Zygote* **30** **(1)**, 48-56. https://doi.org/10.1017/S0967199421000216
4. Farias JG, Bustos-Obregón E, Orellana R, Bucarey JL, Quiroz E & Reyes JG (2005) *Andrologia* **37** **(1)**, 47-52. https://doi.org/10.1111/j.1439-0272.2004.00651.x
5. Cornejo-Guerra C, Salazar-Ardiles C, Morales P & Andrade DC (2024) *Cells* **13** **(7)**, 592. https://doi.org/10.3390/cells13070592.
6. Lord T. *Nat Rev Urol* (2025). https://doi.org/10.1038/s41585-024-00969-6
7. Petrushko M, Yurchuk T, Todorov P, Hristova E, Piniaiev V, Isachenko E, Rahimi G, Mallmann P & Isachenko V (2021) *Cryobiology* **103**, 39-44. https://doi.org/10.1016/j.cryobiol.2021.09.013
8. Whaley D, Damyar K, Witek RP, Mendoza A, Alexander M & Lakey JR (2021) *Cell Transplantation* **30,** https://doi:10.1177/0963689721999617
9. Shaw DM, Cabre G & Gant N (2021) *Front. Physiol.* **12**, 665821. https://doi.org/10.3389/fphys.2021.665821
10. Saxena K & Jolly MK (2019) *Biomolecules* **9** **(8)**, 339. https://doi.org/10.3390/biom9080339
11. Lee SH, Lee MY & Han HJ (2008) *Cell Prolif.* **41** **(2)**, 230-247. https://doi.org/10.1111/j.1365-2184.2008.00516.x
12. Ntekoumes D, Song J, Liu H, Amelung C, Guan Y & Gerecht S (2025) *Adv. Healthcare Materials* **14** **(2)**, e24038HIF. https://doi.org/10.1002/adhm.202403860
13. Semenza GL (2011) *Am. J. Physiol. Cell Physiol.* **301** **(3)**, C550-C552. https://doi.org/10.1152/ajpcell.00176.2011
14. Minoves M, Hazane-Puch F, Moriondo G, Boutin-Paradis A, Lemarié E, Pépin J-L, Godin-Ribuot D & Briançon-Marjollet A (2023) *Int. J. Mol. Sci.* **24** **(8)**, 6875. https://doi.org/10.3390/ijms24086875
15. Chi JT, Wang Z, Nuyten DS, Rodriguez EH, Schaner ME, Salim A, Wang Y, Kristensen GB, Helland A, Børresen-Dale AL, Giaccia A, Longaker MT, Hastie T, Yang GP, van de Vijver MJ & Brown PO (2006) *PLoS Med.* **3** **(3)**, e47. https://doi.org/10.1371/journal.pmed.0030047
16. Lee PWT, Koseki LR, Haitani T, Harada H & Kobayashi M (2024) *Cancers* **16** **(9)**, 1729. https://doi.org/10.3390/cancers16091729
17. Maybin JA (2021) **161** **(1)**, E1-E2. https://doi.org/10.1530/REP-20-0614.
18. Verratti V, Berardinelli F, Di Giulio C, Bosco G, Cacchio M, Pellicciotta M, Nicolai M, Martinotti S & Tenaglia R (2008) *Asian J Androl.* **10** **(4)**, 602-606. https://doi.org/10.1111/j.1745-7262.2008.00346.x
19. Yurchuk TO, Pavlovich OV, Gapon GO, Pugovkin AY & Petrushko MP (2021) *Ukr. Biochem. J.* **93** **(3)**, 24–29. https://doi.org/10.15407/ubj93.03.024
20. Yurchuk T, Petrushko M, Gapon A, Piniaiev V & Kuleshova L (2021) *Cryobiology* S0011-2240(21)00059-6. https://doi.org/10.1016/j.cryobiol.2021.02.009
21. Kopeika EF, Petrushko MP, Piniaiev VI, Yurchuk TO, Pavlovich OV, Mikson KB, Butskyi KI, Hapon HO & Puhovkin AY (2019) *Probl. Cryobiol. Cryomed.* **29** **(1)**, 3-18. https://doi.org/10.15407/cryo29.01.003
22. Yurchuk T, Pavlovich O & Petrushko M (2023) *Probl. Cryobiol. Cryomed.* **32** **(3)**, 171-82 https://doi.org/10.15407/cryo32.03.171
23. Bogdaniuk A, Yurchuk T & Petrushko M (2022) *Cytol. Genet*. **56** **(5)**, 410–416. https://doi.org/10.15407/agrisp9.03.029
24. Janett F, Thun R, Bettschen S, Burger D & Hassig M (2003) *Anim. Reprod. Sci.* **77** **(3-4)**, 213-221. https://doi.org/10.1016/s0378-4320(03)00039-3
25. Moussaoui D, Surbone A, Adam C, Diesch-Furlanetto T, Girardin C, Bénard J, Vidal I, Bernard F, Busiah K, Bouthors T, Primi MP, Ansari M & Vulliemoz N & Gumy-Pause F (2023) *Front. Pediatr.* **11**, 1177389. https://doi.org/10.3389/fped.2023.1177389
26. Goossens E, Jahnukainen K, Mitchell RT, van Pelt A, Pennings G, Rives N, Poels J,

Wyns C, Lane S, Rodriguez-Wallberg KA, Rives A, Valli-Pulaski H, Steimer S, Kliesch S, Braye A, Andres MM, Medrano J, Ramos L, Kristensen SG, Andersen CY, Bjarnason R, Orwig KE, Neuhaus N & Stukenborg JB (2020) *Hum. Reprod. Open.* **3**, hoaa016, https://doi.org/10.1093/hropen/hoaa016

27. Silva AMD, Pereira AF, Comizzoli P & Silva AR (2020) *Biopreserv. Biobank.* **18 (3)**, 235-243. https://doi.org/10.1089/bio.2020.0010
28. Han Z, Liu X, Wang H, Qazi IH, Wang L, Du R, Dai X & Xu C (2025) *Front. Vet. Sci.* **12**, 1572150. https://doi.org/10.3389/fvets.2025.1572150
29. Li JT, Zhang L, Liu JJ, Lu XL, Wang HX & Zhang JM (2021) *Andrologia* **53 (10)**, e14191. https://doi.org/10.1111/and.14191
30. Shen S, Shao Y & Li C (2023) *Cell Death Discov.* **9**, 284 . https://doi.org/10.1038/s41420-023-01581-0
31. Du Plessis SS, Agarwal A, Halabi J & Tvrda E (2015) *J. Assist. Reprod. Genet.* **32**, 509–520. https://doi.org/10.1007/s10815-014-0425-7
32. Gualtieri R, Kalthur G, Barbato V, Di Nardo M, Adiga SK & Talevi R (2021) *Antioxidants (Basel)* **10 (3)**, 337. https://doi.org/10.3390/antiox10030337
33. Arya D, Pawar P, Gajbhiye R, Tandon D, Kothari P, Goankar R & Singh D (2025) *Mol. Cell Endocrinol*. **595**, 112393. https://doi.org/10.1016/j.mce.2024.112393]
34. Meng K, Liu Q, Qin Y, Qin W, Zhu Z, Sun L, Jiang M, Adu-Amankwaah J, Gao F, Tan R & Yuan J (2025) *Chin. Med. J. (Engl).* **138 (4)**, 379-388. https://doi.org/10.1097/CM9.0000000000003126
35. Li S, Liu W, Chen X, Chen Z, Shi J & Hua J (2025) *Reprod. Sci.* **32(2)**, 261-277. https://doi.org/10.1007/s43032-024-01746-x
36. Basheeruddin M & Qausain S (2024) *Cureus* **16 (7)**, e63852. https://doi.org/10.7759/cureus.63852
37. Hu J, Wu J, Liu X, Zhang Y, Mo L, Liu L, Liu S, Ou C & He Y (2024) *Sci. Rep.* **14 (1)**, 8465. https://doi.org/10.1038/s41598-024-59213-1
38. Torres M, Laguna-Barraza R, Dalmases M, Calle A, Pericuesta E, Montserrat JM, Navajas D, Gutierrez-Adan A & Farré R (2014) *Sleep* **37 (11)**, 1757-65. https://doi.org/10.5665/sleep.4166
39. Rana S, Lone FA, Souza-Junior JBF & Bhat GR (2024) *Discov. Appl. Sci*. **6**, 619. https://doi.org/10.1007/s42452-024-06230-4
40. Su LJ, Zhang JH, Gomez H, Murugan R, Hong X, Xu D, Jiang F & Peng ZY (2019) *Oxid. Med. Cell Longev.* **2019***,* 5080843. https://doi.org/10.1155/2019/5080843.
41. Vargas A, Bustos-Obregón E & Hartley R (2011) *Biol. Res*. **44 (2)**, 161-167. http://dx.doi.org/10.4067/S0716-97602011000200008
42. Ghandehari-Alavijeh R, Tavalaee M, Zohrabi D, Foroozan-Broojeni S, Abbasi H & Nasr-Esfahani MH (2019) *Andrologia*. **51 (2)**, e13189. https://doi.org/10.1111/and.13189
43. Li Z, Wang S, Gong C, Hu Y, Liu J, Wang W, Chen Y, Liao Q, He B, Huang Y, Luo Q, Zhao Y & Xiao Y (2021) *Front. Cell Dev. Biol*. **9**, 725933. https://doi.org/10.3389/fcell.2021.725933
44. Giraud MN, Motta C, Boucher D & Grizard G (2000) *Hum Reprod*. **15(10)**, 2160-2164. https://doi.org/10.1093/humrep/15.10.2160
45. Prochowska S, Bonarska-Kujawa D, Bobak Ł, Eberhardt M & Niżański W (2024) *Sci. Rep.* **14**, 10214. https://doi.org/10.1038/s41598-024-61006-5.
46. Luptakova D, Baciak L, Pluhacek T, Skriba A, Sediva B, Havlicek V & Juranek (2018) *Sci Rep* **8**, 6952. https://doi.org/10.1038/s41598-018-25088-2
47. Kim JC, Li Y, Lee S, Yi YJ, Park CS & Woo SH (2008) *Mol Cells* **26 (6),** 558-565. https://doi.org/10.7750/BioDiscovery.2013.9.5
48. Treulen F, Arias ME, Aguila L, Uribe P & Felmer R (2018) *Cryobiology* **83**, 65-74. https://doi.org/10.1016/j.cryobiol.2018.06.001
49. Walke G, Gaurkar SS, Prasad R, Lohakare T & Wanjari M (2023) *Cureus* **15 (7)**, e42583. https://doi.org/10.7759/cureus.42583.
50. Wang Y, Fu X & Li H (2025) *Front. Endocrinol (Lausanne)* **16**, 1520835. https://doi.org/10.3389/fendo.
51. Kodzik N, Ciereszko A, Judycka S, Słowińska M, Szczepkowska B, Świderska B & Dietrich MA (2024) *Sci Rep* **14**, 17707. https://doi.org/10.1038/s41598-024-68395-7.
52. Mahfouz R, Sharma R, Thiyagarajan A, Kale V, Gupta S, Sabanegh E & Agarwal A (2010) *Fertil. Steril.* **94 (6)**, 2141-2146.

https://doi.org/10.1016/j.fertnstert.2009.12.030

53. Le MT, Nguyen TTT, Nguyen TT, Nguyen TV, Nguyen TAT, Nguyen QHV & Cao TN (2019) *Clin. Exp. Reprod. Med.* **46 (2)**, 67-75. https://doi.org/10.5653/cerm.2019.46.2.67
54. Finelli R, Leisegang K, Kandil H, Agarwal A (2022) *World J. Mens Health.* **40 (1)**, 87-103. https://doi.org/10.5534/wjmh.210153.
55. Hahm JY, Park J, Jang ES & Chi SW (2022) *Exp. Mol. Med.* **54 (10)**, 1626-1642. doi: https://doi.org/10.1038/s12276-022-00822-z
56. Kumareswaran R, Ludkovski O, Meng A, Sykes J, Pintilie M & Bristow RG (2012) *J. Cell Sci.* **125 (Pt1)**,189-99. https://doi.org/10.1242/jcs.092262
57. Thomson LK, Fleming SD, Aitken RJ, De Iuliis GN, Zieschang JA & Clark AM (2009) *Hum Reprod*. **24 (9)**, 2061-2070. https://doi.org/10.1093/humrep/dep214
58. Kopeika J, Thornhill A & Khalaf Y (2015) *Hum Reprod Update* **21 (2)**, 209-227. https://doi.org/10.1093/humupd/dmu063
59. Chen W, Zhang Z, Chang C, Yang Z, Wang P, Fu H, Wei X, Chen E, Tan S, Huang W, Sun L, Ni T, Yang Y & Wang Y (2020) *Cell Discov.* **6**, 56. https://doi.org/10.1038/s41421-020-0183-x.
60. Li S & Yang QE (2022) *Gene* **20**, 823:146390. https://doi.org/10.1016/j.gene.2022.146390
61. Fouladvandi R, Masoudi AA, Totonchi M, Hezavehei M & Sharafi M (2024) *Reprod Domest* **59 (5)**, e14570. https://doi.org/10.1111/rda.14570
62. Khosravizadeh Z, Khodamoradi K, Rashidi Z, Jahromi M, Shiri E, Salehi E & Talebi (2022) *J. Assist. Reprod. Genet.* **39**, 1815–1824. https://doi.org/10.1007/s10815-022-02545-6
63. Wang SY, Lau K, Lai KP, Zhang JW, Tse AC, Li JW, Tong Y, Chan TF, Wong CK, Chiu JM, Au DW, Wong AS, Kong RY & Wu RS (2016) *Nat Commun* **7**, 12114. https://doi.org/10.1038/ncomms12114
64. Amin BY, Prasad JK, Ghosh SK, Lone SA, Kumar A, Mustapha AR, Din O & Kumar A (2018) *Reprod. Domest. Anim.* **53 (5)**, 1033-1040. https://doi.org/10.1111/rda.13200
65. Mazur P, Katkov II, Katkova N & Critser JK (2000) *Cryobiology* **40 (3)**, 187-209. https://doi.org/10.1006/cryo.2000.2238
66. Kumar A, Prasad JK, Srivastava N & Ghosh SK (2019) *Biopreserv. Biobank.* **17 (6)**, 603-612. https://doi.org/10.1089/bio.2019.0037
67. Balamurugan B, Ghosh SK, Lone SA, Prasad JK, Ramamoorthy M & Kumar A (2022) *CryoLetters* **43 (3)**, 158-166. https://doi.org/10.54680/fr22310110712
68. Coimbra-Costa D, Alva N, Duran M, Carbonell T & Rama R (2017) *Redox Biol*. **12**, 216-225. https://doi.org/10.1016/j.redox.2017.02.014
69. Hai E, Li B, Zhang J & Zhang J (2024) *Cell Death Discov.* **10 (1)**, 239. https://doi.org/10.1038/s41420-024-02013-3
70. Algieri C, Blanco-Prieto O, Llavanera M, Yeste M, Spinaci M, Mari G, Bucci D & Nesci S (2023) *Reprod. Domest. Anim.* **58 (1)**, 184-188. https://doi.org/10.1111/rda.14261
71. Beetch M, Harandi-Zadeh S, Shen K, Lubecka K, Kitts D, O'Hagan H & Stefanska B (2020) *Br. J. Pharmacol.* **177**, 1382–1408. https://doi.org/10.1111/bph.14888
72. Dwyer KD, Snyder CA & Coulombe KLK (2025) **12 (2)**, 154. https://doi.org/10.3390/bioengineering12020154
73. Ebenezer Samuel King JP, Sinha MK, Kumaresan A, Nag P, Das Gupta M, Arul Prakash M, Talluri TR & Datta TK (2022) *Front. Genet.* **13**, 1025004. https://doi.org/10.3389/fgene.2022.1025004
74. Ghandehari-Alavijeh R, Tavalaee M, Zohrabi D, Foroozan-Broojeni S, Abbasi H & Nasr-Esfahani MH (2019) *Andrologia* **51 (2)**, e13189. https://doi.org/10.1111/and.13189
75. Giraud MN, Motta C, Boucher D & Grizard G (2000) *Hum. Reprod.* **15 (10)**, 2160-2164. https://doi.org/10.1093/humrep/15.10.2160
76. Johnston SD, Satake N, Zee Y, López-Fernández C, Holt WV & Gosálvez J (2012) *Reproduction* **143 (6)**, 787-97. https://doi.org/10.1530/REP-11-0436
77. Kaltsas A, Markou E, Kyrgiafini MA, Zikopoulos A, Symeonidis EN, Dimitriadis F, Zachariou A, Sofikitis N & Chrisofos M (2025) *Genes (Basel)* **16 (1)**, 93. https://doi.org/10.3390/genes16010093
78. Kietzmann T, Petry A, Shvetsova A, Gerhold JM & Gorlach AB (2017) *J. Pharmacol.* **174**, 1533–1554. https://doi.org/10.1111/bph.13792

79. Ortmann BM, Taylor CT & Rocha S (2024) *Trends Biochem. Sci.* **49** (**7**), 573-582. https://doi.org/10.1016/j.tibs.2024.03.008
80. Ouyang Y, Wu Q, Li J, Sun S & Sun S (2020) *Cell Prolif.* **53**, e12891. https://doi.org/10.1111/cpr.1289
81. Silvestris E, Dale B & Elder K (2016) *Reprod. Biomed. Online.* **33**, 668–683. https://doi.org/10.1016/j.rbmo.2016.09.006.
82. Wang W, Todorov P, Pei C, Wang M, Isachenko E, Rahimi G, Mallmann P & Isachenko V (2022) *Cells* **11 (13)**, 2110. https://doi.org/10.3390/cells11132110
83. Wu Q & Ni X (2015) *Curr. Drug. Targets*. **16** (**1**), 13-19. https://doi.org/10.2174/1389450116666150113121054