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PERSPECTIVE

NANOENGINEERED CRYOPROTECTANTS AND SMART BIOMATERIALS FOR PRECISION SPERM CRYOPRESERVATION: A NEW FRONTIER IN MALE FERTILITY PRESERVATION

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Abstract

Sperm cryopreservation is a key technology in reproductive medicine, providing patients the possibility to retain viability before medical interventions or age-related decline. Despite its clinical significance, current cryopreservation procedures suffer substantial limits due to cryoinjury, most notably from intracellular ice formation, osmotic imbalance, membrane instability, and oxidative damage. These conditions significantly affect sperm motility, viability, and genetic integrity post-thaw. To overcome these problems, recent breakthroughs have focused on merging nanotechnology and smart biomaterial science to produce next generation cryoprotectants and preservation systems. Nanoengineered cryoprotectants comprising customized nanomaterials such as liposomes, polymeric nanoparticles, and biologically derived exosomes have shown improved membrane protection, effective antioxidant delivery, and reduction of ice nucleation compared to traditional agents. Early preclinical tests reveal that these alterations considerably enhance post-thaw sperm sustainability, minimize DNA fragmentation, and sustain functional ability for fertilization. Moreover, the combination of individualized cryopreservation protocols leveraging microfluidic technology and embedded biosensors allows unprecedented control and real-time monitoring of cryopreservation quality suited to unique patient demands. Despite these gains, further study into nanotoxicity, long-term safety, and regulatory standards is necessary before widespread clinical adoption. Collectively, nanoengineered cryoprotectants and smart biomaterials constitute a promising new frontier, seeking to enhance male fertility preservation with higher efficiency, safety, and tailored solutions.

Keywords: nanoengineered cryoprotectants; polymeric nanoparticles; reproductive health; smart biomaterials; sperm cryopreservation; trehalose.

INTRODUCTION

It is possible to cryopreserve the organs, cells, tissues, and other biosamples for long-term storage at very low temperatures (-85 °C to -196 °C) (1). Biological materials can be used in future therapeutic applications and scientific research because they preserve their functional integrity and normal structure after thawing by drastically lowering or even halting all biological and chemical processes while in cryogenic storage (2). This is the basic cryopreservation

mechanism. This method has been widely applied, particularly in the preservation of germplasm resources and biological applications. For instance, cryopreservation will enhance transplant results in biomedicine, decrease expenses, reduce waiting times, and boost organ supply (3). In vitro treatment for fertility and reproductive therapies has evolved due to the preservation of gametes, oocytes, sperm, whole reproductive organs, and parts of tissues (such as ovarian and testicular tissue), as well as embryos (4). This allows individuals to continue being fertile for a while, even in extreme situations that may normally lead to infertility. Over the past few decades, there has been a substantial advancement in the preservation of animal genetic material in germplasm banks (5). The preservation of endangered species and biodiversity conservation depend heavily on this effort. Additionally, cryopreservation increases the genetic development of animals by avoiding the spatiotemporal limitations of animal reproduction (6).

Although contemporary therapeutic nanoparticles are suitable up to hundreds of nm in size, the National Institutes of Health (NIH) defines nanoparticles as structures that range from 1 to 100 nm in at least one dimension. Given the 150–200 μm tissue junction between capillaries, nanoscale structures have special qualities that improve reactive regions and overcome tissue or cell barriers. The ideal nanoparticle size for pharmacokinetic characteristics is around 100 nm in hydrodynamic diameter. Larger particles (>200 nm) primarily accumulate in the liver and spleen, indicating that nanocarrier size also influences in vivo destiny. Larger nanoparticles are swiftly eliminated from the bloodstream by the reticuloendothelial system's macrophages, whereas smaller ones are restricted to tissue extravasations and renal clearance. Naturally, surface modification and additional biomaterial composition are necessary for the absorption and removal of nanoparticles in vivo.

Over the past 40 years, a variety of nanopharmaceuticals have been developed for the effective and targeted administration of bioavailable medications. Traditionally, the payload (reagent, medication combination, or imaging agent) has been encapsulated by a liposome, nanocrystal, or polymer-based nanoparticles (NP) (7, 8). One major advantage of employing nanopharmaceuticals is that they reduce systematic cytotoxicity and adverse effects associated with the free medicine by enhancing the payload therapeutic index. Compounds derived from nanomaterials may show in a range of healthcare facilities and laboratory settings, including those related to reproductive health (9). Arousing sexual attraction, producing healthy gametes, facilitating egg fertilization, and providing a nurturing environment for the resulting embryo growth until parturition are all activities of the reproductive system (10). This system's intricacy and sophistication make it more susceptible to different types of pathology, which frequently show up as sub- or infertility phenotypes. Actually, an estimated 50 million couples globally are infertile, which represents 9% of the total population of reproductive age (11). Thus, there are numerous opportunities for the therapeutic use of NPs in the reproductive system. In fact, compared to traditional reagents, Depending on the loaded material, NPs may have greater selectivity, efficacy, and reduced off-target damage when targeting reproductive cells (12). In light of this possibility, this review examines the benefits and drawbacks of some of the NPs thought to be most suitable for use in the reproductive environment. In addition to describing the status of research in this developing subject, intends to give a summary of the most popular biodegradable, biocompatible nanoparticles utilized in cryopreservation practices.

ICE FORMATION DURING CRYOPRESERVATION IN SPERM CELLS

Cryopreservation is a basic and important method of preserving biological specimens that can effectively reduce metabolism and provide essential support for a variety of biological applications (13). Figure 1 shows the primary cryopreservation procedures and cryoinjuries that arise during cooling operations. Importantly, the primary issue that results in a degradation of cell viability throughout the thawing and freezing phases of cryogenic preservation is the growth and formation of ice crystals. The entire cryopreservation process involves ice crystal formation, which must be controlled and suppressed to minimize cellular damage (14). When the freeze-thaw cycle occurs, the ice injury can typically be divided into the negative effects of external and intracellular ice.

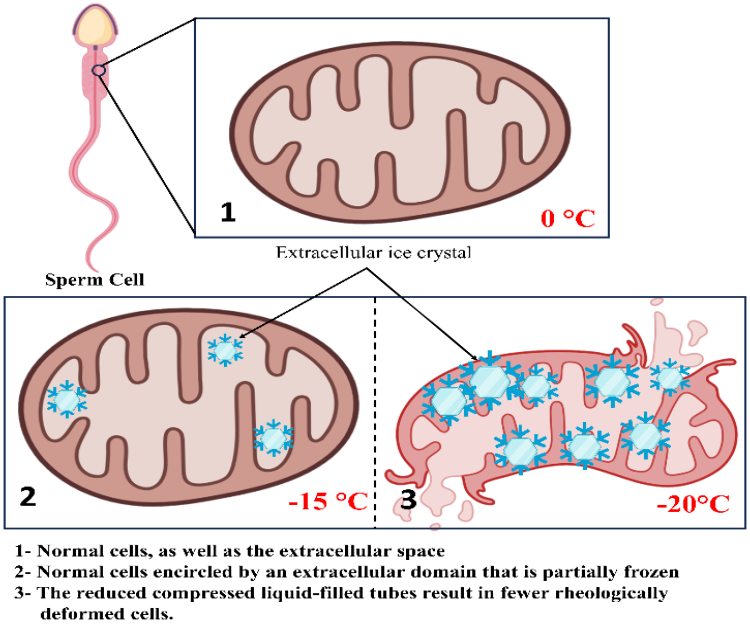

Figure 1. Phases of ice formation in sperm cell cryopreservation.

Cooling rates, that can be defined as progressive freezing, are known to have a significant impact on the ability of cells to survive of cryogenic procedure.

The majority of internal water escapes during slow freezing because its chemical potential is greater than that of the external ice phase. This causes cells to become dehydrated, which in turn produces extracellular ice and changes the cell's osmotic pressure. Intracellular water may not escape rapidly enough if the cooling rate is too fast, resulting in intracellular ice and causing fatal cryoinjury to cells throughout the freezing process (15). Since ice crystals can form under both slow and rapid freezing conditions, it is crucial to promote extracellular ice formation while minimising intracellular ice to reduce cryoinjury (16). In addition to causing mechanical harm to the cell, extracellular ice also raises the concentration of solutes, leading to osmotic injury (17). In other words, the state of the extracellular fluid that results from ice formation is related to the capacity of cells to survive.

There is substantial experimental evidence that the slow-freeze technique can seriously harm cells by reducing the amount of unfrozen water that remains. The process of damage, the liquid-filled tubes in which the cryogenic cells are located, and the mass of the cells all significantly affect the ultimate cryopreservation efficiency because of the interactions between cells (18).

Furthermore, extracellular ice contributes significantly to the warming process. It is well known that during thawing, the hazardous temperature range of -15°C to -160 °C speeds up the transformation of some smaller crystals of ice and water that is liquid into larger ice crystals (19). Therefore, inhibiting the formation and development of ice particles during the procedure of warming is crucial for enhancing cryopreservation effectiveness and continues to be a focus of cryogenic storage research. All things considered, the process of ice recrystallization is intricate and closely linked to various warming scenarios. Temperature and warming rates are crucial factors that affect the formation and growth of extracellular ice (20). Table 1 shows how 10 different types of cryopreservation methods have been used on human sperm cells, and highlights the effects on sperm function.

Table 1. An overview of the mechanisms underlying ice formation and how they affect different cryopreservation methods used on human sperm cells.

Ice formation type	Method used	Cryoprotectant used	Detection technique	Effect on sperm	Ref
Extracellular	Slow freezing	Glycerol	The use of cryomicroscopy	Damage to the membrane	(21)
No ice (glassy state)	The process of vitrification	Trehalose + DMSO	Differential scanning calorimetry	Enhanced viability	(22)
Inside the cell	Standard freezing	Glycerol and sucrose	Raman spectroscopy	DNA breakage	(23)
Preventing ice recrystallization	Nano-heating vitrification system	Nanoparticles coated with PEG	Cryo-TEM	Preserved the integrity of the acrosome	(24)
Controlled extracellular	Programmable freezing	Ficoll + EG	Temperature	Minimal decrease of motility	(25)
Inside the cell	Freezing in two steps	Albumin + glycerol	CryoSEM	Decreased activity of the mitochondria	(26)
Absence of ice crystals	The vitrification of Cryoloop	PVP + ethylene glycol	Microscopy using polarized light	High post-thaw sperm motility	(27)
Outside of cells	Freezing in direction	Fructose + DMSO	Profiling via Calorimetry	Unbalanced osmotic	(28)
Minimal intracellular ice	Extremely quick freezing	Antifreeze proteins + glycerol	Microscopy of electrons	Moderate protection of DNA	(29)
Minimal ice formation	The use of microfluidic preservation	Hydrogel + trehalose matrix	Imaging in infrared	High recovery of functioning sperm	(30)

CHEMICAL STRATEGIES FOR ICE SUPRESSION IN CRYOPRESERVATION

It is clear from the previous examination of ice damage during cryopreservation that effective ice control can reduce the impacts of ice damage and boost the effectiveness of cryogenic preservation of tissues, cells, and organs (31). Due to the quick advancement of chemistry and materials, special compounds with the ability to tune ice have long been found and used as ice inhibitors in cryopreservation processes, offering a wealth of opportunities to improve cryopreservation. Here we provide a brief overview of the ice inhibition materials, emphasising their ice control and inhibition techniques during cryopreservation. These materials include CPAs, AFPs, polymer synthetic nanomaterials, and hydrogels (32).

DMSO, a cryoprotective compound that can enter cells to partially prevent damage by reducing the rise of the solute level after freezing, is the gold standard for the preservation process of mammalian cells (33). Furthermore, before the cells in vials are sufficiently stable to be used in repeatable studies, they must be transmitted forward through multiple passes. Although most cell lines respond well to vial freezing in DMSO, other types are more sensitive

to the substance (34). Enhancing post-thaw viability and reducing processing would be advantageous in many industries if it were possible to reliably maintain all cells and use fewer DMSO dosages. DMSO does not work well on cell monolayers when compared with freezing in solution; usually, only 20–35% of cells are recovered (35). It is evident that we must alter our cryopreservation strategy in order to improve cell function and recovery while reducing processing challenges. Thus, proper ice management may minimize the consequences of ice injury and enhance the effectiveness of cryopreservation of cells, tissues, and organs (36). Due to the advancement of chemistry and materials, new compounds with ice-tuning properties have long been created and used as ice blockers in cryopreservation processes, offering numerous chances to advance cryopreservation. Here we provide a brief overview of the ice-inhibition materials currently used, emphasizing their ice control and inhibition properties during cryopreservation. These materials include hydrogels, synthetic polymers, nanomaterials, cryoprotective agents (CPAs), and antifreeze proteins (AFPs).

Cryoprotective molecules: small solutes and ice-binding proteins in ice regulation

The successful cryopreservation of various biological samples is greatly influenced by the freezing and thawing process and the chemical makeup of the solution. It may be possible to improve the solution's freezing resistance and lessen the ice harm to cryopreserved cells, even though it is challenging to completely eradicate ice formation. In order to prevent ice damage to cells, CPAs are used as supplements. It is widely believed that CPAs play three functions in achieving ice inhibition and improving cryopreservation: freezing point depression, ice shape and growth management, and ice-recrystallization inhibition (IRI) (37). At particular doses, dimethyl sulfoxide (DMSO), the most widely used CPA, exhibits a mechanism of ice-point depression. The freezing process is altered and cryopreserved items are preserved by the chemical reaction between DMSO and water (38). Molecular dynamic modeling involving DMSO and water has been used to investigate the molecular level of DMSO's interaction process. The results showed that DMSO may form hydrogen bonds, increasing the amount of water that is unfrozen and improving freezing tolerance. Furthermore, when the temperature dropped and the CPA concentration rose, the number of hydrogen bonds formed increased continuously, demonstrating that the special interaction stopped the water molecules from dispersing throughout the freezing process. But at high concentrations, the penetrating CPAs undoubtedly exhibit toxicity and even negatively affect the cells' genes, preventing DMSO from being widely used in therapeutic applications (39). Nonreducing sugars, such as sucrose and trehalose, which are biocompatible and nontoxic, are used as ice-inhibition materials in contrast to the usual penetrating CPAs. Furthermore, sugars block ice-recrystallization, allowing for more efficient cryopreservation (40). By infusing CPAs and nonpermeating sugars it is possible to reduce the amount of permeating CPAs and their toxicity. This improves tissue and cell cryopreservation success by resolving the conflict between CPA toxicity and the risk of intracellular ice formation.

In order to survive in extremely cold environments, organisms have a unique ability to adapt to the cold by producing a particular type of protein. The AFPs, or protective proteins, have special ice-crystal control properties. The discovery of the first ice-binding AFP in the late 1960s garnered immediate scientific interest since it may shield against cryoinjury when cells are exposed to extremely cold temperatures. Numerous AFPs have been discovered in the bodies of bacteria, insects, fish, and other natural organisms (41). Three key macroscopic ice-tuning characteristics are typically revealed by AFPs: thermal hysteresis (TH), dynamic ice shaping (DIS), and IRI. Research hotspots and areas of significant scientific interest are the distinct ice-modifying properties of AFPs-related DIS and TH. It was determined that AFPs' absorption-inhibition effect was the fundamental mechanism governing their various ice morphologies. AFPs have the ability to adhere to ice crystals' prism or basal planes and slow down the pace of ice development (42, 43). The curving and flat ice crystals may be the cause of temperature hysteresis, such that there is an elevated melting point and decreased freezing point, as per thermodynamic principles. It is interesting to note that hyperactive AFPs have larger TH gaps, suggesting that TH can gauge how effective AFPs are in ice-tuning. At extremely low temperatures, a process known as "ice recrystallization" occurs whereby small ice crystals gradually grow into larger ones. The process is driven by thermodynamics and results in a decrease in the system's total free energy. Ice

recrystallization results in cellular dehydration and harm to the surrounding tissue, causing structural and functional problems (44). The development of ice crystals is thus restricted by the micro curvatures of the ice surfaces caused by the preferential bonding between the AFPs plane and the ice crystal. The AFPs' binding effect may be molecularly driven by hydrogen bonds formed between their hydroxyl groups and ice. Following the addition of trace amounts of AFPs, the solution exhibits decreased ice-grain area.

Synthetic polymers as biomimetic ice inhibitors: engineered alternatives to natural antifreeze proteins

Although AFPs have distinct modulation and modifying properties for ice crystals, their high cost, potential immunogenicity and cytotoxic effects, large-scale manufacturing challenges, and needle-shaped ice crystals make them unsuitable for cryopreservation. In contrast, antifreeze glycoproteins (AFGPs) can alter the curvature of needle ice crystals, which ultimately results in the formation of round, flat ice crystals (45). The strange surface curvature increases as soon as the AFGPs are absorbed onto the surface of a particular plane of ice. This will cause vapor pressure to rise, which will lower the melting point and prevent water molecules from assimilating into a surface pocket on the ice. In order to reduce needle-shaped ice crystals, the AFGPs' must absorb onto the rapidly expanding prism and promote ice growth from the ice's basal plane orientation. In this way, AFGPs reduce needle-shaped ice crystal formation during cryopreservation which are known to cause serious damage to cells (46). Nonetheless, the effectiveness of AFGPs have limits and the design of an artificial synthetic materials with an ice-tuning capability is highly desirable.

Today, a broad platform for creating AFP-like polymers with scalability and stable ice-modulation capabilities has been made possible by the rapid advancements in polymer chemistry. This has led to significant progress in the design and synthesis of complicated structures and functional groups. Changeable macromolecular and tiny molecular polymers have recently entered the rapidly developing field of ice inhibition, and as a result, they hold great potential for cellular cryopreservation. Our focus here is on synthesizing anti-icing polymer substances, such as ice binding, amphiphilicity, and small molecules.

NATURAL AND SYNTHETIC MATERIALS THAT REGULATE ICE FORMATION AND GROWTH

The nucleation, growth, and melting of ice can be influenced by polymers, surfactants, or other molecules through physical or chemical means. Some molecules interact with water through hydrogen bonding, van der Waals forces, and electrostatic interactions. Certain surfactants can promote ice melting by reducing water's surface tension, whereas other polymers can cling to ice crystal surfaces and form a barrier that stops ice crystals from forming (47). Since several of these ice-interacting compounds have long been developed as CPAs, there have been much potential to enhance cryopreservation results.

Attempts have been made to introduce trehalose, a non-reducing disaccharide, into cryoprotective approaches, e.g., into cells directly through endocytosis or diffusion, because it cannot be generated by mammalian cells (48). Oocytes and other big mammalian cells are subjected to microinjection. Hypochlorite treatment is a helpful method for permeabilizing infectious *Cryptosporidium parvum* oocysts and promoting the intracellular uptake of mixture solutions, such as trehalose, for the cryopreservation of these organisms (49). Trehalose is successfully delivered into tiny cells using platforms based on NP microencapsulation. To avoid cryoinjury, large quantities of trehalose are often needed. Despite being the fundamental principle that regulates intra and extracellular ice during cooling and rewarming, significant ice production takes place in the cryopreservation solution. In contrast, PVA only permits nonvitreous cellular cryopreservation by restricting the growth of extracellular ice, which increases cell recovery (50). This can be as a result of the cryopreserved cells' freeze tolerance or the suspension system's prior optimization. The evidence must be examined more thoroughly. Table 2 summarises 10 synthetic and natural materials that have been used to control ice in cryopreservation applications.

Table 2. A summary of synthetic and natural materials for controlling ice in cryopreservation applications.

Name of material	Type of material	Origin	Ice regulation mechanism	How utilised in cryopreserv-ation	Ref
Proteins known as antifreeze (AFP)	Organic	Insects and fish	Prevent the recrystallization of ice	Sperm and oocyte cryopreservation	(51)
Trehalose	Organic	Plants and Microbes	limits ice and stabilize membranes.	utilized in conjunction with vitrification medium	(52)
Polyvinyl alcohol (PVA)	Organic	Artificial impersonation	Inhibition of ice recrystallization (IRI)	Cryoprotectant Agent Supplement	(53)
Polyampholytes	Artificial	manufactured in a lab	Prevent the nucleation of intracellular ice	Increases the viability after thawing	(54)
Polyethylene glycol (PEG) coated nanoparticles	Artificial	Lab-synthesized	Chains of PEG alter the interfacial interactions between ice and water, decrease the formation and recrystallisation of ice crystals, and enhance heat transmission during freezing and thawing	Incorporated into cryopreserv-ation systems to improve temperature regulation and lessen damage caused by ice during freezing and reheating	(55)
Glycerol	Organic	biological	decreases the production of ice crystals	Typical CPA in freezing sperm	(56)
Hydroxyethyl starch (HES)	Artificial	A polysaccharide modification	restricts the growth of ice crystals	Mixed with DMSO to freeze cells	(57)
Nanosheets made of graphene oxide	Artificial	Designed	changes the dynamics of ice nucleation	Cryomedia nanocarrier experiment	(58)
Proline	Organic	Animals and Plants	Osmoprotectant, which inhibits ice	Used in mixes for cryoprotection	(59)
Zwitterionic substances	Artificial	Designed in a lab	IRI and ice nucleation suppression	New cryoprotectants based on polymers	(60)

Freezing-resistant proteins and their synthetic equivalents

The main mechanisms of action of AFPs are ice inhibition and adhesion. Ice crystals' growth and recrystallization are prevented by AFPs adhering to their surface. The AFPs have ice-binding sites that help with this interaction because their structures are similar to those of the

ice lattice. To effectively attach and inhibit ice formation, type I AFPs, for example, have a helical structure that matches the ice lattice (61). The two faces that make up AFPs are the nonice-binding face (NIBF) and the ice-binding face (IBF). Due to the orderly arrangement of hydrophilic hydroxyl groups and hydrophobic methyl groups within the residue of the IBF, which are made up of a flat array of β-sheets, the ice-like hydrating layer structure is produced. The molecules of water in the hydration region on the NIBF, on the other hand, have a disorderly structure. A better ability to nucleate heterogeneous ice is achieved when methyl and hydroxyl groups are arranged precisely, while large hydrophobic and charged groups have a depressing impact (62). AFPs cause the ice surface to microcurve by binding as separate proteins, which lowers the freezing temperatures below the melting point. One of the most important mechanisms of AFPs is the so-called "thermal hysteresis" phenomenon. Because it keeps the fluid supercooled without freezing, the thermodynamic hysteresis effect is essential for avoiding the formation of massive ice crystals that could harm cells and tissues.

Substances that initiate ice formation

Ice nucleators, a distinct class of ice regulators from AFPs, prevent excessive supercooling by encouraging the phase transitions of solutions at comparatively high subzero temperatures (63). The proteins, lipoproteins, and inorganic crystals are examples of endogenous ice nucleators. Numerous bacterial species are known to produce ice-nucleating proteins. For example, a commercial product utilised as an additive in the production of artificial snow is derived from *Pseudomonas syringae*. Additionally, it has been shown that cell recovery and repeatability are enhanced when nucleation is produced at temperatures close to zero, utilizing ice mimics, including inorganic and mineral particles (64). In order to enhance the phase-change cryoablation process's capacity to eliminate CD 44 high-expression cells, Rao's team produced chitosan-adorned cellulose nanocrystals. Moreover, it has been shown that human induced pluripotent stem cells (iPSCs) may be successfully cryopreserved by sand-mediated ice seeding, even in the absence of serum and with lower quantities of cryoprotectant; the cells retained high levels of pluripotency and survival. Both antifreeze proteins and nucleants can effectively bind to the ice surface and have a tandem array of amino acids, such as β-helices, despite having distinct effects on ice modulation (65). The question then becomes whether it is promotion or inhibition. While some researchers have highlighted the optimal freezing point when nucleators are added, others have suggested that size is the deciding factor. Given the impact of particles on cryopreservation, it is necessary to ascertain how freezing occurs and the exact link between freezing and antifreezing.

ANTIOXIDANT THAT HELPS INHIBIT DAMAGE CAUSED BY OXIDATIVE STRESS

Superoxide dismutases and superoxide oxidoreductases are catalysts that dismutate superoxide anions. There are two different forms of their extracellular and intracellular existence. Two intracellular forms are SOD manganese, which is primarily found in the matrix of mitochondria (MnSOD, SOD-2) with manganese in the active center, and copper-zinc SOD, which is mostly found in the nucleus and contains, as the name implies, both zinc and copper components (Cu, ZnSOD, SOD-1) (66). The physically identical extracellular form of SOD (EC-SOD, SOD-3) is found in the extracellular space; however, its active core contains zinc and copper instead of manganese. SOD is especially active in semen plasma, with 75% of its activity attributable to SOD-1 activity and the remaining 25% to SOD-3. These two isoenzymes have been found to most likely come from the prostate. The conversion of hydrogen peroxide into molecules oxygen and water is then catalyzed by catalase (CT) (67). The heme structure with a centrally located iron atom is characteristic of CT. Its action has been established in peroxisomes, mitochondria , and cytoplasm in various cells. In the form of an ejaculate, it has been detected in human and rodent sperm. It has also been detected in semen plasma, with the prostate as the source. Catalase is involved in the stimulation of sperm capability through nitric oxide, via a complicated mechanism involving hydrogen peroxide. The enzyme initiating the lowering of hydrogen peroxide (H_2O_2) and organic peroxides, especially peroxides of phospholipids in the antioxidant system of semen, is glutathione peroxidase (GPX) (68). It has selenium in the form of selenocysteine in its active location. It is mostly found in the matrix of mitochondria of

sperm, but a nuclear version has also been found that actively participates in the chromatin condensing process and protects sperm DNA from oxidative stress (OS) damage. Along with the enzymes mentioned above, a presence of GPX in semen plasma was also shown, supporting the idea that it originated in the prostate. Enzyme molecules' complex spatial arrangement affects their catalytic capabilities as well as their vulnerability to various environmental stimuli. The main way that enzymes work is through the substrate's spatial adjustment to the enzyme's catalytic center (69).

Table 3. Ten important antioxidants and their functions in mitigating cellular damage caused by oxidative stress.

Name of antioxidant	Type (synthetic or natural)	Action mechanism	Targeting for ROS	Area of application	Ref
Glutathione	Endogenous (natural)	Preserves the redox equilibrium and scavenges free radicals	H_2O_2, OH	Liver function and male fertility	(74)
Ascorbic acid, or vitamin C	Natural (food)	Reduces oxidative stress by acting as an electron donor	H_2O_2, O2•-	Skin health and semen protection	(75)
The α-tocopherol, or vitamin E	Natural (food)	Protects lipid membranes and prevents lipid peroxidation	LOO•, OH•	Stability of the sperm membrane	(76)
Q10 Coenzyme (CoQ10)	Organic (mitochondrial)	Energy generation and scavenging of mitochondrial ROS	H_2O_2 and superoxide	Infertility in men and cardiovascular health	(77)
N-acetylcysteine	Precursor synthetic	Glutathione is replenished, a direct antioxidant	H_2O_2, OH	Detoxification and antioxidant treatment	(78)
Melatonin	Natural (hormone)	Increases antioxidant enzymes and directly scavenges ROS.	OH•, ONOO–	Neuroprotection and sperm cryoprotection	(79)
Resveratrol	Organic (polyphenol)	Lowers ROS and activates antioxidant genes (SIRT1).	Super-oxide, (OH•)	Enhancement of sperm quality and anti-aging	(80)
Selenium	Natural (trace element)	Glutathione peroxidase cofactor	Lipid peroxides and H_2O_2	Activation of antioxidant enzymes	(81)
The enzyme catalase	Enzyme-based natural	Produces oxygen and water from H_2O_2.	H_2O_2.	Defense against cellular antioxidants	(82)
L-carnitine	Natural (derivative of amino acids)	Lipid transport and mitochondrial antioxidants	Lipid radicals and H_2O_2	Increases the viability and motility of sperm	(83)

The cell contains other non-enzymatic antioxidants that are also essential for shielding sperm cells from OS. Coenzyme Q10 (CoQ10), which is particularly abundant in the mitochondria of sperm engaged in cell respiration. CoQ10 plays a crucial role in energy generation. Its use as an antioxidant and motility-stimulating chemical is supported by this advantageous impact. Interestingly, CoQ10 protects against OS-induced sperm loss by inhibiting the production of superoxide. A linear relationship between CoQ10 levels and the number of sperm in the semen and their motility was found, and there was a significant negative association between CoQ10 level and hydrogen peroxide (70, 71).

Through a mechanism that is yet unclear, selenium shows promise in shielding sperm DNA from OS damage. Since selenium is an essential component of selenoenzymes, its ability to enhance glutathione activity is assumed to be the source of its antioxidant properties. There are about 25 selenoproteins that help preserve the structural integrity of sperm, including phospholipid hydroperoxide glutathione peroxidase (PHGPX) and sperm capsular selenoprotein glutathione peroxidase. The most common symptoms of selenium deficiency are structural abnormalities in the sperm midpiece and decreased sperm motility (72).

Whether directly through the acceleration of reactions caused by enzymes or indirectly through the deactivation of free radicals and the halting of chain reactions, the ultimate role of all antioxidant in the body is to counteract the effects of oxidation processes. Since all of the molecules involved in reactions have been utilized and need to be replenished, the antioxidant systems undoubtedly also consist of groups of chemicals that contribute to the ongoing recovery of the antioxidant capacity. Mammalian sperm cells have a reduced capacity for antioxidant defense because of the absence of a part of the cytoplasm, making them less resilient to oxidative damage. Antioxidants found in semen are therefore especially important to mitigate the negative consequences of OS during sperm preservation. Because there aren't enough natural antioxidant reserves to maintain the sperm's adequate biological potential, natural antioxidants must now be added to the semen to dilute it. Nowadays, synthetic and plant-derived compounds are widely used in sperm preservation methods for many species and are regarded as a beneficial source of antioxidants, particularly because of their low incidence of adverse effects (73). Table 3 mentions 10 important antioxidants and their functions in cellular protection**.**

NANOTECHNOLOGY TO DELIVER ANTIOXIDANTS DURING SPERM CRYOPRESERVATION

Developments in encapsulation nanotechnology have aided in the creation of novel nano antioxidant compounds with protective properties for sperm cryopreservation (84). The use of nanotechnology can increase the administration of non water-soluble protectants, co-deliver two or more medications for combination therapy, and deliver the medicine to a specific site (nanocarriers) to carry out its therapeutic activity with maximum safety (85). To prevent OS, lower apoptosis, DNA damage, and lipid peroxidation, and ultimately preserve the integrity of the sperm membrane during cryopreservation. Figure 2 shows how several nanoparticles (polymeric, silver, lipid-based, and gold) deliver antioxidants.

Types: liposomes, cyclodextrins, polymeric nanoparticles

Liposomes are synthetic spherical vesicles made up of two or more lipid layers with an aqueous cavity inside. Water-soluble medications that would not otherwise be able to easily cross the bilayer membrane can be trapped by liposomes, which are made of phospholipid for bio compatibility and cholesterol levels for stability. Lipophilic medications can also be loaded into the lipid layers to become dispersible in aqueous media (86). Applying liposomes has a number of benefits, such as: i) easy synthesis; ii) easy manipulation of pharmacokinetics and pharmacodynamics; iii) suitable for the delivery of drugs with a variety of properties; iv) excellent biocompatibility and resemblance to the biological membrane; v) improved therapeutic index; vi) being bio-degradable; vii) being suitable for large-scale production.

Liposomes are used in several fields, including sperm cryopreservation, cosmetic formulations, vaccine delivery, and nanomedicines. Interest in employing liposomal compounds as preservation diluents has increased recently due to research showing that they reduce the risk of egg yolk contamination and improve semen quality by better protecting sperm from harm (87, 88).

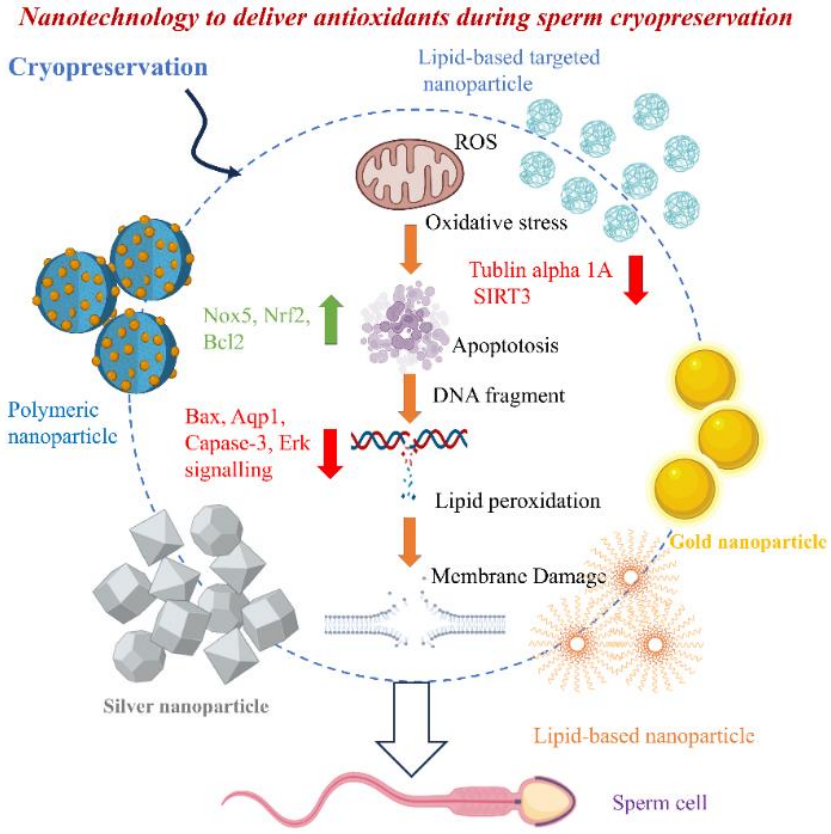

Figure 2. Nanotechnology-driven antioxidant delivery in sperm cryopreservation.

Cyclodextrins (CDs) are types of cyclic non-toxic compounds that are produced when starch is broken down by enzymes. They are called a-, ß-, and γ-CD, respectively, and contain six, seven, or eight glucose units connected by -1-4 bonds. The solubility and/or stability of native CDs might be altered by joining different functional groups from modified CDs (89). They resemble cones in shape and have a hydrophilic external surface, which renders CDs soluble in water. The formation of inclusion complexes of lipophilic molecule guests is aided by the formation of a non-polar inner cavity. CDs are frequently referred to as enabling excipients for pharmaceuticals because of their capacity to influence the physicochemical properties of drugs and other substances (90). Drug molecules can form complexes with one or more CD molecules, and CD molecules can form complexes with one or more drug molecules. Typically, one CD molecule and one drug molecule combine to form a complex. Prior to cryopreservation, it has been demonstrated that treating sperm from different species with cyclodextrins loaded with the right pharmacological molecules (antioxidants, essential oil) may improve sperm quality following the freezing-thawing procedure (91).

Polyethylene glycol (PEG) is a non-toxic, neutral polymer with a variable molecular weight and the HOH_2C (CH_2OCH_2) n CH_2OH structure (92). Nowadays, one of the most widely used polymeric polymers in drug administration is PEG. It may have an impact on the toxicity and pharmacokinetics of bioactive substances. Additionally, PEG might extend the "drug-carrier" assembly's lifespan, allowing for the application of smaller amounts of the "drug-carrier" composite and reducing toxicity (93). PEG has a wide range of advantageous qualities, such as outstanding solubility in both organic and aqueous solvents, which facilitates end-group modification. It is also frequently used to change the carriers that are utilized in medications. PEGs have a positive effect on sperm cryopreservation. Figure 3 shows the division of nanoparticles into three categories: natural, synthetic, and antioxidant based. Each of these types has a unique function in reducing cryoinjury by controlling the development of ice, OS, and membrane damage.

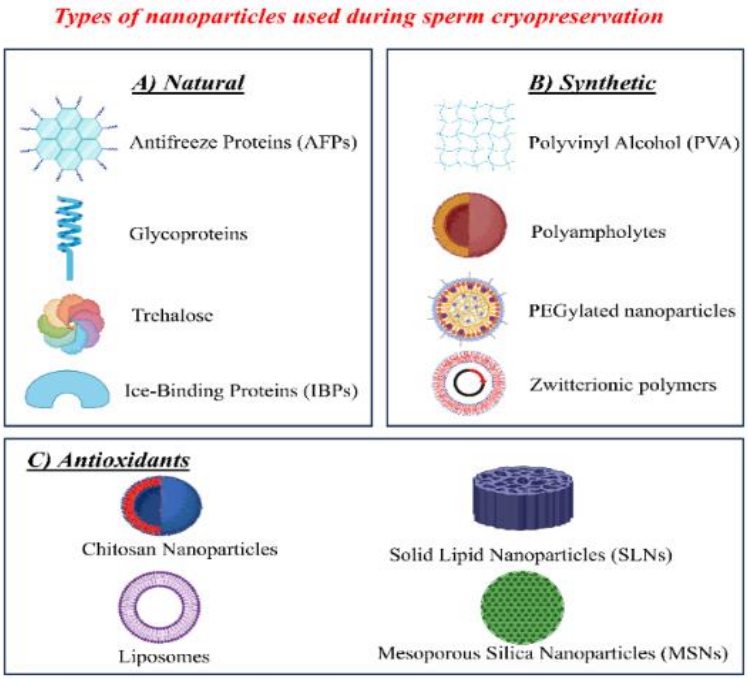

Figure 3. Different kinds of nanoparticles for sperm cryopreservation.

CHALLENGES DURING CRYOPRESERVATION: OVERCOMING CELLULAR DAMAGE AND ICE FORMATION

The formation and growth of ice crystals is a prominent feature of the freeze-thaw process. The main reason for the decline in cell viability is this. Water molecules have a tendency to organize themselves when the temperature drops below the freezing point. This results from the conflict between the ordered organization of water's molecules and their disordered thermal motion (94, 95). Cryoinjury results from the phase transition of extracellular and intracellular water. Extracellular aqueous solutions typically produce ice crystals below their equilibrium freezing point, which reduces the amount of water in the solution and raises the extracellular matrix concentration (96). A lengthy freezing procedure often results in severe dehydration and shrinkage as a result of the higher pressure of osmotic outside the cells. Cryopreservation benefits from a certain level of cellular dryness since it lowers the possibility of excessive intracellular ice accumulation (97). However, extreme dehydration may be irreversible and is a key factor damaging to biological activities.

When the actual crystallization temperature (usually between -15°C and -60°C) falls lower than the theoretical freezing point, sample supercooling ensues. Thus, biosamples do not instantly change from a liquid to a solid phase when the temperature drops below zero. When a supercooled solutions is in a metastable condition, a slight thermal perturbation can easily start the phase transition (98). When ice nucleates, it spreads quickly throughout the biosample, warming the solution and releasing latent heat, which causes more serious mechanical damage to the samples. Using a quick-freezing technique with less cryoprotectant results in a more severe cryoinjury. As the cooling rate increases, intracellular water cannot escape as rapidly resulting in intracellular ice and potentially fatal cell cryoinjury (99).

Rewarming biological materials to physiological temperatures is a problem as well. The main challenge during thawing is avoiding the water-ice transition phase, just like in the freezing process. The cells become more supercooled between -15°C and -60°C, which promotes the conversion of free water to ice. As a result, the ice crystals get bigger as the temperature rises. This process, known as "ice recrystallization," causes mechanical harm during both fast-freezing and slow-freezing processes (100). The extracellular space quickly becomes hypotonic as massive crystals of ice melt into water as the temperature gets closer to the melting point. As a result, hyperosmotic stress

causes water to enter the cells, which causes them to swell and possibly burst.

LIMITATIONS OF CONVENTIONAL CRYOPROTECTANTS

Ice formation and growth is controlled by CPAs during the cooling and warming phases of the cryopreservation protocol. The most popular CPAs, DMSO and glycerol, are particularly good at managing crystals and enhancing cryopreservation results because of their excellent capacity to create hydrogen bonds (HBs) with water. The quantity of CPA-water bonds containing hydrogen increases CPA concentration, which lowers the nucleation temperature and raises the glass transition temperature, hence decreasing the likelihood of ice formation and growth. Their toxicity manifests itself in the interim and has the potential to take over. For instance, DMSO suppresses osteoclast creation, differentiation, and function in vitro; it also encourages dehydration close to lipid membrane interfaces and works in concert with vanadium to hinder zebrafish embryo development and cause pericardial edema. In vitro, it causes significant changes in human cellular functions and the epigenetic environment (101). Ethylene glycol (EG) and DMSO treatment significantly reduces the proportion of oocyte with normal actin microfilaments. Even after many complex washing procedures, glycerol residues remain inside the cells and can create problems because it causes hemolysis or alters the structure of red blood cells.

Cooling technologies are currently divided into two categories: slow freezing and fast freezing. The latter is also known as vitrification, which produces ultra-high viscosity glass by skipping the crystalline phase at extremely high cooling rates. Although the slow freezing method allows for minimum CPA loading, it is still difficult to overcome the challenges of prolonged contact of cells to potentially dangerous CPAs and ice damage (102). The two main concerns for the vitrification process are attaining a fully vitrified state and the use of high-dose CPA which can be toxicity. Together, the search for bio-compatible cryoprotective substances and techniques to regulate the growth, production, and recrystallization of ice during freeze-thaw cycles remains a crucial task.

FUTURE PERSPECTIVES

This review highlights advances in cryopreservation ice inhibition materials and methods. First, we looked at the basic mechanisms of ice cryodamage during the cooling-thawing phase of cryopreservation. We compiled current chemical freezing-inhibition compounds, such as traditional CPAs, AFPs, synthetic polymer nanomaterials, and hydrogels, based on our understanding of ice injury. Examples of cutting-edge ice-blocking engineering solutions that have been emphasized include trehalose delivery, cellular encapsulation, and bioinspired structures. We also explained how to control the growth and production of ice crystals utilizing external physical field techniques. Regardless of extracellular and intracellular ice inhibition, high-efficiency cryopreservation is crucial for both practical therapeutic applications and new basic scientific study.

Materials that suppress ice should be investigated at a molecular level, with a focus on how they interact with molecules of water and ice. AFP stimulates and directs the development of anti-icing materials since it is a natural ice inhibitor. Determining the fundamental process of AFPs on freezing nucleation, form, and recrystallization has advanced significantly up to this point. Even with these developments, some tasks are still challenging. First, more research is required on the three-dimensional form of AFPs and their ability to modify ice formation, growth, and shape. Second, there is disagreement over the universal interpretation of natural AFPs for ice inhibition, and there is currently no theoretical method for measuring ice inhibition quantitatively. For ice inhibition and future cryopreservation applications, these problems will compel scientists to investigate a wide range of bioinspired AFP molecules and/or polymers that share structural or functional characteristics. To help with the cryostorage for biological samples, it should be emphasized that accessibility, cost, and knowledge of toxins are equally important.

In order to suppress ice and avoid freezing damage, advanced engineering techniques such as cell encapsulation, trehalose administration, and bioinspired design for structures have been proposed. As the only nontoxic CPA, trehalose treatment can produce intracellular ice inhibition. Based on cell structures, cell encapsulation can provide a single structure with long-term storage,

freezing control, and cell-based therapy capabilities. Ice suppression can be inspired by a variety of natural species' structures. Despite significant advancements, these methods' primary limitation is their low throughput at laboratory scale. How to accomplish macropreservation using these methods to meet current clinic needs should be one of the future research directions. In future developments of cryopreservation procedures warming will most likely become a combination of synergistic thawing and multiphysical processes. To shatter the ice barrier, for instance, uniform and rapid warming rates can be achieved by combining magnetic and laser fields. More significantly, the physical field allows for warming that is both medically and physically flexible and prevents cryopreserved materials from changing in structure or function.

CONCLUSION

The delicate balance between the generation of ROS and antioxidant defense mechanisms is disturbed by OS. Sperm cells may sustain irreversible oxidative damage as a result of this disturbance, which would lower their viability and functionality. In the end, this may lead to serious problems with male reproductive health. One way to lessen these negative effects is to take exogenous antioxidant supplements. Their inclusion in a cryopreservation procedure make it possible to restore cellular equilibrium, scavenge excess ROS, and shield sperm cells from more oxidative damage. Although a number of antioxidants, such as vitamin C, coenzyme Q L-carnitine, and glutathione, have shown promise, there is still disagreement among scientists on their actual utility because of various research method shortcomings. It is also vital to remember that while antioxidants might offer therapeutic benefits, there remains a possibility for overconsumption, resulting to reductive stress. This counterintuitive result underlines the significance of a balanced approach to antioxidant therapy. The rapid breakthroughs in chemistry, material synthesis, biochemistry, and engineering, anticipate that sophisticated developments in cryopreservation procedures will maintain pace with present and developing needs, delivering a bright future for regenerative medicine. We hope that our critical assessment here of prior efforts will be beneficial in encouraging the development of safe, high-quality, and highly-efficiency cryopreservation of biological samples.

REFERENCES

1. Dannhorn A, Kazanc E, Flint L, Guo F, Carter A, Hall AR, Jones SA, Poulogiannis G, Barry ST, Sansom OJ, Bunch J, Takats Z & Goodwin RJA (2024) *Nat Protoc* **19**, 2685–711. https://doi.org/10.1038/s41596-024-00987-z.
2. Parihar A, Kumar A, Panda U, Khan R, Parihar DS & Khan R (2023) *Adv Biol* **7**, 2200285. https://doi.org/https://doi.org/10.1002/adbi.202200285.
3. Arav A & Natan Y (2024) *Regen Med Reports* **1**, 137-48.
4. Estudillo E, Jiménez A, Bustamante-Nieves PE, Palacios-Reyes C, Velasco I & López-Ornelas A (2021) *Int J Mol Sci* **22**, 10864. https://doi.org/10.3390/ijms221910864.
5. Priyanka V, Kumar R, Dhaliwal I & Kaushik P (2021) *Sustainability* **13**, 6743. https://doi.org/10.3390/su13126743.
6. Vickram AS, Infant SS, Manikandan S, Sowndharya BB, Gulothungan G, Chopra H (2025) *Ann. Med. Surg.* **87(6)**, 3618-34. 10.1097/MS9.0000000000003333
7. Kumbhar PR, Kumar P, Lasure A, Velayutham R & Mandal D (2023) *Discov Nano* **18**, 156. https://doi.org/10.1186/s11671-023-03913-6.
8. Poudel P & Park S (2022) *Pharmaceutics* **14**, 835. https://doi.org/10.3390/pharmaceutics14040835.
9. Fraser B, Peters AE, Sutherland JM, Liang M, Rebourcet D, Nixon B & Aitken RJ (2021) *Front Physiol* **12**, 753686. https://doi.org/10.3389/fphys.2021.753686
10. Andreoli L, Guadagni I, Picarelli G & Principi M (2024) *Autoimmun Rev* **23**, 103507. https://doi.org/https://doi.org/10.1016/j.autrev.2023.103507.
11. Bharath S, Vickram AS, Sowndharya BB, Prasanth C, Rani DJ, Gulothungan G, Chopra H, Malik T (2025) *Transl Res. Anat* **40**, 100410. https://doi.org/10.1016/j.tria.2025.100410
12. Izci M, Maksoudian C, Manshian BB & Soenen SJ (2021) *Chem Rev* **121**, 1746–803.

https://doi.org/10.1021/acs.chemrev.0c00779.
13. Whaley David, Damyar Kimia, Witek Rafal P, Mendoza Alan, Alexander Michael & Lakey Jonathan RT (2021) *Cell Transplant* **30**, 0963689721999617. https://doi.org/10.1177/0963689721999617.
14. Huang M, Hu M, Cai G, Wei H, Huang S, Zheng E & Wu Z (2025) *J Nanobiotechnology* **23**, 187. https://doi.org/10.1186/s12951-025-03265-6.
15. Wang Z, Gao D & Shu Z (2024) *Adv Eng Mater* **26**, 2400800. https://doi.org/https://doi.org/10.1002/adem.202400800.
16. Ribeiro JC, Carrageta DF, Bernardino RL, Alves MG & Oliveira PF (2022) *Animals* **12**, 359. https://doi.org/10.3390/ani12030359.
17. Xie Y, Zhou K, Tan L, Ma Y, Li C, Zhou H, Wang Z & Xu B (2023) *J Agric Food Chem* **71**, 19221–39. https://doi.org/10.1021/acs.jafc.3c06155.
18. Chang T & Zhao G (2021) *Adv Sci* **8**, 2002425. https://doi.org/https://doi.org/10.1002/advs.202002425.
19. Murray KA & Gibson MI (2022) *Nat Rev Chem* **6**, 579–93. https://doi.org/10.1038/s41570-022-00407-4.
20. Bai B, Xue C, Wen Y, Lim J, Le Z, Shou Y, Shin S & Tay A (2023) *Adv Funct Mater* **33**, 2303373. https://doi.org/https://doi.org/10.1002/adfm.202303373.
21. Mahdavinezhad F, Gilani MAS, Gharaei R, Ashrafnezhad Z, Valipour J, Nashtaei MS & Amidi F (2022) *Reprod Biomed Online* **45**, 341–53. https://doi.org/https://doi.org/10.1016/j.rbmo.2022.03.033.
22. Ivanova A, Simonenko E, Yakovenko S & Spiridonov V (2023) *Biophys Rev* **15**, 1223–32. https://doi.org/10.1007/s12551-023-01133-x.
23. Akhtar MF, Ma Q, Li Y, Chai W, Zhang Z, Li L & Wang C (2022) *Animals* **12**, 2277. https://doi.org/10.3390/ani12172277.
24. Esmeryan KD, Fedchenko YI & Chaushev TA (2025) *Cryobiology* **118**, 105195. https://doi.org/https://doi.org/10.1016/j.cryobiol.2025.105195.
25. Sathe S (2021) *Bov Reprod* **20**, 986-99. https://doi.org/https://doi.org/10.1002/9781119602484.ch78.
26. Zhou D, Wang X-M, Li R-X, Wang Y-Z, Chao Y-C, Liu Z-Z, Huang Z-H, Nie H-C, Zhu W-B, Tan Y-Q & Fan L-Q (2021) *Asian J Androl* **23**, 91-6. https://doi.org/10.4103/aja.aja_29_20
27. Aliakbari F, Taghizabet N, Azizi F, Rezaei-Tazangi F, Samadee Gelehkolaee K & Kharazinejad E (2022) *Zygote* **30**, 289–97. https://doi.org/10.1017/S0967199421000071.
28. Ozimic S, Ban-Frangez H & Stimpfel M (2023) *Curr Issues Mol Biol* **45**, 4716–34. https://doi.org/10.3390/cimb45060300.
29. Savvulidi FG, Ptacek M, Malkova A, Kratochvilova I, Simek D, Martinez-Pastor F & Stadnik L (2023) *J Appl Anim Res* **51**, 182–92. https://doi.org/10.1080/09712119.2023.2171045.
30. Milewska AJ, Kuczyńska A, Pawłowski M, Martynowicz I, Deluga-Białowarczuk S, Sieczyński P, Kuczyński W & Milewski R (2024) *J Clin Med* **13**, 7562. https://doi.org/10.3390/jcm13247562.
31. Hu Y, Liu X, Liu F, Xie J, Zhu Q & Tan S (2023) *ACS Biomater Sci Eng* **9**, 1190–204. https://doi.org/10.1021/acsbiomaterials.2c01225.
32. Kang MJ, Ioannou S, Lougheide Q, Dittmar M, Hsu Y & Pastor-Soler NM (2023) *Am J Physiol Physiol* **326**, C229–51. https://doi.org/10.1152/ajpcell.00479.2022.
33. Gore M, Narvekar A, Bhagwat A, Jain R & Dandekar P (2022) *J Mater Chem B* **10**, 143–69. https://doi.org/10.1039/D1TB01449H.
34. Aarattuthodi S, Kang D, Gupta SK, Chen P, Redel B, Matuha M, Mohammed H & Sinha AK (2025) *Vitr Cell Dev Biol - Anim* **23**, 1-24. https://doi.org/10.1007/s11626-025-01027-0.
35. Lomba L, García CB, Benito L, Sangüesa E, Santander S & Zuriaga E (2024) *ACS Biomater Sci Eng* **10**, 178–90. https://doi.org/10.1021/acsbiomaterials.3c00859.
36. Chen J, Liu X, Hu Y, Chen X & Tan S (2023) *Front Vet Sci* **10**, 1201794. https://doi.org/10.3389/fvets.2023.1201794
37. Ma J, Zhang X, Cui Z, Zhao M, Zhang L & Qi H (2023) *J Mater Chem B* **11**, 4042–9. https://doi.org/10.1039/D3TB00131H.
38. Ekpo MD, Boafo GF, Xie J, Liu X, Chen C & Tan S (2022) *Front Immunol* **13**, 1030965.

https://doi.org/10.3389/fimmu.2022.1030965
39. Marcantonini G, Bartolini D, Zatini L, Costa S, Passerini M, Rende M, Luca G, Basta G, Murdolo G, Calafiore R & Galli F (2022) *Molecules* **27**, 3254. https://doi.org/10.3390/molecules27103254.
40. Zhang X, Bi J, Ma Y, Liu K & Yi J (2025) *J Sci Food Agric* **105(13),** 7124-7139. https://doi.org/https://doi.org/10.1002/jsfa.14414.
41. Davies PL (2022) *Biochem Cell Biol* **100**, 282–91. https://doi.org/10.1139/bcb-2022-0029.
42. Drori R & Stevens CA (2023) *Ice Binding Proteins* **10**, 169-181. https://doi.org/10.1007/978-1-0716-3503-2_12.
43. Kannan K & Sivaperumal P (2025) *Food Biosci* **66**, 106134. https://doi.org/https://doi.org/10.1016/j.fbio.2025.106134.
44. Wang D, Wu J, Wu S, Chen X, Li W, Chen X, Gao C & He Z (2024) *Adv Funct Mater* **34**, 2315532. https://doi.org/https://doi.org/10.1002/adfm.202315532.
45. Gharib G, Saeidiharzand S, Sadaghiani AK & Koşar A (2022) *Front Bioeng Biotechnol* **9**, 770588. https://doi.org/10.3389/fbioe.2021.770588
46. Kamat K, Naullage PM, Molinero V & Peters B (2022) *Biomacromolecules* **23**, 513–9. https://doi.org/10.1021/acs.biomac.1c01247.
47. Dhyani A, Wang J, Halvey AK, Macdonald B, Mehta G & Tuteja A (2021) *Science* **373**, eaba5010. https://doi.org/10.1126/science.aba5010.
48. Ou L, Setegne MT, Elliot J, Shen F & Dassama LMK (2025) *Chem Rev* **125**, 2120–83. https://doi.org/10.1021/acs.chemrev.4c00595.
49. Jaskiewicz JJ, Dayao DAE, Girouard D, Sevenler D, Widmer G, Toner M, Tzipori S & Sandlin RD (2023) *PLOS Pathog* **19**, e1011425. https://doi.org/10.1371/journal.ppat.1011425
50. Jiang P, Li Q, Liu B & Liang W (2023) *Cryobiology* **113**, 104786. https://doi.org/https://doi.org/10.1016/j.cryobiol.2023.104786.
51. Ghalamara S, Silva S, Brazinha C & Pintado M (2022) *Bioresour Bioprocess* **9**, 5. https://doi.org/10.1186/s40643-022-00494-7.
52. Wu D, Cao Y & Huang Q (2023) *J Food Eng* **357**, 111657. https://doi.org/https://doi.org/10.1016/j.jfoodeng.2023.111657.
53. Hadi Z, Ahmadi E, Shams-Esfandabadi N, Davoodian N, Shirazi A & Moradian M (2024) *Cryobiology* **114**, 104853. https://doi.org/https://doi.org/10.1016/j.cryobiol.2024.104853.
54. Pabuccuoğlu S, Uğur H & Pabuccuoğlu SK (2025) *J Istanbul Vet Sci* **9**, 47–56.
55. Kommineni N, Butreddy A, Sainaga Jyothi VGS & Angsantikul P (2022) *IScience* **25(10)**, 105127. https://doi.org/10.1016/j.isci.2022.105127.
56. Zhang P-Q, Tan P-C, Gao Y-M, Zhang X-J, Xie Y, Zheng D-N, Zhou S-B & Li Q-F (2022) *Stem Cell Res Ther* **13**, 152. https://doi.org/10.1186/s13287-022-02817-z.
57. Shin DY, Park JS, Lee H-S, Shim W, Jin L, Lee KW, Park JB, Kim DH & Kim JH (2024) *Biochem Biophys Reports* **38**, 101658. https://doi.org/https://doi.org/10.1016/j.bbrep.2024.101658.
58. Li M, Zheng Y, Bai H & Gao W (2025) *ACS Appl Mater Interfaces* **17**, 19247–62. https://doi.org/10.1021/acsami.5c01179.
59. Bailey TL, Hernandez-Fernaud JR & Gibson MI (2021) *RSC Med Chem* **12**, 982–93. https://doi.org/10.1039/D1MD00078K
60. Yuan L, Chen B, Zhu K, Ren L & Yuan X (2024) *Macromol Rapid Commun* **45**, 2400309. https://doi.org/https://doi.org/10.1002/marc.202400309.
61. Gao Y, Han C & Wang J (2025) *Mol Syst Des Eng*. **10**, 692-721. https://doi.org/10.1039/D5ME00045A.
62. Forbes J, Bissoyi A, Eickhoff L, Reicher N, Hansen T, Bon CG, Walker VK, Koop T, Rudich Y, Braslavsky I & Davies PL (2022) *Nat Commun* **13**, 5019. https://doi.org/10.1038/s41467-022-32469-9.
63. Liu Z, Zheng X & Wang J (2022) *J Am Chem Soc* **144**, 5685–701. https://doi.org/10.1021/jacs.2c00203.
64. Daily MI, Whale TF, Kilbride P, Lamb S,

John Morris G, Picton HM & Murray BJ (2023) *J R Soc Interface* **20**, 20220682. https://doi.org/10.1098/rsif.2022.0682.
65. Pal D, Hall R, Nazarenko Y, Barrie L & Ariya PA (2025) *Npj Clim Atmos Sci* **8**, 204. https://doi.org/10.1038/s41612-025-01062-4.
66. Zaaboul F & Liu Y (2022) *Compr Rev Food Sci Food Saf* **21**, 964–998. https://doi.org/https://doi.org/10.1111/1541-4337.12924.
67. Ebrahimi B, Matavos-Aramyan H & Keshtgar S (2022) *Cell Tissue Bank* **23**, 213–225. https://doi.org/10.1007/s10561-021-09953-5.
68. Ghafarizadeh AA, Malmir M, Naderi Noreini S, Faraji T & Ebrahimi Z (2021) *Andrologia* **53**, e13891. https://doi.org/https://doi.org/10.1111/and.13891.
69. Contreras MJ, Arias ME, Silva M, Cabrera P & Felmer R (2022) *Theriogenology* **189**, 1–10. https://doi.org/https://doi.org/10.1016/j.theriogenology.2022.06.005.
70. Kameni SL, Meutchieye F & Ngoula F (2021) *Open J Anim Sci* **11**, 473–500. https://doi.org/10.4236/ojas.2021.113033
71. Hirad AH, Alarfaj AA, Ravindran B & Narasimhamoorthi SP (2025) *Biochem Biophys Res Commun* **742**, 151019. https://doi.org/https://doi.org/10.1016/j.bbrc.2024.151019.
72. Gaikwad KP, Chandak CS, Ambhore JP, Narkhede MB & Ashwini A (2024) *IP Int J Compr Adv Pharmacol* **9**, 166–76. https://doi.org/10.18231/j.ijcaap.2024.024
73. Díaz Ruiz E, Navas González FJ, León Jurado JM, Arando Arbulu A, Delgado Bermejo J V & González Ariza A (2024) *Animals* **14**, 2936. https://doi.org/10.3390/ani14202936.
74. Liu T, Sun L, Zhang Y, Wang Y & Zheng J (2022) *J Biochem Mol Toxicol* **36**, e22942. https://doi.org/https://doi.org/10.1002/jbt.22942.
75. Agwu E, Kaigama G & Ezihe C (2023) in *Ascorbic Acid - Biochemistry and Functions*, (eds) Kükürt A & Gelen V, IntechOpen, https://doi.org/10.5772/intechopen.110589.
76. Meulmeester FL, Luo J, Martens LG, Mills K, van Heemst D & Noordam R (2022) *Antioxidants* **11**, 2322. https://doi.org/10.3390/antiox11122322.
77. Gasmi A, Bjørklund G, Mujawdiya PK, Semenova Y, Piscopo S & Peana M (2024) *Crit Rev Food Sci Nutr* **64**, 3907–3919. https://doi.org/10.1080/10408398.2022.2137724.
78. Kalyanaraman B (2022) *Redox Biol* **57**, 102497. https://doi.org/https://doi.org/10.1016/j.redox.2022.102497.
79. Kopustinskiene DM & Bernatoniene J (2021) *Pharmaceutics* **13**, 129. https://doi.org/10.3390/pharmaceutics13020129.
80. Almatroodi SA, A. Alsahli M, S. M. Aljohani A, Alhumaydhi FA, Babiker AY, Khan AA & Rahmani AH (2022) *Molecules* **27**, 2665. https://doi.org/10.3390/molecules27092665.
81. Ikram M, Javed B, Raja NI & Mashwani Z-R (2021) *Int J Nanomedicine* **16**, 249–68. https://doi.org/10.2147/IJN.S295053.
82. Anwar S, Alrumaihi F, Sarwar T, Babiker AY, Khan AA, Prabhu S V & Rahmani AH (2024) *Biomolecules* **14**, 697. https://doi.org/10.3390/biom14060697.
83. Emran T, Chowdhury NI, Sarker M, Bepari AK, Hossain M, Rahman GMS & Reza HM (2021) *Biomed Pharmacother* **143**, 112139. https://doi.org/https://doi.org/10.1016/j.biopha.2021.112139.
84. Ragavendran C, Kamaraj C, Alam MW & Rajkumar M (2025) *Inorg Chem Commun* **180**, 115035. https://doi.org/https://doi.org/10.1016/j.inoche.2025.115035.
85. Saka R & Chella N (2021) *Environ Chem Lett* **19**, 1097–106. https://doi.org/10.1007/s10311-020-01103-9.
86. Singh S, Khurana K, Chauhan SB & Singh I (2023) *Futur J Pharm Sci* **9**, 78. https://doi.org/10.1186/s43094-023-00530-z.
87. Mohan J, Kolluri G, Srivastava V, Tyagi JS & Tiwari AK (2023) *Worlds Poult Sci J* **79**, 593–617. https://doi.org/10.1080/00439339.2023.2225793.
88. Chelliah R, Rubab M, Vijayalakshmi S, Karuvelan M, Barathikannan K & Oh D-H (2025) *Next Nanotechnol* **8**, 100209. https://doi.org/https://doi.org/10.1016/j.nxnano.2025.100209.
89. Guo J, Hou J, Hu J, Geng Y, Li M, Wang H, Wang J & Luo Q (2023) *Chem Commun* **59**,

9157–66. https://doi.org/10.1039/D3CC01962D.
90. Gu A & Wheate NJ (2021) *J Incl Phenom Macrocycl Chem* **100**, 55–69. https://doi.org/10.1007/s10847-021-01055-9.
91. Chopra H, Bibi S, Goyal R, Gautam RK, Trivedi R, Upadhyay TK, Mujahid MH, Shah MA, Haris M & Khot KB (2022) *Front Oncol* **12**, 925379. https://doi.org/10.3389/fonc.2022.925379
92. Servesh A, Lokesh Kumar S, Govindaraju S, Tabassum S, Raj Prasad J, Kumar N & Ramaraj SG (2024) *Polym Adv Technol* **35**, e6433. https://doi.org/https://doi.org/10.1002/pat.6433.
93. Pei J, Yan Y, Palanisamy CP, Jayaraman S, Natarajan PM, Umapathy VR, Gopathy S, Roy JR, Sadagopan JC, Thalamati D & Mironescu M (2024) *Green Processing and Synthesis* **13**, 20230094. https://doi.org/doi:10.1515/gps-2023-0094.
94. Zhang X, Dai X, Gao L, Xu D, Wan H, Wang Y & Yan L-T (2023) *Chem Soc Rev* **52**, 6806–6837. https://doi.org/10.1039/D3CS00347G.
95. Sudhakaran G, Kesavan D, Kandaswamy K, Guru A & Arockiaraj J (2024) *Reprod Toxicol* **124**, 108531. https://doi.org/https://doi.org/10.1016/j.reprotox.2023.108531.
96. Xiong X, Cao X, Xu X & Zhuo Q (2025) *Food Eng Rev* **8**, 1-22. https://doi.org/10.1007/s12393-025-09412-x.
97. Jaiswal AN & Vagga A (2022) *Cureus* **14(11),** e31564 . https://doi.org/10.7759/cureus.31564
98. Chen Q, Xi Z, Xu Z, Ning M, Yu H, Sun Y, Wang D-W, Alnaser AS, Jin H & Cheng H-M (2025) *Chem Soc Rev* **54,** 4567–616. https://doi.org/10.1039/D5CS00090D.
99. Qi K, Jia D, Zhou S, Zhang K, Guan F, Yao M & Sui X (2024) *Adv Biol* **8**, 2400201. https://doi.org/https://doi.org/10.1002/adbi.202400201.
100. Du X, Wang B, Li H, Liu H, Shi S, Feng J, Pan N & Xia X (2022) *Compr Rev Food Sci Food Saf* **21**, 4812–46. https://doi.org/https://doi.org/10.1111/1541-4337.13040.
101. Sivanesan J, Sivaprakash B, Rajamohan N, Phanindra VSS, Sonne C, Liew RK & Lam SS (2024) *Environ Chem Lett* **22**, 2943–2975. https://doi.org/10.1007/s10311-024-01777-5.
102. Fang Y, Wu R, Lee JM, Chan LHM & Chan KYJ (2023) *TrAC Trends Anal Chem* **160,** 116959. https://doi.org/https://doi.org/10.1016/j.trac.2023.116959.