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PERSPECTIVE

THE ROLE OF CARBOHYDRATES FROM MONO- TO POLYSACCHARIDES IN THE PRESERVATION OF ANIMAL SPERM

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Abstract

BACKGROUND: The rapid anthropogenic deprivation of biodiversity and challenges of intensive selection in animal husbandry require the development of reliable methods for the long-term preservation of genetic material. Sperm cryopreservation is a key technology, but traditional cryoprotectants are cytotoxic, necessitating the search for new, safe, and effective solutions. **OBJECTIVE:** The purpose of this review is to systematize scientific data on the role and mechanisms of action of saccharides as cryoprotectants for sperm, as well as to analyze the prospects for the development of new effective and biocompatible cryopreservation media. **MATERIALS AND METHODS:** We conducted a comprehensive review of the existing literature devoted to the study of the effect of mono-, di-, oligo- and polysaccharides on the survival of spermatozoa of various animal species after cryopreservation. **RESULTS:** An analysis of the literature revealed that the cryoprotective effectiveness of saccharides increases consistently with the increase in the complexity of their molecular structure. Key parameters determining the effect of saccharides include the type of glycosidic bond, hydration number, and conformational flexibility. Among disaccharides, trehalose and sucrose are particularly noteworthy, demonstrating high efficacy for various species. The greatest multifunctional potential, combining membrane-stabilizing, antioxidant, and ice crystallization-inhibiting properties, has been identified in natural polysaccharides (fungi, algae, plants). **CONCLUSION:** Carbohydrates are the basis for creating new generation cryoprotective media. The development of composite media based on these will help solve problems both in genetic resource banks for biodiversity conservation and in practical breeding under simplified, resource-saving storage protocols.

Keywords: cryodamage; cryopreventive agents; saccharides; sperm cryopreservation.

INTRODUCTION

The natural process of evolution on our planet is the disappearance of some species of animals and plants and the appearance of others. However, the aggressive anthropogenic impact of recent decades has caused a significant depletion of the gene pool. The loss of any

biological species can pose a threat to both individual biocenoses and the ecological system as a whole. Species that are endangered due to population decline or habitat loss will not be able to survive on their own without human assistance. There is a need to develop technologies aimed at preserving the genetic material of rare and endangered species.

Cryobanks play a key role in this process. Most cryobanks specialize in the cryopreservation of gametes (primarily sperm) and embryos. Their main goal is to produce offspring using assisted reproductive technologies, which include artificial insemination, in vitro fertilization and embryo transfer. Furthermore, these technologies are of great importance in the development of the livestock industry. In particular, the method of cryopreserving bull sperm is one of the key technological methods for intensifying beef and dairy cattle production based on genotypic selection.

Research in the field of cryobiology of farm animal sperm is being conducted in various directions. Traditional cryopreservation methods rely on ultra-rapid, deep cooling, which suppresses the formation of large ice crystals. However, this process typically requires high concentrations of cryoprotectants and is unsuitable for large volumes of samples, as it is extremely difficult to achieve rapid, deep, and uniform cooling. In addition, ultra-low temperatures (-136°C, ... -196°C) require complex equipment and trained personnel, as well as high energy costs, which negatively impacts scientific, logistical and technological plans. The low temperature range (-70°C, ... -90°C) is promising for the development of new cryopreservation technologies due to the more flexible variation of biomaterial freezing conditions, the possibility of storing large samples, reduced resource intensity, and the accessibility of the procedure, increasing the availability of the method in hard-to-reach regions and in extreme conditions where the deployment of complex cryogenic equipment is impossible. All this is of decisive importance for the practical activities of cryobanks. Temperature ranges (-70°C to -90°C) are currently of little use, since cellular metabolism slows down significantly when using traditional cryoprotectants (glycerol, DMSO, etc.) at these temperatures.However, energy reserves are depleted, metabolic waste products accumulate, and a stress-induced pathooxidative process occurs. Special cryoprotective environments are required to reduce the negative impact of cold.

Cryoprotectants are crucial for cryopreservation. The compounds currently used to regulate phase transitions in intra- and extracellular fluids are organic solvents that can exhibit cytotoxicity at normal physiological temperatures.Therefore, in some cases they must be removed after thawing, which is extremely difficult or impossible for many clinically important biological materials.Furthermore, traditional cryoprotectants (based on organic solvents) are unable to prevent ice recrystallization, which is another serious problem with modern cryopreservation technologies. To date, more than a hundred organic substances have been identified that have cryoprotective properties of varying degrees of severity. Among the wide variety of chemical compounds, several have been found to exhibit a very pronounced cold-protecting effect: glycerin, dimethyl sulfoxide (DMSO), dimethylacetamide, propylene glycol, and ethylene glycol (1). However, none of these are involved in the metabolism of viable cells. To address this deficiency, energy-containing substances, membrane stabilizers, and antihypoxants have been added to cryopreservation solutions. Substances with antioxidant properties have proven to be highly effective in preservatives, as radicals formed during cold stress cause additional cell damage, and their elimination ensures the long-term viability of the biological object. Animal reproductive cells are particularly sensitive to cryopreservation factors. Damage to the lipid matrix leads to loss of membrane integrity, decreased sperm motility, and loss of fertility. (2, 3, 4, 5, 6). Therefore, the composition and physicochemical properties of preservative media are decisive when preparing sperm for low-temperature storage or freezing. An analysis of modern research in the field of cryopreservation of biological objects indicates the relevance of searching for new effective cryoprotectants with low cytotoxicity. The main focus of the research is the isolation and testing of natural polymers whose structure is rich in functional groups that form bonds with water molecules. Carbohydrate components are primarily capable of modulating the ice crystallization process. Particular attention is being paid to carbohydrate-based macromolecules, ranging from glycosides to polysaccharides (7, 8).

THE ROLE OF SUGARS IN SPERM CRYOPRESERVATION PROCESSES

According to studies on sperm cryobiology, the positive effect of sugars in the medium for its dilution and cryopreservation is explained by their diverse actions: stabilization of osmotic pressure and the stability of protein-lipid complexes, a decrease in electrical conductivity, prevention of sperm agglutination, a shift in the oxidation-reduction potential towards its decrease, inactivation of antibodies, structuring of water, a decrease in the temperature of the onset of water crystallization, participation in glycolysis, prevention of the harmful effects of glycerol on the acrosome, etc. (9, 10, 11). Undoubtedly, the described action depends on the molecular weight of the saccharide, the structure of its molecule, the size of the glycosidic bonds and other features (7, 8). To date, there is no single mechanism for cryoprotection, but since sugars are impermeable cryoprotectants, they coat the surface of the outer layer of the sperm membrane, stabilizing and protecting its structure from the outside. Several hypotheses exist regarding the bioprotective function of sugars during cold exposure (11):

i) Water substitution hypothesis. Sugars form hydrogen bonds with charged and polar groups on the surface of biological molecules, replacing water molecules during dehydration and preventing protein denaturation (12).

ii) Headgroup linkage hypothesis. Sugars form a hydrogen bonding framework linking several headgroups of membrane lipids, which prevents the transition from liquid crystalline state to the gel phase upon dehydration (13).

iii) Vitrification hypothesis. Sugars in biological systems are excellent glass-forming agents, protecting biological molecules from the formation of a hexagonal ice structure Thus, they prevent structural fluctuations, protein denaturation, and mechanical disruption of membranes. (14).

iv) Water trapping hypothesis. Sugars surround the remaining water molecules close to the surface of biomolecules, thereby protecting the surface of biomolecules from dissolution by water molecules and preserving their natural properties in a quasi-dry state (15).

It is generally accepted that saccharides in high or low concentrations have a negative osmotic effect, leading to aggressive dehydration or swelling of spermatozoa and destruction of their membranes (16). Compared to diploid cells, sperm contain less water and do not require a high concentration of saccharides to dehydrate them before cooling. Therefore, a specific combination of several types of saccharides is used to maintain the cryoprotective effect.

Sperm freezing protocols most frequently utilize representatives of different saccharide classes: monosaccharides (fructose, glucose, galactose, etc.); reducing disaccharides (maltose, lactose, rabinose, etc.) and non-reducing disaccharides (trehalose, sucrose, etc.); oligosaccharides (cyclodextran, etc.); and polysaccharides (dextrans, glucans, plant and fungal polysaccharides). All of these have multidirectional effects, and their protective effect in synthetic sperm preservation media increases in the following order: monosaccharides – disaccharides – trisaccharides.

STRUCTURAL FEATURES AND IMPORTANCE OF MONOSACCHARIDES IN SPERM CRYOPRESERVATION PROCESSES

Monosaccharides are the simplest saccharides from which all carbohydrates are built. They have a linear, unbranched structure with one carbonyl (C=O) functional group and one hydroxyl (OH) group on each of the remaining carbon atoms.

The use of monosaccharides in sperm preservatives is a historical fact. Currently, this group of saccharides is actively used as an energy substrate, replenishing sperm energy reserves during cooling and thawing.

In particular, it was shown that under conditions of storage temperature of stallion sperm for 5 days at 5°, 10° or 15°C, the minimum concentration of glucose in the diluent required to optimize the characteristics of sperm movement was 40 mM (17).

Adding/removing glucose after chilling affected sperm motility but not viability. Furthermore, adding substrates that readily support oxidative phosphorylation (pyruvate, lactate) did not improve stallion sperm quality compared to adding glucose alone and, over time, led to detrimental effects on sperm quality. These effects are likely related to oxidative stress that develops during chilling (17).

Combination of different monosaccharides: ribose, arabinose, xylose, glucose, mannose, fructose, galactose, rhamnose, maltose, lactose, sucrose in a synthetic sperm extender based on phosphate buffer, NaCl, non-dialyzable skim milk solids, antibiotics showed that ram spermatozoa survive best at 37 °C in extender with 185 mM glucose, mannose, fructose or sucrose, and at 5 °C - ribose, arabinose, xylose and galactose (18).

It was found that monosaccharides are inferior in cryoprotective action to disaccharides, but the best results were obtained when freezing boar sperm with those disaccharides that contained glucose (19).

The effect of a combination of monosaccharides (fructose, galactose, glucose, and xylose) and disaccharides (lactose, trehalose, maltose, and sucrose) on the composition of sperm cryopreservation extenders was evaluated in various animal species. Specifically, it was shown that using a combination of maltose and fructose resulted in higher progressive motility of frozen/thawed rooster sperm and fertility than using a combination of trehalose and fructose in a cryoprotective extender (20).

STRUCTURAL FEATURES AND SIGNIFICANCE OF DI- AND TRISACCHA RIDES IN SPERM CRYOPRESERVATION

Disaccharides consist of two monosaccharide residues linked by an O-glycosidic bond. If one hemiacetal hydroxyl remains free, the disaccharides exhibit aldehyde properties and are called reducing. If the bond between the two monosaccharide residues is via both hemiacetal hydroxyls, then such disaccharides do not exhibit aldehyde properties and are called non-reducing.

Many researchers working with sperm most often use non-reducing disaccharides such as sucrose and/or trehalose. These disaccharides have the same molecular weight as the reducing disaccharide lactose, which has long been used in traditional sperm freezing media. However, these saccharides differ in composition: lactose contains glucose and galactose, sucrose contains glucose and fructose, and trehalose contains two glucose molecules (19). In experiments with freezing frog sperm, the use of 1% (w/v) sucrose together with 15% DMSO was shown to be effective, but increasing the sucrose concentration to 10% promoted cell damage due to excessive plasmolysis and exposure to solutes, as proposed in the two-factor cryodamage hypothesis (21). While in experiments with freezing stallion sperm in a medium based on skim milk, egg yolk, dimethylformamide with the addition of sucrose at concentrations of 25, 50 and 100 mM, the best result in preserving sperm motility was observed when using 50 and 100 mM sucrose, and the preservation of acrosomal membranes and high membrane potential of sperm mitochondria when using 100 mM sucrose (22).

The use of 100 mM sucrose is effective not only for deep freezing of spermatozoa at liquid nitrogen temperatures, but also for preservation in a supercooled state (-10°C). In particular, the use of an oil coating to prevent ice formation at low subzero temperatures in a buffered saline solution supplemented with 2%-20% Ficoll-400, 0.1%-1% xanthan gum, or 2%-8% albumin for stallion sperm proved to be more effective with the addition of 100 mM sucrose (23).

In recent years, a large body of work has been devoted to the use of the non-reducing disaccharide trehalose in sperm cryopreservation. The effectiveness of incorporating this sugar into a medium for freezing and storing sperm at liquid nitrogen temperatures has been demonstrated for axolotl sperm at a concentration of 200 Mm (24), diamond catfish - 100 mm (25), tambaki (*Colossoma macropomum*) – 100-150 mM (26), ‘Assam Hill’ goats – 100 mM (27), ram - 60 мМ (28, 29, 30), or 100 mM (31), stallions – 50 mM (32), as well as in several studies on human sperm (33, 34). A comparative analysis of the physicochemical properties of sucrose and trehalose revealed a number of features that likely explain their differences in cryoprotective action. (35, 36). For example, the hydration number - the average number of water molecules that are tightly bound to a dissolved compound - is higher for trehalose (8.0-12.1) than for sucrose (6.3-11.2) Although both saccharides have similar molecular weights and structures, an aqueous solution of trehalose has a higher specific viscosity than a sucrose solution. Furthermore, trehalose is more evenly distributed in an aqueous solution due to its continuous network of sugar-water hydrogen bonds, whereas sucrose forms clusters and its molecules are tightly bound to each other due to its lower hydration

number. Also, based on the radius of gyration and glycosidic dihedral angles of dissolved disaccharides, trehalose acquires a more flexible structure than sucrose. Consequently, sperm dehydration in trehalose and sucrose media prior to freezing will proceed differently, which will also impact ice formation processes. The difference between the saccharides determines their interaction with the diluent components and directly with the cell's organic structures at various stages of cryopreservation. In particular, due to the hydrophilic nature of disaccharides, proteins can associate with them in a single amorphous phase (37).

The cryoprotective effect depends not only on the concentration of saccharide in the medium, but also on the rate of cooling and thawing. Analyzing the efficiency of using 0.15 M and 0.3 M concentrations of sucrose and trehalose during ultra-fast and slow freezing of bovine sperm in liquid nitrogen, as well as thawing at 37°C or 65°C, it was shown that higher sperm quality (total motility, progressive motility, viability and acrosome integrity) is maintained in the series with slow freezing in the medium of any disaccharide and any thawing temperature (38).

The cryoprotective effect of sucrose or trehalose at the indicated concentrations is likely due to the fact that slow cooling slows the osmotic dehydration of spermatozoa during cooling. However, this is sufficient to prevent intracellular ice formation and minimize the negative effects of increased extracellular osmolality.

Gentiobiose, a structural similarity to trehalose, significantly improved male sperm motility parameters, DNA and membrane integrity, and mitochondrial membrane potential in sperm after thawing compared to those stored in conventional media without additives or in the presence of trehalose as a cryoprotectants (39).

A comparison of these sugars shows that both have the same number of hydroxyl and hydroxymethyl groups (8 groups), and all these groups are oriented cylindrically around two pyranose rings (with a slight difference of trehalose having two hydroxymethyl groups and gentiobiose having only one hydroxymethyl group), as well as the same number of rings (2 rings) and the same number of glycosidic oxygen atoms (1 atom). However, a significant difference between the two sugars is the presence of an additional dihedral angle during the glycosidic linkage of gentiobiose. Trehalose is a non-reducing disaccharide with an alpha-glycosidic linkage (α Glc(1 → 1) α Glc). Gentiobiose is beta-anomeric and is a reducing disaccharide with a beta-glycosidic linkage (β Glc(1→6) β Glc).

There is also a functional difference between these two sugars (11):

i) In the presence of trehalose, a higher rate of residual bilayer coating is observed than in gentiobiose.

ii) Higher levels of sugar-sugar hydrogen bonds (sugar clustering) are observed in the presence of trehalose than in gentiobiose.

iii) Fewer glucose-lipid hydrogen bonds are observed in the presence of trehalose than in gentiobiose. In other words, gentiobiose readily attaches to the membrane. Consequently, hydration of the bilayer membrane is reduced in the presence of gentiobiose, and cell damage during the freezing process is minimal (40).

iv) The penetration depth of trehalose into the bilayer membrane is less than that of gentiobiose due to the hydrogen bonds that gentiobiose forms with esters (such as sphingolipids, phosphatidylcholine, phosphatidylethanolamine, ceramides, etc.).

These observations may be indirectly related to the specific conformational characteristics of trehalose, including a high tendency to self-aggregate, high hydrophilic strength, and lack of flexibility (40).

The high cryopreservation effects of trehalose and gentiobiose compared to other disaccharides and monosaccharides are related to their structure (11).

Trehalose and gentiobiose can form strong hydrogen bonds with polar groups of biomaterials, thereby replacing water molecules at the membrane-liquid interface and maintaining the head groups in their hydrated position (11, 41).

Furthermore, trehalose and gentiobiose have greater cryopreservation strength than other sugars such as sucrose and glucose due to the presence of α ,α -(1 → 1) glycosidic bonds. Studies have shown that α ,α -trehalose stabilizes membranes by binding to cell membrane lipids, thereby increasing cell viability (42), maintaining membrane integrity, and reducing cryopreservation-induced damage (43). The structure of the reducing disaccharide lactose contains two monosaccharides (glucose and galactose) linked by a β-1,4 glycosidic bond formed by the interaction of the hemiacetal

hydroxyl of galactose and the alcoholic hydroxyl of glucose. This disaccharide is widely used in extenders for bull and boar semen (44). In particular, it was shown that boar semen frozen in egg yolk with lactose had higher survival rates of 38% than semen frozen in Beltsville Freezing Extender 5 (BF5) 22% (45). Maltose has become widely known in modern sperm cryopreservation technologies, the positive potential of which has been discovered in various animal species (46). The chemical structure of maltose (a disaccharide) consists of two α-D-glucose units linked by an α(1-4)-glycosidic bond. This means that the carbon of one glucose unit at position 1 in the α-anomeric configuration forms a bond with the carbon of a second glucose unit at position 4. Both glucose residues in the maltose molecule are in the pyranose form. Maltose is classified as a reducing sugar because it contains one potentially free aldehyde group that can be oxidized. The difference between reducing and non-reducing disaccharides lies in the size of their glycosidic bonds and the ability or inability of their molecules to undergo mutarotation, which determines the interaction of the saccharides with the diluent components and directly with the organic structures of the cell at various stages of cryopreservation.

According to the literature, various combinations of non-reducing and reducing saccharides can provide the most effective protection of sperm during freezing/thawing (47). In particular, it has been shown that when using a combination of maltose and fructose, a higher percentage of progressively motile frozen/thawed rooster spermatozoa and a high percentage of egg fertility are achieved than when using a combination of trehalose and fructose (20). However, a higher chromatin integrity level (92.4%) was obtained with trehalose than with maltose (74.5%). However, maltose had a positive effect on the preservation of frozen/thawed sperm in the chicken reproductive tract and on egg fertilization. Thus, despite their similar molecular weights, maltose and trehalose have different physicochemical and biological properties, which determine their function and effectiveness as components of cryoprotective media.

Cryopreservation of stallion semen in a lactose medium supplemented with 50 and 150 mM trehalose showed that the extender containing 50 mM trehalose improved plasma and acrosomal membrane integrity, DNA integrity, increased mitochondrial membrane potential, reduced reactive oxygen species levels, and increased post-thaw progressive sperm motility compared to the control group (without trehalose). Mares inseminated with frozen-thawed semen supplemented with 50 mM trehalose tended to have better pregnancy rates than the control groups (32).

STRUCTURAL FEATURES AND IMPORTANCE OF OLIGOSACCHARIDES IN SPERM CRYOPRESERVATION PROCESSES

Oligosaccharides are polymers containing three to ten monosaccharides. Of these, raffinose has been shown to play a role in cryopreservation processes. It should be noted that raffinose consists of D-galactose, D-glucose, and D-fructose residues and is one of the most common plant reserve carbohydrates.

A simple and rapid method for cryopreservation of mouse sperm at a temperature of -80 °C in microtubes containing a 20% raffinose solution is known (48).

Frozen-thawed sperm retains practical fertilization rates even after three months of storage. However, experiments with freezing avian sperm using liquid nitrogen vapor have shown that raffinose negatively impacts sperm quality and fertility. Thus, a comparative analysis of the effectiveness of using the disaccharide sucrose and the trisaccharide raffinose in cryopreservation of sperm of the local Thai chicken breed Pradu Hang Dum showed that in the presence of 1 mmol sucrose in a medium with dimethylformamide, it significantly increased the motility, membrane integrity, acrosome integrity and mitochondrial functions of frozen-thawed spermatozoa and demonstrated the highest levels of fertility after cryopreservation of sperm. While raffinose had a negative effect on sperm quality and fertility in the concentration range from 1 to 100 mmol (49). The presence of raffinose (1, 5 and 10 mM) in the preservative (4% DMSO) for Kadaknath rooster semen did not affect the sperm parameters after in vitro thawing, but fertility was significantly ($p < 0.05$) higher in the group receiving 10 mM raffinose (50).

Differences in cryoprotective activity were also identified when comparing raffinose with trehalose at equal concentrations. Trehalose was

shown to provide significantly better recovery of cryopreserved mouse sperm (11).

STRUCTURAL FEATURES AND IMPORTANCE OF POLYSACCHARIDES IN SPERM CRYOPRESERVATION PROCESSES

Polysaccharides are polymers containing long chains of monosaccharides linked by glycosidic bonds. One of the first polysaccharides to demonstrate reliable cryoprotective activity was hydroxyethyl starch (HES). This O-alkyl derivative of starch, which has long been widely used as a plasma substitute, is biocompatible, non-toxic, and does not require removal before use. The result depends heavily on the molecular weight, the degree of HES substitution, and the removal of cryopreserved blood components after thawing (51). In most cases, the use of HES produces a moderate to strong positive effect, with survival rates of up to 95% of preserved cells. The mechanism of cryoprotective action of HES, as well as macromolecular cryoprotectants in general, is a subject of debate. It is assumed that, accumulating in the intercellular space, HES potentiates the reorganization of hydrogen bonds between water molecules, inhibits crystal growth, ice recrystallization, and also equalizes osmotic pressure, preventing cell dehydration as a result of excess intracellular pressure with the release of water and membrane damage.

According to literature, polysaccharides of some plants may have a cryoprotective effect on animal sperm, In particular, the introduction of *Lycium barbarum* and *Laminaria japonica* polysaccharides into the extender improves the quality of cashmere goat sperm during freezing (52). and is also the best antioxidant candidate for inclusion in the freezing medium to improve the quality of giant panda sperm during cryopreservation (53). Polysaccharides from *Astragalus membranaceus* have shown high efficiency in cryopreservation of dairy goat sperm (54) and bull sperm polysaccharides *Laminaria japonica* (55), *Apium graveolens* and *Astragalus membranaceus* (56). Sulfated polysaccharides also increase sperm viability. In particular, the use of sulfated polysaccharides from the seaweeds *Gracilaria domingensis* and *Ulva fasciata* in a freezing medium for fish sperm promotes their recovery after thawing (57, 58).

It should be noted that mushroom polysaccharides also have a cryoprotective effect; however, their effect on reproductive cells remains poorly understood at present. The wide spectrum of biological action of basidiomycete polysaccharides (59) indicates their possible practical use also in cryopreservation of reproductive cells. In particular, xylomannan was isolated from the mycelium and fruiting bodies of the basidiomycete fungus *Flammulina velutipes* (60), which had an effect on freezing Chinese hamster ovary cells to -80°C. In our series of studies, we revealed a pronounced cryoprotective effect of polysaccharide fractions from *Trametes ochracea* (Pers.) (61), *Hericium erinaceus* (Pers.) (62, 63), *Ganoderma applanatum* (Pers.) (64) in relation to cells of animal origin (human blood leukocytes, bull sperm) at various negative temperatures (-5°C, -80°C). A possible mechanism of cryoprotective action of xylotrophic fungal polysaccharides is the stabilization of water fractions during cooling and the binding of free radicals (61). Standardization of biotechnological methods for obtaining polysaccharides will provide prospects for their use as new effective cryopreservatives with significant pharmaceutical action.

CONCLUSION

This review systematizes current knowledge on the role of carbohydrates as key components in the development of effective and safe sperm cryopreservation technologies. Carbohydrates represent an essential class of cryoprotectants with a multi-layered mechanism of action. From monosaccharides, which primarily serve as energy substrates, to complex polysaccharides, their protective function evolves from a simple osmotic effect to comprehensive membrane stabilization, inhibition of ice crystallization, and suppression of oxidative stress. The cryoprotective efficacy of saccharides naturally increases with increasing molecular weight and structural complexity. The efficiency of a particular saccharide is determined by its fine chemical architecture, parameters such as the type of glycosidic bond (α, α-1→1 in trehalose, β-1 → 6 in gentiobiose), hydration number, conformational flexibility and ability to form hydrogen bonds, predetermine interactions with the lipid bilayer and water. This explains the

Table 1. Cryoprotective properties and efficacy of saccharides for sperm of different animal species.

Class of saccharides	Key representatives	Main cryoprotective properties and mechanisms	Examples of species-specific efficacy (from the review)
Mono-saccharides	Glucose, Fructose, Galactose, Xylose	Energy substrate, osmotic stabilization, participation in glycolysis. Low independent cryoprotection.	Stallion (5°C): 40 mM glucose to maintain motility. Ram: Best survival at 5°C with ribose, arabinose, and xylose.
Disaccharides			
Non-reducing	Trehalose (α,α-1↔1)	Water substitution, vitrification, high hydration number (8.0-12.1), molecular flexibility, membrane stabilization.	Sheep: 60-100 mM – improved motility, membrane integrity, decreased of osmotic stress. Human: Superior to sucrose in motility and DNA integrity. Axolotl, catfish: 100-200 mM – effective protocol for cryobanks.
	Sucrose (glucose-fructose)	Membrane stabilization, osmotic protection. Tendency to clustering.	Stallion: 100 mM – optimal for motility and acrosomal integrity, effective even at -10°C. Bull: 0.15-0.3 M – effective with slow freezing. Poultry (chickens): 1 mM increases fertility.
Reducing	Maltose (α-1→4)	Osmotic protection, membrane stabilization.	Rooster: When combined with fructose, it gives the highest fertility, superior to the combination with trehalose.
	Lactose (β-1→4)	Osmotic stabilizer in classical environments.	Boar: Shows a 38% survival rate in egg yolk extender.
	Gentiobiosis (β-1→6)	Better binding to membrane lipids than trehalose, effective stabilization.	Human: Superior to trehalose in some parameters (DNA integrity, MMP).
Trisaccharides	Raffinose (galactose-glucose-fructose)	Osmotic protection. Strong species specificity.	Mice: 20% solution for simple freezing at -80°C. Birds: Often has a negative effect on sperm quality and fertility.
Poly-saccharides	Hydroethylstarch	Inhibition of ice recrystallization, equalization of osmotic pressure, viscosity stabilization.	Cell cultures: Survival rate up to 95%. Used as a plasma substitute.
	Plant polysaccharides (*Astragalus, Lycium*)	Antioxidant activity, membrane stabilization, combined action.	Goat, bull: Improved motility, membrane integrity and decreased of osmotic stress after freezing.
	Seaweed polysaccharides (*Laminaria,* sulfated)	Antioxidant and membrane-protective action.	Fish, bull: Increases sperm vitality and motility.
	Polysaccharides of mushrooms (*Trametes*, *Hericium*)	Stabilization of water fractions, binding of free radicals, biocompatibility.	Bull, human leukocytes: Pronounced cryoprotective effect at -5°C and -80°C.

species specificity and the lack of a universal protocol, and opens the way for targeted

molecular design of cryoprotectants (Table 1). Consideration should also be given to recently published data on protein stabilization mechanisms (65) indicating that the processes under consideration may be more complex. Therefore, further in-depth research is required to fully understand the role of sugars in sperm cryopreservation and optimize existing protocols.

Mastering the moderately low temperature range (-70°C to -90°C) is a strategic direction for the development of accessible cryobiology. While this regime reduces resource consumption and simplifies logistics, it requires the creation of fundamentally new cryoprotective environments capable of compensating not only for the physical damage caused by ice but also for the long-term metabolic and oxidative stress in cells. The shortcomings of traditional penetrating cryoprotectants (cytotoxicity, the need for washing) prompt the search for biocompatible alternatives. In this context, natural polysaccharides from plants, algae, and fungi are emerging as among the most promising candidates. They combine macromolecular advantages (viscosity stabilization, crystallization control) with high biocompatibility, low toxicity, and natural antioxidant activity.

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