Cryopreservation of Semen in Domestic Animals: A Review of Current Challenges, Applications, and Prospective Strategies

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Simple Summary: Here we present a comprehensive review of the current literature describing mechanisms of damage incurred by cryopreservation, and we highlight several classical and novel strategies to mitigate this damage and increase sperm survival during the cryopreservation process.

Abstract: Cryopreservation is a way to preserve germplasm with applications in agriculture, biotechnology, and conservation of endangered animals. Cryopreservation has been available for over a century, yet, using current methods, only around 50% of spermatozoa retain their viability after cryopreservation. This loss is associated with damage to! different sperm components including the plasma membrane, nucleus, mitochondria, proteins, mRNAs, and microRNAs. To mitigate this damage, conventional strategies use chemical additives that include classical cryoprotectants such as glycerol, as well as antioxidants, fatty acids, sugars, amino acids, and membrane stabilizers. However, clearly current protocols do not prevent all damage. This may be due to the imperfect function of antioxidants and the probable conversion of media components to more toxic forms during cryopreservation.

Keywords: cryo-damage; sperm banking; sperm cryopreservation; antioxidant; membrane stabilization

1. Introduction

Mammalian sperm are among the first cells to be successfully cryopreserved, and over the last seven decades the use of cryopreserved semen for artificial insemination has come to play a crucial role in animal agriculture [1]. For most animal species, however, a large population of sperm are incapacitated after cooling to and warming from liquid nitrogen temperatures. Thus, to achieve equivalent artificial insemination fertility rates, several times more cryopreserved bull sperm are needed in comparison with fresh samples [2]. Although artificial insemination (AI) in pigs with frozen or fresh semen is common, the fertility of cooled semen remains about 50% of fresh semen [3]. After the insemination of superovulated ewes, pregnancy rates and fertility characteristics with frozen–thawed ram semen are reduced from fresh semen by 20% [4]. The long history of advancements in sperm cryopreservation in livestock has facilitated modern genetic strategies in breeding programs but has also been at the forefront in the assessment and mitigation of cryopreservation-induced damage, including osmotic, oxidative, and epigenetic damage that has expanded to somatic cell cryopreservation research. In this review, we first discuss a brief history and the importance of cryopreservation in livestock; we then highlight the biology, injuries, stresses, and challenges of sperm cryopreservation; and finally, we discuss conventional and modern strategies to prevent cryopreservation-induced damage to sperm. In short, this review aims to capture both classical and modern understanding of the modes and mitigation of damage induced by the cryopreservation process in domestic animal sperm.
It is believed that the first successful AI was performed by Italian physiologist Lazzaro Spallanzani in 1780, who inseminated a dog using frozen–thawed semen [5]. Subsequent researchers repeated this work, including John Hunter in 1799, whose method resulted in human pregnancy [6]. At the same time in France, AI was shown to be a useful method of improving fertility in horses [7]. Following these preliminary reports, horse fertility centers began to spread throughout Europe for the collection and extension of semen and breeding of mares [7]. The various techniques for artificial insemination (AI), technician training, and revised methods for collection and cooling semen continued to increase, and by 1938 around 40,000 mares, 1.2 million cows, and 15 million sheep had been serviced by AI in Russia [8]. In the early 1800s the majority of the AI trials were being conducted in Europe and Russia; however, several successful efforts in horses and cattle had been reported in the USA as well [8]. For example, in 1907 a calf was produced via AI at the Oklahoma Experimental Station. After this achievement, the application of AI in the USA was developed, and in 1937 AI was applied in the cattle herds at the University of Missouri and subsequently at other universities [8].

Though references to the preservation of semen date back to the 1600s [9], it was not until the expansion of (AI) in the late 1950s and early 1960s—a time when the dairy cattle industry realized a need for longer storage of bull semen—that semen cryopreservation became a serious area of research. This continued study was founded on the basic discovery of Polge et al. (1949) that demonstrated that glycerol could enable the conservation of cells at low temperatures [10]. The progress in this front was rapid: the first offspring produced via cryopreserved semen was born in 1951 [11], and today the modern cattle industry is based on the use of AI with cryopreserved sperm. Table 1 shows the first recorded offspring produced via AI using frozen–thawed semen in different species. Beyond agriculture, human cryopreservation has provided flexibility in fertility options not only for couples but also for those undergoing iatrogenic treatments such as cancer therapy [12] or transgender women undergoing gender-affirming surgery [13].

Table 1. First recorded offspring produced via AI using frozen–thawed semen in different species.

| Species | Year | Reference |
|---------|------|-----------|
| Avian   | 1942 | [14]      |
| Bovine  | 1951 | [11]      |
| Human   | 1953 | [15]      |
| Porcine | 1957 | [16]      |
| Equine  | 1957 | [17]      |
| Ovine   | 1967 | [18]      |

2. Cryobiology of Sperm

Cryopreservation is the process by which cells or tissues can survive at low temperatures with reduced or ceased of metabolic activity [19]. During this process, however, cells experience various types of damage to all aspects of cell and tissue anatomy and physiology that consequently lead to reduced cell or tissue function [20]. In particular, semen cryopreservation leads to various physical, biochemical, and oxidative damages to the sperm membrane, leading decreases in the viability and fertilizing capacity of sperm [19]. These cryoinjuries mostly lead to loss of motility, plasma membrane functionality, and acrosome integrity of sperm [19]. Much of this damage occurs at the beginning and end of protocols when cryoprotective agents are added and removed [21], but damage also can happen during freezing and thawing at slow to moderate cooling and warming rates [22]. In a study by Bucak et al., several undesired changes in vacuole-like structure, as well as head, neck, acrosome, and mitochondria damages were observed via a scanning electron microscopy evaluation [23]. Moreover, freeze–thaw-induced damage to aquaporins in the plasma membrane have been found that could affect the plasma membrane’s functionality [24].
In the presence of extracellular ice during cooling, sperm experience very strong hypertonic conditions because the relative amount of water decreases in the extracellular unfrozen fraction [25]. This results in exosmosis to maintain an equilibrium between the intra- and extracellular solutes, leading to cellular dehydration, where the extent of this dehydration is generally inversely proportionate to cooling rate. The reverse process takes place during thawing and sperm face a relatively hypotonic condition resulting in swelling because of water uptake. In contrast, at fast cooling rates, classical cryobiology suggests that cellular dehydration is reduced and that intracellular ice formation is mainly responsible for cell death, a theory thoroughly documented in many somatic cell types [25]. However, sperm may be different: Morris et al. used cryo-scanning electron microscopy and freeze substitution to show that no intracellular ice is formed in sperm [26]. They claim that osmotic tension experienced during thawing is the reason for cell damage. To be clear, there is still debate about the presence of intracellular ice in sperm frozen at too-fast cooling rates. For example, the presence of intracellular ice is central to research by Devireddy et al., who use a calorimetry-based technique that works under the assumption that sperm lyse because of intracellular ice to identify the water permeability of sperm at subzero temperatures [27]. Clearly, more work in this area is justified. Nevertheless, the “two-factor hypothesis” describes two complementary modes of damage occurring at both ends of the cooling rate spectrum [28]: damage due to osmotic dehydration (known as solute effects injury for too-slow cooling protocols), and damage due to intracellular ice formation or, per Morris et al., osmotic imbalance during warming for too-fast cooling protocols [26]. This injury dichotomy suggests that there is an optimal cooling rate where damage is minimal. Membrane permeability to water (and its temperature dependence) is one of the main criteria determining the optimum cooling rate that itself depends on membrane composition as well as the presence of cryoprotective agents [27]. At subzero temperatures, a “water transport model” can be used to calculate cell volume changes during freezing and to predict optimal cooling rates that maximize dehydration (and thus minimize the likelihood of intracellular ice or osmotic imbalances) and minimize hypertonic exposure times [29].

3. Cryopreservation Injuries in Sperm

3.1. Changes in Sperm Plasma Membrane

The sperm plasma membrane is the major site of damage during cryopreservation (Figure 1). This damage can be divided into that which occurs during equilibration with cryoprotectant media and that which occurs during cooling [30]. As a high concentration (5–15% v/v) of permeating cryoprotective agents such as glycerol is almost always included in sperm cryopreservation media in most of the animal species, the equilibration process of sperm with these media can cause large changes in cell volume [22]. To wit, upon exposure to CPA containing media, sperm shrink and gradually reswell (see Figure 1 inset). Sperm are especially sensitive to these osmotically induced volume changes, where damage has been shown through loss of membrane integrity, loss of motility, and loss of acrosome integrity [31].

After CPA equilibration, sperm are cooled, which is another source of injury often seen at the plasma membrane [32]. First and foremost, the freeze–thaw process causes enormous changes in extracellular concentrations of the permeating CPA and other medium components. This freeze- and thaw-induced change in osmolality and the resulting osmosis can cause dramatic changes in cell volume, and thus significant mechanical stress on the cell membranes [33]. The freeze-concentration of the ionic components in the media are hypothesized to be a principle source of damage [34], and this damage is mitigated in some respect by the major nonionic media components, such as permeating (e.g., glycerol or ethylene glycol) or nonpermeating cryoprotectants (e.g., sucrose, raffinose, or trehalose),
that reduce the relative ionic concentrations at subzero temperatures. Even in the absence of extracellular ice and concomitant large osmotic gradients, cooling-induced phase transitions change the ultrastructure of the plasma membrane [35]. In fact, most of the cryodamage in sperm is associated with the structural stability of the plasma membrane and is thus linked to plasma membrane composition [36]. For example, some phospholipids increase membrane flexibility, and cholesterol provides stability that seems to improve the resistance of sperm to freezing damage [37]. Membranes that have a low cholesterol to phospholipid ratio with an asymmetric pattern of cholesterol distribution seem to be more vulnerable to injuries [38]. Furthermore, lateral movement of membrane phospholipids is usually restricted in temperatures lower than 5 °C, and this ultimately results in a transition from the fluid to the gel phase. As a result of this phenomenon, membrane lipids are restructured, some cholesterol molecules are released, and many integral proteins in the plasma membrane such as ion channels become irreversibly clustered [39] in a way that can cause a loss of functionality [40]. All of these changes lead to destabilization of the membrane and a loss of its selective permeability, thereby increasing the influx of ions such as Ca\(^{2+}\) and bicarbonate from the extracellular space [19] that in itself can cause cryopreservation-induced sperm capacitation [41].

Figure 1. Mechanisms and foci of cryopreservation-induced injury (right) in sperm under two regimes: equilibration with and from high concentrations of cryoprotectant media which induces potentially damaging volume excursions (blue line representing volume, and red line representing an osmotic tolerance limit; top left) and cooling in the presence and absence of extracellular ice (bottom left).
3.2. Changes in Sperm Plasma Membrane

The mechanisms responsible for DNA fragmentation after cryopreservation are still not completely understood, though it has been attributed to the increase in oxidative DNA damage [42]. Cryoinjuries on the sperm nucleus should be explored by distinguishing between different sites of injury, such as DNA and nucleoproteins. Sperm chromatin consists of DNA and protamine (P1 and P2) along with the histones that shape the nucleoprotein structure [43]. The distribution of protamine is different between species; for example, bull, boar, and ram have only P1 in the nucleoprotein structure while mouse, horse, and human present both P1 and P2. Additionally, P1 to P2 ratios are different between species, and this ratio affects the resistance of that structure to freeze–thawing procedures [44]: the extent of DNA-induced cryodamage is higher in species having both P1 and P2 rather than those exhibiting only P1 [44].

High DNA fragmentation and low mitochondrial membrane potential are significant contributors to reduced motility after cryopreservation [45]. The mitochondrial dysfunction that occurs following cryopreservation can result in the formation of reactive oxygen species (ROS). Interruption of oxidative phosphorylation and inactivation of the antioxidant enzymes are probably the main reasons for mitochondrial dysfunctionality [46]. Damages to the mitochondrial DNA and inner and outer mitochondrial membranes are the other reason for mitochondrial dysfunction. All these changes reduce the mitochondrial membrane potential, which can result in releasing free oxygen radicals through the membrane pores [47]. Mitochondria play a crucial role in ATP production by regulating oxidative phosphorylation and the tricarboxylic acid cycle important for the motility and fertilizing capacity of sperm [48].

3.3. Proteome Alterations

Studies have demonstrated that cryopreservation can alter the expression level of many proteins related to sperm function [49]. Moreover, the freezing process can cause protein degradation, phosphorylation, and carbonylation [50]. Recently, numerous comparisons of protein profiles have been reported between fresh and frozen sperm in several species to understand this aspect of cryoinjury (see [49] for review). The application of comparative proteomics has led to identifying specific proteins as potential key biomarkers for post-thaw recovery and sperm fertility in general [51]. For example, Chen et al. discovered that 41 proteins changed in boar spermatozoa during cryopreservation. These proteins were related to sperm motility, plasma membrane integrity, energy metabolism, capacitation, and sperm–oocyte fusion [52]. Expression of A-kinase anchoring protein (AKAP)-4, Fibrous sheath interacting protein 2 (FSIP2), Fascin, Ornithine decarboxylase antizyme 3 and Leucine-rich repeat, and coiled-coil centrosomal protein 1 are less present in frozen–thawed sperm [51]. On the other hand, the levels of fifteen proteins related to the reduction of fertility, including Nexin 1, Spermadhesins PSP1, Tetraspanin CD63 (CD63), Complement Factor D (CFD), and Ras GTPase-activating-like protein IQGAP2, were significantly increased after cryopreservation [51]. A recent study on boar sperm found that sperm membrane proteins such as Fc fragment of IgG binding protein, Lactadherin, Arylsulfatase a precursor and F-actin capping protein subunit alpha 1 are biomarkers that predict the likelihood of boar semen post-thaw functionality (also known as freezability) [53].

Similar to the results of studies conducted on boar sperm, proteomic analysis of ram sperm found significant changes in the abundance of 51 proteins. The proteins such as T-complex protein CCT subunits, Casein kinase I isoform gamma-2 isoform X2 (CSNK1G2), and TOM1-like protein 1 isoform X2 (TOM1L1) were decreased while the levels of other proteins such as Leukocyte elastase inhibitor (SERPINB1) and Tyrosine-protein kinase Fer isoform X2 (FER) were significantly increased in frozen sperm compared with fresh sperm [51]. He et al. also showed that cryopreservation decreased the expression of hexokinase1 (HXK1) and Casein kinase II subunit alpha (CSNK2A2) in ram sperm. They demonstrated that these proteins are in the sperm flagellum and thus may be associated with sperm motility and viability [48].
These cryopreservation-induced effects on proteins are now being explored in other species. For example, in rainbow trout sperm, cryopreservation can cause the release of proteins related to structure and metabolism from the mitochondria, cytoskeleton, nucleus, and cytosol to the extracellular fluid [54]. A recent study on Adria gazelle sperm found that 85 proteins differed between fresh and frozen–thawed samples. These proteins were mostly related to sperm metabolic pathways including mitochondrial energy production and glycolysis, which may explain the significant loss of motility in thawed sperm [55]. Ryu et al. found that cryopreservation success (freezability) biomarkers such as voltage-dependent anion-selective channel protein 2 (VDAC2) or glutathione s-transferase mu 5 (GSTM5) were different between bull sperm with high freezing tolerance index and low freezing tolerance index [56]. VDAC2 is a mitochondrial protein and plays an important role in the transportation of ions [57]. Therefore, alteration of this protein may induce ionic imbalance, which has adverse impacts on sperm function. Moreover, GSTM5 as a freezability biomarker plays a key role in regulating sperm resistance to oxidative stress [58]. Possibly relatedly, Gaitskell-Phillips et al. found that mitochondrial proteomics facilitated differentiation between good- and poor-freezing sperm in stallion [59], identifying six key proteins with more than a threefold change. In the chicken, Cheng et al. reported increases in 36 proteins and decreases in 19 proteins during cryopreservation. They found that proteins such as tubulin α-3, outer dense-fiber protein, and tektin5 were increased, while proteins including dynein and axonemal were decreased after freeze–thaw. Tubulin and Tektin are linked with flagellum structure and sperm motility [60], and flagellum-related protein alteration can decrease the motility of rooster sperm after cryopreservation. Furthermore, the activity of enzymes related to glycolysis, including glyceraldehyde-3-phosphate dehydrogenase and L-lactate dehydrogenase, was down-regulated after cryopreservation, which can cause a decrease in ATP content and motility in sperm [61]. In summary, the application of modern proteomics methods has yielded new insights into the full scope of changes and damage induced by sperm cryopreservation protocols.

3.4. Epigenetic Modifications

Epigenetics encompasses heritable mechanisms that include histone modifications, DNA methylation, and non-coding RNAs [62]. Epigenetic modifications could alter the performance of chromatin and help to regulate gene expression. Alterations in epigenetic patterns happen in response to environmental signals [63]. Recent studies demonstrated that increased oxidative stress in sperm cells during the freezing process resulted in epigenetic modifications and are the main reasons for the decrease in their motility and fertilization ability [63]. Importantly, these epigenetic changes could be transmitted to offspring and affect embryonic development [62]. Salehi et al. reported that epigenetics patterns such as DNA methylation (DNMT), histone methylation, and acetylation were reduced in rooster sperm after cryopreservation [64]. There is a negative correlation between free radicals, DNA fragmentation, and DNMT [65] that can reduce the fertilization potential of sperm after thawing.

Flores et al. found that protein-DNA disulfide bonds and histone H1-DNA binding proteins were altered in boar sperm after cryopreservation [66]. It is suggested that osmotic and oxidative tensions can cause an alteration in the structure of the nucleus during freezing and thawing [67]. Moreover, Aurich et al. stated that stallion sperm DNA cytosine methylation was increased by cryopreservation [68]. Another study reported that during cryopreservation, methylation levels increased in several genes, including CXCR4B, DND, POU5F1, VASA, SOX2, and SOX3 in zebrafish sperm, that may be correlated with the down-regulation of these genes [69]. A negative correlation has been reported between DNA fragmentation and DNA methylation that is most likely related to oxidative stress [65]. Increased methylation of some important genes such as POU5F1 and SOX2 in sperm can have adverse effects on early embryo development [70].
4. Prevention of Sperm Cryoinjury

Many methods are available for the cryopreservation of domestic animal sperm. While post-thaw viability varies from species to species and even specimen to specimen, current methods protect only about 50% of sperm against cryoinjury. To improve these results, numerous approaches have been tried to increase sperm resilience during the cryopreservation process. We organize them into two categories. First, we discuss conventional strategies that include cryoprotectants, antioxidants, membrane stabilizers, and other classical media modifications. Second, we introduce some novel recent approaches that have shown some promise. Figure 2 displays a summary of conventional and novel strategies that have been applied for semen cryopreservation in different animal species.

![Figure 2. Main strategies to prevent animal sperm cryo-injuries (note all acronyms are in the abbreviation list).](image)

4.1. Conventional Strategies

During the last 25 years, the uses of various cryoprotectants and antioxidants have been the conventional strategies against cryoinjury that we will discuss in the following sections.

4.1.1. Cryoprotectants

In general, semen extenders must have appropriate osmolarity and buffering capacity along with an adequate pH enabling them to protect sperm cells from cryogenic injury [69]. Generally, sperm cryopreservation extenders include one or both of two types of cryoprotectants: a non-permeating cryoprotectant (milk, egg yolk, soybean lecithin, or raffinose), or a penetrating cryoprotectant (glycerol or dimethyl sulfoxide (Me$_2$SO)) [71].
Classical penetrating cryoprotective agents (CPAs) (e.g., glycerol and ethylene glycol) are known to provide a protective effect due to colligative properties [28]. At any given subzero temperature, the total unfrozen solution osmolality will be the same regardless of initial media constituents. In the absence of (relatively low toxicity) CPAs, the bulk of the osmolytes are salts. These salts cause significant damage during cooling and warming [72]. However, if the cryopreservation medium is supplemented with a significant percentage of penetrating CPA, then both the intra- and extracellular solutions retain the initial ratio of salt to CPA and at any given temperature, and the bulk of the osmolytes are CPA, not salt. This produces a less-damaging subzero environment for sperm. Classical CPAs also confer protection against damaging intracellular ice formation. However, as discussed above, Morris et al. demonstrated that this protection may be unnecessary in sperm, suggesting that the sperm intracellular water volume is too small to allow ice to nucleate and grow in the intracellular space [26]. A second benefit of penetrating cryoprotectants is that they improve the rearrangement of membrane lipids and proteins, membrane fluidity, and greater dehydration at lower temperatures that contribute to improved cryo-survival [38].

Although glycerol has many benefits as a CPA, there are some debates regarding its toxicity when it is applied in high concentrations [73]. Higher doses of glycerol are thought to be harmful in several aspects of cell function. Glycerol impacts plasma membrane coats such as glyocalyx, glycoproteins, and glycolipids and may deteriorate the membrane and increase the viscosity of cytosol [74]. Moreover, toxic levels of glycerol change the polymerization and depolymerization of α and β tubulins, the major proteins of the microtubules in sperm tail [73]. Si et al. assessed the motility and acrosome integrity of Rhesus monkey sperm that was frozen in the presence of different concentrations of glycerol (2%, 5%, 10%, and 15%), and they reported that the highest motility and highest acrosome integrity was achieved in 5% glycerol [75]. In the study by Bucak et al., with the use of trehalose in the extender as the modulator of glycerol toxicity, post-thaw quality indicators of ram sperm were significantly improved, and decreasing the glycerol content in the extender via supplementation with trehalose and taxifolin hydrate resulted in increasing the antioxidant capacity and reducing the oxidative stress [76].

On the other hand, a non-permeating cryoprotectant acts extracellularly without crossing the plasma membrane [77]. Therefore, non-permeating cryoprotectants such as sugars can provide similar colligative protection as their permeating counterparts, but only on the exterior of the cell. This, however, should be carefully considered: the advantage of permeating CPAs in the colligative sense is that they are associated only with a temporary change in water volume upon equilibration, whereas nonpermeating solutes such as sucrose or raffinose in similar molalities cause long-term and large-volume changes that are associated with membrane and cytoskeletal damage. Therefore, in general, species with tight volume tolerances (also known as osmotic tolerance limits) may benefit more from media containing permeating cryoprotectants over those that do not [77].

Egg yolk is the major component of extenders in most of the animal species and human and low-density lipoprotein is the effective fraction of egg yolk that protects sperm during freeze–thaw [78]. However, the challenges associated with using egg yolk that include microbial contamination have encouraged researchers to find a replacement [57]. Moreover, the wide variability of egg yolk compositions makes it difficult to standardize extenders, suggesting that sourcing egg yolk is an important part of any repeatable semen preservation protocol. It must be clear whether the eggs come from the same farm, are from the same breed or species, are being fed the same feed, etc. [79]. Because of the above drawbacks, several egg yolk alternatives have been explored in recent years. For example, soybean contains a high component of low-density lipoproteins such as lecithin, which is a plant-based extender ingredient. Soybean lecithin has recently been successfully used as an extracellular cryoprotectant for cryopreservation of semen in ram [80], goat [81], bull [82], buffalo [83], and human [84]. This non-animal origin protectant has improved the viability, mitochondrial membrane potential, and acrosome integrity in ram sperm [78] and reduced the lipid peroxidation of goat semen [81].
4.1.2. Antioxidants

Sperm contains a high amount of polyunsaturated fatty acid (PUFA) that makes it prone to lipid peroxidation due to the massive production of reactive oxygen species (ROS) during cryopreservation [49]. Sperm regulate and react to this oxidative stress through various intrinsic antioxidant protective systems that exist in both sperm and seminal plasma including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR), as well as non-enzymatic antioxidants such as methionine, vitamin C (ascorbic acid), and vitamin E (α-tocopherol) [85]. The capacity, however, is not enough [86], and the performance of these antioxidants could be reduced by dilution or cooling, resulting in decreased benefits of the endogenous antioxidative defense. Thus, as a cryopreservation strategy, antioxidants are used to reduce the detrimental effect of ROS on sperm during cryopreservation [85].

The two main categories of antioxidants are enzymatic and nonenzymatic and both have been well documented as effective strategies for improving cryorecovery and mitigating cryoinjury. The enzyme superoxide dismutase (SOD) in the cytoplasm (Cu, Zn—SOD) and the mitochondria (Mn-SOD) is responsible for combining with two frequent ROS molecules including the superoxide anion ($O_2^-$) and hydrogen peroxide [85]. However, there are controversial results regarding the beneficial effects of SOD. While the addition of SOD to extenders provided higher-motility sperm during cooling and freezing preservation [87,88], Silva et al. did not find any increase in sperm kinematic parameters after adding SOD to the ram semen extender [89]. Nevertheless, acrosome integrity and mitochondrial activity were improved in the presence of SOD [89]. Roca et al. reported that catalase improves the fertilizing potential of boar sperm after thawing by using catalase alone or in combination with SOD [90]. In addition, Fernandez-Santos et al. demonstrated that catalase inhibits DNA damages during the oxidative stress of cryopreservation [91].

We also can find numerous studies describing the beneficial effects of glutathione for semen cryopreservation in broad ranges of species. In fresh semen, glutathione did not affect the quality of ram sperm [89]. In another study, Silva et al. demonstrated that glutathione (2 mM and 5 mM) preserved the acrosome integrity of ram sperm [89]. Moreover, 5 mM oxidized glutathione increased the motion and velocity characteristics of ram sperm after freeze–thaw [92], and similar results were found at lower concentrations in turkey sperm [93]. Similar results have been found using the multifunctional antioxidant melatonin, where positive effects for sperm cryopreservation were found in pig [94], ram [95], goat [96], canine [97], fish [98], bovine [99], and human [100].

Alpha tocopherol (vitamin E) is one of the major compounds with antioxidative properties that is frequently found in the plasma membrane and seminal plasma. This lipophilic antioxidant protects fatty acid contents of membranes against peroxidation [86] and it has a dose-dependent effect [101]. The trolox analogue of vitamin E, which is soluble in water, improved several quality indicators of boar semen during cooling storage [102]. In addition, it increased fertilizing capacity and reduced the amount of hydrogen peroxide in bull sperm when it was added to the extender [103]. The beneficial effects of vitamin E on reproductive capacity have also been shown in chicken [104], boar [105], rabbit [106], ram [107], and buck [108] when added as supplementation to their diets.

Ascorbic acid (vitamin C) is another water-soluble vitamin attributed with reproduction; however, its exact mechanism is still uncertain [109]. Vitamin C might have a significant effect on the protection of DNA during cryopreservation [110]. Azawi and Hussein reported an improvement in the motility and viability of ram sperm supplemented with 0.9 mg/mL vitamin C during preservation at room temperature [111]. In a contradictory report, vitamin C reduced the motility of ram sperm when extenders were enriched with 50 mM or 100 mM compared to the control group [112]. Vitamin C might be a pro-oxidative compound in the presence of ferrous ions in the extender [85], as it converts Fe$^{3+}$ into Fe$^{2+}$, resulting in a reaction with oxygen or hydrogen peroxide, which then triggers lipid peroxidation [113].
Amino acids are available in seminal plasma and they are counted as non-enzymatic scavengers with antioxidant characteristics. Various types of amino acids such as hypotaurine, glutamine, cysteine, taurine, histidine, proline, and glycine were found to reduce DNA fragmentation and enhance the various post-thaw parameters of ram sperm [114]. Sangeeta et al. found that the addition of 25 mM l-proline and 20 mM l-glutamine in a Tris-based medium decreased lipid peroxidation and enhanced the acrosome integrity of sperm [115]. Fattah et al. found that the addition of 1 mM and 2 mM l-carnitine in cryopreservation media improved the mitochondrial function of sperm and resulted in higher progressive motility after thawing [116]. However, combinations of these amino acids may result in a negative impact on the semen quality, as Zhandi and Sharafi found that combining cysteine and glutathione in soybean-lecithin-based extender increased apoptosis in their post-thaw analysis of ram sperm [117]. Another amino acid that is frequently used in the various types of extenders is bovine serum albumin (BSA), which can protect the membrane integrity of sperm, especially during heat stress [118]. In some trials, 10% or 15% BSA have been attempted as a substitute for egg yolk in ram semen diluents and these demonstrated an equal cryoprotective effect compared with the egg yolk [119, 120].

Finally, in a study by Coyan et al., the addition of 1, 2, and 4 mM ergothioneine reduced the percentage of DNA fragmentation in post-thaw ram sperm [121]. Ergothioneine is a low-molecular-mass thiol that is present in some tissues. It scavenges oxygen hydroxyl radicals and peroxy radicals and acts as a regulator of iron metabolism, and it has been shown that ergothioneine protects sperm from oxidative stress and improved the post-thaw motility of ram [121] and canine [122] sperm.

4.1.3. Sugars

Sugars play multifunctional roles not only for regulating osmotic pressure and reducing relative ion concentrations, but also for stabilizing proteins and phospholipid bilayers during the freezing process [123]. They also have been considered not only as a source of energy for the sperm during cryopreservation but also as a means to prevent damage to different structural, sub-structural, and biochemical organs [124]. Trehalose is a disaccharide formed by binding two D-glucose molecules and is one the most effective sugars for cryopreservation [125]. Trehalose increases the distance between the membrane phospholipids by binding to the polar portion of the phospholipids in the plasma membrane. This distance may inhibit the formation of ice in the plasma membrane by helping the flow of water molecules from the cell and consequently stabilize the plasma membranes. Moreover, trehalose protects the transmembrane ion channels, reducing water leakage both into and out of the cells [126], and has been associated with modifications of the proteins during cryopreservation of ram sperm [127]. While trehalose is a popular nonpermeating cryoprotectant sugar alone or in combination with other additives [128], there has been much success in sperm cryopreservation media recipes that use other types of sugars, most notably in mouse sperm, which relies nearly entirely on the inclusion of raffinose [129]. This being said, some sugars that have positive effects in one species (e.g., raffinose in mouse) have negative effects in others (e.g., raffinose in chicken [130]).

Lactose is another sugar additive that is mainly used in pig semen freezing media [131]. Lactose can modulate the toxicity of solutions as well as interact with main groups of membrane phospholipids, and thus could increase the stability of the membrane during the freeze–thaw process [132]. Freezing media supplemented with lactose and trehalose improved quality of swine sperm post-thawing [133]. Chanapiwat et al. observed that lactose increases recovery of boar sperm compared to other sugars such as fructose, glucose, and sorbitol when they used them in an egg yolk-based media [134]. In another trial, replacement of lactose by trehalose and sucrose reduced oxidative stress markers and improved the quality of the frozen–thawed sperm [132].
A brief survey of the types of sugars used in animal semen cryopreservation extender are shown in Table 2.

Table 2. Example literature for several sugar additives used in animal semen extenders.

| Sugars | Species     | Results                                                                                     | Authors |
|--------|-------------|----------------------------------------------------------------------------------------------|---------|
|        | Ram         | Increased motility of frozen–thawed sperm                                                   | [135]   |
| Trehalose |            | Improved motility of frozen–thawed sperm                                                   | [136]   |
|        |             | Improved viability and membrane integrity of frozen–thawed sperm                            | [137]   |
|        |             | Improved post-thaw parameters                                                               | [126]   |
|        |             | Improved acrosome integrity                                                                 | [138]   |
|        |             | Improved kinetic parameters, morphology, membrane integrity, and mitochondrial activity     | [139]   |
|        |             | Improved post-thaw recovery using 50 and 100 mM trehalose with slow cooling                  | [140]   |
|        |             | Increased post-thaw parameters using combination of 3% glycerol and 60 mM trehalose          | [141]   |
|        | Goat        | Improved survival rate during cold storage using combination of 50 mM taurine and 50 mM trehalose | [142]   |
|        | Buffalo     | Improved ultrastructural morphology of sperm using combination of 1.5% ethylene glycol and 100 mM trehalose | [143]   |
|        | Bull        | Increased post-thaw motility and acrosome integrity                                         | [124]   |
|        | Fructose    | Fructose-based extender improved post-thaw motility and viability                           | [134]   |
|        | Boar        | Improved motility, viability, and membrane integrity                                        | [144]   |
|        |             | Improved post-thaw mitochondrial activity and viability                                      | [145]   |
|        | Bull        | Improved motility and plasma membrane integrity on the 3rd, 5th, and 7th day of storage     | [146]   |
| Glucose | Ram         | Glucose improved post-thaw parameters                                                       | [147]   |
|        | Boar        | Glucose increased post-thaw recovery                                                        | [148]   |
|        |             | Improved motility, viability, mitochondrial activity of frozen–thawed sperm                 | [149]   |
| Raffinose | Ram         | Increased viability and motility and decreased acrosome abnormalities                        | [137]   |
|        | Chicken     | Reduced fertility performance                                                                | [130]   |
|        | Bull        | Improved motility and plasma membrane integrity on 3rd, 5th and 7th day of storage          | [146]   |
|        | Mouse       | Raffinose is required for the standard inbred mouse sperm cryopreservation protocol          | [150]   |
|        | Bull        | Improved motility, acrosome integrity, and plasma membrane functionality                     | [151]   |
|        | Stallion    | Increased several kinetic parameters using 100 mM sucrose                                   | [152]   |

4.1.4. Membrane Stabilizers

During cooling, phospholipids in the membrane undergo a phase transition from a liquid phase to the crystalline gel phase. Integral proteins are excluded from the crystalline gel domains and the membrane becomes unstable [152]. Additionally, cooling-induced efflux of cholesterol from the membrane induces capacitation-like changes in frozen sperm [152]. Several articles have reported that the treatment of sperm before cryopreservation with cyclodextrins loaded with cholesterol leads to improved plasma membrane integrity and increases the osmotic tolerance of sperm [153–155].

There are several reports on improved survival rates after the inclusion of cholesterol during cryopreservation of various species’ sperm [156,157]. For instance, the addition of cholesterol-loaded-cyclodextrin (CLC; also referred to as cholesterol-cyclodextrin complexes) in boar sperm improved plasma membrane and acrosome integrity and decreased lipid peroxidation after cryopreservation [158]. CLC increases the membrane and acrosomal integrity of bull sperm following cryopreservation [159]. Khellouf et al. found cholesterol and vitamin E both preloaded in cyclodextrins can improve protection in frozen bovine sperm against cold shock and oxidative stress [160]. Chuaychu-Noo et al. reported that treatment of rooster sperm with CLC before cryopreservation improved the quality of
Murphy et al. found that pretreating stallion sperm with cholesterol before freezing reduced superoxide generation and increased post-thaw sperm viability [162]. Wojtusik et al. demonstrated that adding CLC to addra gazelle sperm prior to freezing prevented the loss of metabolism and motility-associated proteins such as CAPZB, HS90A, and PGAM2, as well as improving post-thaw sperm motility [55]. Adding CLC in skim-milk-based extender enhanced cryoresistance in ram sperm and improved sperm motility, sperm plasma membrane integrity, and fertility [153]. However, CLC and other cholesterol products are not a panacea: adding cholesterol-loaded cyclodextrin before cryopreservation did not increase fertility in sheep [163], horses [164], and donkeys [165], and exogenous cholesterol incorporated into the sperm membrane using cholesterol-loaded cyclodextrin can impair capacitation-related mechanisms, reducing fertility in frozen–thawed sperm [156].

4.1.5. Using Dietary Additives

As the impact of membrane lipid constituents affects post-thaw viability, it is reasonable to expect that pre-ejaculate diet may impact semen quality, if not cryopreservation success. Towards this, it has been reported that diet components and formulation affect the semen quality in the animal. Zanussi et al. demonstrated that dietary 2% flaxseed oil plus 200 mg/kg vitamin E for 60 days improved aged broiler breeder semen parameters and reproductive performance. This was attributed to possibly increased testosterone, reduced lipid peroxidation, and modification in the content of DPA, DHA, and arachidonic acid of the plasma membrane [166]. In horses, researchers found that complementing stallions’ diet with DHA not only increased the DHA in sperm but also increased the kinetic parameters of cryopreserved sperm [167]. Ansari et al. reported that oral usage of D-aspartic acid increased the concentration of sperm in the rooster and improved the fertility and hatchability rates in hens inseminated with those sperm [168]. Generally, the impact of diet on sperm cryosurvival seems to be marginal, but diet modifications in composition, feeding regimens, and trial length could affect sperm freezability [169]. In other studies, fish oil (3% dry matter of diet) significantly improved different post-thaw ram sperm parameters, while palm and sunflower oil (3% dry matter of diet) neither enhanced nor negatively affected ram semen characteristics [170,171]. In another report, dietary consumption of oleic acid in rams resulted in higher total antioxidant capacity and superoxide dismutase and lowered the amounts of malondialdehyde during liquid storage of semen [172].

4.1.6. Warming/Thawing

There are significant interactions among cooling rate, warming rate, and survival in cryopreserved semen [173]. From a fundamental cryobiology point of view, samples that are cooled quickly must be warmed quickly, because samples that are cooled in a quasi equilibrium or nonequilibrium manner will be subject to deleterious recrystallization upon warming, so warming rates must be sufficient to outrun this process [174]. Semen is no exception [175]. Hernández found that more-rapid warming resulted in increased motion parameters in boar sperm, with significant interactions among warming rates and glycerol concentrations [176]. In the bull, Lysachenko found that cryopreserved sperm had better motility when warmed more rapidly in temperatures up to 70 °C [177], extending studies by Correa et al. who only warmed at temperatures up to 37 °C [178]. However, rapid warming rates are more difficult to achieve. Hernández et al., and subsequently Tomás-Almenar and de Mercado, used a 70 °C water bath to achieve this and noted sensitivity on the scale of seconds between successful warming and killing sperm [175,176].
4.2. Novel Strategies

Classical approaches to improve recovery after cryopreservation have been mostly chemical, with some careful attention to adjusting cooling and warming rates. Here we highlight several novel approaches outside of these paradigms that have been applied to increase sperm cryosurvival. Some of these approaches include mild sublethal stress induced before freezing, induction of a magnetic field before freezing, nanoparticle-enhanced cryopreservation, sperm preservation via freeze-drying, and monolayer centrifugation.

4.2.1. Induction of Mild Sublethal Stress before Freezing

Mild sublethal stresses induced before freezing, including hydrostatic [179], osmotic [180], or oxidative stress [181], increase sperm resistance against cryo-injury. All the above-mentioned studies intended to induce responses that can prevent the apoptotic pathway during cryopreservation. For example, Huang et al. stated that the induction of very low hydrostatic pressure before cryopreservation enhanced the ubiquinol-cytochrome C reductase complex protein that is thought to play a critical role in regulating sperm motility [179]. This protein also participates in various cellular functions, such as cell cycle control, protein stabilization, redox regulation, fatty acid metabolism, and scavenging damaged proteins [182]. In another study, using a very low concentration of nitric oxide (NO) as a free radical that can induce mild oxidative stress significantly reduced the caspase-3 activity in cryopreserved bull sperm [181]. Similarly, Hezavehei et al. reported that the moderate stress generated by using 0.01 µM nitric oxide in human sperm resulted in increased post-thaw motility and viability and reduced apoptosis-like changes after cryopreservation [183]. Furthermore, they found that the effect of sublethal nitrosative stress on human sperm is a time-dependent response [183]. Their results demonstrated that the application of sublethal nitrosative stress (0.01 µM) before the cryopreservation process for 60 min could protect sperm against cryo-injuries. Another interesting sublethal stress recently applied to semen cryopreservation is photobiomodulation, where sublethal laser light was applied to human [184], turkey [185], bovine [186], and rabbit [187] sperm with improved post-thaw recovery. While the mechanism of success is not well understood, Safian et al. found reduced levels of ROS and lipid peroxidation in the laser-irradiated pretreatment groups compared to controls [184].

4.2.2. Induction of a Magnetic Field before Freezing

The use of “magnetized” extenders treated with a high magnetic field has increased the cryo-survival of sperm in some species. This approach has been employed to interrupt the regular network of water molecules in the cryopreservation medium to reduce the formation of hexagonal ice crystal, which is the most destructive form of ice for sperm. Askarianzadeh et al. observed a higher quality of rooster sperm when the freezing media were prepared under a 2000–4000 gauss magnetic field [190]. The magnetized extender also decreased ROS concentrations in post-thaw boar sperm [190]. In a recent study, cryopreserved human sperm was improved by exposing the freezing diluents to a 1000 Hz ELEF [191].

4.2.3. Nanoparticle-Enhanced Cryopreservation Media

As their name suggests, nanoparticles (NPs) are particles at the nanometer scale, with flexible fabrication and a high surface area to volume ratio [192]. In the reproductive system, some nanoparticles and nanomaterials have been demonstrated to improve fertility outcomes [193]. Recently, studies reported that the application of NPs with antioxidative properties has considerable capability to improve the results of cryopreservation protocols (Table 2) [194–196]. Falchi et al. have shown that cerium oxide (CeO₂) nanoparticles have beneficial effects on ram spermatozoa during cooling [192]. They found that the
cerium oxide (CeO$_2$) NPs act as ROS scavengers to protect the integrity of DNA and plasma membranes.

It was reported that selenium nanoparticles (SeNPs) have been used as a scavenger of ROS to protect sperm cells against oxidative stress [197]. Safa et al. demonstrated that the addition of vitamin E combined with SeNPs into rooster semen extender could increase the total antioxidant capacity (TAC) level and reduce malondialdehyde (MDA) concentration, compared to a control extender after freezing [197]. Adding SeNPs to semen extender improved bull sperm quality and the in vivo fertility rates after the freeze–thawing process [196]. Moreover, Mehdipour et al. found that lecithin nanoliposome extender could decline the percentage of apoptotic sperm and improved the quality of ram sperm after thawing [198]. Recently, Nadri et al. investigated the effect of vitamin E and glutathione peroxidase (GPx) in a lecithin-nanoliposome-based extender for bull sperm quality after cryopreservation [195]. They found that sperm viability and blastocyst formation rate were increased in nano-lecithin-based extender with 1.0 mM GPx [195].

4.2.4. Sperm Preservation via Freeze-Drying (Lyophilization)

The freeze-drying of sperm is a simple preservation technology that has been investigated for decades but has sparked much recent enquiry. It is an alternative, low-cost storage option for biodiversity preservation of domestic species [199]. An attractive feature of freeze-drying is that after drying sperm can be stored at 4°C and transported at room temperature, requiring no liquid nitrogen [200]. In theory, their small size and low water volume suggest that sperm may be good candidates for successful freezing-drying [201]. At the least as a preservation or conservation tactic, even nonviable freeze-dried sperm have intact chromatin and can be used for fertilization via intracytoplasmic sperm injection [202]. Wakayama and Yanagimachi reported that freeze-dried mouse spermatozoa were able to create normal embryos and generate normal offspring, although the sperm were motionless and appeared dead [203].

Moreover, other studies reported live offspring in mice [204], rat [200], rabbit [205], and horse [206] via fertilization with lyophilized sperm. Restrepo et al. compared three cryopreservation methods including freezing, vitrification, and freeze-drying on the quality parameters of equine spermatozoa; they found that freeze-drying produced a decline in lipid peroxidation (LPO) and enhanced mitochondrial membrane potential ($\Delta\Psi_M$) when compared to other methods [46].

4.2.5. Monolayer Centrifugation

Single-layer centrifugation (SLC) has been shown to separate robust sperm from the rest of the ejaculates; therefore, it can increase the percentage of cells with higher resistance against cryo-damages. This approach, which is based on colloid centrifugation, can select sperm with desirable characteristics and consequently could end up with more viable sperm post-thawing [207]. In this approach, sperm is layered over a colloid and gently centrifuged, then robust sperm move through the colloid to form a pellet, while seminal plasma is retained at the top and cells with lower quality or reduced cryo-resistance are retained at the interface between the sample and colloid [208]. In previous studies, sperm were selected with better chromatin integrity and a higher proportion of high mitochondrial membrane potential [208]. This approach can potentially select the good quality semen that might not reach the minimum cryopreservation threshold for freezing. It should be noted that SLC has produced varied results depending on the density of colloid used in trials; lowering the density of the colloid tends to reduce its selective capacity [209].
5. Sperm Cryobanking Expansion in Research and Industry

We are in the midst of a biodiversity crisis that has been termed the sixth mass extinction event in the history of Earth [210]. Loss of genetic diversity of livestock and disease outbreak due to limited genetic resources is threatening the current production farming system. One way to address this issue is through the technology of cryopreservation: the genetics of many local or endangered breeds/species with dwindling or valuable genetic pools may be stored under the form of cryopreserved reproductive cells [211]. Beyond the frequently considered wild species conservation efforts, domesticated animal genetic resource conservation is an important challenge to maintain domestic biodiversity and adaptation of animal species to global changes or breeding accidents or epidemics [212]. Reproductive cell cryopreservation also represents security for farm animal genetics and may be a useful tool to contribute to the measurement of genetic progress.

Biobanking is a rapidly growing industry, covering diverse fields such as human medicine, farm animal production, laboratory animal record-keeping, and wildlife. Different reproductive cell types are now stored in cryobanks: mainly semen and embryos in mammalian species, semen and primordial germ cells in birds, semen and germ cells in fish, and semen and larvae in shellfish. Some cryobanks also include somatic cells with the hope that, in the future, these cells will be reprogrammed to become inefficient reproductive cells [213]. In France, the National Cryobank of Domestic Animals contains reproductive cells and somatic tissues of avian species, mammals, fish, or shellfish conserved as semen, embryos, or larvae depending on the species. This progress in reproductive physiology and biotechnologies has made possible the extension of the range of species available in cryobanks.

6. Conclusions and Future Perspective

The cryopreservation of domestic animal sperm is a complex procedure that involves the regulation of many agents to achieve desirable results. To ensure even minimal success, suitable diluents, sperm dilution rates, cooling rates, and thawing rates are needed. Moreover, complete knowledge of the complex sperm physiology for each species is required to maximize the post-thaw recovery of sperm and consequently, fertility.

Although the first successful sperm cryopreservation was over 60 years ago, current methods result in post-thaw spermatozoa with about 50% post-thaw viability for most species. Likely due to the wide variation among species’ sperm, a standardized cryopreservation process has not been established. Some measures, including specific cryoprotectants, have been applied to improve the survival and fertility of frozen animal sperm. Factors leading to spermatozoa cryoinjury are complicated and have not been fully explored, and the extensive and severe cryoinjury to the structure and physiological function of domestic animal sperm have been confirmed. Currently, the cryoinjury mechanisms in mammalian and avian spermatozoa are of considerable interest in the field of cryobiology and reproductive science. Because the functional role of spermatozoa is regulated by proteins and nucleic acids, a molecular explanation may be necessary. Research related to cryopreservation of domestic animal semen has progressed greatly in recent years; however, significant room for improvement still exists. The current technological advances, such as vitrification, freeze-drying, and high-throughput sequencing technologies, can provide a new perspective to improve the cryopreservation efficiency of animal sperm.

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References
1. Curry, M.R. Cryopreservation of mammalian semen. Methods Mol. Biol. 2007, 368, 303–311. [CrossRef] [PubMed]
2. Shannon, P.; Vishwanath, R. The effect of optimal and suboptimal concentrations of sperm on the fertility of fresh and frozen bovine semen and a theoretical model to explain the fertility differences. Anim. Reprod. Sci. 1995, 39, 1–10. [CrossRef]
3. Crabo, B. Preservation of boar semen: A worldwide perspective. Reprod. Dom. Anim., Suppl 1990, 1, 3–9.
4. Maxwell, W.M.C.; Evans, G.; Rhodes, S.L.; Hillard, M.A.; Bindon, B.M. Fertility of superovulated ewes after intrauterine or oviductal insemination with low numbers of fresh or frozen-thawed spermatozoa. Reprod. Fertil. Dev. 1993, 5, 57–63. [CrossRef] [PubMed]
5. Spallanzani, L. Opuscoli di fisca, Anim. e Veg. Opuscolo I. Oss e Sperienze Intorno Ai Vermicelli Spermatici Dell’Uomo e Degli Anim. Modena 1776, 15–109.
6. Ombrelet, W.; Van Robays, J. History of human artificial insemination. F V V ObGyn 2010, 1–5.
7. Foote, R. The history of artificial insemination: Selected notes and notables. J. Anim. Sci. 2010, 80, 1–10. [CrossRef]
8. Herman, H.A. Improving cattle by the millions. In NAAB and the Development and Worldwide Application of Artificial Insemination; University of Missouri Press: London, UK, 1981.
9. Sherman, J.K. Research on frozen human semen: Past, present, and future. Fertil. Steril. 1964, 15, 485–500. [CrossRef]
10. Polge, C.; Smith, A.U.; Parkes, A.S. Revival of spermatozoa after vitrification and dehydration at low temperatures. Nature 1949, 164, 666. [CrossRef]
11. Stewart, D. Storage of bull spermatozoa at low temperature. Vet. Rec. 1951, 63, 65–66.
12. Mulder, R.L.; Font-Gonzalez, A.; Green, D.M.; Loeffen, E.A.H.; Jacqueline, M.M.H.; Loonen, J.; Yu, R.C.; Ginsberg, J.P.; Mitchell, R.T.; Byrne, J.; et al. Fertility Preservation in Childhood, Adolescent, and Young Adult Cancer 2 Fertility preservation for male patients with childhood, adolescent, and young adult cancer: Recommendations from the PanCareLIFE Consortium and the International Late Effects of Childhood Cancer Guideline Harmonization Group. Lancet Oncol. 2021, 22, E57–E67. [CrossRef] [PubMed]
13. Rodriguez-Wallberg, K.-A.; Haljestej, J.; Arver, S.; Johansson, A.L.V.; Lundberg, F.E. Sperm quality in transgender women before and after gender affirming hormone therapy-A prospective cohort study. Andrology 2021, 9, 1773–1780. [CrossRef]
14. Shaffriner, C. Longevity of fowl spermatozoa in frozen condition. Science 1942, 96, 337. [CrossRef]
15. Bunge, R.G.; Sherman, J.K. Fertilizing capacity of frozen human spermatozoa. Nature 1953, 172, 767–768. [CrossRef]
16. Hess, E.; Teague, H.; Ludwig, T.; Martig, R. Swine can be bred with frozen semen. Ohio Farm. Home Res. 1957, 42, 100.
17. Barker, C.; Gandier, J. Pregnancy in a mare resulting from frozen epididymal spermatozoa. J. Reprod. Infertil. 2007, 21, 47.
18. Salamon, S.; Lightfoot, R.J. Fertilization and embryonic loss in sheep after insemination with deep frozen semen. Nature 1967, 216, 194–195. [CrossRef]
19. Watson, P.F. The causes of reduced fertility with cryopreserved semen. Anim. Reprod. Sci. 2000, 60, 481–492. [CrossRef]
20. Parks, J.E.; Graham, J.K. Effects of cryopreservation procedures on sperm membranes. Theriogenology 1992, 38, 209–222. [CrossRef]
21. Johnson, L.A.; Weitze, K.F.; Fiser, P.; Maxwell, W.M.C. Storage of boar semen. Anim. Reprod. Sci. 2000, 62, 143–172. [CrossRef]
22. Mazur, P.; Fuller, B.J. Life in the frozen state. In Principles of Cryobiology; CRC Press: Boca Raton, FL, USA; Academic Press: Boca Raton, FL, USA, 2004; pp. 3–65.
23. Bucak, N.; Erdoğan, C.; Ili, A.; Başpınar, N.; Dursun, Ş.; Gündoğar, Ş. Effects of cryoprotectants and trehalose on electron microscopic evaluation of cryopreserved semen. J. Reprod. Infertil. 2017, 18, 25–26.
24. Zhmakin, A.I. Physical aspects of cryobiology. Phys. Uspekhi 2008, 51, 231. [CrossRef]
25. Pegg, D.E. Principles of Cryopreservation. In Cryopreservation and Freeze—Drying Protocols, 3rd ed.; Wolters, W.F., Oldenhof, H., Eds.; Methods in Molecular Biology; Humana Press Inc: Totowa, NJ, USA, 2015; Volume 1257, pp. 3–19.
26. Morris, G.J.; Faszer, K.; Green, J.; Draper, D.; Groat, B.; Fonseca, F. Rapidly cooled horse spermatozoa: Loss of viability is due to osmotic imbalance during thawing, not intracellular ice formation. Theriogenology 2007, 68, 804–812. [CrossRef] [PubMed]
27. Devireddy, R.; Swanlund, D.; Olin, T.; Vincente, W.; Troedsson, M.; Bischof, J.; Roberts, K. Cryopreservation of equine sperm: Optimal cooling rates in the presence and absence of cryoprotective agents determined using differential scanning calorimetry. Biol. Reprod. 2002, 66, 222–231. [CrossRef] [PubMed]
28. Mazur, P.; Leibo, S.; Chu, E. A two-factor hypothesis of freezing injury: Evidence from Chinese hamster tissue-culture cells. Exp. Cell Res. 1972, 71, 345–355. [CrossRef]
29. Toner, M.; Cravalho, E.; Armant, D. Water transport and estimated transmembrane potential during freezing of mouse oocytes. J. Membr. Biol. 1990, 115, 261–272. [CrossRef]
30. Hammerstedt, R.H.; Graham, J.K.; Nolan, J.P. Cryopreservation of mammalian sperm: What we ask them to survive. J. Androl. 1990, 11, 73–88. [CrossRef]

31. Oldenhof, H.; Gojowsky, M.; Wang, S.P.; Henke, S.; Yu, C.J.; Rohn, K.; Wolkers, W.F.; Sieme, H. Osmotic Stress and Membrane Phase Changes During Freezing of Stallion Sperm: Mode of Action of Cryoprotective Agents. Biol. Reprod. 2013, 88, 11. [CrossRef]

32. Holt, W.; North, R. Effects of temperature and restoration of osmotic equilibrium during thawing on the induction of plasma membrane damage in cryopreserved ram spermatozoa. Biol. Reprod. 1994, 51, 414–424. [CrossRef]

33. Noiles, E.E.; Bailey, J.L.; Storey, B.T. The temperature dependence in the hydraulic conductivity, Lp, of the mouse sperm plasma membrane shows a discontinuity between 4 and 0 °C. Cryobiology 1995, 32, 220–238. [CrossRef]

34. Lovelock, J. The haemolysis of human red blood-cells by freezing and thawing. Biochim. Biophys. Acta 1953, 10, 414–426. [CrossRef]

35. Sieme, H.; Oldenhof, H.; Wolkers, W. Sperm membrane behaviour during cooling and cryopreservation. Reprod. Domest. Anim. 2015, 50, 20–26. [CrossRef]

36. De Leeuw, F.; De Leeuw, A.; Den Daas, J.; Colenbrander, B.; Verkleij, A. Effects of various cryoprotective agents and membrane-stabilizing compounds on bull sperm membrane integrity after cooling and freezing. Cryobiology 1993, 30, 32–44. [CrossRef]

37. Dufourc, E.J. Sterols and membrane dynamics. J. Chem. Biol. 2008, 1, 63–77. [CrossRef]

38. Holt, W. Basic aspects of frozen storage of semen. Anim. Reprod. Sci. 2000, 62, 3–22. [CrossRef]

39. Vadnais, M.L.; Althouse, G.C. Characterization of capacitation, cryoinjury, and the role of seminal plasma in porcine sperm. Theriogenology 2011, 76, 1508–1516. [CrossRef]

40. Yeste, M. Sperm cryopreservation update: Cryodamage, markers, and factors affecting the sperm freezability in pigs. Theriogenology 2016, 85, 47–64. [CrossRef]

41. Casas, I.; Flores, E. Gene banking: The freezing strategy. In Boar Reproduction; Springer: Berlin/Heidelberg, Germany, 2013; pp. 551–588.

42. Paoli, D.; Lombardo, F.; Lenzi, A.; Gandini, L. Sperm cryopreservation: Effects on chromatin structure. Genet. Damage Hum. Spermatozoa 2014, 791, 137–150. [CrossRef]

43. Ward, W.S. Function of sperm chromatin structural elements in fertilization and development. MHR Basic Sci. Reprod. Med. 2009, 16, 30–36. [CrossRef]

44. De Leeuw, F.; De Leeuw, A.; Den Daas, J.; Colenbrander, B.; Verkleij, A. Effects of various cryoprotective agents and membrane-stabilizing compounds on bull sperm membrane integrity after cooling and freezing. Cryobiology 1993, 30, 32–44. [CrossRef]

45. Irvine, D.S.; Twigg, J.P.; Gordon, E.L.; Fulton, N.; Milne, P.A.; Aitken, R.J. DNA integrity in human spermatozoa: Relationships with semen quality. J. Androl. 2000, 21, 33–44. [CrossRef] [PubMed]

46. Restrepo, G.; Varela, E.; Duque, J.E.; Gomez, J.E.; Rojas, M. Freezing, Vitrification, and Freeze-Drying of Equine Spermatozoa: Changes in Human Spermatozoa throughout the Cryopreservation Process. Mol. Reprod. Dev. 2011, 84, 321–330. [CrossRef] [PubMed]

47. Kurland, C.G.; Andersson, S.G.E. Origin and evolution of the mitochondrial proteome. Microbiol. Mol. Biol. Rev. 2000, 64, 786–820. [CrossRef] [PubMed]

48. He, Y.X.; Wang, K.; Zhao, X.X.; Zhang, Y.; Ma, Y.J.; Hu, J.J. Differential proteome association study of freeze-thaw damage in ram sperm. Cryobiology 2016, 72, 60–68. [CrossRef] [PubMed]

49. Hezavehei, M.; Sharafi, M.; Kouchesfahani, H.M.; Henkel, R.; Agarwal, A.; Esmaeili, V.; Shahverdi, A. Sperm cryopreservation: A review on current molecular cryobiology and advanced approaches. Reprod. Biomed. Online 2018, 37, 327–339. [CrossRef] [PubMed]

50. Bogle, O.A.; Kumar, K.; Attardo-Parrinello, C.; Lewis, S.E.M.; Estayonol, J.M.; Ballesca, J.L.; Oliva, R. Identification of protein changes in human spermatozoa throughout the cryopreservation process. Andrology 2017, 5, 10–22. [CrossRef]

51. Parrilla, I.; Perez-Patino, C.; Li, J.; Barranco, I.; Padilla, L.; Rodriguez-Martinez, H.; Martinez, E.A.; Roca, J. Boar semen proteomics and sperm preservation. Theriogenology 2019, 137, 23–29. [CrossRef]

52. Chen, X.L.; Zhu, H.B.; Hu, C.H.; Hao, H.S.; Zhang, J.F.; Li, K.P.; Zhao, X.M.; Qin, T.; Zhao, K.; Zhu, H.S.; et al. Identification of differentially expressed proteins in fresh and frozen-thawed boar spermatozoa by iTRAQ-coupled 2D LC-MS/MS. Reproduction 2014, 147, 321–330. [CrossRef]

53. Guimaraes, D.B.; Barros, T.B.; van Tilburg, M.F.; Martins, J.A.M.; Moura, A.A.; Moreno, F.B.; Monteiro-Moreira, A.C.; Moreira, R.A.; Tonioli, R. Sperm membrane proteins associated with the boar semen cryopreservation. Anim. Reprod. Sci. 2017, 183, 27–38. [CrossRef]

54. Nynca, J.; Arnold, G.J.; Frohlich, T.; Ciereszko, A. Cryopreservation-induced alterations in protein composition of rainbow trout semen. Proteomics 2015, 15, 2643–2654. [CrossRef]

55. Wojtusik, J.; Wang, Y.; Pukazhenthi, B.S. Pretreatment with cholesterol-loaded cyclodextrins prevents loss of motility associated proteins during cryopreservation of bull spermatozoa (Nanger dama ruficollis) spermatozoa. Cryobiology 2018, 81, 74–80. [CrossRef]

56. Nynca, J.; Arnold, G.J.; Frohlich, T.; Ciereszko, A. Cryopreservation-induced alterations in protein composition of rainbow trout semen. Proteomics 2015, 15, 2643–2654. [CrossRef]

57. Wyntjes, J.; Wang, Y.; Pukazhenthi, B.S. Pretreatment with cholesterol-loaded cyclodextrins prevents loss of motility associated proteins during cryopreservation of bull spermatozoa (Nanger dama ruficollis) spermatozoa. Cryobiology 2018, 81, 74–80. [CrossRef]

58. Ryu, D.Y.; Song, W.H.; Pang, W.K.; Yoon, S.J.; Rahman, M.S.; Pang, M.G. Freezability biomarkers in bull epididymal spermatozoa. Sci. Rep. 2019, 9, 9. [CrossRef]
Animals 2022, 12, 3271

57. Hinsch, K.D.; De Pinto, V.; Aires, V.A.; Schneider, X.; Messina, A.; Hinsch, E. Voltage-dependent anion-selective channels VDAC2 and VDAC3 are abundant proteins in bovine outer dense fibers, a cytoskeletal component of the sperm flagellum. J. Biol. Chem. 2004, 279, 15281–15288. [CrossRef]

58. Hemachand, T.; Shaha, C. Functional role of sperm surface glutathione S-transferases and extracellular glutathione in the haploid spermatozoa under oxidative stress. FEBS Lett. 2003, 538, 14–18. [CrossRef]

59. Gaitskell-Phillips, G.; Martin-Canó, F.E.; Ortiz-Rodriguez, J.M.; Silva-Rodriguez, A.; Gil, M.C.; Ortega-Ferrusola, C.; Pena, F.J. Differences in the proteome of stallion spermatozoa explain stallion-to-stallion variability in sperm quality post-thaw. Biol. Reprod. 2021, 104, 1097–1113. [CrossRef]

60. O’Donnell, L.; O’Bryan, M.K. Microtubules and spermatogenesis. In Seminars in Cell & Developmental Biology; Academic Press: Cambridge, MA, USA, 2014; pp. 45–54.

61. Cheng, C.Y.; Chen, P.R.; Chen, C.J.; Wang, S.H.; Chen, C.F.; Lee, Y.P.; Huang, S.Y. Differential protein expression in chicken spermatozoa before and after freezing-thawing treatment. Anim. Reprod. Sci. 2015, 152, 99–107. [CrossRef]

62. Urrego, R.; Rodriguez-Osorio, N.; Niemann, H. Epigenetic disorders and altered gene expression after use of Assisted Reproductive Technologies in domestic cattle. Epigenetics 2014, 9, 803–815. [CrossRef]

63. Chao, S.B.; Li, J.C.; Jin, X.J.; Tang, H.X.; Wang, G.X.; Gao, G.L. Epigenetic reprogramming of embryos derived from sperm frozen at-20A degrees C. Sci. China-Life Sci. 2012, 55, 349–357. [CrossRef]

64. Salehi, M.; Mahdavi, A.H.; Sharafi, M.; Shahverdi, A. Cryopreservation of rooster semen: Evidence for the epigenetic modifications of thawed sperm. Theriogenology 2020, 142, 15–25. [CrossRef]

65. Tunc, O.; Tremain, K. Oxidative DNA damage impairs global sperm DNA methylation in infertile men. J. Assist. Reprod. Genet. 2009, 26, 537–544. [CrossRef]

66. Flores, E.; Ramí-Lluç, L.; Bucci, D.; Fernández-Novell, J.M.; Pena, A.; Rodriguez-Gil, J.E. Freezing-thawing induces alterations in histone H1-DNA binding and the breaking of protein-DNA disulfide bonds in boar sperm. Theriogenology 2011, 76, 1450–1464. [CrossRef] [PubMed]

67. Oganesyan, N.; Ankoudinova, I.; Kim, S.H.; Kim, R. Effect of osmotic stress and heat shock in recombinant protein overexpression and crystallization. Protein Expr. Purif. 2007, 52, 280–285. [CrossRef] [PubMed]

68. Aurich, C.; Schreiner, B.; Ille, N.; Alvarenga, M.; Scarlet, D. Cytosine methylation of sperm DNA in horse semen after cryopreservation. Theriogenology 2016, 86, 1347–1352. [CrossRef] [PubMed]

69. Riesco, M.F.; Robles, V. Cryopreservation Causes Genetic and Epigenetic Changes in Zebrafish Genital Ridges. PLoS ONE 2013, 8, 9. [CrossRef]

70. Vassena, R.; Boue, S.; Gonzalez-Roca, E.; Aran, B.; Auer, H.; Veiga, A.; Belmonte, J.C.I. Waves of early transcriptional activation and pluripotency program initiation during human preimplantation development. Development 2011, 138, 3699–3709. [CrossRef] [PubMed]

71. Evans, G.; Maxwell, W.C. Salamons’ Artificial Insemination of Sheep and Goats; Butterworths: London, UK, 1987.

72. Lovelock, J.E. The protective action of neutral solutes against haemolysis by freezing and thawing. Biochem. J. 1954, 56, 265–270. [CrossRef] [PubMed]

73. Alvarenga, M.A.; Papa, F.O.; Landim-Alvarenga, F.C.; Medeiros, A.S.L. Amides as cryoprotectants for freezing stallion semen: A method: Cryoprotective effect on frozen-thawed bull semen. Theriogenology 2002, 57, 1695–1706. [CrossRef]

74. Bucak, M.N.; Keskin, N.; Ili, P.; Bodu, M.; Akalin, PP.; Ozturk, A.E.; Ozkan, H.; Topragaleh, T.R.; Sari, F.; Baspinar, N.; et al. Decreasing glycerol content by co-supplementation of trehalose and soyabean lecithin-based extenders for cryopreservation of ram semen. Anim. Reprod. Sci. 2015, 142, 15–25. [CrossRef] [PubMed]

75. Si, W.; Zheng, P.; Li, Y.H.; Dinneys, A.; Ji, W.Z. Effect of glycerol and dimethyl sulfoxide on cryopreservation of rhesus monkey (Macaca mulatta) sperm. Am. J. Primatol. 2004, 62, 301–306. [CrossRef]

76. Bucak, M.N.; Keskin, N.; Ili, P.; Bodu, M.; Akalin, PP.; Ozturk, A.E.; Ozkan, H.; Topragaleh, T.R.; Sari, F.; Baspinar, N.; et al. Decreasing glycerol content by co-supplementation of trehalose and soyabean lecithin hydrate in ram semen extender: Microscopic, oxidative stress, and gene expression analyses. Cryobiology 2020, 96, 19–29. [CrossRef]

77. Aisen, E.G.; Alvarez, H.L.; Venturino, A.; Garde, J.J. Effect of trehalose and EDTA on cryoprotective action of ram semen diluents. Theriogenology 2000, 53, 1053–1061. [CrossRef]

78. Moussa, M.; Martine, V.; Trimeche, A.; Tainturier, D.; Anton, M. Low density lipoproteins extracted from hen egg yolk by an easy method: Cryoprotective effect on frozen-thawed bull semen. Theriogenology 2002, 57, 1695–1706. [CrossRef]

79. Emamverdi, M.; Zhandi, M.; Shahne, A.Z.; Sharafi, M.; Akbari-Sharif, A. Optimization of Ram Semen Cryopreservation Using a Chemically Defined Soybean Lecithin-Based Extender. Reprod. Domest. Anim. 2013, 48, 899–904. [CrossRef]

80. Forouzanfar, M.; Sharafi, M.; Hosseini, S.M.; Ostadhosseini, S.; Hajian, M.; Hosseini, L.; Abedi, P.; Nili, N.; Rahmani, H.R.; Nasr-Esfahani, M.H. In vitro comparison of egg yolk-based and soybean lecithin-based extenders for cryopreservation of bovine semen. Theriogenology 2010, 73, 480–487. [CrossRef]

81. Salmani, H.; Nabi, M.M.; Vaseghi-Dodaran, H.; Rahman, M.B.; Mohammadi-Sangcheshmeh, A.; Shakeri, M.; Towhidi, A.; Shahne, A.Z.; Zhandi, M. Effect of glutathione in soybean lecithin-based semen extender on goat semen quality after freeze-thawing. Small Rumin. Res. 2013, 112, 123–127. [CrossRef]

82. Aires, V.A.; Hinsch, K.D.; Mueller-Schloesser, F.; Bogner, K.; Mueller-Schloesser, S.; Hinsch, E. In vitro and in vivo comparison of egg yolk-based and soybean lecithin-based extenders for cryopreservation of bovine semen. Theriogenology 2003, 60, 269–279. [CrossRef]
83. Akhter, S.; Ansari, M.S.; Andrabi, S.M.H.; Rakha, B.A.; Ullah, N.; Khalid, M. Soya-lecithin in Extender Improves the Freezability and Fertility of Buffalo (Bubalus bubalis) Bull Spermatozoa. *Reprod. Domest. Anim.* 2012, 47, 815–819. [CrossRef]

84. Reed, M.L.; Ezeh, P.C.; Hamic, A.; Thompson, D.J.; Caperton, C.L. Soy lecithin replaces egg yolk for cryopreservation of human sperm without adversely affecting post thaw motility, morphology, sperm DNA integrity, or sperm binding to hyaluronate. *Fertil. Steril.* 2009, 92, 1787–1790. [CrossRef]

85. Amidi, F.; Pazhohan, A.; Nashtaei, M.S.; Khodarahmian, M.; Nekoonam, S. The role of antioxidants in sperm freezing: A review. *Cell Tissue Bank.* 2016, 17, 745–756. [CrossRef]

86. Aitken, R.J. Free radicals, lipid peroxidation and sperm function. *Reprod. Fertil. Dev.* 1995, 7, 659–668. [CrossRef]

87. Maxwell, W.M.C.; Stojanov, T. Liquid storage of ram semen in the absence or presence of some antioxidants. *Reprod. Fertil. Dev.* 1996, 8, 1013–1020. [CrossRef] [PubMed]

88. Forouzanfar, M.; Ershad, S.F.; Hosseini, S.M.; Hajian, M.; Ostad-Hosseini, S.; Abid, A.; Tavalaee, M.; Shahverdi, A.; Dizaji, A.V.; Esfahani, M.H.N. Can permeable super oxide dismutase mimetic agents improve the quality of frozen-thawed ram semen? *Cryobiology* 2013, 66, 126–130. [CrossRef] [PubMed]

89. Uysal, O.; Bucak, M.N. Effects of oxidized glutathione, bovine serum albumin, cysteine and lycopene on the quality of frozen-thawed pig spermatozoa. *Reprod. Domest. Anim.* 2011, 46, 874–881. [CrossRef] [PubMed]

90. Roca, J.; Rodriguez, M.J.; Gil, M.A.; Carvajal, G.; Garcia, E.M.; Cuello, C.; Vazquez, J.M.; Martinez, E.A. Survival and in vitro fertility of boar spermatozoa frozen in the presence of superoxide dismutase and/or catalase. *J. Androl.* 2005, 26, 15–24. [CrossRef] [PubMed]

91. Fernandez-Santos, M.R.; Dominguez-Rebolledo, A.E.; Esteso, M.C.; Garde, J.J.; Martinez-Pastor, F. Catalase supplementation on thawed bull spermatozoa abolishes the detrimental effect of oxidative stress on motility and DNA integrity. *Int. J. Androl.* 2009, 32, 353–359. [CrossRef] [PubMed]

92. Izanloo, H.; Soleimanzadeh, A.; Bucak, M.N.; Imani, M.; Zhandi, M. The effects of glutathione supplementation on post-thawed turkey semen quality and oxidative stress parameters and fertilization, and hatching potential. *Theriogenology* 2022, 179, 32–38. [CrossRef]

93. Pezo, F.; Zambrano, F.; Uribe, P.; Moya, C.; de Andrade, A.F.C.; Risopatron, J.; Yeste, M.; Burgos, R.A.; Sanchez, R. Oxidative and nitrosative stress in frozen-thawed pig spermatozoa. I: Protective effect of melatonin and butylhydroxytoluene on sperm function. *Res. Vet. Sci.* 2021, 136, 143–150. [CrossRef]

94. Pool, K.R.; Rickard, J.P.; de Graaf, S.P. Melatonin improves the motility and DNA integrity of frozen-thawed ram spermatozoa likely via suppression of mitochondrial superoxide production. *Domest. Anim. Endocrinol.* 2021, 74, 8. [CrossRef]

95. Tanhaei Vash, N.; Nadri, P.; Karimi, A. Synergistic effects of myo-inositol and melatonin on cryopreservation of goat spermatozoa. *Reprod. Domest. Anim.* 2022, 57, 876–885. [CrossRef]

96. Hull, M.G.; North, K.; Taylor, H.; Farrow, A.; Ford, W.C.L. Delayed conception and active and passive smoking. *BMC Vet. Res.* 2021, 17, 36. [CrossRef]

97. Felix, F.; Oliveira, C.C.V.; Cabrita, E. Antioxidants in Fish Sperm and the Potential Role of Melatonin. *Antioxidants* 2021, 10, 36. [CrossRef]

98. Su, G.H.; Wu, S.S.; Wu, M.L.; Wang, L.N.; Yang, L.; Du, M.X.; Zhao, X.Y.; Su, X.H.; Liu, X.F.; Bai, C.L.; et al. Melatonin improves sperm without adversely affecting postthaw motility, morphology, sperm DNA integrity, or sperm binding to hyaluronate. *Fertil. Steril.* 2019, 112, 85–90. [CrossRef] [PubMed]

99. Divar, M.R.; Azari, M.; Mogheiseh, A.; Ghahramani, S. Supplementation of melatonin to cooling and freezing extenders improves canine spermatozoa quality measures. *Anim. Reprod. Sci.* 2022, 235, 1–9. [CrossRef]

100. Minucci, S.; Venditti, M. New insight on the in vitro effects of melatonin in preserving human sperm quality. *Int. J. Mol. Sci.* 2022, 23, 5128. [CrossRef]

101. Rezaei, M.; North, K.; Taylor, H.; Farrow, A.; Ford, W.C.L. Delayed conception and active and passive smoking. *BMC Vet. Res.* 2021, 17, 36. [CrossRef]

102. Cerolini, S.; Maldjian, A.; Surai, P.; Noble, R. Viability, susceptibility to peroxidation and fatty acid composition of boar semen during liquid storage. *Anim. Reprod. Sci.* 2000, 58, 99–111. [CrossRef]

103. Dalvit, G.C.; Cetica, P.D.; Beconi, M.T. Effect of alpha-tocopherol and ascorbic acid on bovine in vitro fertilization. *Theriogenology* 1998, 49, 619–627. [CrossRef]

104. Khan, R.U.; Rahman, Z.U.; Javed, I.; Muhammad, F. Effect of vitamins, probiotics and protein on semen traits in post-molt male broiler breeders. *Anim. Reprod. Sci.* 2012, 135, 85–90. [CrossRef]

105. Brzezinskaslebodzinška, E.; Slebodzinski, A.B.; Petras, B.; Wieczorek, G. Antioxidant Effect of Vitamin-E and Glutathione on Lipid-Peroxidation In Boar Sema Plasma. *Biol. Trace Elem. Res.* 1995, 47, 69–74. [CrossRef]

106. Yousef, M.I. Vitamin E modulates reproductive toxicity of pyrethroid lambda-cyhalothrin in male rabbits. *Food Chem. Toxicol.* 2010, 48, 1152–1159. [CrossRef]

107. Masoudi, R.; Sharafi, M.; Shahneh, A.Z.; Towhidi, A.; Kohram, H.; Esmaeili, V.; Shahverdi, A.; Davachi, N.D. Fertility and flow cytometry study of frozen-thawed sperm in cryopreservation medium supplemented with soybean lecithin. *Cryobiology* 2016, 73, 69–72. [CrossRef] [PubMed]
Animals 2022, 12, 3271

21 of 24

135. Molinia, F.; Evans, G.; Casares, P.Q.; Maxwell, W. Effect of monosaccharides and disaccharides in Tris-based diluents on motility, acrosome integrity and fertility of pellet frozen ram spermatozoa. Anim. Reprod. Sci. 1994, 36, 113–122. [CrossRef]

136. Bucak, M.N.; Atessahin, A.; Varish, O.; Yuce, A.; Tekin, N.; Akcay, A. The influence of trehalose, taurine, cysteamine and hyaluronic acid on ram semen—Microscopic and oxidative stress parameters after freeze-thawing process. Theriogenology 2007, 67, 1060–1067. [CrossRef]

137. Jafaroghi, M.; Khalili, B.; Farshad, A.; Zamiri, M.J. The effect of supplementation of cryopreservation diulents with sugars on the post-thawing fertility of ram semen. Small Rumin. Res. 2011, 96, 58–63. [CrossRef]

138. Aisen, E.G.; Medina, V.H.; Venturino, A. Cryopreservation and post-thawed fertility of ram semen frozen in different trehalose concentrations. Theriogenology 2002, 57, 1801–1808. [CrossRef]

139. Najafi, A.; Zhandi, M.; Towhid, A.; Sharafi, M.; Sharif, A.A.; Motlagh, M.K.; Martinez-Pastor, F. Trehalose and glycerol have a dose-dependent synergistic effect on ram semen cryopreserved in a soybean lecithin-based extender. Cryobiology 2013, 66, 275–282. [CrossRef]

140. Uysal, O.; Bucak, M.N. The role of different trehalose concentrations and cooling rates in freezing of ram semen. An. Univ. Vet. Fak. Derg. 2009, 56, 99–103.

141. Ozturk, A.E.; Bodu, M.; Bucak, M.N.; Agir, V.; Ozcan, A.; Keskin, N.; Ili, P.; Topraggaleh, T.R.; Sidal, H.; Baspinar, N.; et al. The synergistic effect of trehalose and low concentrations of cryoprotectants can improve post-thaw ram sperm parameters. Cryobiology 2020, 95, 157–163. [CrossRef]

142. Bucak, M.N.; Tekin, N. Protective effect of taurine, glutathione and trehalose on the liquid storage of ram semen. Small Rumin. Res. 2007, 73, 103–108. [CrossRef]

143. Keskin, N.; Erdogan, C.; Bucak, M.N.; Ozturk, A.E.; Bodu, M.; Ili, P.; Baspinar, N.; Dursun, S. Cryopreservation Effects on Ram Sperm Ultrastructure. Biopreserv. Biobank. 2020, 18, 441–448. [CrossRef]

144. Reddy, N.S.S.; Mohanarao, G.J.; Atreja, S.K. Effects of adding taurine and trehalose to a tris-based egg yolk extender on buffalo (Bubalus bubalis) sperm quality following cryopreservation. Anim. Reprod. Sci. 2010, 119, 183–190. [CrossRef]

145. Ozturk, C.; Sungur, S.; Ataman, M.B.; Bucak, M.N.; Baspinar, N.; Ili, P.; Inanc, M.E. Effects of Arginine and Trehalose on Post-Thawed Bovine Sperm Quality. Acta Vet. Hung. 2017, 65, 429–439. [CrossRef]

146. Akhter, S.; Ansari, M.S.; Rakha, B.A.; Andradi, S.M.H.; Anwar, M.; Ullah, N. Effect of Fructose Addition in Skim Milk Extender on Survival of Ram Spermatozoa on the Quality of Liquid Nili-Ravi Buffalo (Bubalus bubalis) Semen. Pak. J. Zool. 2010, 42, 227–231.

147. Salamon, S.; Visser, D. Effect of composition of Tris-based diluent and of thawing solution on survival of ram spermatozoa frozen by the pellet method. Aust. J. Biol. Sci. 1972, 25, 605–618. [CrossRef]

148. De los Reyes, M.; Saenz, L.; Lapierre, L.; Crosby, J.; Barros, C. Evaluation of glucose as a cryoprotectant for boar semen. Vet. Rec. 2002, 151, 477–480. [CrossRef]

149. Bucak, M.N.; Keskin, N.; Taspinar, M.; Cohan, K.; Baspinar, N.; Cenariu, M.C.; Dursun, A.N. Raffinose and hypotaurine improve the post-thawed Merino ram sperm parameters. Cryobiology 2013, 69, 74–78. [CrossRef] [PubMed]

150. Sztei, J.M.; Noble, K.; Farley, J.S.; Mobraaten, L.E. Comparison of permeating and nonpermeating cryoprotectants for mouse sperm cryopreservation. Cryobiology 2001, 42, 28–39. [CrossRef] [PubMed]

151. Buyukkeblebici, S.; Tuncer, P.B.; Bucak, M.N.; Tasdemir, U.; Eken, A.; Buyukkeblebici, O.; Durmaz, E.; Sanozkan, S.; Endirlik, B.U. Comparing ethylene glycol with glycerol and with or without diithiothreitol and sucrose for cryopreservation of bull semen in egg-yolk containing extenders. Cryobiology 2014, 69, 74–78. [CrossRef] [PubMed]

152. Consuegra, C.; Crespo, F.; Bottrel, M.; Ortiz, I.; Dorado, J.; Diaz-Jimenez, M.; Pereira, B.; Hidalgo, M. Stallion sperm freezing with sucrose extenders: A strategy to avoid permeable cryoprotectants. Cryobiology 2018, 81, 85–91. [CrossRef] [PubMed]

153. Salmon, V.M.; Castonguay, F.; Demers-Caron, V.; Leclerc, P.; Bailey, J.L. Cholesterol-loaded cyclodextrin improves ram sperm cryoresistance in skim milk-extender. Anim. Reprod. Sci. 2017, 177, 1–11. [CrossRef]

154. Yang, S.X.; Adams, G.P.; Zwiefelhofer, E.M.; Rajapaksha, K.; Anzar, M. Cholesterol-cyclodextrin complex as a replacement for cryoprotectants in skim milk extender: Sperm characteristics post-thawing and in vivo fertility. Anim. Reprod. Sci. 2021, 225, 106691. [CrossRef]

155. Glazar, A.I.; Mullen, S.F.; Liu, J.; Benson, J.D.; Critser, J.K.; Squires, E.L.; Graham, J.K. Osmotic tolerance limits and membrane permeability characteristics of stallion spermatozoa treated with cholesterol. Cryobiology 2009, 59, 201–206. [CrossRef]

156. Lone, S.A. Possible mechanisms of cholesterol-loaded cyclodextrin action on, sperm during cryopreservation. Anim. Reprod. Sci. 2018, 192, 1–5. [CrossRef]

157. Moce, E.; Blanch, E.; Tomas, C.; Graham, J.K. Use of Cholesterol in Sperm Cryopreservation: Present Moment and Perspectives to Future. Reprod. Domest. Anim. 2010, 45, 57–66. [CrossRef]

158. Baishya, S.K.; Biswas, R.K.; Govindasamy, K.; Deka, B.C.; Sinha, S.; Singh, M. Pre-freezing treatment with butylated hydroxytoluene and cholesterol-loaded methyl-beta-cyclodextrin improves quality of cryopreserved boar semen. Cryoletters 2018, 39, 336–344.

159. Yadav, H.P.; Kumar, A.; Shah, N.; Chauhan, D.S.; Lone, S.A.; Swain, D.K.; Saxena, A. Effect of cholesterol-loaded cyclodextrin on membrane and acrosome status of hariana bull sperm during cryopreservation. Cryoletters 2018, 39, 386–390.

160. Khellouf, A.; Benhemia, K.; Fatami, S.; Igier-Ouada, M. The complementary effect of cholesterol and vitamin E preloaded in cyclodextrins on frozen bovine semen: Motility parameters, membrane integrity and lipid peroxidation. Cryo Lett. 2018, 39, 113–120.
Animals 2022, 12, 3271

161. Chuaychu-noo, N.; Thananurak, P.; Chankitisakul, V.; Vongpralub, T. Supplementing rooster sperm with Cholesterol-Loaded-Cyclodextrin improves fertility after cryopreservation. Cryobiology 2017, 74, 8–12. [CrossRef]

162. Murphy, C.; English, A.M.; Holden, S.A.; Fair, S. Cholesterol-loaded-cyclodextrins improve the post-thaw quality of stallion sperm. Anim. Reprod. Sci. 2014, 145, 123–129. [CrossRef]

163. Purdy, P.H.; Moce, E.; Stobart, R.; Murdoch, W.J.; Moss, G.E.; Larson, B.; Ramsey, S.; Graham, J.K.; Blackburn, H.D. The fertility of ram sperm held for 24 h at 5 degrees C prior to cryo5egeration. Anim. Reprod. Sci. 2010, 118, 231–235. [CrossRef]

164. Spizziri, B.E.; Fox, M.H.; Bruemmer, J.E.; Squires, E.L.; Graham, J.K. Cholesterol-loaded-cyclodextrins and fertility potential of stallions spermatozoa. Anim. Reprod. Sci. 2010, 118, 255–264. [CrossRef]

165. Oliveira, R.R.; Rates, D.M.; Pugliesi, G.; Ker, P.G.; Arruda, R.P.; Moraes, E.A.; Carvalho, G.R. Use of Cholesterol-Loaded Cyclodextrin in Donkey Semen Cryopreservation Improves Sperm Viability but Results in Low Fertility in Mares. Reprod. Domest. Anim. 2014, 49, 845–850. [CrossRef]

166. Brinsko, S.P.; Varner, D.D.; Love, C.C.; Blanchard, T.L.; Day, B.C.; Wilson, M.E. Effect of feeding a DHA-enriched nutriceutical on the quality of fresh, cooled and frozen stallion semen. Theriogenology 2005, 63, 1519–1527. [CrossRef]

167. Hashem, E.Z.; Haddad, R.; Esfandi, M.; Esfandi, M.; Sharafi, M.; Mofarahe, Z.S.; Abdollahifar, M.A.; Jajarmi, V.; Karimi, S.; Kazemi, M.; Chien, S.F.; Bayat, M. Photobiomodulation preconditioned human semen protects sperm cells against detrimental effects of cryopreservation. Cryobiology 2021, 98, 239–244. [CrossRef]

168. Ansari, M.; Zhandi, M.; Kohram, H.; Zaghari, M.; Sadeghi, M.; Sharafi, M. Improvement of post-thawed sperm quality and fertility of Arian rooster by oral administration of D-aspartic acid. Theriogenology 2017, 92, 69–74. [CrossRef]

169. Díaz, R.; Torres, M.A.; Paz, E.; Quíones, J.; Bravo, S.; Farias, J.G.; Sepulveda, N. Dietary inclusion of fish oil changes the semen lipid composition but does not improve the post-thaw semen quality of ram spermatozoa. Anim. Reprod. Sci. 2017, 183, 132–142. [CrossRef] [PubMed]

170. Esmaeili, V.; Shahverdi, A.H.; Alizadeh, A.R.; Alipour, H.; Chehrazi, M. Saturated, omega-6 and omega-3 dietary fatty acid effects on the characteristics of fresh, frozen-thawed and blood parameters in rams. Andrologia 2014, 46, 42–49. [CrossRef] [PubMed]

171. Masoudi, R.; Sharafi, M.; Shahneh, A.Z.; Towhid, A.; Kohram, H.; Zhandi, M.; Esmaeili, V.; Shahverdi, A. Effect of dietary fish oil supplementation on ram semen freeze ability and fertility using soybean lecithin- and egg yolk-based extenders. Theriogenology 2016, 86, 1583–1588. [CrossRef] [PubMed]

172. Hashem, E.Z.; Haddad, R.; Esfandi, M. Evaluation of ram semen enrichment with oleic acid on different spermatozoa parameters during low temperature liquid storage. Small Rumin. Res. 2017, 150, 30–39. [CrossRef]

173. Henry, M.A.; Noiles, E.E.; Gao, D.; Mazur, P.; Critser, J.K. Cryopreservation of human spermatozoa. IV. The effects of cooling rate and warming rate on the maintenance of motility, plasma membrane integrity, and mitochondrial function. Fertil. Steril. 1993, 60, 911–918. [CrossRef] [PubMed]

174. Seki, S.; Jin, B.; Mazur, P. Extreme rapid warming yields high functional survivals of vitrified 8-cell mouse embryos even when suspended in a half-strength vitrification solution and cooled at moderate rates to –196 °C. Cryobiology 2014, 68, 71–78. [CrossRef]

175. Tomás-Almenar, C.; De Mercado, E. Optimization of the Thawing Protocol for Iberian Boar Sperm. Animals 2022, 12, 2600. [CrossRef]

176. Hernandez, M.; Roca, J.; Gil, M.A.; Vazquez, J.M.; Martinez, E.A. Adjustments on the cryopreservation conditions reduce the incidence of boar ejaculates with poor sperm freezability. Theriogenology 2007, 67, 1436–1445. [CrossRef]

177. Lyashenko, A. Effect of different thawing procedures on the quality and fertility of the bull spermatozoa. Theriogenology 2009, 79, 129–168. [CrossRef]

178. Lyashenko, A. Effect of different thawing procedures on the quality and fertility of the bull spermatozoa. Theriogenology 2009, 79, 129–168. [CrossRef]

179. Huang, S.Y.; Pribenszky, C.; Kuo, Y.H.; Teng, S.H.; Chen, Y.H.; Chung, M.T.; Chiu, Y.F. Hydrostatic pressure pre-treatment affects the quality of fresh, cooled and frozen stallion semen. Theriogenology 2017, 118, 231–235. [CrossRef]

180. Murphy, C.; English, A.M.; Holden, S.A.; Fair, S. Cholesterol-loaded-cyclodextrins improve the post-thaw quality of stallion sperm. Anim. Reprod. Sci. 2014, 145, 123–129. [CrossRef]

181. Sharafi, M.; Zhandi, M.; Shakeri, M. Beneficial effects of nitric oxide induced mild oxidative stress on post-thawed bull semen quality. Int. J. Fertil. Steril. 2015, 9, 230. [CrossRef]

182. Csermely, P.; Schnaider, T.; Soti, C.; Prohaszka, Z.; Nardai, G. The 90-kDa molecular chaperone family: Structure, function, and clinical applications. A comprehensive review. Pharmacol. Ther. 1998, 79, 129–168. [CrossRef]

183. Hezavehei, M.; Kouchesfahani, H.M.; Shahverdi, A.; Sharafi, M.; Salekdeh, G.H.; Eftekhar-Yazdi, P. Induction of Sublethal Oxidative Stress on Human Sperm before Cryopreservation: A Time-Dependent Response in Post-Thawed Sperm Parameters. Cell J. 2019, 20, 537–543. [CrossRef]

184. Safian, F.; Novin, M.G.; Nazarian, H.; Mofarahae, Z.S.; Abdollahifar, M.A.; Jajarmi, V.; Karimi, S.; Kazemi, M.; Chien, S.F.; Bayat, M. Photobiomodulation preconditioned human semen protects sperm cells against detrimental effects of cryopreservation. Cryobiology 2021, 98, 239–244. [CrossRef]
185. Iaffaldano, N.; Meluzzi, A.; Manchisi, A.; Passarella, S. Improvement of stored turkey semen quality as a result of He-Ne laser irradiation. Anim. Reprod. Sci. 2005, 85, 317–325. [CrossRef]

186. Fernandes, G.H.C.; de Carvalho, Pd.T.C.; Serra, A.J.; Cresphilho, A.M.; Peron, J.P.S.; Rossato, C.; Leal-Junior, E.C.P.; Albertini, R. The Effect of Low-Level Laser Irradiation on Sperm Motility, and Integrity of the Plasma Membrane and Acrosome in Cryopreserved Bovine Sperm. PLoS ONE 2015, 10, e0121487. [CrossRef]

187. Iaffaldano, N.; Rosato, M.P.; Faventi, G.; Pizzuto, R.; Gambacorta, M.; Manchisi, A.; Passarella, S. The irradiation of rabbit sperm cells with He–Ne laser prevents their in vitro liquid storage dependent damage. Anim. Reprod. Sci. 2010, 119, 123–129. [CrossRef]

188. Malkin, T.L.; Murray, B.J.; Brukhno, A.V.; Anwar, J.; Salzmann, C.G. Structure of ice crystallized from supercooled water. Proc. Natl. Acad. Sci. USA 2012, 109, 1041–1045. [CrossRef]

189. Huebinger, J.; Han, H.M.; Hofnagel, O.; Vetter, I.R.; Bastiaens, P.I.H.; Grabenbauer, M. Direct Measurement of Water States in Cryopreserved Cells Reveals Tolerance toward Ice Crystallization. Biophys. J. 2016, 110, 840–849. [CrossRef] [PubMed]

190. Askarianzadeh, Z.; Sharafi, M.; Torshizi, M.A.K. Sperm quality characteristics and fertilization capacity after cryopreservation of rooster semen in extender exposed to a magnetic field. Anim. Reprod. Sci. 2018, 198, 37–46. [CrossRef] [PubMed]

191. Gholami, D.; Ghaffari, S.M.; Riazi, G.; Fathi, R.; Benson, J.; Shahverdi, A.; Sharafi, M. Electromagnetic field in human sperm cryopreservation improves fertilizing potential of thawed sperm through physicochemical modification of water molecules in freezing medium. PLoS ONE 2019, 14, e0214119. [CrossRef] [PubMed]

192. Falchi, L.; Khalil, W.A.; Hassan, M.; Marei, W.F.A. Perspectives of nanotechnology in male fertility and sperm function. Int. J. Vet. Sci. Med. 2018, 6, 265–269. [CrossRef] [PubMed]

193. Teli, M.K.; Mutalik, S.; Rajanikant, G.K. Nanotechnology and Nanomedicine: Going Small Means Aiming Big. Curr. Pharm. Des. 2010, 16, 1882–1892. [CrossRef] [PubMed]

194. Feugang, J.M.; Rhoads, C.E.; Mustapha, P.A.; Tardif, S.; Parrish, J.J.; Willard, S.T.; Ryan, P.L. Treatment of boar sperm with nanoparticles for improved fertility. Theriogenology 2013, 79, 57–61. [CrossRef] [PubMed]

195. Nadri, T.; Towhidi, A.; Zeinoaldini, S.; Martinez-Pastor, F.; Mousavi, M.; Noei, R.; Tar, M.; Sangcheshmeh, M.A. Lecithin nanoparticles enhance the cryosurvival of caprine sperm. Theriogenology 2019, 139, 38–44. [CrossRef] [PubMed]

196. Khalil, W.A.; El-Harairy, M.A.; Zeidan, A.E.B.; Hassan, M.A.E. Impact of selenium nano-particles in semen extender on bull sperm quality characteristics and fertilization capacity after cryopreservation of rooster semen in extender exposed to a magnetic field. Anim. Reprod. Sci. 2018, 198, 37–46. [CrossRef] [PubMed]

197. Safa, S.; Moghaddam, G.; Jozani, R.J.; Kia, H.D.; Jazani, M.A.E. Effect of vitamin E and selenium nanoparticles on post-thaw variables and oxidative status of rooster semen. Anim. Reprod. Sci. 2016, 174, 100–106. [CrossRef] [PubMed]

198. Mehdipour, M.; Kia, H.D.; Nazari, M.; Najafi, A. Effect of lecithin nanoliposome or soybean lecithin supplemented by pomegranate extract on post-thaw flow cytometric, microscopic and oxidative parameters in ram semen. Cryobiology 2017, 78, 34–40. [CrossRef]

199. Anzalone, D.A.; Palazzese, L.; Iuso, D.; Martino, G.; Loi, P. Freeze-dried spermatozoa: An alternative biobanking option for endangered species. Anim. Reprod. Sci. 2018, 190, 85–93. [CrossRef]

200. Kaneko, T.; Serikawa, T. Successful Long-Term Preservation of Rat Sperm by Freeze-Drying. PLoS ONE 2012, 7, 4. [CrossRef]

201. Saragusty, J.; Loi, P. Exploring dry storage as an alternative biobanking strategy inspired by Nature. Theriogenology 2019, 126, 17–27. [CrossRef]

202. Oldenhoff, H.; Zhang, M.; Narten, K.; Bigalk, J.; Sydykov, B.; Wolkers, W.F.; Sieme, H. Freezing-induced uptake of scavengers for preservation of chromatin in freeze-dried stallion sperm during accelerated aging. Biol. Reprod. 2017, 97, 892–901. [CrossRef]

203. Wakayama, T.; Yanagimachi, R. Development of normal mice from oocytes injected with freeze-dried spermatozoa. Nat. Biotechnol. 1998, 16, 639–641. [CrossRef]

204. Ward, M.A.; Kaneko, T.; Kusakabe, H.; Biggers, J.D.; Whittingham, D.G.; Yanagimachi, R. Long-term preservation of mouse spermatozoa after freeze-drying and freezing without cryoprotectant. Biol. Reprod. 2003, 69, 2100–2108. [CrossRef]

205. Liu, J.L.; Kusakabe, H.; Chang, C.C.; Suzuki, H.; Schmidt, D.W.; Julian, M.; Peffer, R.; Borrmann, C.L.; Tian, X.C.; Yanagimachi, R.; et al. Freeze-dried sperm fertilization leads to full-term development in rabbits. Biol. Reprod. 2004, 70, 1776–1781. [CrossRef]

206. Choi, Y.H.; Varner, D.D.; Love, C.C.; Hartman, D.L.; Hinrichs, K. Production of live foals via intracytoplasmic injection of lyophilized sperm and sperm extract in the horse. Reproduction 2011, 142, 529–538. [CrossRef]

207. Lima-Verde, I.; Hurri, E.; Ntalasiris, T.; Johannisson, A.; Stålhammar, H.; Morrell, J.M. Sperm Quality in Young Bull Semen Can Be Improved by Single Layer Centrifugation. Animals 2022, 12, 2435. [CrossRef] [PubMed]

208. Goodla, L.; Morrell, J.M.; Yusnizar, Y.; Stålhammar, H.; Johannisson, A. Quality of bull spermatozoa after preparation by single-layer centrifugation. J. Dairy Sci. 2014, 97, 2204–2212. [CrossRef]

209. Morrell, J.; Johannisson, A.; Dalin, A.M.; Rodriguez-Martinez, H. Morphology and chromatin integrity of stallion spermatozoa prepared by density gradient and single layer centrifugation through silica colloids. Reprod. Domest. Anim. 2009, 44, 512–517. [CrossRef] [PubMed]

210. Barnosky, A.D.; Matzke, N.; Tomiya, S.; Wogan, G.O.U.; Swartz, B.; Quental, T.B.; Marshall, C.; McGuire, J.L.; Lindsey, E.L.; Maguire, K.C.; et al. Has the Earth’s sixth mass extinction already arrived? Nature 2011, 471, 51–57. [CrossRef] [PubMed]

211. Svoradova, A.; Kuzelova, L.; Vasicek, J.; Olekxkova, L.; Chrenek, P. Cryopreservation of chicken blastodermal cells and their quality assessment by flow cytometry and transmission electron microscopy. Biotechnol. Prog. 2018, 34, 778–783. [CrossRef]
212. Joost, S.; Bruford, M.W.; Genomic-Resources, C. Editorial: Advances in Farm Animal Genomic Resources. *Front. Genet.* 2015, 6, 4. [CrossRef]

213. Canovas, S.; Campos, R.; Aguilar, E.; Cibelli, J.B. Progress towards human primordial germ cell specification in vitro. *Mol. Hum. Reprod.* 2017, 23, 4–15. [CrossRef]