Current Trends in the Application of Stem Cells and Their Derivatives in Animal Sperm Cryopreservation

Article *in* Biochemistry (Moscow) Supplement Series A Membrane and Cell Biology · December 2023

DOI: 10.1134/S1990747823050112

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REVIEWS

Current Trends in Stem Cells and Their Derivatives Application in Animal Sperm Cryopreservation

M. A. Tambovsky[a], *, A. M. Aimaletdinov[a], and E. Yu. Zakirova[a]

[a] *Kazan (Volga Region) Federal University, Institute of Fundamental Medicine and Biology, Kazan, 420008 Russia*

**e-mail: maxim.tambovsky.kfu@gmail.com*

Received February 14, 2023; revised May 4, 2023; accepted May 26, 2023

Abstract—Sperm cryopreservation is an important part of preserving the germ cells of various organisms. However, when gametes are frozen, various damage often occur, which have a significant impact in artificial insemination. After thawing, spermatozoa usually have ultrastructural, biochemical, and functional changes such as damage of cell membrane and chromatin and oxidative stress. Since spermatozoa have a limited capacity for biosynthetic activity, they have a low capacity for regeneration. The current trend is to improve sperm cryopreservation using natural extracellular vesicles and stem cells. Extracellular vesicles and stem cells have potential regenerative effects because they contain various bioactive molecules to affect sperm repair. The present review focuses on current strategies to improve sperm health after cryopreservation. In particular, this review describes the results of studies on the use of extracellular vesicles and stem cells as cryoprotectants during sperm freezing and thawing.

Keywords: cryopreservation, cryodamage, sperm, extracellular vesicles, stem cells

DOI: 10.1134/S1990747823050112

INTRODUCTION

Artificial insemination and sperm cryopreservation are among the greatest achievements that have revolutionized not only the breeding industry but also reproductive medicine [1]. Using frozen semen and artificial insemination, semen from the best breeding bulls can be used to inseminate cows worldwide [2]. Freezing animal semen is considered mandatory to preserve genetic material from individuals with high breeding value, as well as for endangered animals.

However, high-quality sperm cryopreservation increases the efficiency and success of artificial insemination. Despite the long history of using cryopreservation as semen preservation, freezing procedures are not always effective [3]. It is known that cryopreservation negatively affects the qualitative characteristics of sperm, causing changes at the structural and molecular levels due to thermal, mechanical, osmotic, and oxidative damage (Fig. 1), which in turn affects the ability to fertilization and subsequent early embryonic development [4].

The preservation of germ cells in rare individuals/animal species that do not tolerate cryopreservation well is also an urgent issue. Rehabilitation of damaged spermatozoa would be a crucial step to increase the number of fertile spermatozoa. Methods to improve the results of artificial insemination are needed for the reproduction of wild cats, especially snow leopards, which reproduce poorly even in their natural habitat and hardly reproduce.

One of the determinants of the efficiency of artificial insemination is the improvement of methods for storing animal semen in a chilled or deep-frozen state. Much of the research work on semen cryopreservation has focused on methods/approaches to improve the efficiency of freezing, which are based on the protection of spermatozoa from the damaging effects of the freezing procedure, with various diluents, cryoprotectants, antioxidants and nutritional components [6].

In the current state of cryobiology, special attention has been paid to the repair of sperm damage during freezing and thawing. This review shows strategies to protect and/or repair sperm damage that occurs during cryopreservation using animal cells or their derivatives in conjunction with traditional cryoprotectants.

EFFECT OF MESENCHYMAL STEM CELLS ON THE CHARACTERISTICS OF ANIMAL SEMEN DURING CRYOPRESERVATION

Mesenchymal stem cells (MSCs) are an integral part of regenerative veterinary medicine [7, 8] and veterinary medicine [9, 10]. It is known that repair of

Abbreviations: MSCs, mesenchymal stem cells; ADSCs, adipose-derived stem cells; MVs, microvesicles; EVs, extracellular vesicles; SPEs, seminal plasma exosomes.

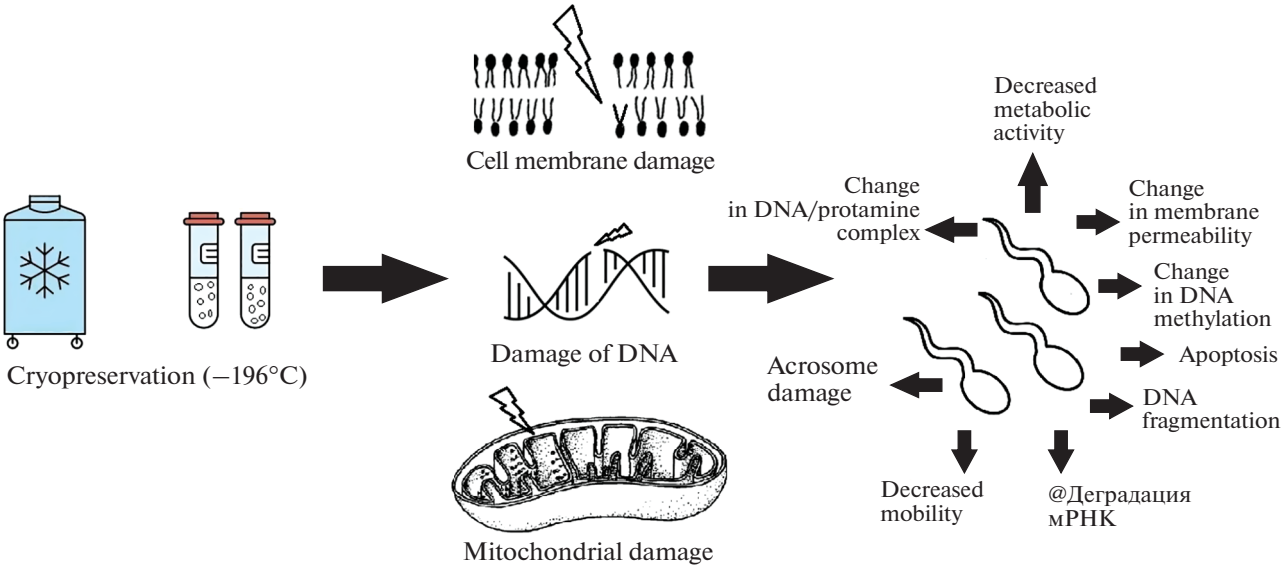

Fig. 1. Possible damage to spermatozoa after cryopreservation.

damaged tissue occurs due to the multipotent nature of MSCs and associated paracrine mechanisms, including the secretion of various signaling factors, mainly proteins [11].

Qamar et al. showed that the use of MSCs in sperm cryopreservation can be an effective biological approach to enhance sperm fertility and viability, as well as by supporting repair mechanisms through the secretion of various MSCs proteins [12]. The study investigated the effect of canine MSCs isolated from adipose tissue (Adipose Derived Stem Cells, ADSCs) on canine sperm survival. In this study, ADSCs were added before sperm were frozen, mixed and then the mixture was cryopreserved in a cryopreservation medium. After thawing, the authors observed improved sperm quality in samples with ADSCs. This was probably due to the effect of stem cells on repair mechanisms that affect spermatozoa at both cellular and molecular levels. A possible reason for the improvement could be proteins secreted by ADSCs, such as annexin, dysferlin and fibronectin, which are normally involved in repair at the cellular level [12].

Annexins are known to play an important role in exocytosis, endocytosis, aggregation, membrane fusion, and their binding to Ca^{2+}-dependent membrane makes them suitable for membrane repair [13]. In addition, annexin 1 and 2 together with the protein dysferlin are involved in Ca^{2+}-dependent sarcolemma repair of skeletal myocytes [14].

Fibronectin regulates cellular processes and the maintenance and repair of damaged tissues. In addition, this protein found in seminal plasma influences proteasome activation, acrosome reaction, condensation, gamete interaction, and embryonic development [15].

The authors' assumption about the effect of annexin, dysferlin, and fibronectin on greater preservation of frozen sperm membranes was based on the high levels of gene expression of these proteins in sperm from experimental samples compared to control samples [12].

Consequently, ADSCs supported repair mechanisms in sperm by interacting with spermatozoa through the production of various bioactive substances that are integral to repair mechanisms. Further studies in this direction are needed to identify other factors secreted by ADSCs that may play an active role in preserving sperm quality after cryopreservation.

Further in vivo studies would also help to demonstrate the efficacy of ADSCs in sperm cryopreservation. Perhaps the use of sperm supplemented with ADSCs for artificial insemination could also be useful in solving problems related to the female reproductive tract due to their regenerative and multipotent nature [12].

EFFECT OF EXTRACELLULAR VESICLES ON ANIMAL SEMEN CHARACTERISTICS DURING CRYOPRESERVATION

Exosomes, microvesicles (MVs) are microscopic extracellular vesicles (EVs), a few nanometers in diameter, released into the intercellular EVs space by cells of various tissues. There are several types of EVs, differing in origin, size, etc. (Table 1). It is known that EVs carry out communication between cells, as they can transport proteins, nucleic acids, and lipids, mediating a paracrine mechanism of regulation of tissue or recipient cells [16]. And also, MVs secreted by MSCs participate in the regeneration of damaged endogenous cells through the movement of trophic and regulatory molecules [17].

Table 1. Classification of extracellular vesicles

Extracellular vesicles	Size, nm	Formation peculiarities	Internal content	Presumed functions
Apoptotic cells	1000–5000	Formed at the terminal stages of cell death during cell fragmentation, surrounded by cell membrane. Distinguishing apoptotic corpuscles from other types of extracellular vesicles is a permeable membrane	Genomic DNA, whole organelles, rRNA. The main marker is annexin 5	Carry out intercellular interaction. Can mediate horizontal transfer of DNA, RNA and can be considered as signal transporters between cells
Microvesicles	100–1000	Formed by protrusion and detachment of sections of the cell plasma membrane	Generally similar to exosomes. There is data on the content of various cell adhesion proteins, cytoskeleton components, matrix metalloproteinases, glycoproteins, mitochondrial, centrosomal and ribosomal proteins. The obligatory components of exosomes – tetraspanins, flotillins, annexins and ESRT proteins – are absent. The main markers are CD40, CD62	They carry out intercellular interaction. Their role as biological markers of diseases is suggested
Exosomes	30–150	Membrane particle of endocytotic origin formed inside multivesicular endosomal cells	The contents of exosomes reflect the cytosolic composition of donor cells: nonspecific and tissue-specific proteins, various types of RNA. It is characterized by the presence of a greater number of oncogenic proteins and influence on cell proliferation and migration to a greater extent than for microvesicles. The main markers are CD63, CD9, Alix, TSG101	Carry out intercellular interaction

In the studies of Qamar et al. [18] it was shown that exosomes derived from ADSCs improve the quality of dog sperm after thawing during co-cryopreservation.

The addition of optimal concentration of exosomes (50 μg/mL) significantly increased the motility and the proportion of live spermatozoa after thawing. The percentage of spermatozoa with intact plasma membrane was statistically higher in samples with exosomes than in controls.

The EVs of seminal plasma in different animal species also have an ambiguous effect on sperm preservation after cryopreservation. EVs are produced in the male genital tract, including the testicular appendage and prostate. EVs have been shown to be involved in the regulation of spermatozoa function through binding and subsequent fusion with their membrane, integrating cytosolic and membrane components into the germ cell. At present, all the necessary conditions for binding and fusion of these vesicles with the recipient cell have not been clearly defined yet. In addition, it has been reliably established that the transfer of protein molecules from seminal plasma vesicles to sper-

matozoa is possible only in the presence of a certain pH value, temperature, and in the presence of zinc [27].

It has been shown that seminal plasma exosomes (SPEs) have a cryoprotective effect on spermatozoa [22, 28]. Studies by Du et al. demonstrated that boar SPEs can enhance antioxidant properties of spermatozoa, reduce malonic dialdehyde content, maintain the integrity of the sperm plasma membrane, improve sperm motility, and inhibit premature capacitation. Fluorescence and scanning electron microscopy demonstrated that the exosomes directly bind to the membrane of the sperm head. The authors suggest that this improves the integrity of the spermatozoon plasma membrane but does not affect in vitro induced capacitation [29].

It is known that there is certain specificity in the binding of SPEs to spermatozoa, depending on the specificity of the binding sites. For example, Zhou et al. showed that exosomes from the testis appendage have a certain affinity for the postacrosomal region of the sperm head [30], whereas exosomes obtained from the accessory gonads will have affinity for all domains of the head cell membrane: the acrosomal ridge, acrosome, and postacrosome [20]. Researchers believe that exosomes that bind to the sperm head will affect the capacity, acrosomal response, and ability to fuse with the oocyte, while those that fuse to the middle and main part of the tail will have a greater effect on mitochondrial activity, energy metabolism, and motility [31].

At the same time, Goericke-Peschab et al. [32] demonstrated the absence of a positive effect of EVs seminal plasma on the quality of dog sperm on the quality of spermatozoa both when they were cryopreserved together and when EVs was added after the samples were thawed. In control ejaculate samples, EVs was removed to obtain pure semen. The experiments showed a short-term beneficial effect of EVs only on sperm velocity parameters after thawing compared to the control group. However, this effect leveled off 30 min after thawing. In their conclusions, the researchers questioned the feasibility of using EVs of seminal plasma during cryopreservation to improve the quality of dog spermatozoa after thawing [32].

The data obtained by Ferraz et al. [33] demonstrate the possibility of using EVs from domestic animals of one species for successful cryopreservation of sperm samples from rare and endangered animals of another species. The authors found that EVs from the oviducts of dogs and cats improved the condition of red wolf and cheetah spermatozoa after cryopreservation. Specifically, red wolf and cheetah sperm thawed with EVs from dog and cat oviducts had more intact acrosomes than control samples. In addition, red wolf sperm in the presence of EVs from dog oviducts better supported sperm motility over time. However, this effect was not observed in cheetah sperm samples. The positive effect of the vesicles was attributed to the fact that they can carry proteins important for sperm function, which not only improve sperm motility after thawing, but also increase the preservation of the acrosome of red wolf and cheetah sperm in vitro. The results of this study indicate that these EVs may be a valuable tool to improve sperm cryopreservation conditions in endangered species [33].

CONCLUSIONS

It is a recognized fact that semen from different animal species has different sensitivity to cryopreservation [34]. The success of sperm cryopreservation is determined by the complex interaction between the sperm membrane and seminal plasma, the developer, and the selection of acceptable freezing methods. The integrity of the membrane is very important for the normal functioning of spermatozoa after thawing. Loss of surface proteins such as the progesterone receptor, especially from the acrosome, can impair the post–thaw quality and fertilizing ability of the sperm [35]. Therefore, the structural integrity of spermatozoa is essential for proper sperm function and fertilization and subsequent embryonic development of the organism [36].

Most mammalian sperm freezing protocols include removal of seminal plasma before cryopreservation by centrifugation [37], which practically minimizes the protective effect of antioxidant enzymes of seminal plasma against oxidative stress [38].

The biosynthetic capacity of sperm is limited, which is the main obstacle for their self-recovery after damage [38]. However, many external factors control sperm function by acting through surface and membrane components, so it has been hypothesized that the use of MSCs/microvesicles in sperm cryopreservation can be an effective biological approach to enhance sperm fertility and viability by supporting repair mechanisms through the secretion of various proteins. However, when using MSCs and EVs, it is necessary to take into account that stem cells and their derivatives from different tissues produce substances with different bioactivity in relation to recipient cells. Therefore, standards of quality control measurements and safety protocols should be developed for MSCs and EVs, as well as the efficiency of their use for commercial purposes in artificial insemination.

FUNDING

This work was supported by the Russian Science Foundation (project no. 23-26-00172) and by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

COMPLIANCE WITH ETHICAL PRONCIPLES

The authors declare no conflict of interest.

This article does not contain any studies involving animals or human participants performed by any of the authors.

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Translated by E. Zakirova

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