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REVIEW

Sperm cryopreservation: A review on current molecular cryobiology and advanced approaches

BIOGRAPHY

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KEY MESSAGE

Understanding the new aspects of sperm cryobiology, such as epigenetic and proteomic modulation, as well as novel techniques, is essential for clinical applications and the improvement of ART protocols. Long-term follow-up studies on the resultant offspring obtained from cryopreserved spermatozoa is recommended for future studies.

ABSTRACT

The cryopreservation of spermatozoa was introduced in the 1960s as a route to fertility preservation. Despite the extensive progress that has been made in this field, the biological and biochemical mechanisms involved in cryopreservation have not been thoroughly elucidated to date. Various factors during the freezing process, including sudden temperature changes, ice formation and osmotic stress, have been proposed as reasons for poor sperm quality post-thaw. Little is known regarding the new aspects of sperm cryobiology, such as epigenetic and proteomic modulation of sperm and trans-generational effects of sperm freezing. This article reviews recent reports on molecular and cellular modifications of spermatozoa during cryopreservation in order to collate the existing understanding in this field. The aim is to discuss current freezing techniques and novel strategies that have been developed for sperm protection against cryo-damage, as well as evaluating the probable effects of sperm freezing on offspring health.

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INTRODUCTION

The first record of semen cryopreservation dates back approximately 200 years, when Lazaro Spallanzani (1776) attempted to preserve spermatozoa by cooling it in snow (*Royere et al., 1996*). Further scientific progress was made considerably later with Polge's discovery of glycerol's cryoprotectant properties (*Polge et al., 1949*). This advance marked a turning point in the field of fertility preservation. Since that advance, there have been considerable improvements in techniques for cryopreservation of semen of different species. The earliest offspring produced from cryopreserved spermatozoa were reported in 1951 (cow), 1953 (human), 1957 (pig, horse) and 1967 (sheep) (*Curry, 2000*). Sperm cryobanks were developed in the 1960s for cattle and in the 1970s for humans (*Sanger et al., 1992*). Today, artificial insemination of animals and human assisted reproductive technology routinely use cryopreserved semenatozoa (*Kopeika et al., 2015; Yeste, 2016*). However, despite numerous achievements in sperm cryobiology, the search continues for methods that can optimally recover viable spermatozoa after cryopreservation.

Sperm cryopreservation is an effective route to the management and preservation of male fertility in humans and domestic animals (*Sharma, 2011*). Cytotoxic treatments, such as chemotherapy and radiotherapy, as well as surgical treatments, may lead to testicular failure or ejaculatory dysfunction (*Agarwal et al., 2014b; Rousset-Jablonski et al., 2016*). In such situations, freezing of spermatozoa can be a suitable solution to preserve fertility; the frozen-thawed semen can be used for intrauterine insemination (IUI), IVF or intracytoplasmic sperm injection (ICSI) (*Dohle, 2010*). Cryopreservation is widely used to preserve spermatozoa obtained from azoospermic patients who have undergone testicular sperm extraction (*Di Santo et al., 2012*) and can also be routinely used in men who want to begin assisted reproduction treatment and have a back-up sperm source. Furthermore, cryopreservation facilitates the storage of donor semen, while infectious disease screening can be completed and confirmed negative (*Anger et al., 2003*). In animals, artificial insemination is an extensively employed technique that uses frozen-thawed spermatozoa to manage or accelerate the rate of genetic improvement (*Flores et al., 2011; Masoudi et al., 2016*) by inseminating select or multiple females, respectively, with the semen obtained from a male of desired genetic quality (*Comizzoli, 2015*).

BIOLOGY OF SPERM CRYOPRESERVATION

A complete understanding of sperm physiology during cryopreservation is mandatory to ensure maximum success. A key factor in sperm cryobiology is that they are small cells with a large surface area (*John Morris et al., 2012; Morris, 2006*). These characteristics affect the viscosity and glass transition temperature of the intracellular cytosol in sperm cells, which makes them less susceptible to potential damage (*Isachenko et al., 2003*). In the absence of cryoprotective agents, cold shock and the induction of ice crystal formation can lead to the destruction of organelles in the sperm cells (*AbdelHafez et al., 2009*). This event may manifest in the oxidation of cellular compounds, as well as disruption and damage of cellular structures, such as the DNA, acrosome and plasma membrane, which ultimately reduces fertility (*O'Connell et al., 2002*). Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anions (O_2_) and hydroxyl radicals (OH^-) may induce apoptosis, membrane lipid peroxidation, disruption of mitochondria and DNA damage (Bollwein *et al.*, 2008). Conventionally, the concentration of cryoprotective agents, as well as membrane-stabilizing additives, is correlated with the rate and sensitivity of spermatozoa to sub-zero temperatures. The lipid composition of the sperm plasma membrane is a major factor that can influence the cryotolerance and cold sensitivity of spermatozoa. Differences in the fatty acid profile and omega-3/omega-6 ratio in spermatozoa of different species results in different levels of cryotolerance (*Esmaeili et al., 2015*). Moreover, spermatozoa obtained from different species may be different in size, shape and lipid composition, potentially affecting their resistance to cryo-injuries (*Esmaeili et al., 2015; Fattah et al., 2017; Maldjian et al., 2005*). Pre-freezing semen quality parameters, such as sperm motility and the abstinence period of sperm donors, can also affect the cryosurvival rate of post-thaw sperm (*Zhou et al., 2014*). Spermatozoa with abnormal motility traits (e.g. asthenozoospermic, oligoasthenozoospermic) are particularly susceptible to cryo-damage, possibly reducing their fertilizing ability (*Borges et al., 2007*). The following sections discuss the effects of freezing on the structures and macromolecules (proteins, transcriptome and epigenome) of spermatozoa, as well as the effects of cryopreservation on post-thaw sperm parameters.

ULTRASTRUCTURAL CHANGES OF SPERMATOZOA DURING FREEZING

Several studies have examined cryo-damage in spermatozoa of different species (*Ozkavukcu et al., 2008; Yeste, 2016*). Acrosome disintegration and partial removal of the outer acrosomal membrane with depletion of acrosomal content are common alterations that are attributed to physical freezing events (*Barthelemy et al., 1990*). These defects are probably attributable to ice crystal formation during the freezing of extracellular fluids, which results in expansion of the sub-acrosomal region. Alternatively, osmotic changes may cause damage to the lipid membrane structure, leading to tension changes in water canal proteins and ionic leakage in plasma membranes and resulting in morphological changes (*Sa-Ardrit et al., 2006*). Interestingly, it has been shown that rapid freezing markedly reduced the ultrastructural changes and preserved the integrity of sperm heads compared with slow freezing (*Serafini et al., 1986*). Studies have shown that glycerol is preferable to dimethyl sulfoxide (DMSO) as a cryoprotectant to protect sperm structures (*Oettle and Soley, 1986; Serafini et al., 1986*).

Woolley and Richardson have observed that extenders containing glycerol and egg yolk improved the apical segment of the acrosome and circular mitochondria after thawing (*Woolley and Richardson, 1978*). Cytoskeleton proteins, such as vimentin and actin, are other sperm structures that may incur damage during freezing. Transmission electron microscopy (*Harvey et al., 2013*) of post-thaw spermatozoa showed an incremental increase in wrinkling of the plasmalemma and sub-acrosomal swelling, as well as loss of acrosomal content and the appearance of vesiculations (*Ozkavukcu et al., 2008*).