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# Seminal Proteins and Cryopreservation of Semen: A Mini-Review

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**Abstract**

This study aimed to analyze the effects of semen cryopreservation on seminal proteins. Initially, we analyzed the effects of cold shock and the advent of proteomics and its benefits on the characterization of seminal proteins. Subsequently, studies reporting losses of some sperm proteins and increase in the expression of others during the cooling and freezing of the semen of different species are presented. Finally, some questions were raised to motivate the study of semen proteomics aiming to predict quality losses during the cryopreservation of semen in breeding animals of greater genetic merit.

**Keywords:** Inflammation; Cardiotoxicity; Interleukins

## Introduction

There have been reports of resistance of sperm to the freezing process since 1776 with Spallanzani. However, it was only with the discovery of the cryoprotectant effects of glycerol by Polge et al. [1] that sperm cryopreservation began to be disseminated. The process consists of dilution, cooling, freezing, storage, and defrosting. Moreover, the interaction between the diluent, cooling and freezing curves and thawing is required to obtain semen with good fertilizing capacity Amann et al. [2]. Cold shock occurs when semen is rapidly cooled from room temperature to 0°C. It is characterized by circular movement of spermatozoa, premature loss of motility, decreased energy production, and increased membrane permeability with consequent loss of intracellular ions and molecules. Damage to the sperm membrane is responsible for these changes Amann et al. [2]. One way to reduce these effects is to slowly cool the diluted semen and add milk or egg yolk proteins to semen diluents Amann et al. [2]. With the advent of proteomics, many studies aiming to characterize seminal proteins were conducted, which allowed identification of changes in protein composition after cryopreservation and correlation of these changes with the fertility and freezing patterns of the semen of each animal.

Ollero et al. [3] observed through western blot of sperm extracts that cryopreserved semen had four protein bands less than fresh semen, and that therefore, the freezing/thawing process could lead to protein loss. It was assumed for a long time that cryopreservation led to the release of proteins, especially enzymes, from the sperm surface to the extracellular fluid. However, recent studies demonstrated that there is an increase in the expression of other proteins, both in the spermatozoon and in the seminal plasma, in addition to the loss of some proteins by spermatozoa. Ardon et al. [4] observed a higher expression of bind sperm protein (BSP) 1, 3 and 5 in bull spermatozoa after the cryopreservation process when compared to fresh semen. Wang et al. [5], in turn, demonstrated an increase in the expression of human spermatozoa proteins subjected to freezing/thawing. The study by Westfalewicz et al. [6] showed a clear difference in protein response to the cryopreservation process. The authors analyzed the changes in protein composition of fresh bovine semen during the equilibration period (4ºC for 2.5 hours) and of frozen semen using 2D electrophoresis. They observed that the expression of 25 protein spots (representing 16 proteins) were altered after the cryopreservation process.

While 18 spots had decreased expression, five spots had increased expression. In the seminal fluid, in turn, the expression of six protein spots (representing four proteins) was altered, of which two had increased expression, and four had decreased expression. Most of the sperm proteins affected by cryopreservation are membrane binding proteins, and, according to the authors, the loss of these proteins may alter the natural sperm coating. They also observed that the greatest changes occur in the equilibrium period, when the sperm interact with the diluent. Semen protein composition varies among individuals of the same species, and some studies found a correlation between the expression of some proteins and semen freezability. Roncoletta et al. [7] observed, using gel electrophoresis, that animals with high semen freezability

had a protein band of approximately 61.8 kDa while animals with low semen freezability did not. Jobim et al. [8] found higher protein expression (15–16 kDa, 11–12 kDa, 13–14 kDa and 18–20 kDa) in the seminal plasma of bulls with semen of high freezability, and, according to the authors, these proteins are the BSP-A1/-A2, acidic seminal fluid protein (aSFP), BSP-A1/A2 or A3, and alpha-chain clusterin, respectively.

In contrast, they found an association between the 25–26 kDa protein and low semen freezability, and this protein might be the lipocalin-type prostaglandin d synthase. The cryopreservation process also causes changes in transmembrane proteins. Severe damage was observed in the acrosome of 60% of the cryopreserved bull spermatozoa, and more than half of these animals showed incorrect localization of the IZUMO1 protein, a transmembrane protein responsible for the fusion of the spermatozoid to the oocyte membrane after acrosome reaction. Furthermore, after incubation for 360 minutes, a higher percentage of cryopreserved spermatozoa lost IZUMO1 compared to fresh semen Fukuda et al. [8], Mohan et al. [10] evaluated the enzymatic activity of the angiotensin-converting enzyme (ACE) in the spermatozoa and seminal plasma of bulls and buffaloes and found that the activity of the enzyme decreased in spermatozoa and increased in seminal plasma after semen cryopreservation in both species. They attributed these findings to changes due to cold shock.

## Final considerations

Many questions about the effects of cryopreservation on seminal proteins remain unanswered. Which proteins undergo changes with cryopreservation and how do these changes affect fertility? What can be changed in the cryopreservation method to mitigate the negative effects of cooling and freezing on seminal proteins? It seems clear that the semen of some breeding animals is more resistant to this process than that of others, but would it be possible to determine molecular markers to identify these individuals early? Therefore, several such aspects remain unaddressed, and require further study.

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