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Freezing-thawing induces alterations in histone H1-DNA binding and the breaking of protein-DNA disulfide bonds in boar sperm

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Abstract

The main aim of this work is to gain insight into the mechanisms by which freezing-thawing alters the nucleoprotein structure of boar sperm. For this purpose, the freezing-thawing-related changes of structure and location of histones-DNA domains in the boar sperm head were analyzed through Western blot and immunocytochemistry. Afterwards, it was analyzed whether freezing-thawing induced changes in tyrosine phosphorylation levels of both protamine 1 and histone H1, through Western blot analyses in samples previously subjected to immunoprecipitation. This analysis was completed with the determination of the changes induced by freezing-thawing on the overall levels of sperm-head disulfide bonds through analysis of free-cysteine radicals levels. Freezing-thawing induced significant changes in the histones-DNA structures, which were manifested in the appearance of a freezing-thawing-linked histone H1-DNA aggregate of about a 35-kDa band and in the spreading of histone H1-positive markings from the caudal area of the sperm head to more cranial zones. Freezing-thawing did not have any significant effect on the tyrosine phosphorylation levels of either protamine 1 or histone H1. However, thawed samples showed a significant ($P < 0.05$) increase in the free cysteine radical content (from 3.1 ± 0.5nmol/μg protein in fresh samples to 6.7 ± 0.8 nmol/μg protein). In summary, our results suggest that freezing-thawing causes significant alterations in the nucleoprotein structure of boar sperm head by mechanism/s linked with the rupture of disulfide bonds among the DNA. These mechanisms seem to be unspecific, affecting both the protamines-DNA unions and the histones-DNA bonds in a similar way. Furthermore, results suggest that the boar-sperm nuclear structure is heterogeneous suggesting the existence of a zonated pattern, differing in their total DNA density and the compactness of the precise nucleoprotein structures present in each zone.

Keywords: Boar sperm; Freezing-Thawing; Histone H1; Disulfide bonds

1. Introduction

In the last few years there has been an increasing amount of information regarding freezing-thawing-related alteration of sperm nuclear structure. These alterations can strongly affect the fertilizing ability of the sperm without modifying other aspects of sperm function, such as motility or oocyte penetration ability [1]. In the majority of cases and species, the most-studied nuclear sperm alteration linked to freezing-thawing has been DNA fragmentation. In this way, freezing-thawing-induced DNA fragmentation has been observed in several species, such as human and horse [1–2]. Nevertheless, in other species reports regarding DNA frag-

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mentation during freezing-thawing are conflicting. In this regard, no effect was observed in frozen-thawed ram sperm [3]. Centering on boar, reports vary from the presence of a clear increase in freezing-linked DNA damage to the absence of any effect on DNA fragmentation [4–7]. It is noteworthy that these discrepancies have been observed even after the utilization of similar techniques to detect DNA fragmentation, such as Neutral Comet Assay and SCSA [3–7]. This opens doubts about the importance and mechanisms of freezing-thawing-induced alterations of sperm head-DNA structure.

However, although DNA fragmentation would be the clearest effect related to changes in nuclear sperm structure during freezing-thawing, it is not the only possible effect. In this way, our laboratory has shown that freezing-thawing of boar sperm in conditions in which there was not any significant increase of DNA fragmentation induces alterations in the protamine 1-DNA structural interaction instead [7]. This is of importance, since this alteration causes significant changes in the overall boar-sperm nuclear structure after thawing, with the possible consequences of an overall loss of sperm fertilizing ability. Notwithstanding, the exact nature that causes the alteration of the protamine 1-DNA structure is not known. In this respect, it should be remembered that nuclear proteins can modulate their union to DNA through two main mechanisms. The first is through changes in the phosphorylation levels of the nuclear proteins. It is well known that both protamines and histones are present in different degrees of phosphorylated forms, from unphosphorylated forms to highly phosphorylated ones, in mature mammalian spermatozoa, although there are many differences among species. In this sense, protamines are in a non-phosphorylated form in mature sperm [8]. On the contrary, histones are present in eucaryotic cells in various degrees of phosphorylation, with maximal phosphorylation levels during the interphase and mitotic phases of the cell cycle [9]. Changes in the phosphorylation levels of nuclear proteins induce variations in the strength of the proteins-DNA bonds of the nuclear structure. In this way, it has been described that a decrease in the protamine phosphorylation levels induces a loosening in their capacity to link DNA, thus inducing an increased flexibility in the protamines-DNA structure [10]. On the contrary, an increase in histone phosphorylation induces a loosening of the histones-DNA unions [11]. These changes lead histones to play an important role as regulatory elements of gene activity during the cell cycle [11]. Thus, it is possible that the observed changes in the nucleoprotein structure of boar sperm during freezing-thawing are related to changes in the phosphorylation levels of the present nucleoproteins.

Nevertheless, there is another mechanism that can account for the alterations observed. This would be alterations of the disulfide bonds, which are one of the most important union mechanisms between nucleoproteins and DNA [8,12,13]. It is well known that mechanisms such as osmotic oxidative stress induced by osmotic disturbances are able to destroy disulfide bonds in a whole series of cellular structures [14,15]. This is important, since osmotic stress is one of the most important mechanisms, together with oxidative stress, responsible for the main freezing-thawing-induced alterations in sperm, including structural alterations in cellular membranes and mitochondria volume and shape, as well as the rupture of the peri-mitochondrial and head actin network [1,16–25]. Hence, these mechanisms could be at the basis of the already observed freezing-thawing-linked alterations of the boar-sperm nuclear structure.

The study of DNA-proteins structural changes in the sperm head is further complicated, however, by the existence of a heterogeneous structure. It is well known that, although the majority of nuclear sperm proteins are protamines, there are several specific domains in the sperm nucleus in which DNA is associated with histones [26,27]. In this way, the percentage of human sperm DNA that is structured around histones is about 15% [27]. This is important, since the structure associated with the histones-linked DNA domains are much less compact and rigid than that related to the protamines-linked DNA domains [26,27]. Taking into account that the histones-linked domains are located at the telomeric sequences [27], it is reasonable to assume that the freezing-thawing-related alterations of the sperm head-structure would of different intensity in both the histones-linked nuclear domains and the protamines-linked DNA ones. However, there is not any direct evidence that supports this hypothesis, since there are no published data regarding freezing-thawing-related alterations of the histones-DNA structures in sperm.

Taking into account all of the data described above, the aim of this manuscript is centered on two objectives. The first objective is the study of the freezing-thawing-related alterations of the histones-DNA domains in the boar sperm head. This was performed through both Western blot analysis and immunocytochemistry. The second objective is to study the freez-