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Activity, substrate detection and immunolocalization of glutathione peroxidase (GPx) in bovine reproductive organs and semen

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Abstract

The purpose of the current study was to further investigate the role of the antioxidant selenium-dependent enzyme glutathione peroxidase (GPx) in reproductive organs and semen from bulls. To this end a fast and convenient combined method for immune detection and substrate localization was adapted, which allows the assessment of both molecular weight and peroxidase activity of proteins on one and the same SDS-PAGE gel plate. After routine semen analysis of ejaculates, a spectrophotometrical assay of GPx activity in bovine semen was performed. For the immunological analyses performed, a rabbit polyclonal monospecific antibody against GPx was raised. Substrate detection and immunolocalization of GPx in lysates from bovine testis, epididymis, spermatozoa, and seminal plasma was performed. In order to determine the localization of GPx in spermatozoa, immunofluorescence analysis was performed. A positive correlation was established between GPx activity in semen and the number of motile spermatozoa. A negative correlation was observed between GPx activity and the number of immotile spermatozoa. The combined method for immunodetection and substrate localization was tested and proved reliable. Both tetramer and monomer forms of GPx were detected in lysates from testis, epididymis, and spermatozoa. We found no GPx activity in seminal

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plasma. Immunofluorescence shows the presence of GPx chiefly in the mitochondrial and in the acrosome regions of spermatozoa. GPx activity remained stable under the extreme experimental conditions.

Keywords: Glutathione peroxidase (GPx); Bull; Antioxidant; Fertility; Substrate staining

1. Introduction

The cell membrane of mammalian spermatozoa has a high content of polyunsaturated fatty acids (PUFA) [1]. Almost 50% of the fatty acid content of human spermatozoa comprises docosohexanoic acid, with 6 unsaturated complex double bonds [2]. The abundance of unsaturated fatty acids in the sperm plasma membrane helps this structure acquire the fluidity it needs in order to engage in the membrane fusion events associated with fertilization. The lipids incorporated in the sperm plasma membrane amount up to 60–65% of its content, and are of crucial importance for its properties, due not only to the quantity and quality of the lipids [3,4], but also to their spatial arrangement and dynamics, as well as to the physical status of individual lipid molecules [5,6].

The high PUFA content of the sperm plasma membrane renders spermatozoa particularly vulnerable to the deleterious effects of reactive oxygen species and free radicals that may initiate a lipid peroxidation (LPO) cascade. It has been shown that LPO eventually leads to loss of sperm function and apoptotic cell changes [7,8]. Protection from ongoing and progressive LPO is attributable mainly to catalase and glutathione peroxidase.

Glutathione peroxidase (GPx) is present in all eukaryotes. One of its characteristic features is that it is a glutathione-dependent enzyme. In the presence of GPx, glutathione, an electron donor, scavenges various peroxide radicals (ROO·) [9]. Indeed, in spermatozoa, a high ratio glutathione/glutathione disulfide (GSH/GSSG) is known to be a protective factor against oxidative stress [10].

Recent research has shown that a high affinity for lipid peroxides is characteristic of one isoform of GPx–phospholipid hydroperoxide glutathione peroxidase (PHGPx). PHGPx has been found to suppress hydroperoxide-associated cell apoptosis [11]. PHGPx is a selenium-dependent multifunctional protein with antioxidant as well as structural function in the process of sperm maturation [12,13]. Deficiency of PHGPx is considered one of the causes of male infertility [14]. The expression of GPx and its isoenzymes in the reproductive organs of the male rat has been studied in great detail [15]. In the rat, PHGPx and the classic cytosol GPx are found predominantly in the testis, while in the epididymis, the epididymal GPx is basically expressed.

In general, however, it would not be unjustified to say that the roles of the glutathione-dependent enzymes and their respective mechanisms of antioxidant action, having a bearing on fertility preservation, are not, on the whole, adequately explored in man, and even less so in farm animals.

The purpose of the present study was to assess the level of GPx activity in bovine semen with respect to semen quality, and to perform immunolocalization and substrate detection of GPx in bovine testis, epididymis, spermatozoa, and seminal plasma.