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Antioxidant protection and lipid peroxidation in testes and different parts of epididymis in boars

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ABSTRACT

The antioxidative ability of testes and epididymis to protect spermatozoa from detrimental effects of oxidation processes induced by excessive generation of reactive oxygen species has not been previously studied in detail in boar reproductive tissues. The aim of this study was to investigate differences in level of antioxidative protection, intensity of lipid peroxidation, and values of biochemical parameters in testes and different parts of epididymis in sexually mature boars. The study was performed on five Swedish landrace boars from the same litter aged 10 months kept under same ambient conditions. After slaughtering performed at the end of November; tissue samples of testes and the head, body and tail of epididymis were taken. The activity of glutathione peroxidase, glutathione reductase, superoxide dismutase, γ-glutamyltransferase, acid phosphatase, and lactate dehydrogenase, and concentrations of triacylglycerol, phospholipids, cholesterol, free fatty acids were determined in obtained supernatants from homogenized tissues spectrophotometrically; the concentration of malondialdehyde was determined by high-performance liquid chromatography. Significantly higher activities of glutathione peroxidase and glutathione reductase ($P < 0.05$) were found in testes compared with epididymis. In testes, a significantly higher activity of superoxide dismutase was found than in the head and tail of epididymis ($P < 0.05$). Malondialdehyde concentration in head of epididymis was significantly higher than in testes, or the body and tail of epididymis ($P < 0.05$). Simultaneously, malondialdehyde concentration in testes was significantly higher than in body and tail of epididymis ($P < 0.05$). In tail of epididymis, significantly higher activities of γ-glutamyltransferase, acid phosphatase, and lactate dehydrogenase were recorded than in testes. Significantly lower concentrations of triacylglycerol and free fatty acids were recorded in epididymis tail in comparison to epididymis head ($P < 0.05$). It could be concluded that high activities of antioxidative enzymes in testes of boars are essential for the appropriate protection of spermatozoa and cells of testes tissue against oxidative damages. The tissues of testes and epididymis head in boars were more susceptible to lipid peroxidation. Results

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of the present study indicated physiological importance of antioxidative enzymes in reproductive system in boars, and thus may serve for better understanding the mechanisms of male infertility.

1. Introduction

A high cell division rate is immanent to the process of spermatogenesis, demanding huge oxygen consumption in the mitochondria of cells residing in the germinative epithelium [1]. Poor vascularization of testes predisposes them to low-oxygen pressure in their microenvironment. Despite this, testes are subject to oxidation stress due to high concentrations of polyunsaturated fatty acids (PUFAs) and the presence of reactive oxygen species (ROS) generating systems.

Posttesticular maturation of spermatozoa in the epididymis comprises complex biochemical, physiological, and morphological processes primarily associated with the spermatozoa cell membrane. In addition, the epididymis provides an optimal microenvironment for the concentration, transport, maturation, protection, and storage of spermatozoa [2,3]. Highly pronounced secretory activity of the epididymis is specific for each part of the epididymis (head, body, and tail) in breeding mammals [4], and several hundred proteins have been recognized in its secretions. For example, the enzyme acid phosphatase (ACP) in the epididymis is involved in posttesticular processes of spermatozoa maturation [5], whereas the enzyme lactate dehydrogenase (LDH) is important in processes providing energy for survival and motility of spermatozoa [6,7]. Further, it is well known that ROS are of crucial importance in the process of spermatozoa maturation and appropriate quantities are essential for their physiological function [8,9]. However, during the processes of production, maturation, and storage in the epididymis until fertilization, spermatozoa are exposed to damage due to the consequences of oxidation stress, since they are substantially more susceptible than other cells [1,10]. Moreover, spermatozoa are extremely susceptible to oxidation stress induced by the excessive production of ROS due to the presence of high proportions of PUFA in their cell membrane and scarce cytoplasm, which consequently results in an inadequate quantity of antioxidative enzymes and their protection against ROS [11]. All lipid components of the spermatozoa membrane are responsible for their physiological functions and processes, whereas they are also target substrates for lipid peroxidation [12].

Despite the fact that oxidation stress is known to be the dominant etiological factor for male infertility, its basic mechanisms of induction have not yet been fully elucidated [13]. Lipid peroxidation is a process in which the oxidation of PUFA occurs in phospholipids (PHL) and low-density lipoproteins within the spermatozoa cell membranes. Lipid peroxidation may induce loss of cell membrane integrity, disturbance in spermatozoa function, and total cessation of spermatogenesis and steroidogenesis [14,15]. The intensity of oxidation stress may be monitored by measuring the products of biological molecule oxidation and/or by determining enzymatic and nonenzymatic antioxidants [16]. The most common product of lipid peroxidation is malondialdehyde (MDA) and measuring its concentration in tissues is used to determine the intensity of oxidation stress [17–19]. Exposure of spermatozoa to excessive ROS is associated with their abnormal morphology, decreased number in ejaculate, decreased motility, and reduced capacity for oocyte penetration [20,21]. Thus, antioxidative capacity of spermatozoa and the tissue microenvironment in which they were produced and/or stored is an important factor for determining the etiology of male fertility or infertility [1,13,22]. Antioxidative protection of the boar reproductive system comprises enzymatic and nonenzymatic systems which act interactively in the prevention of induction and/or reduction of oxidative stress. Primary antioxidative enzymes involved in ROS scavenging in spermatozoa and different parts of the male reproductive system are: superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase. Many others, such as secondary antioxidative enzymes glutathione reductase (GSH-RD) and γ-glutamyltransferase (GGT), are also associated with the mechanism of antioxidative protection [23,24].

The ability of the testes and epididymis to protect spermatozoa from the damaging effects of oxidative processes by locally synthesized antioxidants has not been fully studied in boars as well as molecular and biochemical mechanisms occurring during their production, storage, and maturation in the epididymis. Thus, the aim of the present study was to investigate differences in the level of antioxidative protection, intensity of lipid oxidative damages, and values of biochemical variables (GGT, LDH, ACP, free fatty acids [FFAs], triacylglycerol [TAG], PHL, and cholesterol [CHOL]) in testes and different parts of the epididymis in reproductive mature boars. Correlations were also established among the tested variables, which could contribute to better understanding of spermatozoa maturation processes and mechanisms of spermatozoa antioxidative protection during their production in the testes and maturation during transport and storage in the epididymis.

2. Materials and methods

2.1. Boar management

The investigation was performed on five Swedish Landrace boars from the same litter kept at the swine farm of the Požega Penitentiary in Požega, Croatia and reared for the economic utilization of porcine meat. From weaning until 4 months of age, pigs were housed in pens 3 × 2 m (0.42 m^2/animal) in size and had accessed to feed and water *ad libitum*. They were fed with complete feed mixture for weaners (Starter for pigs 20%-pelleted, TSH, Čakovec, Croatia). At the age of 6 months, pigs were transferred to pens 2.5 × 4.3 m (1.5 m^2/animal) in size and fed 3 times per day with complete feed mixture for growing and fattening