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Differences in seminal plasma and spermatozoa antioxidative systems and seminal plasma lipid and protein levels among boar breeds and hybrid genetic traits

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ABSTRACT

The objectives of this study were to determine the influence of breed and hybrid genetic traits of boars on lipid and protein concentrations and antioxidative system variables in seminal plasma (SP) and spermatozoa and their correlations with semen quality variables. Semen samples from 27 boars: Swedish Landraces (SL), German Landraces (GL), Large Whites (LW), Pietrains (P) and Pig Improvement Company hybrids (PIC-hybrid), aged from 1.5 to 3 years old, were collected. SP was spectrophotometrically analyzed to determine total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triacylglycerol (TAG), total protein (TP), albumin, and zinc concentrations. The antioxidative system in SP and spermatozoa was established spectrophotometrically by determining total antioxidative status (TAS), total superoxide dismutase (TSOD) and glutathione peroxidase (GSH-Px) parameters, as well as copper-zinc superoxide dismutase (CuZnSOD) and manganese superoxide dismutase (MnSOD) activity in spermatozoa. The hybrid boars had higher ($P < 0.05$) SP concentrations of: TC, LDL-C and TAG than P and GL; HDL-C than P, GL and SL; and TP than P and LW. PIC-hybrid had lower values ($P < 0.05$) in spermatozoa of: TAS and CuZnSOD than SL; TSOD and GSH-Px than SL and P; and MnSOD than SL and LW. Differences in SP and spermatozoa antioxidative system variables and the significant differences in SP protein and lipid variables exist among boars of different breeds and hybrid. Novel data and observed differences in semen variables among boar breeds and hybrids and their correlations with semen quality parameters in this study could contribute to better assessment of boar semen quality.

1. Introduction

Boars included in the artificial insemination (AI)-programs are carefully selected and safeguarded to provide ejaculates with a large number of motile, viable and morphologically normal spermatozoa (Barranco et al., 2015a). Despite these rigorous selection criteria, differences among the AI-boars are still noteworthy regarding *in vivo* fertility and the ability of their spermatozoa to tolerate preservation and oxidative stress (OS) (Parrilla et al., 2012; Guthrie and Welch, 2012).

The OS results from an increased generation of reactive oxygen species (ROS) and/or a decreased available antioxidant defence system. Further, OS is characterized

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by peroxidative damage that can lead to spermatozoa damage, impairing critical spermatozoa functions and, consequently, male infertility (Guthrie and Welch, 2012). Furthermore, boar spermatozoa are particularly susceptible to OS because their plasma membrane is rich in polyunsaturated fatty acids and because the handling and processing of semen often leads to increased levels of ROS (Cerolini et al., 2000; Barranco et al., 2015b). However, to minimize the negative effects of ROS, spermatozoa and seminal plasma (SP) have enzymatic and non-enzymatic antioxidative systems that neutralize excessively generated amounts of ROS (Roca et al., 2005). In this way, these systems provide the optimal amounts of ROS which are necessary for the physiological functions of spermatozoa (Ogbuewu et al., 2010). Thus, antioxidative enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), have important functions in protecting spermatozoa from the ROS generated in semen (Cerolini et al., 2001).

Sperm structure, function or fertilizing potential depend on the lipid composition in spermatozoa and SP (Barranco et al., 2015b). Cholesterol is a substantial component of the spermatozoa membrane, playing a key role in modulating of signalling pathways involved in spermatozoa functionality, particularly capacitation (Visconti et al., 2011). The ejaculated spermatozoa obtain additional cholesterol from the SP, which is incorporated into the cell membrane. Boar spermatozoa and their cell membranes which contained a greater amount of cholesterol were more resistant to stress induced osmotic "shock" (Jacyno et al., 2009). The SP affects spermatozoa function, demonstrated both *in vivo* and *in vitro* studies with a focus on the SP proteins (Caballero et al., 2008; Rodriguez-Martinez et al., 2011; Barranco et al., 2015c). The SP proteins cover and protect the spermatozoa during ejaculation and the higher protein concentration results in the better protection and preservation of spermatozoa cell membrane stability (Strzeżek, 2002).

Until now, the differences in the values of lipids and proteins in SP and the variables of the antioxidative system in SP and spermatozoa among boar breeds have been lacking in veterinary medicine. We assumed that these values/variables may be of a practical relevance for the identification and selection of boar breeds which may contribute to a better assessment of boar semen quality with over average semen preservation/storage capability.

Therefore, the aims of this study were to determine the presence and quantities of lipids and proteins in SP and antioxidative system variables in SP and spermatozoa and to establish eventually differences among boar breeds and PIC hybrids. In addition, we wanted to reveal eventual correlation of abovementioned variables with semen quality parameters.

2. Materials and methods

2.1. Animals

The study was performed on 27 boars, aged between 1.5 and 3 years old (2.51 ± 0.71; mean $\pm$ SD), which were selected in the Centre for Reproduction in Croatian Livestock, at the production work unit in Križevci in Croatia. The animals were divided into five groups according to their breed. The German Landrace and Pietrain groups comprised 6 boars, while the Swedish Landrace, Large White and Pig Improvement Company hybrid (PIC-hybrid) groups consisted of 5 boars. During the experimental period, the animals were kept in separate pen compartments (sized 12 m^2), equipped with a covered outlet in which hygienic measures were regularly conducted. The animals were fed once daily, before the time for semen collection, with 3 kg of commercial feed for boars (crude protein 16%, crude fat 16%, crude fiber 7%, Phosphorus 0.6%, Calcium 1%, Sodium 0.25%, Zinc 50 mg/kg, Vitamin A 5000 IU/kg and Vitamin D_3 625 IU/kg). Water was provided to the boars *ad libitum* by automatic watering.

2.2. Semen sampling and processing

Semen samples were collected using routine procedures twice weekly to obtain semen for artificial insemination. Semen samples were taken once in the early autumn (late September or early October). Following semen sampling, an evaluation of the semen purity and macroscopic properties (volume, colour, consistency and odour) was performed. Following this evaluation, the ejaculate was placed into a water bath heated at 37 °C for 30 s, where the samples remained during the microscopic evaluation and the determination of the semen density. Fresh semen samples were evaluated microscopically for sperm motility and morphologic spermatozoa features by visualization using a phase-contrast system microscope with a warm stage (Olympus BX50F, Tokyo, Japan). The concentration of spermatozoa was objectively determined using a Photometer SDM 5 (MiniTüb, Landshut, Germany). Semen samples meeting the criteria of the macroscopic and microscopic evaluations (concentration $150–250 \times 10^6$ spermatozoa/mL; spermatozoa motility more than 70%) were processed further. The total number of spermatozoa per ejaculate per boar was calculated by multiplying the spermatozoa concentration and ejaculate volume as described previously by Žura Žaja et al. (2016).

2.3. Separation of seminal plasma from spermatozoa

Each fresh ejaculate sample was centrifuged at 1000 g for 15 min at room temperature (about 20 °C). After centrifugation, separated seminal plasma and spermatozoa were obtained, and the seminal plasma was stored at −80 °C until analysis. The separated spermatozoa were washed three times in saline, centrifuged at 500*g* for 5 min at room temperature (about 20 °C), and stored at −80 °C until analysis.

2.4. Seminal plasma and spermatozoa analyses

The concentrations of the following biochemical variables were determined in the seminal plasma samples: total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triacylglycerol (TAG), total proteins (TP), albumin (ALB), and zinc (Zn). The ALB, TC and TAG concentrations