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Antioxidant Defence System of Boar Cauda Epididymidal Spermatozoa and Reproductive Tract Fluids

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Contents

Antioxidants secreted by the reproductive tract protect spermatozoa against the toxic effects of reactive oxygen species (ROS) after ejaculation. This study aimed at characterizing the level of antioxidant protection in boar cauda epididymidal spermatozoa and fluids of the cauda epididymidis, vesicular and prostate glands. Also, this study investigated the effect of a 5-h period of dialysis on the antioxidant capacity of boar seminal plasma. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione transferase (GST) and phospholipid hydroperoxide glutathione peroxidase (PHGPx) activities were monitored in the cauda epididymidal spermatozoa or reproductive tract fluids. Also, the concentrations of total glutathione (GSH + GSSG), L-ergothioneine (ERT) and L-ascorbate and the total antioxidant status (TAS) of the fluids were measured. It was found that the cauda epididymidal spermatozoa exhibited high SOD activity and relatively low activity of PHGPx. The relative amounts of GPx, GR and GST activities in the cauda epididymidal spermatozoa were negligible, whereas CAT activity was undetectable. Greater SOD activity was found in the fluids of the cauda epididymidis and prostate gland. Furthermore, the prostate gland fluid appeared to be the main source of CAT activity in the seminal plasma, whereas the highest level of GPx activity was derived from the cauda epididymidal fluid. The reproductive tract fluids exhibited negligible amounts of GR and GST activities. It seemed that the significant amounts of GSH + GSSG, ERT and L-ascorbate in the reproductive tract fluids could have an ameliorative effect on the level of TAS in the seminal plasma. Dialysis had a marked effect on the total antioxidant capacity of the seminal plasma, which was manifested in greater activity of SOD and GPx. The findings of this study confirmed that the scavenging potential of the seminal plasma is dependent on the contributions of different antioxidants, originating in various fluids of boar reproductive tract.

Introduction

Evidence has been shown that spermatozoa are capable of generating reactive oxygen species (ROS), which is an essential prerequisite for normal cell function; however, excessive ROS production can lead to cellular pathology (Alvarez et al. 1987; Aitken 1994; Aitken et al. 1996). Moreover, spermatozoa are highly susceptible to oxidative stress induced by excessive ROS because their plasma membrane contains large quantities of polyunsaturated fatty acids and their cytoplasm contains inadequate antioxidant enzymes (Aitken et al. 1996; Saleh and Agarwal 2002).

Spermatozoa must rely on epididymal antioxidants and their own intrinsic antioxidant capacity for protection during epididymidal transit and storage and during their presence in the female reproductive tract (Saleh and Agarwal 2002). Moreover, the epididymidis and accessory sex glands play a major role in protecting spermatozoa against oxidative stress by removing excessive ROS and secreting antioxidants into the seminal plasma (Tramer et al. 1998; Vernet et al. 2004). The seminal plasma is endowed with a battery of antioxidant enzymes comprising superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST), which have been detected in several animal species (Alvarez et al. 1987; Beconi et al. 1993; Strzeżek et al. 1999, 2009; Marti et al. 2007). Additionally, the seminal plasma is equipped with diverse non-enzymatic antioxidants, such as glutathione (GSH), L-ergothioneine (ERT), L-ascorbate ascorbate, urate, alpha-tocopherol, pyruvate, taurine and hypo-taurine, which have been shown to combat oxidative attack (Storey 1997; Strzeżek et al. 1999). It should be emphasized that spermatozoa depend largely on the protection afforded by the seminal plasma because they possess limited antioxidant defence mechanisms (Alvarez et al. 1987; Vernet et al. 2004).

The antioxidants of boar seminal plasma are derived from a number of components originating in various reproductive organs, contributing in different ways to the overall function of the antioxidant defence system (Strzeżek et al. 1999; Strzeżek 2002). Despite the purported importance of the antioxidant defence mechanisms in boar seminal plasma, no comprehensive studies regarding the contributions of antioxidant components by the different reproductive tract fluids have been reported. Therefore, the aim of this study was to characterize the activities of the enzymatic antioxidants and the relative concentrations of non-enzymatic antioxidants in the cauda epididymidal spermatozoa or fluids of the cauda epididymidis, vesicular and prostate glands in the boar. Also, this study investigated the effect of dialysis on the antioxidant capacity of the seminal plasma.

Materials and Methods

Animals, reproductive fluids, epididymidal spermatozoa and semen collections

This study was divided into two experiments: (i) Experiment 1 (Exp. 1) was based on the analysis of the antioxidant status in the cauda epididymidal spermatozoa and reproductive tract fluids and (ii) Experiment 2 (Exp. 2) comprised assay of the antioxidant capacity of post-dialysed seminal plasma.

In Exp. 1, the fluids of the cauda epididymidis, vesicular and prostate glands were collected from 36 clinically healthy, sexually mature Polish Landrace boars (aged 1.5–2 years) stationed at the Experimental Research Laboratory of the Department of Physiology and Animal Reproduction, Rzeszow, Poland. The boars were fed a commercial porcine ration and were kept in individual pens, under standard environmental conditions. Water was available *ad libitum*. All experiments were carried out in accordance with the guidelines set out by the Local Ethics Committee for Experimentation with Animals.

In Exp. 1, reproductive fluids and cauda epididymidal spermatozoa were collected from three boars per week during a 3-month period, from October through December. Boars (n = 36) were anaesthetized with 2.5% thiopental (Sandoz GmbH, Kundl, Austria) after premedication with atropine (Atropinum sulfuricum, Polfa, Poland) and Stresnil (Jansen, Pharmaceutica, Beerse, Belgium). Following the induction of anaesthesia, the testis and epididymis were removed through a scrotal incision. After exposing the epididymis, a narrow plastic cannula (1.2 mm in diameter) was inserted into the cauda epididymidis, which was gently squeezed and massaged to extrude the epididymal contents. The epididymal contents were collected into plastic test tubes and washed twice ($1000 \times g$, 5 min at room temperature), after which the fluid was separated from the spermatozoa. The fluid was decanted, re-centrifuged ($10\,000 \times g$, 10 min at room temperature) and stored at −80°C prior to analysis of antioxidant assays. The sperm pellets were used for the preparation of the homogenates. Following surgical laparotomy, the vesicular glands were incised, and the fluid was collected by aspirations, using sterile plastic syringe, whereas the prostatic fluids were harvested from the boars 15 min after slaughter. The total amounts of fluids harvested from each boar were approximately 7, 10 and 0.2 ml from the cauda epididymidis, vesicular and prostate gland, respectively. The reproductive tract fluids were centrifuged ($15\,000 \times g$, 10 min at room temperature) and stored at −80°C, until required.

In Exp. 2, whole ejaculates were collected from 11 Polish Landrace and Large White boars (aged 1–2 years) stationed at the Artificial Insemination Stations in Olecko, Pętkowice and Slawecinek (Poland). The gloved-hand technique was used to collect semen from the boars, once weekly for a period of 3 weeks. The semen was dialysed against Kortowo 3 extender (K3) for a 5-h period at room temperature, using semi-permeable cellulose tubing of 12–14 kDa molecular weight cut-off (Visking Dialysis Tubing, Serva Electrophoresis, Heidelberg, Germany), as described in a previous method (Fraser et al. 2007). Following dialysis, the semen samples were centrifuged ($3000 \times g$, 10 min at room temperature), and the recovered seminal plasma was re-centrifuged ($10\,000 \times g$, 5 min at room temperature). Besides total protein measurements, the activity of SOD, CAT and GPx and the concentrations of total glutathione (reduced and oxidized GSH + GSSG) and total antioxidant status (TAS) were monitored in the non-dialysed and dialysed seminal plasma.

Processing of epididymal spermatozoa for antioxidant assays

The epididymal sperm pellets were re-suspended in 0.85% NaCl and adjusted to a final concentration of 3×10^9 spermatozoa/ml. Sperm samples were homogenized at 4°C, using an Ultra-turrax T8 homogenisator (IKA-Werke, Staufen, Germany). Following homogenization, the sperm suspensions were centrifuged ($10\,000 \times g$, 10 min at 4°C temperature), and the supernatants were used for the measurements of the activity of SOD, CAT, GPx, GR, GST and phospholipid hydroperoxide glutathione peroxidase (PHGPx). The enzyme activities in the sperm extracts were measured spectrophotometrically, using a Beckman DU-62 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA, USA), and expressed as U/10^9 spermatozoa.

Protein content analysis

Total protein content in the reproductive tract fluids was measured, according to the method of Weichselbaum (1946), using serum bovine albumin, BSA (Serum and Vaccine Production, Cracow, Poland), as a standard.

Enzymatic antioxidant assays

The activities of the enzymatic antioxidants, SOD, CAT, GPx, GR and GST, were measured in the fluids of the cauda epididymidis, vesicular and prostate glands or seminal plasma. In some cases, the enzyme assays were performed using commercial kits according to the manufacturer's recommendation. The enzyme activities in the reproductive tract fluids were measured spectrophotometrically and expressed as U/ml. All assays were run in duplicate.

SOD activity

Superoxide dismutase activity was determined according to Beauchamp and Fridovich (1971), with some modifications (Kowalowka et al. 2008). The assay was based on the inhibition of nitroblue tetrazolium (NBT) (Serva Feinbiochemica GmbH & Co, Heidelberg, Germany) caused by the superoxide anion generated by xanthine and xanthine oxidase (Roche Diagnostic, Indianapolis, IN, USA). One unit of SOD activity was defined as the amount of the enzyme capable of decreasing the reduction of NBT by 50% at 25°C (pH 10.2).

CAT activity

A colorimetric assay was used to quantify the CAT activity, using a commercial kit (Sigma Aldrich Corp., St. Louis, MO, USA). This assay was based on the measurements of hydrogen peroxide (H_2O_2), remaining after the action of CAT. The colorimetric method used a substituted phenol, which coupled oxidatively 4-aminoantipyrine in the presence of H_2O_2 and horseradish. The quinoneimine dye coupling product, which correlated with the amount of H_2O_2 remaining in the reaction mixture, was measured at 520 nm. One unit of CAT

decomposed 1 μM H_2O_2 to oxygen and water per minute at 25°C at a substrate concentration of 50 mM H_2O_2 (pH 7.0). Catalase activity was calculated from the change in absorbance.

GPx activity

Glutathione peroxidase activity was measured using a Ransel kit (Randox Laboratories, Ltd., London, UK). In this assay, GPx catalysed the oxidation of GSH by cumene hydroperoxide. In the presence of GR and NADPH, GSSG was converted to GSH with a concomitant oxidation of NADPH to $NADP^+$. The decrease in the absorbance was measured at an absorbance of 340 nm at 37°C (pH 7.2).

GR and GST activity

A commercial assay kit (Sigma Aldrich Corp.) was used to measure GR activity. This assay was based on the reduction of GSSG by NADPH in the presence GR. The enzyme activity was measured by a decrease in absorbance caused by the oxidation of NADPH at 340 nm. One unit of GR was required to oxidize 1.0 μM NADPH at 25°C (pH 7.5).

The activity of GST was measured using a commercial assay kit (Sigma Aldrich Corp.). In this assay, GST catalysed GSH conjugation to 1-chloro-2,4-dinitrobenzene (CDNB) substrate through the thiol group of GSH. The rate of increase in the absorption of the reaction product (GS-DNB) was directly proportional to GST activity and an absorbance of 340 nm at 25°C (pH 7.4).

Phospholipid hydroperoxide glutathione peroxidase (PHGPx) activity

Cauda epididymidal spermatozoa (3×10^9 spermatozoa/ml), suspended in 0.85% NaCl, were added to a solution (1 ml) containing 100 mM potassium phosphate, 1 mM EDTA, 0.05% Triton X-100, 0.01% leupeptin (Boehringer Mannhiem GmbH, Mannheim, Germany), 0.014% pepstatin and 0.07% phenylmethylsulphonyl fluoride (PMSF) (pH 7.8) and incubated for 1 h at 4°C. Following incubation, the sperm suspensions were centrifuged ($10\,000 \times \boldsymbol{g}$, 10 min, 4°C), and the supernatants were used to measure PHGPx activity by a coupled enzymatic assay, using GSH, GR, phosphatidylcholine hydroperoxide (PCOOH) and NADPH, as previously described (Imai et al. 2001). The PCOOH substrate was prepared by enzymatic hydroperoxidation of phosphatidylcholine, according to a previously described method (Roveri and Maiorino 1994).

Non-enzymatic antioxidant assays

The level of TAS and the concentrations of GSH + GSSG, ERT and L-ascorbate were measured spectrophotometrically in the fluids of the cauda epididymidis and vesicular glands or seminal plasma. For reasons of limited quantities of prostatic gland fluid, GR and GST activities, as well as the concentrations of the non-enzymatic antioxidants, were not assayed. All assays were measured in duplicate.

Total GSH + GSSG concentration

The concentration of total GSH + GSSG was determined using a Bioxytech GSH-400 assay kit (Oxis International, Inc., Portland, OR, USA). This assay was based on the conjugation of a substitution product (thioethers), obtained with GSH into a chromophoric thione. The GSH + GSSG concentration was calculated from the change in the absorbance of 400 nm at 25°C (pH 7.8).

ERT concentration

The concentration of ERT was determined according to a previously described method (Carlsson et al. 1974). In this assay, the reaction of ERT with 2,2' dipyridyl disulphide (Merck KGaA, Darmstad, Germany) formed 2-chromophoric pyridine-2-thione (Py-2-SH), which was measured from the change in the absorbance at 343 nm at 26°C (pH 1.0).

L-ascorbate concentration

The concentration of L-ascorbate was determined according to the method of Roe (1954). In this assay, the coupling reaction of dehydroascorbic acid with 2,4 dinitrophenylhydrazine (DNPH) in the presence of thiourea formed a chromogen, which was measured at 515 nm (37°C, pH 8.0).

Analysis of TAS

Total antioxidant status was determined using an assay kit (Randox Laboratories, Crumlin, UK). This method was based on the reaction of ABTS® (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) with peroxidase and H_2O_2 to produce a radial cation $ABTS^+$. Total antioxidant status was measured from a change in $ABTS^+$ in the presence of antioxidants at 600 nm (37°C, pH 7.4).

Statistical analysis

All results are expressed as mean ± SEM. Data were analysed by ANOVA followed by the Tukey's *post hoc* test ($p < 0.05$) for multiple comparisons. The Student's *t*-test was used to analyse differences between pairwise comparisons. The STATISTICA software package (StatSoft Incorporation, Tulsa, OK, USA) was used to analyse the data.

Results

Experiment 1

Enzymatic antioxidants of the cauda epididymidal spermatozoa

The activity of antioxidant enzymes occurring in the cauda epididymidal spermatozoa is shown in Table 1. Spermatozoa harvested from the cauda epididymidis exhibited considerable high SOD activity and low

activity of PHGPx. However, PHGPx activity was approximately sevenfold greater than GPx and GR activities. Furthermore, GPx and GR activities were found at similar concentrations in the sperm cells. It was found that there were negligible amounts of GST activity in the sperm extracts, ranging from 0.04 to 0.06 U/10^9 spermatozoa, whereas CAT activity remained undetectable.

Antioxidant status of the reproductive tract fluids

Table 2 shows total protein content and the activity of enzymatic antioxidants in the fluids of the cauda epididymidis, vesicular and prostate glands. There were wide variations in total protein content in the analysed reproductive tract fluids, being significantly higher ($p < 0.05$) in the vesicular gland fluid, which is a distinguishing feature of these glands. The fluids of the cauda epididymidis and prostate gland exhibited greater ($p < 0.05$) SOD activity. Furthermore, CAT activity was highest in the prostatic gland fluid and lowest in the cauda epididymidis or vesicular glands. It was found that CAT activity in the prostate gland fluid was approximately two- to threefold higher than in the fluids of the cauda epididymidis or vesicular glands. There were no detectable differences ($p > 0.05$) in CAT activity between the fluids of the cauda epididymidis and vesicular glands. In addition, GPx activity was detected in the reproductive tract fluids, being significantly higher ($p < 0.05$) in the cauda epididymidal fluid. The activity of GPx in the cauda epididymidal fluid was approximately threefold higher in the prostatic gland fluid and approximately twofold higher in the vesicular gland fluid. Negligible amounts of GR and GST activities were detected in the fluids of the cauda epididymidis and vesicular glands.

The concentrations of non-enzymatic antioxidants and TAS of the fluids of the cauda epididymidis and vesicular glands are shown in Table 3. The vesicular gland fluid showed higher ($p < 0.05$) concentration of total GSH + GSSG, whereas ERT and L-ascorbate concentrations were greater ($p < 0.05$) in the cauda epididymidal fluid. Higher levels of TAS were detected in the vesicular gland fluid than in the cauda epididymidal fluid.

Table 1. Antioxidant enzyme activity (U/10^9) in cauda epididymidal boar spermatozoa

SOD	CAT	GPx	GR	PHGPx
412.00 ± 24.90	Not detected	0.10 ± 0.03	0.11 ± 0.03	0.70 ± 0.02

Values represent the means (±SEM) of 36 boars.
SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; PHGPx, phospholipid hydroperoxide glutathione peroxidase.

Experiment 2

Effect of dialysis on antioxidant status of the seminal plasma

Dialysis caused various changes in the total protein content and affected the overall antioxidant capacity of the seminal plasma, as shown in Table 4. However, marked changes in the antioxidant capacity of the dialysed seminal plasma were manifested in enhanced ($p < 0.05$) SOD and GPx activities.

Discussion

Spermatozoa mature as they transit from the testis to the cauda epididymidis and undergo a series biochemical modifications, including protein removal or absorption events (Strzeżek 2002; Rodríguez-Martínez et al. 2009). Moreover, proteins and antioxidant components absorbed on the sperm surface and those present in the seminal plasma shield spermatozoa from oxidative damage when they have been deposited in the female reproductive tract (Vernet et al. 2004; Marti et al. 2007).

This study is the first, to our knowledge, to characterize the antioxidant system in the cauda epididymidal spermatozoa and the reproductive tract fluids in the

Table 2. Protein content and enzymatic antioxidant activity in the fluids of boar reproductive tract

Source	Protein content (mg/ml)	SOD (U/ml)	CAT (U/ml)	GPx (U/ml)	GR (U/ml)	GST (U/ml)
Cauda epididymidis	32.19 ± 1.92[a]	242.0 ± 24.45[a]	11.08 ± 3.18[a]	0.79 ± 0.006[a]	0.02 ± 0.003	0.06 ± 0.002
Vesicular glands	127.32 ± 2.13[b]	67.51 ± 15.04[b]	13.47 ± 3.20[a]	0.45 ± 0.001[b]	0.02 ± 0.006	0.04 ± 0.009
Prostate gland	101.0 ± 4.67[c]	307.0 ± 26.34[a]	27.28 ± 3.76[b]	0.24 ± 0.003[c]	n.a.	n.a.

Values represent the means (±SEM) of 36 boars. Within the same columns, values with different letters (a, b, c) are significantly different at $p < 0.05$.
SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; GST, glutathione S-transferase; n.a., not analysed.

Table 3. Concentrations of non-enzymatic antioxidants and total antioxidant status (TAS) of boar reproductive fluids. Values represent the means (±SEM) of 36 boars

Source	Total GSH + GSSG (μM)	ERT (μM)	L-ascorbate (μM)	TAS (μM)
Cauda epididymidis	1150.80 ± 68.30[a]	93.01 ± 2.97[a]	447.86 ± 8.57[a]	1928.00 ± 126.43
Vesicular glands	2131.61 ± 189.90[b]	69.50 ± 5.51[b]	166.21 ± 8.06[b]	2261.00 ± 137.24

Within the same columns, values with different letters (a, b) are significantly different at $p < 0.05$.
GSH + GSSG, total L-glutathione content; ERT, L-ergothioneine.

Table 4. Effect of dialysis on protein content and antioxidant status of boar seminal plasma

Sperm treatment	Total protein content (mg/ml)	SOD (U/ml)	CAT (U/ml)	GPX (U/ml)	GSH + GSSG (μM)	TAS (μM)
Non-dialysis	24.93 ± 0.88	55.73 ± 5.88[a]	3.37 ± 0.86	0.30 ± 0.002[a]	1091.63 ± 83.14	715.70 ± 52.00
Dialysis	20.83 ± 1.66	81.40 ± 5.84[b]	2.50 ± 0.13	0.44 ± 0.003[b]	1126.93 ± 83.60	746.50 ± 48.50

Values represent the means (±SEM) of 33 ejaculates, 3 each from 11 boars. Within the same columns, values with different letters (a, b) are significantly different at $p < 0.05$.
SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GSH + GSSG, total glutathione content; TAS, total antioxidant status.

boar. In the current study, among the multiple redox enzymes detected in the cauda epididymidal spermatozoa and the reproductive tract fluids, SOD is the principal scavenging enzyme that plays a major role in protecting tissue and sperm cells against ROS-mediated attack. There is strong evidence indicating that SOD and its isoenzymes are primarily implicated in suppressing free superoxide anion radicals (Marklund 1984; Peeker et al. 1997; Kowalowka et al. 2008). It should be emphasized that H_2O_2, which is derived from the dismutation of superoxide anion radicals by SOD, is degraded into harmless products by scavenging enzymes, CAT and GPx (Alvarez et al. 1987; Aitken et al. 1996). As CAT activity was not detected in the cauda epididymidal spermatozoa, it seems that PHGPx, an isoform of GPx, could provide the major protection against free superoxide anion radicals. Accumulating evidence has been shown that PHGPx, a selenium-dependent enzyme, is expressed in different male reproductive organs, having multiple functions (Roveri and Maiorino 1994; Ursini et al. 1999; Vernet et al. 2004). It has been well documented that PHGPx reduces phospholipid hydroperoxides and scavenges H_2O_2 (Imai et al. 2001). Moreover, PHGPx has been shown to catalyse a peroxidase-dependent oxidation of protein thiols through a reaction contributing to cross-linking within the capsules enclosing sperm mitochondria (Vernet et al. 2004). This is supported by the observation that PHGPx-defective spermatozoa failed to incorporate rhodamine 123, suggesting a loss of mitochondrial membrane potential (Imai et al. 2001). Even though spermatozoa possess a wide variety of antioxidants, their effectiveness is impaired owing to their limited concentration and distribution (Aitken 1994). Results based on this study reaffirmed that, because of the limited capacity of the antioxidant system inherent in spermatozoa, the scavenging capacity of the seminal plasma plays a key role in protecting sperm cells against ROS-mediated attack. Boar seminal plasma exhibits high extracellular SOD activity (Kowalowka et al. 2008), which is derived mainly from the prostate gland, as shown in the current study. Such results are in agreement with those of several authors, who demonstrated that a major portion of SOD activity in the human seminal plasma is originated in the prostate gland (Jeulin et al. 1989; Peeker et al. 1997).

The presence of measurable activity of CAT in spermatozoa of different species is still uncertain. Several authors detected extremely low levels of CAT activity in human and rat spermatozoa (Alvarez et al. 1987; Jeulin et al. 1989; Tramer et al. 1998). However, in our study, CAT activity was not detected in boar epididymidal spermatozoa, and in other studies, the enzyme activity was not detected in either bovine or canine spermatozoa (Bilodeau et al. 2000; Strzeżek et al. 2009). This discrepancy is somewhat difficult to explain, but might be probably related to the dual pathway reactions exhibited by CAT. It has been suggested that a catalytic pathway of CAT, which only uses H_2O_2 as a substrate, is predominant when there are greater H_2O_2 concentrations, as in case of an oxidative burst or overexpression of SOD, whereas a peroxidative pathway of the enzyme is dominant at lower H_2O_2 concentrations (Vernet et al. 2004). Another explanation for this discrepancy could be the contamination of sperm samples by blood cells (Tramer et al. 1998). It was interesting to find that high SOD activity in the prostatic gland fluid or cauda epididymidal fluid was associated with large amounts of CAT activity or GPx activity in the respective fluid. It has been postulated that CAT activity in human seminal plasma is primarily of prostatic origin (Jeulin et al. 1989). Such observation corroborates the findings of our study, indicating that a major portion of CAT activity in boar seminal plasma appeared to originate in the prostate gland. On the contrary, the bulk of GPx activity in boar seminal plasma appeared to derive mainly from the cauda epididymidal secretions. Furthermore, it seems that the contribution of low amounts of vesicular CAT and GPx activities to the seminal plasma could be probably associated with the relatively low SOD activity in the fluid. In addition, other scavenging enzymes, such as GR and GST, which were detected in considerably low quantities in the cauda epididymidal spermatozoa and reproductive tract fluids, could be implicated in the redox-regulated events and act synergistically to avert oxidative stress (Vernet et al. 2004).

Besides the presence of antioxidant enzymes and seminal plasma proteins, low molecular weight substances containing thiol groups, such as GSH and ERT, constitute the antioxidant defence system of boar seminal plasma (Strzeżek et al. 1999; Strzeżek 2002). It is worth pointing out that the relative contributions of total GSH + GSSG, ERT and L-ascorbate by the reproductive tract fluids could have an ameliorative effect on the level of TAS of the seminal plasma.

Even though the mechanisms responsible for cryo-induced damage to spermatozoa are considered to be multifactorial, excessive production of ROS has been suggested as a major contributing factor, which compromises sperm function (Gadea et al. 2004; Roca et al. 2005). Several authors have applied different antioxidant strategies to overcome cryo-damage to boar spermatozoa (Peña et al. 2003; Gadea et al. 2004, 2005; Roca et al.

2005). However, in spite of these promising results, the use of antioxidant strategies in boar semen cryopreservation is still limited. It can be suggested that, besides the removal of low molecular components from boar seminal plasma by dialysis, enhanced post-thaw sperm quality characteristics could be attributed to the high antioxidant capacity of the seminal plasma (Fraser et al. 2007). Likewise, improvement in the freezability of boar semen and a reduction in cryo-induced sperm DNA damage have been ascribed to the high antioxidant capacity of boar seminal plasma (Hernández et al. 2006; Peña et al. 2006; Fraser and Strzeżek 2007). The results of the present study demonstrate that dialysis enhances the antioxidant capacity of the seminal plasma.

Taken together, the findings of this study confirm that varying amounts of antioxidants, originating in the reproductive tract fluids, with SOD demonstrating the highest activity, constitute the antioxidant defence system in boar seminal plasma. We suggest that a better understanding of the antioxidant defence mechanisms in the boar reproductive tract will help to unravel the origin of oxidative damage to ejaculated spermatozoa.

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Conflict of interest

None of the authors have any conflict of interest to declare.

Author contributions

All authors have been involved in designing the study, analysing the data and drafting of the paper.

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