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The effect of dialysis on the lipid peroxidation and antioxidant status of boar seminal plasma during liquid preservation

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ABSTRACT

The aim of this study was to assess the effect of dialysis on the lipid peroxidation and antioxidant status in liquid stored boar semen. Seventeen ejaculates obtained from eight boars were divided into two aliquots. One aliquot was dialyzed; the other was used as a control. Semen was then stored at 16-17 °C for three days. Sperm quality parameters, capacitation status, thiobarbituric acid reactive substances (TBARS), superoxide dismutase activity (SOD) and total antioxidant capacity (TAC) of dialyzed and non-dialyzed semen were measured. The effect of lipid peroxidation and antioxidant status on semen quality was evaluated. Removal of low molecular weight components (LMWC) from seminal plasma by dialysis resulted in a significantly higher percentage of morphologically normal spermatozoa ($P = 0.002$). Dialysis moderated the increasing level of acrosomal reacted spermatozoa, showing a significantly lower level of acrosomal reacted spermatozoa in dialyzed semen samples according to each day of storage ($P<0.05$). SOD increased with storage time in both groups, whereas control samples showed significantly higher levels of SOD than dialyzed ones ($P<0.05$). Dialyses yielded a significant decrease in TAC and an increase in the level of TBARS ($P<0.05$). Our results show that elevated levels of SOD in dialyzed semen are not able to counterbalance decreased levels of TAC and prevent oxidative injures to the spermatozoal sperm membrane.

Key words: semen, porcine, dialysis, antioxidative status, lipid peroxidation

Introduction

In the past few years the use of preserved semen for artificial insemination in pigs has increased drastically. In practice, boar semen used for insemination is stored at 15-

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20 °C for 1 to 5 days in the liquid state, following the addition of appropriate extender (JOHNSON et al., 2000). However, due to diminished motility, viability and alterations in membrane permeability of stored spermatozoa, the fertility of boar sperm is decreased after long-term liquid storage when compared to fresh liquid semen (WABERSKI et al., 1994). It has been proven that part of this reduction in sperm fertility may be due to the low molecular weight components (LMWC) of seminal plasma (STRZEZEK et al., 1992), oxidative damage from inappropriate formation of reactive oxygen species (ROS), membrane lipid peroxidation (CEROLINI et al., 2000; KUMARESAN et al., 2009) and also apoptotic-like phenomena (KUMARESAN et al., 2009).

The influence of different fractions in seminal plasma on sperm viability is controversial. Seminal plasma has important regulatory functions in various processes before penetration of oocytes by the spermatozoa. These functions refer to nutrition, protection, regulation of motility and capacitation of spermatozoa, gamete recognition and binding (WABERSKI et al., 1996). On the other hand, LMWC have been isolated from boar seminal plasma and found to have a detrimental effect on sperm function (STRZEZEK et al., 1992). Removal of LMWC from boar seminal plasma by dialysis prior to cooling (MRKUN et al., 2009) and freezing (FRASER et al., 2007) improved sperm characteristics after storage, such as sperm motility and plasma membrane integrity.

Boar spermatozoa contain large quantities of polyunsaturated fatty acids (PUFA) in the phospholipids of the membrane (CEROLINI et al., 2000) and possess relatively low antioxidant capacity, making them, thereby, very sensitive to oxidative damage (SANOCKA et al., 2004). At low concentrations, ROS have a positive effect on spermatozoa; they induce sperm capacitation (FORD, 2004), hyperactivation, acrosomal reaction (AITKEN et al., 1995), and signalling processes that ensure fertilization (AITKEN et al., 1989). However, excessive amounts of ROS disrupt the membrane structure and function of mammalian spermatozoa (AITKEN, 1995) and cause damage to the lipid matrix, which is related to the impairment of sperm motility (CHATTERJEE et al., 2001).

In ejaculate there are multiple protective mechanisms designed to protect spermatozoa against oxidative injury (STRZEZEK et al., 2000). While spermatozoa themselves contain predominately antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GP) (STRZEZEK, 2002), seminal plasma is also rich in low molecular weight non-enzymatic antioxidants (i.e. L-glutathione (GSH), L-ergothioneine (ERT)) (ISHII et al., 2005). In stallion semen, GSH and ERT are present in large quantities (MRUK et al., 2002). In boar seminal plasma, however, lower concentrations of GSH and ERT have been detected. It has therefore been proposed that the specific antioxidant properties of seminal plasma proteins and the presence of high SOD activity in boar semen compensate for the lack of low molecular weight antioxidants (STRZEZEK et al., 2002).

Recent studies suggest that removal of LMWC using dialysis prior to cooling improves the quality of liquid-stored boar semen (MRKUN et al., 2009). On the other hand, removal of low molecular weight antioxidants in seminal plasma could influence the total antioxidant status and antiperoxidant activity of the seminal plasma. The aim of the present study, conducted on boar semen, was to investigate the effect of semen dialysis prior to cooling and storing on the total antioxidant capacity (TAC), SOD activity and lipid peroxidation status in seminal plasma. To our knowledge this is the first evaluation of oxidative status in boar semen after dialysis.

Materials and methods

Semen samples and assessment of pre-dialysis sperm parameters. Seventeen ejaculates obtained from eight boars, aged 12 to 24 months old of various breeds that are routinely used at the local AI centre, were included in the study. Semen samples were collected by the gloved-hand technique using a clean semen collecting flask that filters out gel, dust and bristles. Following collection, the filtered semen of each ejaculate was extended with Beltsville Thawing Solution (BTS, Truadeco, Netherlands) at a ratio of 1:2.

Dialysis and storage. Each diluted ejaculate was divided into two aliquots. The first aliquot was dialyzed against BTS twice at room temperature for a total period of 3 hours (first dialysis 1 hour, second dialysis two hours; 1:50 semen:dialysate) using a semi-permeable cellulose tubing of 12-14 kDa molecular weight cut-off (Visking Dialysis Tubing, Serva Electrophoresis, Heidelberg, Germany). The second aliquot was not dialyzed and served as a control (i.e. non-dialyzed semen).

The semen was then stored in closed plastic containers in a thermal box at 16-17 °C for three days. The semen samples were constantly gently agitated.

Basic semen parameters. In the first hour after collection semen parameters (motility, progressive motility, sperm morphology and concentration) were determined, using assisted semen analysis (Hamilton Thorne IVOS 10.2; Hamilton Thorne Research, MA, USA) with a Makler counting chamber (Sefi Medical Instruments, Israel). Concentration was measured with a photometer (Photometer SDM 5, Minitüb, Germany). Sperm morphology of 200 sperms was assessed in diluted semen samples after fixation in Giemsa stain. The sperm characteristics (motility, progressive motility) of dialyzed and non-dialyzed semen samples were then evaluated on the day of collection (day 0, approximately 5 hours after collection), on day 1 (24 h), on day 2 (48 h) and on day 3 (72 h) of semen preservation, respectively.

Other semen analysis. The capacitation status, lipid peroxidation, superoxide dismutase (SOD) and total antioxidant capacity (TAC) of dialyzed and non-dialyzed semen samples were evaluated on the day of collection (day 0, approximately 5 hours after

collection), on day 1 (24 h), on day 2 (48 h) and on day 3 (72h) of semen preservation, respectively. The metabolic activity was only evaluated on day 0 and on the third day of semen storage.

Metabolic activity. The metabolic activity of the spermatozoa was assayed using a spectrophotometric application of the resazurin reduction assay (ZRIMŠEK et al., 2004; ZRIMŠEK et al., 2006), which depends on the ability of metabolically active spermatozoa to reduce the resazurin redox dye to resorufin. Briefly, 30 µL of 1.8 mmol/L resazurin (Sigma, Steinheim, Germany), diluted in physiological saline, was added to 3 mL of semen sample diluted 1:2 with Beltsville thawing solution semen extender (Beltsville Thawing Solution, Truadeco, the Netherlands) and incubated at 37 ºC in a water bath for 10 min. After incubation, two sub-samples of 1 mL were added to 1.5 mL of butanol (Merc, Germany). After rapid vortexing, samples were centrifuged at 3000g for 10 min. Absorbance in the clear upper layer of the butanol extracts was measured at 610 nm (UV/VIS Spectrometer Lambda 12; Perkin Elmer Corp., Analytical Instruments, Norwalk, CT, USA).

Chlortetracycline (CTC) staining analysis. CTC staining analysis was used to identify the progress of capacitation and acrosome reaction of boar spermatozoa. 25 mL of the sperm suspension was treated with a 12.5 mL of a CTC solution for 10 s, followed by the addition of 0.5 mL 12.5% glutaraldehyde in Tris buffer (pH 7.8). The CTC solution was composed of 20 mL of 500 mM CTC in a chilled buffer of 20 mM Tris, 130 mM sodium chloride, and 5mM L-cysteine (VADNAIS et al., 2005). 10 mL of 1.4-diazabicyclo-(2,2,2) octane (Sigma) dissolved in glycerol:PBS (9:1) was added for anti-fading effect. 3 mL of the fixed sperm suspension was placed on a glass slide and a cover slip was applied. The slides were prepared in duplicate and placed in a black box until read. One hundred spermatozoa on each preparation were assessed under an Olympus BX40 microscope equipped with a 400-440 nm excitation filter, a 475 nm emission filter and a 455 nm dichromatic mirror. Assessment of capacitation status was made as described previously (VADNAIS et al., 2005) with sperm being classified as un-capacitated (UC), capacitated (C) and acrosome-reacted (AR).

Oxidative stress biomarkers.

Preparation of seminal plasma. The semen was centrifuged at 818 x g for 10 minutes at room temperature. Supernatant was removed and further centrifuged at 13,000 g for 15 min at 4 ºC to separate seminal plasma, which was then aliquoted and frozen at -80 ºC until assayed for TBARS, TAC and SOD.

Measurement of lipid peroxidation. Membrane lipid peroxidation was estimated by the end-point generation of malondialdehyde TBARS using the thiobarbituric acid (TBA) assay (TBARS assay Kit, Cayman Chemical Company). 100 µl of seminal plasma of each sample was mixed with 2 mL of the TBA-TCA reagent (15%, w/v TCA; 0.375%, w/v

TBA and 0.25 N HCl). The mixture was treated in a boiling water bath for 60 min and then cooled in an ice bath for 10 minutes. After cooling, the suspension was centrifuged at 1600g and 4 °C for 10 min. The supernatant was then separated and the absorbance was measured at 532 nm at room temperature within 30 min after centrifugation (Tecan, Safir 2). The assay was conducted in duplicate. The TBARS concentration was determined by comparing the sample's absorbance at 532 nm with a standard curve created, using malondialdehyde (MDA) as standard.

Determination of TAC levels in seminal plasma. Seminal plasma samples for TAC were assayed in duplicates by an automated biochemistry analyser RX Daytona (Randox, Crumlin, UK), using commercially available Total Antioxidant Status (TAS) kit (Randox, Crumlin, UK). The assay (MILLER et al., 1993) is based on the reduction of free radicals ($ABTS^{*+}$-2,2'-azinobis-(3-ehylbenzothiazoline-6-sulfonate) measured as a decrease of absorbance at 600 nm at 3 min by antioxidants. The $ABTS^{*+}$ radical cation is formed by the interaction of ABTS with ferrylmyoglobin radical species, generated by the activation of metmyoglobin with hydrogen peroxide. The suppression of the absorbance of the $ABTS^{*+}$ radical cation by seminal plasma antioxidants was compared with that from a Trolox (6-hydroxy-2,5,7,-tetramethylchroman-2-carboxylic acid), which is included as part of the TAS kit. The results are expressed as μmol/L of Trolox equivalents.

Measurement of SOD activity. SOD activity was determined spectrophotometrically (550 nm) in duplicates with an automatic biochemical analyser RX Daytona (Randox, Crumlin, UK), using a commercially available Ransod kit (Randox, Crumlin, UK) that is based on the original method of McCORD and FRIDOVICH (1969). According to the method, the superoxide radicals were generated by the xanthine and xanthine oxidase reaction. The amount of superoxide radical produced was determined by 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) as an indicator, which reacts with a superoxide radical to form formazan dye. The SOD activity was determined by the grade of inhibition of the described reaction. The standard calibration curve of the percentage of inhibition by standard solutions and log concentrations (U/mL) was used to determine SOD activity in our specimens.

Statistical analysis. Data are presented as mean ± standard error of the mean (SEM). The normal distribution of data was tested by Kolmogorov-Smirnov-Test. In the case of normal distribution the results obtained for groups of semen samples of different treatment were compared using One Way Repeated Measures Analysis of Variance. If the data were not normally distributed, values were compared with Friedman repeated measures analysis of variance on ranks. When a significant difference between or within the groups was revealed, values were compared by the Tukey test or the Holm-Sidak method. Differences between sperm treatments (dialyzed and non-dialyzed semen) were compared using a paired Student's *t*-test for normal distribution. If the data were not

normally distributed the Wilcoxon test was preformed. Differences with values of $P<0.05$ were considered to be statistically significant. Correlations between TAC, SOD and TBARS in semen samples of different treatments were calculated using the Spearman rank correlation coefficient. SigmaStat 3.5 (SYSTAT Software Inc.) software was used for all tests.

Results

A significant decrease in sperm motility was observed after 3 days of storage in comparison to native samples ($P<0.05$), but there was no significant effect ($P>0.05$) of dialysis on motility. The progressive motility of non-dialysed and dialysed semen decreased after one day of storage in comparison to native samples ($P<0.05$) and remained at the same level until the 3rd day of storage. There was no significant difference between non-dialysed and dialysed samples on each day of semen storage according to both parameters mentioned above ($P>0.05$). Progressive motility on the 2nd and 3rd days of analysis was higher when semen was dialysed before storage (Table 1), but the differences in comparison to non-dialysed samples did not reach the limit of significance ($P = 0.064$ and $P = 0.057$, respectively).

Morphological changes in spermatozoa increased during storage and reached a significantly higher level in comparison to native samples after 3 days of storage ($P<0.05$), whereas the percentage of acrosomal damage already reached a significant difference from native samples on the second day of storage ($P<0.05$). However, dialysis yielded a significantly lower percentage of spermatozoa with abnormal morphology and acrosomal damage ($P<0.05$) in semen after 3 days of storage (Table 2).

The level of capacitated spermatozoa increased in non-dialysed semen from day 0 to day 3 ($P<0.05$). In dialysed semen, the percentage of capacitated spermatozoa increased from day 0 to day 2 but remained the same until the 3rd day of storage.

The percentage of acrosomal reacted spermatozoa in dialysed and non-dialysed semen increased during the liquid storage; a significant difference was observed after 2 days of storage ($P<0.05$), whereas the increase from day 2 to day 3 was not significant ($P>0.05$). Dialysis moderated the increasing level of acrosomal reacted spermatozoa, showing a significantly lower percentage of acrosomal reacted spermatozoa in dialysed versus non-dialysed semen according to each day of storage ($P<0.05$). A difference between dialysed and non-dialysed semen according to non-capacitated and capacitated spermatozoa was observed only after 3 days of storage ($P<0.05$) (Table 2).

TAC and concentration of TBARS in non-dialysed samples remained on the same level throughout the experiment ($P>0.05$) (Fig. 1 and 2). Dialyses resulted in a significant decrease in TAC and increase in TBARS ($P<0.05$); a significant difference was observed between native and dialysed samples after one day of storage, but these parameters

remained unchanged from the 1st day on (P>0.05). A significant positive correlation was observed between non-dialysed and dialysed semen samples according to TAC and TBARS on each day of storage (P<0.05). TAC and TBARS were strongly negatively correlated in dialysed and non-dialysed samples on the 3rd day of storage (P<0.05).

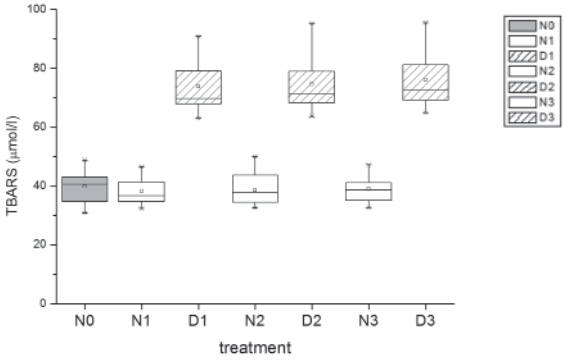

Fig. 1. Effect of dialysis of boar semen on the level of lipid peroxidation in seminal plasma. NO: native semen; N1, N2, N3: non-dialysed semen after 1, 2 and 3 days of liquid storage, respectively; D1, D2, D3: dialysed semen after 1, 2 and 3 days of liquid storage, respectively

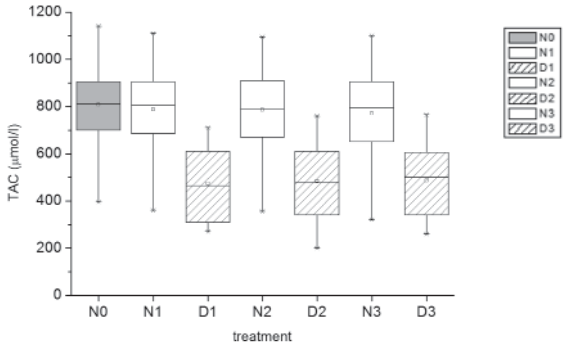

Fig. 2. Effect of dialysis of boar semen on total antioxidant capacity (TAC) in seminal plasma NO: native semen; N1, N2, N3: non-dialysed semen after 1, 2 and 3 days of liquid storage, respectively; D1, D2, D3: dialysed semen after 1, 2 and 3 days of liquid storage, respectively

A significant increase in the level of SOD was observed in non-dialysed and dialysed semen samples on the first day of storage (P<0.05). SOD increased further with storage time, but the only significant increase was observed in non-dialysed samples on the third

day of storage compared to SOD activity on day one (P<0.05). Non-dialysed samples showed a significantly higher level of SOD activity than dialysed ones according to all days of storage (P<0.05) (Fig. 3). A significant positive correlation was observed between non-dialysed and dialysed semen samples according to SOD on each day of storage (P<0.05). TAC and SOD in non-dialysed and dialysed samples on each day of storage were correlated with the TAC and SOD levels in native samples (P<0.05), as well as TBARS, with the exception of day 3 for both samples.

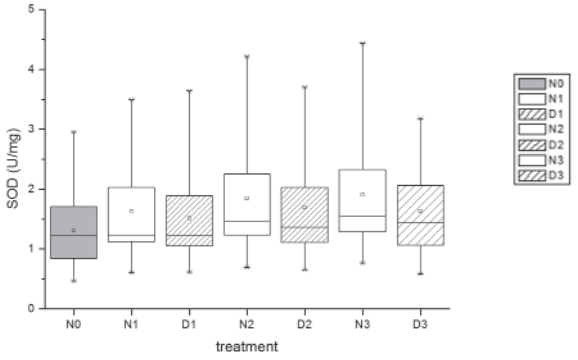

Fig. 3. Effect of dialysis of boar semen on the level of SOD in seminal plasma. NO: native semen; N1, N2, N3: non-dialysed semen after 1, 2 and 3 days of liquid storage, respectively; D1, D2, D3: dialysed semen after 1, 2 and 3 days of liquid storage, respectively

Discussion

The present study characterizes the effects of dialysis on motility and semen morphology, capacitation status, occurrence of peroxidative changes, SOD activity, and antioxidative status in sperm during liquid preservation in dialyzed boar semen.

Liquid storage of boar semen decreases sperm viability, motility and progressive motility due to the susceptibility of sperm cells to thermal, mechanical and osmotic stress during cooling (CEROLINI et al., 2000). In our study, dialysis improved progressive motility after 3 days of storage when compared to non-dialyzed semen, although the difference was a little above the limit of significance (P = 0.057). Previously it has been shown that removing LMWC from boar seminal plasma significantly improves motility and the progressive motility of liquid stored semen (MRKUN, 2009) as well as post-thaw sperm motility (FRASER et al., 2007).

In our study, dialysis decreased the negative effect that storage has on sperm morphology; namely, after dialysis the percentage of spermatozoa with abnormal morphology and acrosomal damage decreased significantly compared to non-dialysed semen, which is in agreement with a previous study (MRKUN et al., 2009). Removal of

LMWC from boar seminal plasma prior to freezing-thawing also significantly improved plasma integrity and mitochondrial function (FRASER et al., 2007). At present, the mechanism by which LMWC may cause damage to spermatozoa is not completely understood. It has been suggested that low-molecular weight components may exert their damaging effect by binding to the sperm membrane, and dialysis induces a redistribution of the sperm surface proteins, resulting in the formation of protective sperm-coating layers that maintain plasma membrane integrity. This phenomenon might be associated with the increased resistance of dialyzed spermatozoa during semen preservation (METZ et al., 1990).

In a recent study the percentage of spontaneously reacted spermatozoa and damaged spermatozoa increased during storage (VALLORANI et al., 2010). The same trend was observed in our study, where the percentage of acrosomal reacted spermatozoa in dialysed and non-dialysed semen increased during liquid storage. However, dialysis significantly lowered the level of acrosomal reacted spermatozoa in comparison to non-dialyzed semen, thereby improving the quality of stored semen. On the other hand, dialysis had no significant effect on the recovery of the spermatozoa intact acrosomes after freezing-thawing (FRASER et al., 2007). Removal of LMWC, that can trigger capacitation, could be the cause of the observed decrease in acrosomal reaction. Also, it has been proposed, dialysis participates in sperm surface protein rearrangement and forms a protective layer (FRASER et al., 2007), which can lead to a lower level of acrosomal reacted spermatozoa.

Dialysis has been found to be an additional step in liquid (MRKUN et al., 2009) and frozen semen preservation (FRASER et al., 2007) providing a decrease in semen capacitation and morphology changes. However, LMWCs also contain thiol groups, such as L-glutathione (GSH) and L-ergothioneine (ERT), and constitute, together with antioxidant enzymes, the antioxidant defence system of boar seminal plasma. They contribute to the chain-breaking antioxidant capacity and have an important role in protecting boar spermatozoa against oxidative damage (STRZEZEK 2002). Therefore, an aim of this study was also to study the effect of dialysis on TAC, TBARS and SOD . Removal of LMWC from seminal plasma by dialysis (molecular weight cut-off of 12-14 kDa) significantly decreased the level of TAC and increased the level of TBARS, which might have a detrimental effect on boar semen, because the higher antioxidant capacity of boar seminal plasma improves freezability and reduces cryo- induced sperm DNA damage (FRASER et al., 2007). In contrast, in a recent study (KOZIOROWSKA-GILUN et al., 2011), the authors concluded that dialysis enhanced the total antioxidant capacity of the seminal plasma, despite the fact that they reported comparable levels of total antioxidant status in dialyzed and non-dialyzed semen. Nevertheless, the obvious decline in TAC after dialysis and the strong negative correlation between TAC and TBARS levels found in our study suggest that removal of low molecular weight non-enzymatic antioxidant

factors (GSH; ERT, L-ascorbate, α-tocopherol) could be responsible for the decrease in total antioxidant status and antiperoxidant activity of the seminal plasma, leading to increased lipid peroxidation. Thus, changes in the total antioxidant defence mechanisms of the seminal plasma (deficiency of low molecular weight antioxidants) affect the overall protection of the spermatozoa from oxidative damage.

Liquid preservation of boar semen induces lipid peroxidation (KUMARESAN et al., 2009), which was also confirmed in our study with increased levels of TBARS. The results of increased SOD activity and TBARS in non-dialysed and dialysed semen on each day of storage are in agreement with the study by STRZEZEK et al. (2004), showing high TBARS production and enhanced SOD activity in boar seminal plasma, in association with increased membrane fluidity. Despite the very low antioxidant capacity of boar spermatozoa (SANOCKA et al., 2004), boar seminal plasma possesses a wide variety of antioxidants with SOD demonstrating the highest activity (STRZEZEK et al., 2002). SOD, derived mainly from the prostate gland (KOZIOROWSKA-GILUN et al., 2011), is the principal scavenging enzyme that plays a major role in protecting tissue and sperm cells against ROS-mediated attack. Therefore higher SOD activity during storage might indicate that SOD makes a significant contribution to the protection of boar spermatozoa against peroxidative damage. However, it has been proposed that the presence of high SOD activity in boar semen compensates for the lack of low molecular weight antioxidants (KOWALOWKA et al., 2008). In our study, SOD activity increased in all samples during storage, but it was significantly higher in non-dialysed samples in comparison to dialysed ones, whereas in the study by KOZIOROWSKA-GILUN et al. (2011) SOD and GP significantly increased after dialysis. These contradictory results are difficult to explain and further research is needed for a better understanding of the effects of dialysis on SOD in boar semen.

Variations of TBARS levels, SOD and TAC activity between dialysed and non-dialysed semen samples in our study suggest that higher SOD activity, together with TAC, is effective in exerting some compensatory protection against the detrimental effect of liquid storage on boar semen, while in dialysed semen, because of the removal of LMWC antioxidants, TAC was not able to minimize oxidative injures leading to an increased level of TBARS. Since we found higher content of lipid peroxidation products (TBARS) in dialysed semen we suggest that removal of non-enzymatic antioxidants makes boar spermatozoa more prone to oxidative stress.

Removal of LMWC in our study caused a higher level of oxidative stress in seminal plasma. Due to the well-known negative effects of ROS on DNA and sperm-oocyte fusion, the effect of dialysis on DNA damage and ways to modify these negative effects by, for example, adding antioxidants to dialyzed semen, should be investigated in the future.

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References

AITKEN, R. J., J. S. CLARKSON, S. FISHEL (1989): Generation of reactive oxygen species, lipid peroxidation and human sperm function. Biol. Reprod. 41, 183-197.

AITKEN, R. J. (1995): Free radicals, lipid peroxidation and sperm function. Reprod. Fertil. Dev. 7, 659-668.

CEROLINI, S., A. MALDJIAN, P. SURAI, R. NOBLE (2000): Viability, susceptibility to peroxidation and fatty acid composition of boar semen during liquid storage. Anim. Reprod. Sci. 58, 99-111.

CHATTERJEE, S., C. GAGNON (2001): Production of reactive oxygen species by spermatozoa undergoing cooling, freezing and thawing. Mol. Reprod. Dev. 59, 451-458.

FORD, W. C. (2004): Regulation of sperm functions by reactive oxygen species. Hum. Reprod. Update 10, 387-399.

FRASER, L., A. DZIEKONSKA, R. SRTZEZEK, J. STRZEZEK (2007): Dialysis of boar semen prior to freezing-thawing: Its effects on post-thaw sperm characteristics. Theriogenology 67, 994-1003.

ISHII, T., S. MATSUKI, Y. IUCHI, F. OKADA, S. TOYOSAKI, Y. TOMITA, Y. IKEDA, J. FUJII (2005): Accelerated impairment of spermatogenic cells in SOD1 knockout mice under heat stress. Free. Radical. Res. 39, 697-705.

JOHNSON, L. A., K. F. WEITZ, P. FISER, W. M. C. MAXWELL (2000): Storage of boar semen. Anim. Reprod. Sci. 62, 143-172.

KOWALOWKA, M., P. WYSOCKI, L. FRASER, J. STRZEZEK (2008): Extracellular superoxide dismutase of boar seminal plasma. Reprod. Dom. Anim. 43, 490-496.

KOZIOROWSKA-GILUN, M., M. KOZIOROWSKI, L. FRASER, J. STRZEZEK (2011): Antioxidant defence system of boar cauda epididymial spermatozoa and reproductive tract fluids. Reprod. Domest. Anim. 46, 527-533.

KUMARESAN, A., G. KADIRVEL, K. M. BUJARBARUAH, R. K. BARDOLOI, A. DAS, S. KUMAR, S. NASKAR (2009): Preservation of boar semen at 18 °C induces lipid peroxidation and apoptosis like changes in spermatozoa. Anim. Reprod. Sci. 110, 162-171.

McCORD, J. M., I. FRIDOVICH (1969): The utility of superoxide dismutase in studying free radical reactions. J. Biol. Chem. 244, 6056-6063.

METZ, K. W., T. BERGER, E. D. CLEGG (1990): Adsorption of seminal plasma proteins by boar spermatozoa. Theriogenology 34, 691-700.

MILLER, N. J., C. A. RICE-EVANS, M. J. DAVIES, V. GOPINATHAN, A. MILNER (1993): A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin. Sci. 84, 407-412.

MRKUN, J., K. POTOČNIK, M. KOSEC, P. ZRIMŠEK (2009): Improvement of biotechnological process for conservation of liquid-stored boar semen using dialysis prior to cooling. Acta. Vet. Bulgaria 62, 849-854.

MRUK, D. D., B. SILVESTRINI, M. Y. MO, C. Y. CHENG (2002): Antioxidant superoxide dismutase - A review: Its function, regulation in the testis, and role in male fertility. Contraception 65, 305-311.

SANOCKA, D., M. KURPISZ (2004): Reactive oxygen species and sperm cells. Reprod. Biol. Endocrinol. 2, 1-7.

STRZEZEK, J., W. KORDAN, H. KOSTYRA, A. ZABORNIAK (1992): Purification and partial characterization of a 5700 Da sperm motility inhibitor factor from seminal plasma of boar. Anim. Reprod. Sci. 29, 35-42.

STRZEZEK, J., L. FRASER, W. DEMIANOWITCZ, W. KORDAN, P. WYSOCKI, D. HOLODY (2000): Effect of depletion tests (DT) on the composition of boar semen. Theriogenology 54, 949-963.

STRZEZEK, J., F. SAIZ-CIDONCHA, P. WYSOCKI, A. TYSZKIEWICZ, M. JASTRZEBSKI (2002): Seminal plasma proteins as markers of biological value of boar semen. Anim. Sci. Pap. Rep. 20, 255-266.

STRZEZEK, J., L. FRASER, M. KUKLINSKA, A. DZIEKONSKA, M. LECEWICZ (2004): Effects of dietary supplementation with polyunsaturated fatty acids and antioxidants on biochemical characteristics of boar semen. Reprod. Biol. 4, 271-287.

VADNAIS, M. L., R. KIRKWOOD, D. SPRECHER, K. CHOU (2005): Effects of cooling and seminal plasma on the capacitation status of fresh boar sperm as determined using chloreteracycline assay. Anim. Reprod. Sci. 87, 121-132.

VALLORANI, C., M. SPINACI, D. BUCCI, C. TAMANINI, G. GALEATI (2010): Effect of antioxidants on boar spermatozoa during sorting and storage. Animal. Reprod. Sci. 122, 58-65.

WABERSKI, D., K. F. WEITZE, T. GLEUMES, M. SCHWARZ, T. WILLMEN, R. PETZOLDT (1994): Effect of time of insemination relative to ovulation on fertility with liquid and frozen boar semen. Theriogenology 42, 831-840.

WABERSKI, D., J. A. GUILEN-SOAREZ, E. BANDEIRA DE ARRUDA, K. F. WEITZE (1996): Effect of a transcervical infusion of seminal plasma on the fertilizing competence of low numbers of boar spermatozoa under controlled AI-ovulation intervals. Anim. Reprod. Sci. 44, 165-173.

ZRIMŠEK, P., J. KUNC, M. KOSEC, J. MRKUN (2004): Spectrophotometric application of resazurin reduction assay to evaluate boar semen quality. Int. J. Androl. 27, 57-62.

ZRIMŠEK, P., M. KOSEC, J. KUNC, J. MRKUN (2006): Determination of the diagnostic value of the resazurin reduction assay for evaluating boar semen by receiver operating characteristic analysis. Asian J. Androl. 8, 343-348.

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SAŽETAK

Cilj istraživanja bio je procijeniti učinak dijalize na peroksidaciju lipida i antioksidacijski status nerastova sjemena pohranjena u tekućem stanju. Sedamnaest ejakulata od osam nerastova bilo je podijeljeno u dvije skupine. Jedna skupina bila je podvrgnuta dijalizi, a druga je bila kontrolna. Sjeme je nakon toga bilo pohranjeno na 16 do 17 °C tijekom tri dana. Izmjereni su pokazatelji kvalitete sjemena: kapacitiranje spermija, reaktivne tvari tiobarbiturne kiseline, aktivnost superoksidne dismutaze i ukupna antioksidacijska sposobnost dijaliziranog i nedijaliziranog sjemena. Procijenjen je učinak peroksidacije lipida i antioksidacijski status na kvalitetu sjemena. Uklanjanje sastojaka male molekulske mase dijalizom iz sjemene plazme dovelo je do značajno povećanog postotka morfološki normalnih spermija ($P = 0,002$). Dijaliza je utjecala na razinu akrosomski reaktivnih spermija pokazujući njihovu značajno manju razinu reaktivnosti u dijaliziranim uzorcima po svakom danu pohrane ($P<0,05$). Aktivnost superoksidne dismutaze povećavala se s vremenom pohrane u obje skupine, ali su kontrolni uzorci pokazivali značajno veću aktivnost nego dijalizirani ($P<0,05$). Dijaliza je značajno smanjila antioksidacijsku sposobnost, a povećala reaktivne tvari tiobarbiturne kiseline ($P<0,05$). Rezultati pokazuju da povećane razine aktivnosti superoksidne dismutaze u dijaliziranom sjemenu ne mogu uravnotežiti smanjenu razinu antioksidacijske sposobnosti i spriječiti oksidativna oštećenja membrane spermija.

Ključne riječi: sjeme, nerast, dijaliza, antioksidacijski status, peroksidacija lipida