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THE RELATIONSHIP BETWEEN SEMINAL PLASMA ASPARTATE AMINOTRANSFERASE ACTIVITY, SPERM OSMOTIC RESISTANCE TEST VALUE, AND SEMEN QUALITY IN BOARS

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The relationship between the activity of aspartate aminotransferase (AspAT) in seminal plasma and the values of the osmotic resistance test (ORT) of acrosomal membranes and semen traits was examined on 120 young hybrid Pietrain and Duroc boars. The following semen quality traits were determined: the volume of the ejaculate, the percentage of spermatozoa with progressive motility, sperm concentration and the total number of spermatozoa in the ejaculate, percentage of spermatozoa with normal acrosome, the percentage of spermatozoa with major and minor morphological defects, ORT, and the activity of AspAT in seminal plasma.

The activity of AspAT in seminal plasma was negatively correlated ($p \leq 0.01$) with the spermatozoa concentration and total number per ejaculate, percentage of spermatozoa with progressive motility and percentage of spermatozoa with a normal acrosome, while positively with the percentage of spermatozoa with major ($p \leq 0.001$) and minor ($p \leq 0.01$) morphological defects. The ORT values negatively correlated with the percentage of spermatozoa with major ($p \leq 0.05$) and minor ($p \leq 0.01$) morphological defects, while positively ($p \leq 0.001$) with the percentage of spermatozoa with a normal acrosome.

Key words: AspAT, boars, ORT, semen quality

INTRODUCTION

Prediction of boar sperm fertilizing ability is of great economic importance in swine breeding. Commonly, it is projected on the basis of the in-vitro evaluation of semen quality. Sperm fertilizing capacity is closely bound to its metabolic function (Rigau *et al*., 1996). The cell membrane plays an important role in both sperm metabolism and capacitation. Therefore, the sperm osmotic resistance test (ORT), which allows the determination of the sensitivity of the sperm acrosomal membranes to osmotic pressure changes, is used to assess the quality of semen. Incubation of fresh semen under different osmotic pressures reduces the percentage of sperm with a normal acrosome. This shows a reduction of the

osmotic resistance in the acrosomal membranes, and thus deterioration of the quality of semen (Schilling and Vengust, 1987). The ORT grades the spermatozoa depending on the functional state of the cell membrane. Scarce literature data have shown a positive relationship between the ORT and fertility of boars (Schilling, 1989; Udala *et al*., 1996) and some semen traits (Strzezek *et al*., 1992; Rigau *et al*., 1996).

Another parameter related to the cell membrane, especially its integrity, is the activity of aspartate aminotransferase (AspAT) in the seminal plasma. AspAT is an intracellular enzyme located mainly in the mid-piece of the sperm cell (Bronicka and Dembinski, 1999). The level of enzyme activity in the seminal plasma reflects the degree of spermatozoa damage or–more specifically–to the mid-piece (Larson *et al*., 1996; Strzezek, 1996). The release of AspAT from the sperm to the seminal plasma is associated with increased permeability of the plasma membrane of the sperm cell, and leads to a decrease in the sperm biological value (Ciereszko *et al*., 1992; Frydrychová *et al*., 2010). A relationship was found between the enzyme activity in seminal plasma and some of the semen quality characteristics (Gaczarzewicz *et al*., 2000; Jacyno *et al*., 2002; Kozdrowski, 2004; Frydrychová *et al*., 2010).

Since some authors demonstrated the relationship between ORT and AspAT activity in seminal plasma and semen quality, in this study we estimated the correlation between the value of these indicators and the value of semen characteristics in boars. The results will determine the usefulness of the indicators for routine evaluation of the quality of semen carried out in insemination facilities.

MATERIAL AND METHODS

Animals and experimental procedures

The studies were carried out at the State Center of Pig Hybridization in Poland on 120 young Piertain and Duroc hybrid boars. Until the age of 35 days, the piglets were kept with their mothers, and after weaning the sows remained in farrowing pens until 70 days of age. During that time the pigs were fed prestarter and grower diets, according to Polish standards of feeding. After routine selection, boars were transferred to individual pens without bedding, being 1x2 m in dimension with hard and slatted flooring (respectively 60:40), where they stayed until the end of the evaluation. During that period of time the animals were fed pellets mixed as specified in Table 1. The daily ration grew with an increase in the body weight of the animals.

Semen collection and analysis

After teaching the boars to mount a phantom, their semen was evaluated at 230, 250, and 270 days of age. Shortly after collection and filtration of the ejaculate, the following characteristics were determined: ejaculate volume, percentage of spermatozoa with progressive motility (subjective method with Nikon microscope), concentration of spermatozoa in 1 mL (cytometric method in Bürker's chamber), and the total number of spermatozoa in the ejaculate.

Table 1. Nutritive value of diet

Specification	In 1 kg diet
Metabolic energy (MJ)	12.7
Crude protein (g)	191
Crude fibre (g)	27
Lysine (g)	10.1
Methionine+cystine (g)	6.4
Threonine (g)	6.7
Tryptophan (g)	2.0
Mineral and vitamin mixture	*

*The mineral and vitamin mixture supplied the following per kg diet: 7700 IU vit. A, 2100 IU vit. D_3, 30 mg vit. E, 1.5 mg vit. K_3, 1.05 mg vit. B_1, 3.6 mg vit.B_2, 2.1 mg vit. B_6, 0.021 mg vit. B_{12}, 15 mg nicotynic acid, 1.05 mg calcium pantothenate, 0.45 mg folic acid, 0.021 mg biotin, 300 mg cholin chloride, 100.5 mg Zn, 30 mg Mn, 21 mg Cu, 75 mg Fe, 0.6 mg J_2, 0.2 mg Se.

Sperm minor and major morphological defects (according to Blom, 1981) and the percentage of spermatozoa with a normal acrosome ridge, NAR (according to Pursel *et al*., 1972), were determined in the preparations stained with eosin and nigrosin. The osmotic resistance test (ORT) of acrosomal membranes was performed according to Schilling and Vengust (1987). To carry out the osmotic resistance test, two samples of semen were collected, 0.2 mL each. One sample was thinned out with 3 mL BTS-Beltsville Thawing Solution (300 mOsm/kg) and incubated for 15 min at 39°C. The other sample was infused with 3 mL BTS and then diluted with distilled water to 150 mOsm/kg and incubated for 120 min at 39°C. After incubation of samples and preparation of stained smears, the percentage of spermatozoa with a normal acrosome ridge (NAR) was determined. The ORT was calculated according to the formula:

ORT = 1/2 [%NAR in 300 mOsm (for 15 minutes) + %NAR in 150 mOsm (for 120 minutes)]

The activity of aspartate aminotransferase (AspAT) in seminal plasma was determined by kinetic method with spectrophotometer Model PRO–Bio, Marcel (reagents Bio Merieux Corp.) AspAT activity was converted as per $1x10^9$ of spermatozoa.

Statistics

Basic statistical characteristics of the results arithmetic means standard ($\overline{X}$), standard error of means (SEM) and correlation coefficients between activity of AspAT and ORT and semen traits including significance (p) were calculated using the Statistica 8.0 PL package.

RESULTS

Table 2. Mean values of semen traits of experimental boars (n=120)

Semen traits	$\bar{X}$	SEM*
Ejaculate volume (cm^3)	171.0	1.92
Concentration of spermatozoa (n x 10^6 x cm^{-3})	192.8	9.56
Total number of spermatozoa (n x 10^9)	28.9	1.69
Spermatozoa with progressive motility (%)	74.9	0.93
Spermatozoa with major defects (%)	7.31	1.34
Spermatozoa with minor defects (%)	6.95	1.08
Spermatozoa with normal acrosome (%)	76.9	1.28
ORT (%)	64.9	1.23
AspAT (mU/10^9 spermatozoa)	73.9	6.56

SEM* – standard error of means

Mean values of semen characteristics given in Table 2 are typical of young males of the species. AspAT activity in seminal plasma reached the level of 73.9 mU/10^9 sperm, and the value of ORT was 64.9%. The studied ejaculates were characterized by a relatively small proportion of sperm with major (7.31%) and minor (6.95%) morphological defects.

Table 3. Correlation coefficients between activity of aspartate aminotransferase in seminal plasma and the values of the osmotic resistance test (ORT) and semen traits

Semen traits	AspAT	ORT
Ejaculate volume	- 0.04	- 0.13
Concentration of spermatozoa	- 0.37**	0.09
Total number of spermatozoa	- 0.28**	- 0.11
Spermatozoa with progressive motility	- 0.31**	0.08
Spermatozoa with major defects	0.41***	- 0.18*
Spermatozoa with minor defects	0.30**	- 0.21**
Spermatozoa with normal acrosome	- 0.19**	0.66***

*$p \leq 0.05$; **$p \leq 0.01$; ** *$p \leq 0.001$

Estimated coefficients (Table 3) showed a significant negative correlation between the activity of AspAT in seminal plasma and semen concentration ($p \leq 0.01$), and between the total number of sperm ($p \leq 0.01$) and the percentage of progressive motile sperm ($p \leq 0.01$). We found that the activity of seminal plasma AspAT is strictly positively correlated with the percentage of spermatozoa with

major ($p \leq 0.001$) and minor ($p \leq 0.01$) morphological defects. However, significant ($p \leq 0.01$) negative correlations were present between the level of seminal plasma AspAT activity and the percentage of sperm with normal acrosome.

It has been shown (Table 3) that there is a significant negative correlation between the ORT values and the percentage of spermatozoa with major ($p \leq 0.05$), and minor ($p \leq 0.01$) morphological defects. Between the ORT values and the percentage of sperm with a normal acrosome, a strict positive correlation occurred ($r = 0.66$, $p \leq 0.001$). A slight (statistically insignificant) relationship was observed between the ORT and the remaining characteristics of semen.

DISCUSSION

The results indicate a high quality of the semen of young boars. Boar semen parameters improve most extensively at the age of 10-14 months (Gregor and Hardge, 1995), whereas in the present study we evaluated 8-9-month-old boars. We found that ejaculate volume and sperm concentration was characteristic of young boars and similar to values obtained for young boars of various breeds in other studies (Kawecka *et al.*, 1997; Kawecka, 2002; Kolodziej and Jacyno, 2005). The percentage of progressive motile sperm and those with morphological defects, AspAT activity in seminal plasma, and the sperm ORT value did not deviate significantly from those found for adult insemination boars (Rigau *et al*., 1996; Udala *et al*., 1996; Kondracki *et al*., 2004; Kozdrowski, 2004; Pokrywka *et al*., 2009; Szostak and Przykaza, 2010).

The small proportion of sperm with morphological defects, found in this study, demonstrates an adequate development of the spermatogenesis and spermiogenesis processes. In other studies (Kawecka *et al*., 1997; Kawecka *et al*., 2000; Jacyno *et al*., 2009), the authors found twice as high the percentage of morphologically altered sperm in ejaculates of young (6 months old) boars.

The estimated correlation coefficients indicate that the permeability of the membranes of the mid-piece and greater AspAT "leakage" into seminal plasma result in a significant deterioration of the quality of semen. The significant negative correlation between the activity of this enzyme and the concentration and the total number of sperm in the ejaculate is not fully reflected by other studies. Studies on hybrids of wild boars and domestic swine found no significant correlation between the activity of seminal plasma AspAT and sperm concentration, but showed a significant ($p \leq 0.05$) negative correlation between the activity of this enzyme and the total number of sperm in the ejaculate and ejaculate volume (Kozdrowski, 2004). Negative significant ($p \leq 0.05$) correlation between AspAT and the volume of the ejaculate of boars of various breeds were also shown by Gaczarzewicz *et al*. (2000). However, the authors showed no significant association of the enzyme activity with sperm concentration. Contrary to the above-cited authors, no association of AspAT with ejaculate volume was observed in the present study.

The level of AspAT activity in seminal plasma indicates the degree of disorders in the mitochondrial system of sperm, which adversely affects the

sperm motion aparatus (Strzezek *et al*., 1987). This has been confirmed in this study, which reveals a strict negative correlation between the activity of this enzyme and the percentage of sperm with progressive motility. Frydrychová *et al*. (2010) also found a close negative correlation ($r=-0.53$; $p\leq0.001$) between AspAT and sperm motility. also Johnson *et al*. (2000) indicate the association between sperm progressive motility and cell membrane integrity.

The estimated correlation coefficients indicate that a more extensive AspAT „leakage" from the sperm cell to the seminal plasma significantly increase the proportion of sperm morphological defects in the ejaculate. Sperm fertilizing ability depends not only on the number of morphologically altered spermatozoa, but also on the nature of the malformations. According to Blom (1981), sperm major morphological defects correlate with male fertility more closely than the minor defects. The ejaculates evaluated in the present study reveal a closer relationship between the activity of AspAT in seminal plasma and the percentage of spermatozoa with major ($r=0.41$, $p\leq0.001$) than minor ($r=0.30$, $p\leq0.01$) morphological defects. We also found, as had been demonstrated by Gaczarzewicz *et al*. (2000), that the higher activity of this enzyme in plasma is accompanied by a significantly reduced percentage of sperm with a normal acrosome. Some authors report a close relationship between the seminal plasma activity of AspAT and in-vivo sperm fertilizing ability (Slaweta, 1981; Glogowski *et al*., 1996).

An important sperm assessment criterion is the morphology of the acrosome. Only spermatozoa with intact acrosomes and intact membranes and are able to fertilize oocytes in vivo (Yanagimachi, 1994; Lechniak *et al.*, 1998). In these studies we found a strong positive correlation between the ORT and the percentage of sperm with a normal acrosome, as well as a significant negative correlation between the ORT and the percentage of spermatozoa with morphological defects. Similar correlations were also shown by Rigau *et al*. (1996). The authors also showed a positive ($p\leq0.01$) correlation between the ORT and the percentage of sperm with progressive motility, which was neither confirmed in this study nor by Udala *et al*. (1996). Some studies have shown a high correlation between the ORT and the performance achieved in reproduction (Schilling *et al.*, 1989; Udala *et al*., 1996; Kaka *et al*., 2012).

SUMMARY

The correlation coefficients estimated in this study indicate a close relationship between both the activity of AspAT in seminal plasma and the sperm ORT value and the quality characteristics of fresh boar semen. This suggests the usefulness of these relatively readily and easily obtained indicators for routine evaluation of semen quality and for obtaining objective results in the insemination practice.

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ODNOS KVALITETA SEMENA NERASTOVA, AKTIVNOSTI SEMINALNE ASPARTAT TRANSFERAZE I REZULTATA TESTA OSMOTSKE REZISTENCIJE

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SADRŽAJ

U ovom radu su prikazani rezultati ispitivanja odnosa aktivnosti aspartat aminotransferaze (AspAT) u semenoj plazmi i vrednosti osmotskog testa rezistencije (ORT) membrane akrozoma i karakteristika ejakulata 120 mladih nerastova, meleza pijetrena i duroka. Određivani su sledeći parametri: zapremina ejakulata, procenat progresivno pokretnih spermatozoida, koncentracija i ukupan broj spermatozoida u ejakulatu, procenat spermatozoida sa normalnim akrozomom, procenat spermatozoida sa manjim i većim morfološkim oštećenjima, ORT vrednost i aktivnost AspAT u semenoj plazmi.

Aktivnost AspAT u semenoj plazmi je bila u negativnoj korelaciji ($p \leq 0,01$) sa koncentracijom spermatozoida i njihovim ukupnim brojem po ejakulatu, procentom progresivnih spermatozoida i procentom spermatozoida sa normalnim akrozomima. Ona je istovremeno bila u pozitivnoj korelaciji sa procentom spermatozoida sa većim ($p \leq 0.001$) i manjim ($p \leq 0.01$) morfološkim oštećenjima. Vrednosti ORT su bile u negativnoj korelaciji sa procentom spermatozoida sa većim ($p \leq 0.05$) i manjim ($p \leq 0.01$) morfološkim oštećenjima, a u pozitivnoj ($p \leq 0.001$) sa procentom spermatozoida sa normalnim akrozomima.