THE RELATIONSHIP BETWEEN ENZYMATIC ACTIVITY OF ASPARTATE AMINOTRANSFERASE AND SEMEN QUALITY PARAMETERS IN BOARS

Frydrychová S., Lustyková A., Lipenský J. and Rozkot M.

Institute of Animal Science, Praha Uhříněves, Czech Republic

Abstract

The objective of this study was to examine the relationship between the enzymatic activity of aspartate aminotransferase (AST) in seminal plasma and in spermatozoa to semen quality parameters in boars. Semen volume, sperm motility, sperm concentration, percentage of morphologically abnormal spermatozoa (MAS), total number of spermatozoa per ejaculate and the AST activity in supernatants and in spermatozoa was assessed in 102 collected samples of semen fertile AI boars. AST activity in supernatant correlated negatively with sperm motility ($P<0.05$) and positively with MAS, sperm concentration ($P<0.05$) and semen volume. AST activity in spermatozoa positively correlated with sperm motility and sperm concentration and negatively with MAS and semen volume ($P<0.05$). Significant differences were recorded for sperm motility, total number of spermatozoa, AST in supernatant and in spermatozoa between compared groups with low and high occurrence MAS ($P<0.05$).

In conclusion, our results demonstrate significant interaction of the enzymatic activity of aspartate aminotransferase (AST) and semen quality parameters in boars in particular to rate occurrence MAS and sperm motility.

Key Words: Boar, aspartate aminotransferase, semen quality

The quality of semen is extremely important while using hybrid boars for insemination, where the amount of insemination doses are very important, as well as fertilization of the highest possible number of ovulating egg cells, which depends on the good quality of the semen (Strzezek, 1998). The cell membrane plays an important role in both sperm metabolism and capacitation. Aspartate aminotransferase (AST) is an intracellular enzyme related with sperm membrane integrity and it is a determinant of cellular damage spermatozoa (Larson et al. 1996). The release of AST from the spermatozoa to the seminal plasma is associated with increased permeability of the plasma membrane and its leads to a decrease in the sperm biological value (Ciereszko et al., 1992).

The objective of this study was to examine the relationship between the enzymatic activity of aspartate aminotransferase (AST) in seminal plasma and in spermatozoa to semen quality parameters in boars.

Material and Methods

A total of 102 ejaculates (sperm-rich fraction) from hybrid AI boars aged 1 to 3 years were collected with using the gloved-hand technique. Ejaculates with motility $\geq$ 70% were used for this study. The following parameters were evaluated in fresh native boar semen: semen volume, sperm motility, sperm concentration, percentage of morphologically abnormal spermatozoa (MAS), total number of abnormal spermatozoa per ejaculate, enzymatic activity of AST in supernatant and in spermatozoa. The volume of the sperm-rich fraction of the ejaculate was determined using a graduated cylinder. The sperm motility was subjectively assessed using phase contrast microscopy with a heating stage (38°C) at 200× magnification. Each sample was examined for three different microscopic fields and motility was expressed as percentage of sperm showing normal forward progressive movement. The sperm concentration was determined by a cytometric method using Bürker's chamber. Morphologically abnormal spermatozoa were assessed according to the staining method of Čeřovský (1976) and evaluated microscopically under oil immersion and 1 500× magnification. The following categories of abnormalities were determined per 200 evaluated spermatozoa: head defects (pyriform, rounded head, narrow at the base etc.), proximal protoplasmic droplet, distal protoplasmic droplet, tail defects (folded tail, bent tail, coiled tail etc.), acrosome defects (loosen and swollen) and the others (degenerative spermatozoa). The AST activity was measured with a BIOLATEST kit (Lachema, Brno, Czech Republic) and with an ENCORE spectrophotometer and calculated per 10^9 spermatozoa. A 2ml sample of native semen was centrifuged at 1 700 rpm for 10min and the supernatant was used to assess the AST activity.

The rest of sperm in a test tube was added 2ml of distilled water and frozen (-22°C). After thawing, the sample was centrifuged at 1 700 rpm for 10min and the AST activity in spermatozoa was determined.

Basic statistical characteristics of the results arithmetic means, standard deviation, correlation coefficient and significance (P) were calculated by the QC Expert program (TriloByte Statistical Software s.r.o., Pardubice, Czech Republic).

The semen samples were divided into groups where group A had ≤25% MAS and group B >25% MAS. Percentage MAS ≤25% is required for AI in the Czech Republic. Statistical significance was determined using the unpaired t-test at level of ($P<0.05$).

Results and Discussion

The initial parameters of boar semen quality in this study are presented in Table 1. In fresh semen was assessed the mean values AST 104.30±41.02 mU/10^9 in supernatant and 161.56±38.30 mU/10^9 in spermatozoa. The proportions of the various MAS are represented in Table 1 also. Increased incidence proportion defect on spermatozoa was represented proximal protoplasmic droplet, tail defects and distal protoplasmic droplet. These morphologically abnormalities are the most common defects incident in spermatozoa (Čeřovský et al. 2005; Kawecka et al., 2008; Lipenský et al. 2010).

Table 1. The mean values of boar semen quality parameters (n=102)

Item	x±SD
Semen volume (ml)	246.42±101.23
Sperm motility (%)	77.16±5.74
Sperm concentration (10^3/mm^3)	409.70±152.91
Total number of spermatozoa (×10^9)	95.71±40.02
AST in supernatant (mU/10^9 spermatozoa)	104.30±41.02
AST in spermatozoa (mU/10^9 spermatozoa)	161.56±38.30
Morphologically abnormal spermatozoa - total (%)	17.79±14.65
Head defects	1.11±0.20
Proximal protoplasmic droplet	3.72±4.81
Tail defects	4.77±7.12
Distal protoplasmic droplet	6.74±9.06
Acrosome defects	1.09±1.87
Other abnormalities	0.43±1.00

Table 2. Correlation coefficients between boar semen quality and AST

	AST in supernatant	AST in spermatozoa
Semen volume	0.05	-0.21*
Sperm motility	-0.37*	0.38*
Sperm concentration	0.24*	0.22*
Morphologically abnormal spermatozoa	0.76*	-0.53*
Head defects	0.15	-0.18*
Proximal protoplasmic droplet	0.37*	-0.34*
Tail defects	0.56*	-0.28*
Distal protoplasmic droplet	0.55*	-0.46*
Acrosome defects	0.004	-0.19
Other abnormalities	0.08	-0.22
AST in spermatozoa	-0.37*	-

* $P<0.05$

Table 2 shows the correlation between boar semen quality and AST activity in supernatant and in spermatozoa. AST activity in supernatant correlated negatively with sperm motility (P<0.05) and positively with MAS, sperm concentration (P<0.05) and semen volume. AST activity in spermatozoa positively correlated with sperm motility and sperm concentration and negatively with MAS and semen volume (P<0.05). Leakage of this enzyme into external environment decreased the sperm quality parameters. Kozodrowski (2004) and Gaczarzewicz et al. (2000) also found significant negative correlation between AST and semen volume (P<0.05). Contrary to Jacyno et al. (2013) and Kawecka et al. (2008) noted significant negative correlation between AST and sperm concentration (P<0.05) and negative correlation between AST and semen volume (P<0.05). Gaczarzewicz et al. (2010) and Kawecka et al. (2008) determined negative correlation between AST and sperm motility (P<0.05). The estimated correlation coefficients indicate that permeability of the membranes and leakage AST into seminal plasma had result in significant deterioration of semen quality.

In Table 2. also presented relation AST enzyme to monitored morphological abnormalities of spermatozoa. The highest significant positive correlations (P<0.05) were between AST in supernatant and proximal protoplasmic droplet (r=0.37), tail defects (r=0.56), distal protoplasmic droplet (r=0.55). Significant negative correlations (P<0.05) were between AST in spermatozoa and head defects (r=-0.18), proximal protoplasmic droplet (r=-0.34), tail defects (r=-0.28) and distal protoplasmic droplet (r=-0.46). Kawecka et al. (2008) assessed positive correlation between the activity of AST in seminal plasma and different forms of abnormal spermatozoa with the highest value at small abnormal head (r=0.44). Churg et al. (1974) demonstrated occurrence of this AST enzyme in protoplasmic droplet which confirms our observed value of this correlation.

Comparison of mean values of semen quality parameters between group A and B divided according rate of occurrence MAS is summarized in Table 3. Significant differences were recorded for sperm motility, total number of spermatozoa, AST in supernatant and in spermatozoa between compared groups (P<0.05).

Table 3. Comparison of mean values of semen quality parameters between group A and B divided according rate of occurrence MAS (x±SD)

Item	Group	
	A (n=79)	B (n=23)
Semen volume (ml)	240.25±101.85	267.61±98.29
Sperm motility (%)	78.29±5.66^{a}	73.26±4.16^{b}
Sperm concentration (10^3/mm^3)	400.87±151.14	440.02±158.47
Total number of spermatozoa ($\times 10^9$)	91.33±39.76^{a}	110.76±37.96^{b}
AST in supernatant (mU/10^9 spermatozoa)	89.88±29.69^{a}	153.81±35.90^{b}
AST in spermatozoa (mU/10^9 spermatozoa)	170.88±34.33^{a}	129.56±34.19^{b}

Data with different letters (a and b) in the same column indicates significant difference at P<0.05

Conclusion

In conclusion, our results demonstrate significant interaction of the enzymatic activity of aspartate aminotransferase (AST) and semen quality parameters in boars. In this study was found the important relationship between enzymatic activity AST and rate occurrence MAS.

References

STREZEZEK J. (1998): The current of problems insemination of sows - factors to have influenced for its effectiveness. Pig Production and Breeding. Anim. Prod. Rev., App. Sci. Rep., 39, 49-73.

CHURG A., ZANEVELD L.J.D. AND SCHUMACHER G.F.B. (1974): Detergent Treatment of Human and Rabbit Spermatozoa: Ultrastructural Changes and Release of Midpiece Enzymes, Biol. Reprod., 10, 429-437.

CIEREZSKO A., GLOGOWSKI J., STRZEZEK J., DEMIANOWICZ W. (1992): Low stability of aspartate aminotransferase activity in boar semen, Theriogenology, 37, 1269-1281.

ČEŘOVSKÝ J. (1976): Metoda barvení kančích spermií pro morfologické hodnocení, Živočišná Výroba, 21, 361-366.

ČEŘOVSKÝ J., FRYDRYCHOVÁ S., LUSTYKOVÁ A., ROZKOT M. (2005): Changes in boar semen with a high and low level of morphologically abnormal spermatozoa. Czech J. Anim. Sci., 50, 289-299.

GACZARZEWICZ D., PIASECKA M., UDALA J., BLASZCYK B., STANKIEWICZ T. AND LASZCZYNSKA M. (2010): Plasma membrane changes during the liquid storage of boar spermatozoa:a comparison of methods, Acta Vet. Hung., 58, 105-116.

GACZARZEWICZ D., UDALA J., LASOTA B., BLASZCYK B. (2000): Anylysis of the selected parameters of qualitative and biochemical evaluation of the semen of boars, used in AI centre, Anim. Prod. Rev., App. Sci. Rep., Pig Production and Breeding, 48, 93-97.

JACYNO E., KAWECKA M., KOLODZIEJ-SKALSKA A., PIETRUSZKA A., MATYSIAK B., NAPIERALA D. (2013): The relationship between seminal plasma aspartate aminotransferase activity, sperm osmotic resistence test value and semen quality in boars, Acta Vet-Beograd, 63, 397-404.

KAWECKA M., PIETRUSZKA A., JACYNO E., CZARNECKI R., KAMYCZEK M. (2008): Quality of semen of young boars of the breeds Pietrain and Duroc and their reciprocal crosses, Arch. Tierz., 51, 42-54.

KOZDROWSKI R. (2004): Activity of aspartate aminotransferase, alkaline and acid phosphatase of wild boar/domestic pigs hybrids semen in annual cycle, Acta Sci. Pol., Med. Vet., 3, 79-85.

LARSON K., EINARSSON S., NICANDER L. (1996): Influence of thawing diluents on vitality, acrosome morphology ultrastructure and enzyme release on deep frozen boar spermatozoa, Livest. Prod. Sci., 5, 293.

LIPENSKÝ J., LUSTYKOVÁ A., ČEŘOVSKÝ J. (2010): Effect of season on boar semen morphology, J. Cent. Eur. Agr., 11, 465-468.

Corresponding Address:
Ing. Soňa Frydrychová, Ph.D.
Institute of Animal Science Prague
Department of Pig Breeding
Komenského 1239, 51741 Kostelec nad Orlicí
Czech Republic
Email:frydrychova.sona@vuzv.cz

This study was supported by research project QJ1510138.