EFFECT OF SEASON ON BOAR SEMEN QUALITY AND ENZYMATIC ACTIVITY OF ASPARTATE AMINOTRANSFERASE

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Abstract

The objective of this study was to find out the effects of season on semen quality parameters and enzymatic activity of aspartate aminotransferase (AST) in the fertile hybrid AI boars (n=139 ejaculates) in a period of 4 years. 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. Boars were divided into four groups according to season period of the year: winter (1st-3rd month), spring (4th-6th month), summer (7th-9th month) and autumn (10th-12th month). Sperm motility was significantly higher ($P<0.05$) in winter and spring vs. summer and autumn. Percentage of MAS was significantly higher ($P<0.05$) in summer and autumn vs. winter and spring. Sperm concentration was significantly the lowest ($P<0.05$) in autumn vs. winter and spring. Activity of AST in supernatant was significantly the higher in summer and autumn ($P<0.05$) which could be due to lower motility and higher incidence of MAS. The highest AST values in spermatozoa were found in winter and spring ($P<0.05$). In conclusion, results of this study detected effect of season on semen quality parameters particularly sperm motility and MAS and on AST activity in supernatant and in spermatozoa.

Key Words: Boar, aspartate aminotransferase, seasonal effect, semen quality

Seasonality is a considerable factor affecting reproduction in pigs because boars and swine are very sensitive to seasonal changes. Changes in the composition of semen can vary between 25-30% over the year (Colenbrander and Kemp, 1990). Therefore, control of qualitative and quantitative traits of boar semen has great economic importance for pig breeders (Smital, 2010). Rozeboom (2000) noted that determining the initial quality of a boar ejaculate is the first step in semen processing and should ensure that prior to further processing a high quality artificial insemination dose of semen will be produced. Examination of semen characteristics such as sperm progressive motility, concentration, volume and morphology are routine procedures for assessing semen quality in AI centres. There are many other tests evaluating sperm quality as is assessment of the enzymatic activity of aspartate aminotransferase (AST) in seminal plasma that related with the metabolic performance and sperm membrane integrity. AST is an intracellular enzyme that distinguishes as cytoplasmic isoenzyme from spermatozoa (Ciereszko et al., 1992) and as mitochondrial isoenzyme located mainly in the mid-piece of the sperm cell (Bronicka and Dembinski, 1999). The increase in the level of AST enzyme activity in seminal plasma is considered as a determinant of cellular damage (Larson et al., 1996) incurred during liquid storage of semen (Pandey et al., 2001; Frydrychova et al., 2010; Lalrintluanga et al., 2012) or during cryopreservation (Cierezsko et al., 1992; Bielas et al., 2003; Frydrychova et al., 2013). Activity of AST during years between wild boar and domestic pigs studied only Kozdrowski (2004) who *no* found effect of the duration of light period on the activity of AST and alkaline phosphatase. The objective of this study was to find out the effect of season on semen quality parameters and activity of AST.

Material and methods

A total of 129 hybrid AI boars were used for this study. Ejaculates (n=139) from healthy and fertile mature boars were collected with using the gloved-hand technique during 4-year period. The ejaculates were divided into 4 groups according to season period of the year: winter (1st-3rd month), spring (4th-6th month), summer (7th-9th month) and autumn (10th-12th month).

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 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 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 error of mean, and significance) were calculated by the QC Expert program (TriloByte Statistical Software s.r.o., Pardubice, Czech Republic). Statistical significance was determined using analysis of variance ANOVA – *Fisher's* test at significance level of ($P<0.05$).

Results and Discussion

The mean values of semen quality parameters and AST activity in supernatant and in spermatozoa during season are presented in Table 1. Activity of AST in supernatant was significantly the higher in summer 124.30±7.52 mU/10^9 spermatozoa and autumn 129.95±8.28 mU/10^9 spermatozoa ($P<0.05$) contrary to the AST in spermatozoa where the highest values were found in winter 154.95±5.50 mU/10^9 spermatozoa and spring 157.97±10.21 mU/10^9 spermatozoa ($P<0.05$). AST affects sperm fertilizing ability when Kocvin-Podsiadla and Adamska-Jarecka (1984) states that leak AST into seminal plasma affects the chemical composition and metabolic activity of sperm which have an adverse effect on the number of fertilized eggs and embryos survivability.

Table 1. Effect of season on boar semen quality and AST activity (x±SEM)

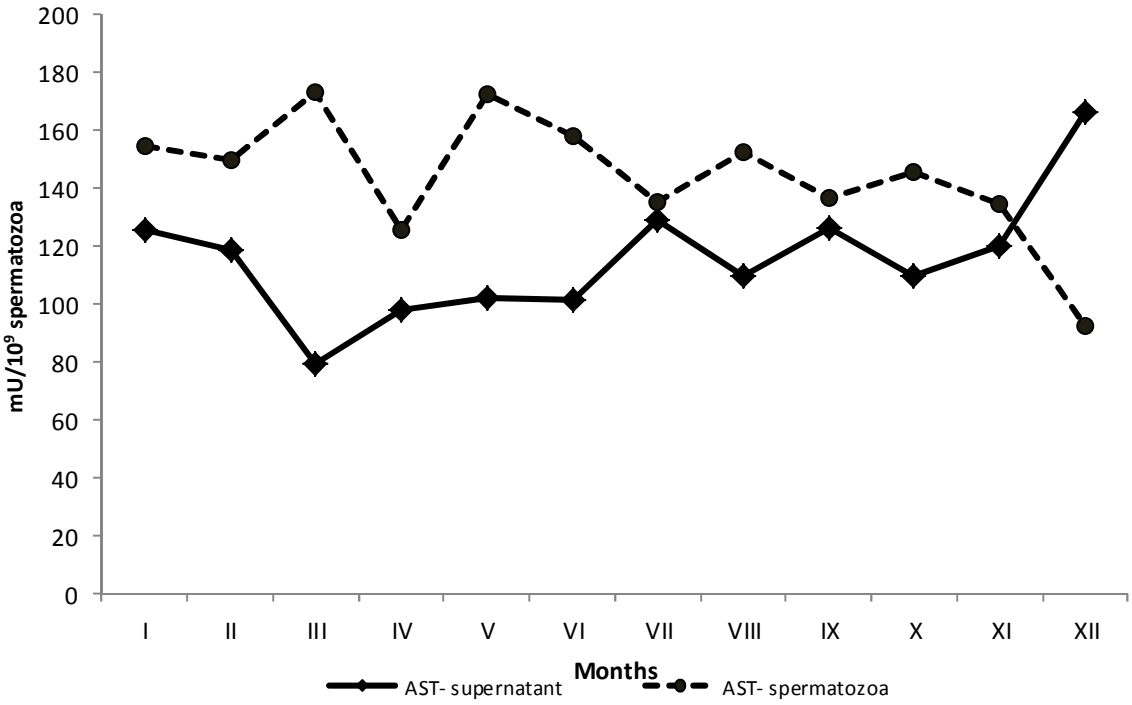
	Months	1st-3rd	4th-6th	7th-9th	10th-12th
Item	n	36	33	34	36
Semen volume (ml)		266.94±23.48	231.55±11.34	253.83±18.25	292.78±17.49
Sperm motility (%)		74.31±1.65^a	76.67±1.37^a	66.61±2.62^b	67.64±2.27^b
Sperm concentration (10^3/mm^3)		401.69±25.86^a	416.11±22.48^a	399.02±27.33^{ab}	339.36±27.39^b
TNS (×10^9)		97.02±6.60	93.30±5.25	100.36±9.96	92.36±6.73
AST in supernatant (mU/10^9 sp.)		113.86±7.56^{ab}	101.10±7.85^a	124.30±7.52^b	129.95±8.28^b
AST in spermatozoa (mU/10^9 sp.)		154.95±5.50^a	157.97±10.21^a	139.18±7.29^{ab}	126.04±7.56^b
MAS (%)		19.42±2.54^a	18.44±3.12^a	23.81±3.48^b	31.99±4.04^b

TNS - Total number of spermatozoa per ejaculate
MAS - Morphologically abnormal spermatozoa
Data with different letters (a and b) in the same column indicates significant difference at $P<0.05$

The percentage of motile sperm, ejaculate volume, sperm concentration and on the basis of these three parameters, laboratory staff assess whether the semen meets the minimum criteria for suitability for AI (Brucka-Jastrzebska et al., 2008). Sperm motility was significantly higher in winter 74.31±1.65% and spring 76.67±1.37% vs. summer 66.61±2.62% and autumn 67.64±2.27% ($P<0.05$). Sperm concentration was significantly the lowest in autumn 339.36±27.39×10^3/mm^3 vs. winter 401.69±25.86×10^3/mm^3 and spring 416.11±22.48×10^3/mm^3 ($P<0.05$). Kunowska-Slósarz and Makowska (2011) also found that the highest concentration of sperm and a high % of live spermatozoa were noticed in ejaculates collected during the late winter and spring time. Different results in sperm concentration found

Bronicka and Dembinski (1999) where ejaculates collected in the autumn has the highest concentration of sperm as a result of restoring the functionality of testis after the summer. The total number of spermatozoa in ejaculate is determining the number of insemination doses, which can be made from one ejaculate (Kondracki et al., 2003). Our result indicated that season did not influence semen volume and the total number of spermatozoa per ejaculate. The some results in semen volume also reported Cheon et al. (2002). The mean values of AST in supernatant and in spermatozoa during year according to months are in Figure 1. In March was assessed the lowest value of AST in supernatant 79.23±12.74 mU/10^9 spermatozoa and the highest value 166.22±13.22 mU/10^9 spermatozoa was noted in December (P<0.05). This is contrary to the findings of Kozodrowski (2004) who reported the lowest value of AST in November (66.27±24.15 mU/10^9) and the highest in July (135 mU/10^9 spermatozoa). The lowest value of AST in spermatozoa was found in December 92.72±14.47 mU/10^9 spermatozoa and the highest was in March 173.41±8.86 mU/10^9 spermatozoa and in May 172.91±18.73 mU/10^9 spermatozoa (P<0.05).

Figure 1. Effect of months on activity of AST during year



Conclusion

In conclusion, our results demonstrate significant interaction of season on semen quality parameters and enzymatic activity of AST. According to results of the boar semen quality in season was the best in winter and in spring particularly sperm motility, sperm concentration, incidence of MAS and AST activity in supernatant as well as in spermatozoa.

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