Relationship between abnormal spermatozoa and seminal plasma free amino acids in boars

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ABSTRACT: The objective of this study was to provide some data on concentrations of seminal plasma free amino acids (FAAs) in order to compare these values with different total contents of morphologically abnormal spermatozoa (MAS) in the semen of 37 experimental adult boars. The boars were kept in the same housing, feeding and breeding conditions. Immediately after collection, semen gel free volume, sperm concentration and total MAS were determined microscopically. The boars were divided into two groups (A – n = 24, B – n = 13) according to the significantly different total MAS content ($\bar{x}$: A = 9.46 ± 4.95, B = 46.00 ± 14.54%, $P < 0.01$). Deproteinized seminal plasma samples (supernatant) were analysed for concentrations of 13 FAAs: Tau, Asp, Thr, Ser, Glu, Gly, Ala, Val, Met, Ile, Leu, Tyr and Lys by liquid chromatography (AAA 339 M analyser). A highly significant correlation was determined between total MAS and total FAA content in all experimental boars (n = 37, r = –0.60, $P < 0.01$). Glutamic acid was a predominant FAA in seminal plasma in both groups of boars ($\bar{x}$: A = 28.49 vs. B = 17.60μM/100 ml) but its concentration was significantly higher in group A ($P < 0.01$). The proportion (%) of glutamic acid concentration in the total content of FAAs in both groups of boars was nearly equal (A = 38.1 vs. B = 38.9%, $P > 0.05$) as well as the proportion index of the individual FAAs calculated from glutamic acid (= 100%). The differences in the particular FAAs recorded between group A and group B were statistically significant for 11 out of the 13 FAAs observed ($P < 0.05$ and $P < 0.01$) in favour of group A as well as in the total calculated content of FAAs per boar ($\bar{x}$: 74.70 vs. 45.23μM/100 ml, $P < 0.01$). A significantly negative relationship between the MAS content and the concentration of seminal plasma FAAs (r = –0.60, $P < 0.01$) is the main result of this study with regard to the markers for potential boar semen fertility estimation.

Keywords: boars; semen; morphologically abnormal spermatozoa; seminal plasma free amino acids

The identification of semen abnormalities is of primary importance from the economic and genetic aspect especially for pig units practising artificial insemination. Visual estimates of the percentage of morphologically abnormal (altered) spermatozoa by light microscopy are the most frequently used and acceptable method for semen quality assessment (Rozeboom, 2000). The sperm of fertile boars generally has less than 10% of morphologically abnormal spermatozoa and the other ejaculates with more than 20–25% must be discarded (Gadea, 2002). Moreover, the persistence of morphologically abnormal spermatozoa (Čeřovský et al., 2005) can be included in inherited traits (Andersson et al., 2002; Corcuera et al., 2002). Čeřovský (1979), Stemmler et al. (1982), Krajňák (1995) and Grandjot (1997) reported an evidently negative significant influence of the higher incidence of morphologically abnormal spermatozoa on pregnancy rate and litter size of inseminated sows. According to the results of Waberski et al. (1990), two criteria are sufficient for the selection of boars or ejaculates for AI: sperm motility and the percentage of morphologically abnormal spermatozoa. In addition to spermatozoa, there is an increasing abundance of evidence that other components in semen participate in physiological processes associated with fertilization (Claus, 1990; Waberski et al., 1997;

Supported by the Ministry of Agriculture of the Czech Republic (Project No. MZe 0002701401).

Ottensmeier, 1998; Maňásková et al., 2000; Flowers, 2001; Strzežek, 2002). The isolation and detailed examination of semen plasma components, particularly protein substances, deserve more experimental attention (Strzežek, 2002).

The research on free amino acid content in boar seminal plasma is not as attractive as in the case of seminal protein studies. The results of free amino acid analysis for the various fractions of seminal plasma were presented for example by Milovanov and Žilcov (1969), Johnson et al. (1972) and Louis et al. (1994). Hypotaurine, glutamic acid, glycine and taurine were determined as predominant free amino acids in boar seminal plasma (Johnson et al., 1972; Louis et al., 1994). The specific roles of these amino acids in seminal plasma have not been fully identified yet. Although the total free amino acid output in seminal plasma was lower for boars on the low protein feed treatment, this seems to be due to the difference in semen volume (Louis et al., 1994).

We did not find any published data that characterize the effect of morphologically abnormal spermatozoa on the free amino acids in boar seminal plasma. Therefore, the objective of our study was to provide data on the relationship between abnormal spermatozoa and seminal plasma free amino acids in boars.

MATERIAL AND METHODS

Thirty-seven ejaculates from 37 AI adult breeding boars older than eighteen months were collected during the first week of November. The boars were kept under the same housing, feeding and breeding conditions. All the boars were penned individually in pens with the partially slatted floor.

The semen was collected by the gloved-hand technique into a sterilized bottle with the opening covered with two layers of sterile cotton gauze to separate the gelatinous fraction from the liquid part of the ejaculate. Immediately after the collection the semen volume (gel free fraction) and the sperm cell concentration were determined. The sperm concentration was determined by means of colorimetric method. The daily output of sperm cells was calculated from the total sperm output per ejaculate and the length of the previous collection interval. The determination of morphologically abnormal spermatozoa (MAS) was carried out microscopically (magnification 1 500×) in each ejaculate on stained smears of the native semen on slides according to Čeřovský (1976).

This basic experimental material was used to assess the relation of the MAS content to the concentration of semen plasma free amino acids (FAAs). For this purpose, moreover, the boars were divided into two groups according to the different MAS content: group A (n = 24) boars with a significantly lower incidence of MAS and group B (n = 13) with a significantly higher proportion of MAS ($P < 0.01$). The seminal plasma samples from all individual boars of this experiment were analysed for 13 FAAs: taurine (Tau), aspartic acid (Asp), threonine (Thr), serine (Ser), glutamic acid (Glu), glycine (Gly), alanine (Ala), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), tyrosine (Tyr) and lysine (Lys). The seminal plasma samples were frozen immediately after their collection and centrifugation (595 g) and stored at –20°C until later analysis.

A 1 ml portion of previously stored seminal plasma from individual boars was deproteinized by adding 200 mg of sulphosalicylic acid and 1 ml of acid buffer solution (8.96 g citric acid +50.02 g lithium citrate +7.02 g lithium chloride per 1 000 ml distilled water) and by centrifuging at 1.610 g for 20 min. The deproteinized solution (supernatant) was filtered and FAA concentrations were determined by liquid chromatography and photometrically by means of ninhydrin detection on an automatic analyser of amino acids (Model AAA 339 M).

On the basis of published information (Johnson et al., 1972; Louis et al., 1994) the following additional correlations were determined between FAAs content and semen volume and sperm concentration.

Basic statistical characteristics of the results, arithmetic mean ($\bar{x}$), standard deviation (sd), correlations (r), significances (P) were obtained using the QC Expert program. Mean values were compared by the unpaired t-test. Significance was declared at $P < 0.05$ and $P < 0.01$ level.

RESULTS AND DISCUSSION

A comparison of the mean data on semen quality parameters in group A and group B is presented in Table 1. There were no significant differences in these parameters with the exception of sperm abnormalities only. The percentage of MAS was sig-

Table 1. Comparison of the experimental groups of boars according to average sperm production values ($\overline{x} \pm sd$)

Group	*n*	Sexual rest (days)	Semen volume (ml)	Sperm concentration (10^3/mm^3)	Total number of spermatozoa per ejaculate ($\times 10^9$)	Daily spermatozoa output ($\times 10^9$)	Morphologically abnormal spermatozoa (%)**
A	24	7.63 ± 1.93	416 ± 119.78	308.75 ± 130.23	119.91 ± 41.90	16.26 ± 5.89	9.46 ± 4.95
B	13	7.15 ± 0.99	465.39 ± 119.99	241.54 ± 105.58	109.17 ± 52.50	15.17 ± 6.18	46.00 ± 14.54

**Differences in the mean values of this parameter between groups are statistically significant ($P < 0.01$)

Table 2. Comparison of the concentrations of semen plasma free amino acids in group A and group B ($\overline{x} \pm sd$)

Group	*n*		Free amino acids (μM/100 ml)													
			Tau**	Asp*	Thr**	Ser	Glu**	Gly**	Ala	Val*	Met**	Ile**	Leu**	Tyr*	Lys**	Total
A	24	$\overline{x}$	5.01	2.56	1.59	1.62	28.49	14.53	3.16	4.02	2.59	2.75	2.33	1.23	4.84	74.70**
		sd	1.72	0.68	0.39	0.55	10.35	6.80	3.48	2.13	1.17	1.20	0.73	0.69	2.41	23.24
B	13	$\overline{x}$	3.20	2.01	1.05	1.65	17.60	8.47	1.64	2.35	1.09	1.49	1.32	0.75	2.60	45.23
		sd	1.24	0.74	0.50	1.50	7.82	5.08	0.77	0.98	0.61	0.79	0.44	0.27	0.79	17.18

*Differences in the mean concentration of this FAA between groups are statistically significant ($P < 0.05$)
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Table 3. Correlations between basic semen characteristics and seminal plasma free amino acids (FAAs)

Semen characteristics	Seminal plasma free amino acids*		
	n	*r*	*P*
Volume	37	–0.27	n.s
Sperm concentration	37	0.44	$P < 0.01$
Morphologically abnormal spermatozoa	37	–0.60	$P < 0.01$

*Total FAAs per each ejaculate

Table 4. Correlations between the seminal plasma free amino acids within group A and group B (*r*)

		Group B													
		Tau	Asp	Thr	Ser	Glu	Gly	Ala	Val	Met	Ile	Leu	Tyr	Lys	
Group A	Tau	–	0.39	0.54	0.22	0.30	0.45	0.59*	0.55	0.37	0.29	0.34	0.25	0.39	Tau
	Asp	0.63**	–	0.46	0.35	0.60*	0.35	0.38	0.70**	0.36	0.46	0.65*	0.39	0.27	Asp
	Thr	0.17	0.02	–	–0.10	0.30	0.07	0.76**	0.54	0.41	0.34	0.62*	0.64*	0.25	Thr
	Ser	0.25	0.31	0.20	–	0.64*	0.70**	–0.05	0.20	0.19	0.29	0.32	–0.43	0.47	Ser
	Glu	0.73**	0.81**	–0.03	0.14	–	0.77**	0.03	0.80**	0.81**	0.87**	0.88**	0.18	0.48	Glu
	Gly	0.61**	0.52**	0.02	–0.03	0.78**	–	–0.11	0.57*	0.59*	0.67*	0.60*	0.06	0.47	Gly
	Ala	–0.11	0.01	0.22	0.01	0.11	0.23	–	0.27	0.07	–0.03	0.29	0.40	0.11	Ala
	Val	0.81**	0.31	0.07	0.13	0.42*	0.42*	–0.32	–	0.85*	0.87**	0.86**	0.52	0.36	Val
	Met	0.00	–0.04	0.19	0.04	0.30	0.32	0.37	–0.17	–	0.97**	0.82**	0.31	0.43	Met
	Ile	0.85**	0.59**	0.05	0.16	0.80**	0.75**	0.10	0.56*	0.30	–	0.86**	0.30	0.47	Ile
	Leu	0.40	0.59**	0.31	0.18	0.69**	0.63**	0.37	0.09	0.55**	0.68**	–	0.56*	0.45	Leu
	Tyr	0.19	0.24	0.50*	0.16	0.42*	0.41*	0.22	–0.43	0.49*	0.44*	0.77**	–	–0.08	Tyr
	Lys	0.28	0.29	–0.10	0.40	0.37	0.35	0.21	0.22	0.01	0.26	0.20	0.18	–	Lys
		Tau	Asp	Thr	Ser	Glu	Gly	Ala	Val	Met	Ile	Leu	Tyr	Lys	
		Group A													

*Interaction is statistically significant ($P < 0.05$)
**Interaction is statistically significant ($P < 0.01$)

Table 5. Concentrations of the free amino acids in proportion to glutamic acid

Group	*n*	Free amino acids (%)												
		Glu	Tau	Asp	Thr	Ser*	Gly	Ala	Val	Met*	Ile	Leu	Tyr	Lys
A	24	100.00	18.56	9.70	6.68	6.56	50.84	12.28	15.20	10.40	9.73	8.69	4.63	18.29
B	13	100.00	22.77	13.43	7.17	9.55	48.39	11.66	14.43	6.06	8.43	8.16	5.09	18.72

*Differences in the proportion of this FAA to Glu concentrations between groups are statistically significant ($P < 0.05$)

nificantly higher in the semen of animals in group B (B = 46.00 vs. A = 9.46, $P < 0.01$). This situation is a methodical advantage for our purpose of this study according to the results published by Johnson et al. (1972) and Louis et al. (1994) .

Differences in FAAs concentrations between group A and group B of boars are presented in Table 2. Significantly higher FAA concentrations in 11 out of the 13 acids observed in group A ($P < 0.05$ and $P < 0.01$) and significantly higher total FAAs content ($\bar{x}$ = 74.7 vs. 45.23μM/100 ml, $P < 0.01$) were determined in group A of boars with a significantly lower MAS content ($P < 0.01$, Table 1). According to Johnson et al. (1972) the quantities of FAAs appeared to be related to the sperm concentration prior to the plasma and sperm separation and to semen volume according to Louis et al. (1994). A statistically positive and significant relation was found out between sperm concentration and FAAs content and a higher negative significant relation between FAAs content and MAS content for all the boars taken together ($r = 0.44$, $P < 0.01$; $r = –0.60$, $P < 0.01$, Table 3). We did not find any significant correlation between FAAs content and semen volume ($r = –0.27$, $P = 0.12$, $P > 0.05$, Table 3). The highest correlation between MAS and total FAAs content ($r = –0.60$) supports a possible substantial relation between these two monitored traits.

Glutamic acid was a predominant free amino acid in seminal plasma for both experimental groups ($\bar{x}$: A = 28.49 and B = 17.60μM/100 ml, Table 2) while its concentration was significantly higher in group A ($P < 0.01$). It is interesting that the proportion of its concentration in the total content of FAAs is nearly the same in both groups of boars (A = 38.1% vs. B = 38.9%, $P > 0.05$).

Table 4 presents correlations between the seminal plasma FAAs concentrations within group A and group B separately. Positive correlations between the monitored FAAs in both groups clearly prevail. The highest number of significant correlations with the other FAAs was determined in group A in glutamic acid, glycine, isoleucine, leucine and tyrosine; in group B in leucine, glutamic acid, glycine, valine and isoleucine. Out of the five FAAs mentioned above, both groups share four FAAs, i.e. glutamic acid, leucine, glycine and isoleucine. As it was already mentioned, glutamic acid is a dominant FAA with the equal proportion in the total content of all the monitored FAAs (A = 38.1, B = 38.9%). Glutamic acid is also the FAA with the highest number of significant correlations (Table 4).

Having been inspired by an "ideal protein composition" (the proportion of amino acids to lysine = 100%) we similarly calculated the proportion of FAA content to glutamic acid average content (= 100%) separately for both groups (A and B). Table 5 presents the results. Evident relative stability in the mutual proportion of FAAs as the whole in seminal plasma (Table 4) and a very close proportion of FAAs to the glutamic acid content in both groups were determined (Table 5) in spite of significant differences in the content of FAAs and significant difference in MAS incidence between the two groups of boars (A vs. B, Tables 1 and 2).

The concentration of glutamic acid in seminal plasma could be utilized to estimate the concentration of the other FAAs and possibly also the semen quality in relation to MAS in boars kept in the same conditions. There is no doubt that an increased content of MAS in ejaculate indicates a lower quality and fertilizing ability of the sperm, especially when the sperm is diluted and used for insemination (Krajňák, 1995; Grandjot, 1997; Andersson et al., 2002; Corcuera et al., 2002). The determined significantly negative relation between MAS and FAAs content indicated also the impairment of the seminal plasma quality, i.e. the whole ejaculate. In this connection, the positive effect of reciprocal exchange of seminal plasma between ejaculates with different sperm quality reported by Flowers (1997) deserves to pay attention to this information.

CONCLUSION

The present study was conducted to provide more quantitative data on the variability of seminal plasma free amino acids and to compare these values with different incidence of morphologically abnormal spermatozoa in boar ejaculates. The main result of this study is the evident negative relationship between the content of morphologically abnormal spermatozoa and the concentration of seminal plasma free amino acids in the AI boars observed and kept under the same conditions.

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Received: 2006–04–28
Accepted after corrections: 2006–12–19

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