

## RELATION OF SEMINAL PLASMA COMPONENTS AND PRLR GENE INCIDENCE TO MORPHOLOGICALLY ABNORMAL SPERMATOZOA OF BOARS

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### Abstract

The objective of this study was to find out the differences and relation of morphologically abnormal spermatozoa incidence (MASI) to seminal plasma (SP), testosterone (T), oestradiol – 17 beta ( $E_2$ ), concentration ions (Ca, Mg, Zn, K, Na) and proportion of allele (A, B) prolactin receptor (PRLR) gene. Thirty-eight ejaculates from 38 AI boars were used. The boars were kept under the same housing and feeding conditions. There were no significant differences between boars in semen volume and sperm concentration. Boars were divided into two groups (I and II) according to significant different of MASI,  $P < 0.01$ . In the group I were MASI up to 25 % ( $x = 5.6$  %) and the group II were more than 25 % MASI ( $x = 53.1$  %). Statistically significant differences were not found for all measure parameters between boar group I and II ( $x$ : T =  $0.93 \pm 1.04$  vs.  $0.61 \pm 0.54$  ng/ml;  $E_2$  =  $350.9 \pm 452.07$  vs.  $169.8 \pm 126.15$  pg/ml; Ca =  $0.98 \pm 0.62$  vs.  $0.81 \pm 0.37$  mM/L; Mg =  $16.28 \pm 7.92$  vs.  $14.75 \pm 8.70$  mM/L; Zn =  $0.48 \pm 0.15$  vs.  $0.46 \pm 0.18$  mM/L; K =  $10.64 \pm 2.76$  vs.  $11.40 \pm 3.90$  mM/L; Na =  $96.96 \pm 22.67$  vs.  $94.58 \pm 16.21$  mM/L), except of different PRLR alleles frequency proportion within the group II (allele A = 34.4 % vs. allele B = 56.6 %,  $P < 0.05$ ). Concentration of Zn and K ions in SP of total observed boars ( $n = 38$ ) was negatively related to MASI (Zn:  $r = -0.34$ , K:  $r = -0.34$ ,  $P < 0.01$ ). The results of this study lead to a conclusion that the differences between boar SP components and frequency of PRLR alleles correlate with MASI variability.

### Introduction

Seminal plasma, the fluid in which mammalian spermatozoa are suspended in semen, is a complex mixture of secretions that originate from the testes, epididymis, and the male accessory sexual glands. It contains a variety of both inorganic and organic components. There are proteins, sugars, minerals, citric acid, ascorbic acid, enzymes and also biologically active components as a prostaglandin, estrogens and androgens in the seminal plasma (Jelínek et al., 2003). The seminal plasma contains factors that influence both spermatozoa and the female genital tract during sperm transport (Yanagimachi, 1994; Waberski et al., 1995).

Seminal plasma is important for progressive motility of sperm cells (Rodríguez-Martínez et al., 1990) and might be of importance to protect membranes and maintain fertilizing capacity during storage (Harrison et al., 1978). Sperm function is highly dependent on ionic environment (Hamamah et al., 1998). Zinc is one of the most important ions in the seminal plasma. Wong et al. (2002) reported that zinc influences the process of spermatogenesis, plays a major role in sperm motility (Wroblewski et al., 2003; Henkel et al., 1999), stabilizes sperm membrane (Lewis-Jones et al., 1996), exerts protective, antioxidant-like activity (Gavella et al., 1998), preserves the ability of sperm nuclear chromatin to undergo decondensation and modulates sperm function (Suruki et al., 1995).

Testosterone is also necessary for spermatogenesis. It is androgenic hormone primarily responsible for normal growth and development of male sex and reproductive organs and secondary characteristic and the behaviour consistent with the male's role in reproduction (Ptaszynska, 2006).

The identification of sperm abnormalities has had a primary importance from the economic and genetic aspects especially for pig units practising artificial insemination. Many morphological abnormalities have been related to cases of infertility (Bonet et al., 1991). Numbers of studies have also shown a significant negative correlation between the percentage of spermatozoa with cytoplasmic droplets and farrowing rate and number of live born piglets (Zeuer, 1992).

The development of pig genome map offers the opportunity to identify individual genes controlling reproduction (Thuy et al., 2006). Research of genetic markers for the semen quality in boars will be beneficial to the improvement of porcine fertility (Huang et al., 2006). The prolactin receptor gene (PRLR), located on chromosome 16 in pigs, is a candidate gene for reproductive traits (Kmieć et al., 2006).

The objective of this study was to find out the differences and relation of seminal plasma components and PRLR gene incidence to morphologically abnormal spermatozoa of boars.

### Material and methods

We used thirty-eight ejaculates from 38 AI boars. The boars were kept under the same housing and feeding conditions.

Ejaculates were collected from boars using the gloved hand technique on a phantom mount. The semen gel free volume and sperm concentration were determined immediately after the collection. The sperm concentration was determined by spectrophotometric method.

MASI was evaluated after making fresh semen smears on microscopic slides stained according to the method of Čeřovský (1976). Seminal plasma was separated from spermatozoa by centrifugation and frozen at  $-20^{\circ}\text{C}$  for later analysis. Concentration ions in seminal plasma was assessed by the atomic absorption spectrophotometer SP9 and samples of seminal plasma for the determination proportion of allele (A, B) prolactin receptor (PRLR) gene were processed in the special genetic laboratory (MZLU Brno).

Ejaculates were divided into two groups (I, II) according to significantly different of MASI. In the group I were MASI up to 25 % ( $x = 5.6\%$ ) and the group II were more than 25 % MASI ( $x = 53.1\%$ ),  $P < 0.01$ .

Basic statistical characteristics of the results, arithmetic means, standard deviations and significances (P) were obtained using the QC Expert program. The statistical significance was checked by the t-test at significance levels of  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$ .

## Results and discussion

A comparison of the results of the components monitored of seminal plasma is presented in Table 1. There were no statistically significant differences for all parameters measure between boar group I and II ( $x$ :  $T = 0.93 \pm 1.04$  vs.  $0.61 \pm 0.54$  ng/ml;  $E_2 = 350.9 \pm 452.07$  vs.  $169.8 \pm 126.15$  pg/ml;  $Ca = 0.98 \pm 0.62$  vs.  $0.81 \pm 0.37$  mM/L;  $Mg = 16.28 \pm 7.92$  vs.  $14.75 \pm 8.70$  mM/L;  $Zn = 0.48 \pm 0.15$  vs.  $0.46 \pm 0.18$  mM/L;  $K = 10.64 \pm 2.76$  vs.  $11.40 \pm 3.90$  mM/L;  $Na = 96.96 \pm 22.67$  vs.  $94.58 \pm 16.21$  mM/L),  $P > 0.05$ .

This study only noted that concentration ions of Zn and K in seminal plasma of total boars observed ( $n = 38$ ) were negatively significant related to MASI,  $P < 0.01$  (Table 2).

Strzeżek et al. (1995) reported that the level of  $Zn^{2+}$  ions in the seminal plasma was rapidly decreased in boars with a high ejaculation frequency. A zinc deficiency may result in lower fertility due to increasing sperm fragility. Se and Zn seem to have vital roles in spermatogenesis because a substantial deficiency in either results in structural abnormalities in the sperm produced (Wayene et al., 1997).

Figure 1. shows representation genotypes of prolactin receptor (PRLR) gene. Genotypes AB was the most present in both groups and genotypes AA was in the least present.

Frequency alleles PRLR gene presents Table 3. There was statistically significant different in B allele frequency of PRLR gene in the group II ( $P < 0.05$ ). Huang et al. (2006) revealed that boars with the AA genotype in PRLR had significantly better semen quality with a higher percentage of normal sperm and a lower percentage of immature sperm than those with other genotypes. Kmiec et al. (2006) found association between PRLR genotype and ejaculate volume, sperm concentration, percentage of live sperm and number of live sperm in the ejaculate ( $P < 0.01$ ) but Lin et al. (2006) did not record significant effects between prolactin receptor (PRLR) and semen quality parameters.

**Table 1.: Mean values and standard deviations for monitored components of seminal plasma in boars**

Group	n	Testosterone (ng/ml)	Oestradiol 17 beta (pg/ml)	Ca (mM/L)	Mg (mM/L)	Zn (mM/L)	K (mM/L)	Na (mM/L)
I	22	$0.93 \pm 1.04$	$350.9 \pm 452.07$	$0.98 \pm 0.62$	$16.28 \pm 7.92$	$0.48 \pm 0.15$	$10.64 \pm 2.76$	$96.96 \pm 22.67$
II	16	$0.61 \pm 0.54$	$169.8 \pm 126.15$	$0.81 \pm 0.37$	$14.75 \pm 8.70$	$0.46 \pm 0.18$	$11.40 \pm 3.90$	$94.58 \pm 16.21$

Differences between the groups are not significant  $P > 0.05$

**Table 2.: Correlation coefficients in observed traits**

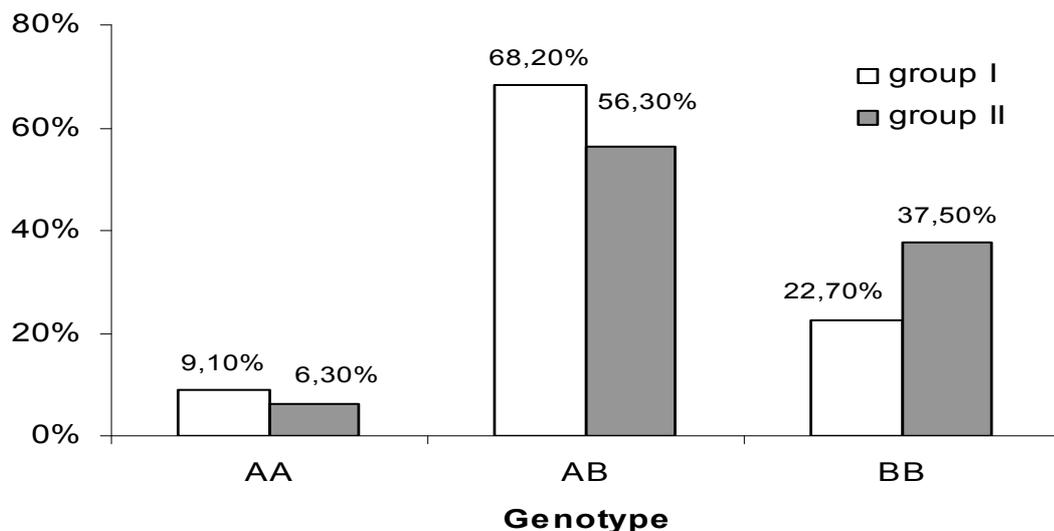
Traits	Ca	Mg	Zn	K	Na
MASI	-0,14	- 0.27	- 0.34*	- 0.34*	- 0.21

\*  $P < 0.01$

**Table 3.: Allele proportion of PRLR gene**

Group	n	Allele			
		A		B	
		n	%	n	%
I	22	19	43.2	25	56.8
II	16	11	34.4 <sup>a</sup>	21	65.6 <sup>b</sup>

a vs. b  $P < 0.05$

**Figure 1. Genotypes of PRLR gene in the experimental groups**

## Conclusion

It seems that the differences between boar seminal plasma components and frequency of PRLR alleles correlate with MASI variability and should be further investigated.

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