THE EFFECT OF GENETIC MARKERS ASSOCIATED WITH BOAR TAINT ON THE BASIC BOAR SPERM QUALITY PARAMETERS

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Abstract

Boar taint is mainly caused by androstenone (A) and skatole (S) which are stored in fat tissue and negatively affect meat quality. The objective of this study was to find out whether various genetic markers for possible reducing the occurrence of boar taint have an effect to the basic quality indicators of boar sperm of the Přeštice black-pied breed (PC).

Native ejaculates were collected from 12 AI fertile boar's PC breed during 2 years. The following basic boar sperm quality parameters were evaluated: sperm volume, sperm concentration, total number spermatozoa per ejaculate, % morphologically of abnormal spermatozoa, sperm motility (velocity and sperm kinematic parameters), % of live spermatozoa and sperm viability during 96h of storage. Genetic markers that are associated for boar taint CYP2E1 genotype CC increases the level of S, TEAD3 genotype CC increases the level of A, HSD3B genotype GG decreases the amount of A, CYB5A genotype TT and GT decrease the level of A, FMO1 and FMO5 genotype GG increases the level of A were used for this study. Statistically significant difference ($p < 0.05$) was noted for CYP2E1 marker between TC and CC genotypes in sperm volume in the basic boar sperm quality parameters. No statistically significant differences ($p > 0.05$) were found among viability, velocity and sperm kinematic parameters of boar sperm and the genotypes of individual markers associated with boar taint.

In conclusion, it can be said that according to the results obtained so far, the differences among genotypes for individual genetic markers associated with boar taint appear to be statistically inconclusive. It can therefore be concluded that breeding to reduce boar taint should not have a negative effect on the fertility of breeding boars.

Key Words: Boar semen quality; taint; genetic marker

Boar taint is mainly caused by androstenone, skatole and indole which are stored in fat tissue and negatively affect meat quality. Androstenone is a pheromonal steroid that is synthesized in the testes and metabolized in the liver. Skatole is produced in the colon by bacterial degradation of tryptophan and metabolized by liver enzymes (Zamaratskaia et al., 2009). Sexual maturity can affect levels of both androstenone and skatole, while skatole is more affected by diet and environment and management factors than androstenone (Squires, 2006). Castration is used in piglets to reduce the boar taint, which reduces the concentration of skatole and androstenone in the fat below threshold values that start at 0.20 to 0.25 μg/g for skatole and 0.5 to 1 μg/g for androstenone (Strathe et al., 2013). The heritability of androstenone and skatole ranges from medium to high values, i.e. in the range of 0.55–0.88 for androstenone and 0.23–0.55 for skatole (Parois et al., 2015). The current trend is to eliminate boar taint by castration (EU legislation) and so other alternatives are being sought. One possibility is the use identification of genetic factors related to boar taint can be implemented into breeding programs to select

animals that produce low levels of taint. A number of candidate genes have already been identified that eliminate boar taint in different pig populations. Candidate genes for androstenone are e.g. CYP17A, CYB5, CYP21, SULT2A1, TEAD3, FMO1 and FMO5 and for skatole e.g. CYP2E1, SULT1A1 (Bai et al., 2015; Falková et al., 2019; Kim et al., 2013; Neuhoff et al., 2015; Robic et al., 2012; Squires, 2006).

The objective of this study was to find out whether various genetic markers for possible reducing the occurrence of boar taint have an effect to the basic quality indicators of boar sperm of the Přeštice black-pied breed (PC).

Material and Methods

Sperm ejaculate (n=230) from 12 fertile AI boars of Přeštice black-pied pig aged 3.5 to 5 years were used in this study. Ejaculate were collected using the gloved-hand technique during 2 years. The boars were kept in the same housing, feeding and breeding conditions.

The following parameters were evaluated in the fresh native boar semen: semen volume, sperm motility, sperm concentration and morphologically abnormal spermatozoa (MAS). Sperm motility (rapid, medium, slow and immotile) and kinetic characteristics – straight line velocity (VSL, μm/ s), average path velocity (VAP, μm/s), curvilinear velocity (VCL, μm/s), straightness (STR, VSL/ VAP, %), linearity (LIN, VSL/VCL, %), wobble (WOB, VAP/VCL, %) were assessed using SCA software (Sperm Class Analyzer, version 5.4. Microptic S.L., Spain). Evaluation was performed using a 2 μl sample placed in a Leja 20 chamber and 500 sperm were evaluated by negative phase contrast microscopy with a heating stage (38 ° C) at 160x magnification. Sperm concentration was measured with IMV AccuRead (manufactured in USA for Biochrom Ltd. Cambridge UK). Morphologically abnormal spermatozoa (MAS) were assessed according to the staining method of Čeřovský (1976) and evaluated microscopically under oil immersion and 1500× magnification. Percentage of live spermatozoa was estimated by supravital staining technique using the eosin-nigrosin stain mixture (Věžník et al., 2004). One drop from each sample was mixed with 1 drop of 1% eosin Y, then 2 drops of 10% nigrosine were added after 30 s. Two hundred spermatozoa per slide were evaluated under a light microscope (1500×). The boar semen was diluted in dilution rate 1+2 in extender Androstar plus (AS+, Minitüb, Germany) stored at 17 °C. Sperm viability was evaluated at 24 h, 48 h, 72 h and 96 h storage time with SCA software. Genetic markers CYP2E1, TEAD3, HSD3B, CYB5A, FMO1 and FMO5 were determined in boar semen in Mendel agrogenomics laboratory in Brno. Genetic markers that are associated for boar taint CYP2E1 genotype CC increases the level of scatol (S), TEAD3 genotype CC increases the level of androstenone (A), HSD3B genotype GG decreases the amount of A, CYB5A genotype TT and GT decrease the level of A, FMO1 and FMO5 genotype GG increases the level of A were used for this study.

Basic statistical characteristics of the results of arithmetic means, standard deviations (SD) and significance (p) were calculated by the QC Expert program (TriloBite Statistical Software s.r.o., Pardubice, Czech Republic). The data were analysed by statistical analysis of variance ANOVA followed by the Fisher test ($p<0.05$).

Results and Discussion

The initial quality of native semen was as follows: semen volume 249.38±116.81 ml, progressive sperm motility 85.05±6.28 %, sperm concentration $447.61±192.97×10^3/mm^3$, MAS 28.03±12.69 % and live spermatozoa 75.41±2.91 %. The basic boar sperm quality parameters according to individual markers and genotypes are noted in the Table 1. Statistically significant difference ($p <0.05$) was noted for CYP2E1 marker between TC and CC genotypes in sperm volume. The results of the genetic markers in Table 1 also show that the GG genotype at the HSD3B marker, the TT genotype at the CYB5A

marker, the AA genotype at the FMO1 marker and the GG genotype at the FMO5 marker were not detected in 12 PC boars. Oh et al. (2006) found that selection for increased muscle depth and reduced backfat may result in reduced boar fertility and it in semen volume, total number spermatozoa and sperm concentration.

The results of sperm viability up to 96 hours of storage time according to individual markers and genotypes are shown in Table 2. The results show that no statistically significant differences were found in sperm survival during 96h of storage time ($p>0.05$).

No statistically significant differences ($p>0.05$) were found between velocity (Table 3) and sperm kinematic parameters (Table 4) of boar sperm and the genotypes of individual markers associated with boar taint. Arsenakis et al. (2017) selection of boars for reduced backfat thickness might negatively influence semen motility, whereas selection for increased lean meat percentage and loin eye depth would not necessarily compromise semen quality traits. Merks et al. (2010) found low and negative correlation values between androstenone and sperm motility, volume and concentration. On the other hand, Bergsma et al. (2007) recorded low genetic correlations with positive correlation between androstenone and sperm volume, motility and viability, whereas negative values were found between skatole and the traits of motility and longevity. Strathe et al. (2013) noted that skatole and androstenone can be reduced through selection without affecting important economical production and litter size traits. This implies that estimations of genetic correlations between semen and meat quality traits could only be indicative and may not always predict phenotypic performance (Arsenakis et al., 2017).

Table 1. Mean values and standard deviation (SD) the basic boar sperm quality parameters according to individual markers and genotypes

Marker	Genotype	Frequency of genotype	Sperm volume (ml)	Sperm motility (%)	MAS (%)	LS (%)	SC ($mm^3 \times 10^3$)	TNS $\times 10^3$
	TC	0.25	156.13±22.80*	81.14±10.13	38.84±19.56	72.71±1.36	607.73±214.57*	75.45±39.01
CYP2E1	TT	0.33	404.39±56.40	82.73±5.15	30.74±13.35	74.04±4.79	314.83±105.15	129.26±51.91
	CC↑	0.42	248.83±89.90*	87.34±5.17	23.37±7.41	76.96±1.08	441.20±163.04	100.24±20.91
	TC	0.84	288.17±120.92	83.65±7.11	31.27±14.01	74.74±3.48	445.98±203.92	104.21±40.97
TEAD3	TT	0.08	316.52±0.00	86.7±0.00	16.43±0.00	77.35±0.00	377.49±0.00	119.48±0.00
	CC↑	0.08	131.40±0.00	87.80±0.00	27.14±0.00	74.28±0.00	451.20±0.00	59.29±0.00
	AA	0.63	250.58±114.07	82.55±6.11	29.56±15.10	74.33±2.90	463.88±204.41	91.21±40.47
HSD3B	AG	0.36	272.29±97.49	89.41±4.12	25.37±8.12	77.28±2.00	419.14±197.11	101.19±17.57
	GG↓	0						
	GG	0.73	226.06±103.74	84.75±1.96	21.60±7.66	76.80±1.05	304.15±112.65	102.46±34.05
CYB5A	**GT↓**	0.27	213.57±117.81	85.16±7.43	30.44±13.75	74.88±3.26	501.41±193.49	91.81±35.04
	TT↓	0						
	GA	0.18	267.38±150.89	84.48±2.34	29.18±1.73	73.78±2.55	513.38±179.33	102.92±30.99
FMO1	**GG↑**	0.82	217.38±121.60	85.17±6.97	27.78±14.16	75.77±2.99	433.00±128.47	101.1±50.70
	AA	0						
	AA	0.59	224.94±101.13	82.86±6.92	31.71±15.09	75.66±3.45	492.58±193.05	100.54±28.60
FMO5	GA	0.41	298.72±102.25	87.66±4.81	23.62±8.56	75.10±2.45	393.65±199.58	105.06±39.09
	GG↑	0						

*$p<0.05$

MAS - Morphologically abnormal spermatozoa, **LS** - Live spermatozoa, **SC** - Sperm concentration

TNS - Total number of spermatozoa per ejaculate

Table 2 Mean values and standard deviation (SD) sperm viability up to 96 hours of storage time according to individual markers and genotypes

Marker	Genotype	Frequency of genotype	Sperm viability (%)			
			24 h	48 h	72 h	96 h
CYP2E1	TC	0.25	74.80±10.18	70.48±10.18	62.97±12.07	61.09±12.96
	TT	0.33	75.04±7.25	70.37±8.17	65.66±8.54	62.09±9.74
	CC↑	0.42	77.79±4.85	73.39±5.91	68.88±5.23	65.16±5.16
TEAD3	TC	0.84	75.42±7.09	70.84±7.90	65.30±8.33	61.88±8.61
	TT	0.08	79.34±0.00	75.87±0.00	71.42±0.00	67.61±0.00
	CC↑	0.08	80.00±0.00	75.59±0.00	71.56±0.00	71.00±0.00
HSD3B	AA	0.63	76.02±6.32	71.16±7.13	64.77±8.10	61.96±8.78
	AG	0.36	79.07±5.42	75.23±6.51	71.48±5.12	67.85±5.34
	GG↓	0				
CYB5A	GG	0.73	76.78±2.64	71.23±4.79	65.62±5.71	61.76±6.28
	GT↓	0.27	77.26±6.94	73.16±7.75	67.80±8.55	64.98±8.74
	TT↓	0				
FMO1	GA	0.18	77.70±5.14	72.08±8.17	64.09±5.79	60.48±7.57
	GG↑	0.82	77.00±3.37	72.76±7.13	67.90±8.14	64.91±8.27
	AA	0				
FMO5	AA	0.59	76.20±6.76	71.76±7.28	65.55±8.56	62.99±8.93
	GA	0.41	78.23±5.29	73.69±7.06	69.19±6.79	65.44±7.44
	GG↑	0				

p>0.05

Table 3. Mean values and standard deviation (SD) percentage motility expressed as velocity according to individual markers and genotypes

Marker	Genotype	Frequency of genotype	Velocity			
			Rapid (%)	**Medium (%)**	**Slow (%)**	**Immotile (%)**
CYP2E1	TC	**0.25**	66.93±26.22	18.83±13.72	7.83±5.70	5.58±3.42
	TT	**0.33**	61.71±15.58	17.21±6.18	8.71±6.10	6.42±6.80
	CC↑	**0.42**	55.97±12.47	21.65±1.21	15.21±7.01	7.07±4.50
TEAD3	TC	0.84	67.21±16.52	17.08±6.05	10.74±6.97	4.97±4.21
	TT	0.08	55.09±0.00	21.83±0.00	17.08±0.00	6.00±0.00
	CC↑	0.08	48.38±0.00	28.58±0.00	1.86±0.00	11.23±0.00
HSD3B	AA	0.63	68.00±15.34	18.22±7.43	8.69±4.88	5.08±3.95
	AG	0.36	49.53±7.87	22.30±0.65	19.17±2.96	9.00±4.25
	GG↓	0				
CYB5A	GG	0.73	65.94±19.43	17.59±8.22	10.52±7.16	5.96±5.25
	GT↓	0.27	59.01±8.58	21.64±1.19	13.60±5.23	5.75±2.44
	TT↓	0				
FMO1	GA	0.18	69.28±22.89	15.92±9.62	9.97±8.72	4.82±4.45
	GG↑	0.82	61.36±15.03	20.17±6.07	12.24±6.23	6.23±4.44
	AA	0				
FMO5	AA	0.59	67.58±15.17	18.79±8.03	8.71±3.82	4.91±4.30
	GA	0.41	60.43±21.11	18.62±7.14	13.82±8.83	7.13±5.40
	GG↑	0				

P>0.05

Table 4. Mean values and standard deviation (SD) sperm kinematic parameters of sperm motility to individual markers and genotypes of boar taint

Marker	Genotype	Frequency of genotype	Sperm kinematic parameters					
			VCL (μm/s)	VAP (μm/s)	VSL (μm/s)	STR (%)	LIN (%)	WOB (%)
CYP2E1	TC	0.25	64.62±29.49	34.93±16.29	23.93±0.83	53.25±1.76	37.12±10.21	59.85±6.26
	TT	0.33	61.08±15.14	32.27±10.34	22.59±11.72	44.22±8.53	35.65±15.68	52.93±5.49
	CC↑	0.42	56.96±6.64	26.47±9.43	29.48±8.28	45.62±8.74	53.00±17.11	62.71±4.89
TEAD3	TC	0.84	67.56±14.5	34.42±11.15	26.90±8.28	48.65±8.07	41.88±14.37	60.02±4.27
	TT	0.08	55.18±0.00	24.15±0.00	37.04±0.00	45.02±0.00	65.57±0.00	68.19±0.00
	CC↑	0.08	43.77±0.00	23.41±0.00	23.35±0.00	54.49±0.00	44.34±0.00	64.28±0.00
HSD3B	AA	0.63	66.56±16.41	37.30±9.82	22.46±8.18	53.91±5.97	35.09±11.06	59.60±4.62
	AG	0.36	53.29±2.67	21.28±4.06	33.91±4.43	41.11±5.53	62.74±4.01	64.67±4.98
	GG↓	0						
CYB5A	GG	0.73	66.02±18.91	31.79±13.25	28.97±7.69	47.20±8.10	44.93±15.02	60.27±4.74
	GT↓	0.27	58.08±5.40	31.71±6.69	25.65±9.88	51.81±5.91	45.51±17.48	63.73±4.71
	TT↓	0						
FMO1	GA	0.18	70.11±21.73	40.30±8.70	21.90±3.70	53.89±2.67	33.67±5.34	59.82±6.21
	GG↑	0.82	60.68±14.16	28.92±10.21	29.67±8.35	47.27±7.74	48.98±15.06	62.12±4.71
	AA	0						
FMO5	AA	0.59	64.62±17.03	32.93±10.82	27.55±9.57	50.21±7.12	42.01±13.64	60.94±4.64
	GA	0.41	63.56±18.24	37.73±13.67	24.86±5.75	48.53±9.94	42.53±15.47	60.21±4.55
	GG↑	0						

P>0.05

VCL – Curvilinear velocity, **VAP** – Average-path velocity, **VSL** – Straight-line velocity

STR – Straightness index (VSL/VAP×100), **LIN** – Linearity index (VSL/VCL×100)

WOB – Oscillation index (VAP/VCL×100)

Conclusion

In conclusion, it can be said that according to the results obtained so far, the differences among genotypes for individual genetic markers associated with boar taint appear to be statistically inconclusive. It can therefore be concluded that breeding to reduce boar taint should not have a negative effect on the fertility of breeding boars.

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