MORPHOLOGICALLY ABNORMAL SPERMATOZOA CHANGES AS A TOOL FOR SEMEN QUALITY ASSESSMENT OF THE BOARS

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Owing to the fact, that the specific practical method for the boar sperms staining did not exists at the beginning of the pigs AI introduction in our country, first the development of a new staining method was done. Following parameters were defined: simplicity, good tinction of sperms parts, elimination of wet fixation of the semen smear with specific solutions and to speed up the staining procedure which is important for evaluation of morfology abnormal spermatozoa (MAS) content before the distribution of insemation doses to the pThe **new staining method** of the native semen smear on the glass slide by saturated solution of congo red (approximately for 20 to 30 sec.) and then by 0.5 % solution of crystal violet for 5 or 10 sec., is a speed simple method for laboratory practise. Dry samples can be viewed microscopically under oil immersion.

A precise evaluation may be obtained by performing one separate count for sperm head morphology, droplets and tail morfology.
Deformities in head shape (head-cap), tail formation a cytoplasmic droplets (proximal- near the head, distal – middle of tail) should be counted as abnormal spermatozoa. Some authors consider level of MAS occurrence to be an important marker for the semen quality (BLOM and ANDERSEN, 1975; LARSSON et al. 1988; KRAJIŇÁK, 1995; VĚŽNÍK, 2000; FEITSMA et al., 2005). Some research suggest that acrosome integrity may be a better indication of sperm quality than motility.
This above staining method allowed us, at first, to perform **comparative study in the field of morphologically altered spermatozoa occurrence in the normal fertile and infertile boars.**

Table 1.: Comparison of an average occurrence level (%) of the ten obvious frequent morphological abnormalities of spermatozoa in the sperm of infertile boars with the occurrence level in fertile boars used in artificial insemination (AI) and in fertile boars from natural breeding

Abnormality		Infertile boars (n = 16)	Normal fertile boars			
			In AI (1000 ejaculates)		In natural breeding (n = 29)	
		%	%	±	%	±
Proximal protoplasmic droplet	a	41.50	4.05	- 37.45	1.67	-39.83
Acrosome defect	b	17.78	0.73	-17.05	0.67	-17.11
Distal protoplasmic droplet	a	17.44	3.70	-13.74	3.51	-13.93
Bent tail	c	9.13	2.33	-6.80	1.87	-7.26
Persistent acroblast	b	5.47	0.02	-5.45	0.00	-5.47
Coiled tail	c	2.72	0.34	-2.38	0.24	-2.48
Unstained acrosome	b	2.13	0.00	-2.13	0.00	-2.13
Condensation of acrosome content	b	1.72	0.35	-1.37	0.46	-1.26
Acrosome swelling	b	0.78	0.44	-0.34	1.06	+0.28
Tail deteached	c	0.78	0.05	-0,73	0.01	-0.47

a = protoplasmic droplet, b = acrosome defects, c = tail defects

In this case level and structure of MAS in ejaculates of infertile boars are characterized by higher incidence of residual protoplasmic droplets, defects of acrosome and tail anomalies. This knowledge clearly suggest that essessment morfology abnormal spermatozoa level in ejaculate may play an important role in potential fertility estimation of the boars in particular in the boars used for AI.
According to ROZEBOOM (2000) the incidence of proximal droplets in fertile boars is quite low but fertility as measured by farrowing rate and litter size gradually decreases as the prevalence of proximal droplets increase. The same effect appears to be true, if not more severe for distal droplets. However, distal droplets are more commonly found in ejaculates than proximal droplets. Although there are limited scientific references regarding the impact of cytoplasmic droplets in boar ejaculates, it has bean suggested that the incidence of plasmas droplets should not exceed 15 % when semen is stored for extended periods of time-at least 2 days (ROZEBOOM, 2000).
ALM et al. (2006) escertained that the proportion of normal (abnormal) spermatozoa in the sperm morphology analysis significantly influenced fecundity of swine artificially inseminated with a dose 2 and 3 billion spermatozoa.
The new staining method was used also to **monitoring developmental (pubertal) stages of MAS incidence** in relation to 48 LW and 41 L boars at the age of five, six and seven months. The semen was collected on farms by gloved-hand technique into 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. Semen collection were performed on the „live-phantom" – on the gilts in oestrus.

Table 2 .: Morphologically abnormal spermatozoa incidence (`x / %) in the LW and L breed in the course of sexual maturation

LW/n = 49			**L/n = 41**		
age /months			**age/months**		
5	6	7	5	6	7
67.79	21.61	12.17	51.19	28.34	23.36
a	b	c	d	e	f

Statistical significance: a-b**, b-c**, d-e**, e-f**; **P < 0.01
Limit: up to 25 %

The highest decrease of MAS was found out between the fifth and sixth months of age in both breeds. Five – month – old boar ejaculates could not be used for insemination regardless of the experimental breeds.
In both groups all together (n = 89) there were 49 % ejaculates of six-months-old boars and 83,4 % ejaculates of seven – months – old ones that did not exceed determined limit of MAS maximum incidence (up to 25 %) in ejaculates for AI usage according to Czech norm.
Further study was focused on significant increase of proximal **protoplasmic droplet in the boar ejaculates in „summer season"** (7th to 9th month).

Table 3.: Changes of protoplasmic droplets content in boars ejaculates according to season of the year

Morphologically abnormal spermatozoa (%)	S e a s o n					
	Summer months (7 th – 9 th)			Remaining months (10 th – 6 th)		
Total incidence within the range	0 - 15	15.1-30	< 30	0 - 15	15.1 –30	< 30
Proximal protoplasmic droplets	2.11	7.40*	17.58**	1.90	5.41*	8.05**
Distal protoplasmic droplets	2.33	5.89**	15.85	2.46	9.70**	20.54

* P = < 0.05; ** P < 0.01

Proximal protoplasmic droplet was the most frequent kind of deviation characterizing the morphological changes of the spermatozoa in this summer period (P< 0.05, P< 0.01). These ejaculates using for AI is problematic, at least, when we take into consideration the fact that an increase occurrence of spermatozoa with proximal protoplasmic droplet characterizes the spermiogram of the infertile boars in our previous study. It is interesting to note, that development of distal protoplasmic droplets incidence have had apposite seasonal trend in comparison with proximal protoplasmic droplets development.

Recently, we performed a study with the aim to find out the level of **changes in two groups of boars in the insemination with diametrically different content of MAS** in semen collection I (group A up to 10 % and group B above 40 % P< 0.01), with an interval of 25 weeks between semen collection I and collection II. According to WABERSKI et al. (1990) two criteria are sufficient for the selection of boars for insemination or ejaculate: sperm motility and percentage of MAS especially when semen is preserved and used for the insemination of sows after a longer time, i.e. three to five days. Some abnormalities of spermatozoa can be a result of pathological processes that affect testicles and epididymis tract, others can be caused genetically, some may be caused by unsuitable rearing conditions.

Spermiogenesis is a finely regulated process. In the porcine, the percentage of abnormally formed sperm cells is relatively higher than in other male production animals.

According to FEITSMA et al. (2005) it was confirmed that morphological aberrations in boar sperms have a negative effect on both farrowing rate and litter size.

GADEA (2002) believed, that the aim of the examination of the MAS content was to find out, if the spermatozoa developed in the testes normally and if they matured completely in the epididymis.

Table 4 shows development of the frequency of MAS in both groups (A and B). In the total occurrence of MAS the difference between groups A and B is significant in both collections (I a II), in favour of group A (I:5.59 vs. 53.13, II:12.14 vs. 40.88; P< 0.001).

The differences in the occurrence of spermatozoa with proximal and distal protoplasmic droplet between groups A and B in the semen from collection I and II are statistically significant (P < 0.01) and are the main portion of the total number of MAS in both groups. In the group B in the semen from collection I a significantly higher content of degenerative spermatozoa was detected than in the group A (P < 0.05, Table 4). In other forms of MAS the differences between groups A and B according to collection I and II were not statistically significant.

Table 5 presents MAS with the highest occurrence frequency in proportion to the total MAS content. Four forms of MAS represent the main part of the total MAS content (from 89.51 to 93.88%). This situation changes resembles the values obtained in our workplace in the past: the boars in insemination stations 90.72 % and in natural breed-ing 90.84% (ČEŘOVSKÝ, 1978).

Table 4.: Difference in the occurrence of morphologically abnormal spermatozoa between the groups of boars A and B accordingly to ejaculate collection order

Group	Order of sperm collection	Morphologically abnormal spermatozoa -MAS (x)									
		Total AS	Proximal protoplasmic droplet	Distal proto-plasmic droplet	Bent tail	Folded tail	Coiled tail	Acrosome defect	Narrowing of the head basis	Degenerative forms	Other abnor-malities
A	I	5.591	1.682	2.273	1.000	0.000	0.136	0.136	0.045	0.045	0.273
B		53.125**	22.750**	14.563**	7.500	0.750	1.063	3.000	0.438	2.063*	1.000
A	II	12.136	4.136	3.818	2.273	0.136	0.273	0.636	0.364	0.136	0.364
B		40.875**	20.313**	10.313**	7.313	0.375	0.563	0.438	0.563	0.375	0.625

*P<0,05 ; **P<0,01

Table5 Changes and differences in the representation of morphologically abnormal spermatozoa with the highest noted occurrence frequency (x)

Abnormalities	Group A				Group B			
	Order of sperm collection							
	1		2		1		2	
	n	%	n	%	n	%	n	%
Total	123	100	267	100	850	100	654	100
Proximal protoplasmic droplet	37	30.08	91	34.08	364	42.82	325	49.69
Distal protoplasmic droplet	50	40.65	84	31.46	233	27.41	165	25.23
Bent tail	22	17.89	50	18.73	120	14.12	117	17.89
Acrosome defect	3	2.44	14	5.24	48	5.65	7	1.07
Total	112	91.06	239	89.51	765	90.00	614	93.88**

**P<0,01

In the group A the MAS occurrence increased in 7 boars (31.8%) and in the group B the MAS oc-currence decreased in 7 boars (43.7%). It means that in 15 boars from the group A and in 9 boars from the group B there was no significant change in the MAS occurrence in comparison with the situation in collection I.

Significant changes (+ and –) in the MAS oc-currence were observed in collection II in 14 boars (36.8%), i.e. practically in one third of boars out of the total number of the monitored ones (A + B = 38 animals). In 24 boars (63.2%) there were no significant changes in any of the monitored MAS form and in the total AS occur-rence..

In conclusion, the situation in aduld boars group A and B did not change from the aspect of the comparison of the phenotypic MAS development with the permitted occurence limit up to 25 % in the Czech Republic for AI in the course of the monitored period. In the group A all boars remained below the limit and in the group B, on the other hand, all boars remained above the limit, i.e. without the applicability for insemination. It gives evidence of considerable persistence of the initial status in the MAS content observed, probably with hereditary background. These conclusion could be useful for possible study of MAS genetics, for AI practice and breeding of pigs.

Not long ago we have been surprised by MAS level persistence in accordance to seasonal MAS changes. The summer MAS elevation is obviously falling to the end of the year but in this case there is no end to it. Probably it is caused by global climatic changes or summer extremly hot climate in the recent years.

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