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IMPORT OF BOARS - SEMEN QUALITY CONTROL AND POSSIBILITY OF COMPLAINTS

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Abstract: Serbia is one of the countries with the continuous import of breeding sows and boars. Boars are usually imported at the age of 4 to 6 months, in the period when the quality of the breeding males cannot be determined due to sexual immaturity (prepubertal and pubertal age). In this paper, the method and results of semen quality control in 40 imported young boars are described, and also the method of documenting the cause for action claim. In the case of suspicious semen quality it is necesseary to perform at least 3 consecutive controls in one month intervals in order to establish a final estimation of quality and usability of semen. Of 40 imported boars, 4 boars (10%) were subject of complaint due to: azoospermia (1 boar), absence or reduction of total and progressive motility, present sperm agglutination (2 boars), and increased number of pathological forms of spermatozoa (78%, 1 boar). Increased proportion of sperm with unstable chromatin structure (SCSA test - 33.2% and 37.1%) was established in two boars. To initiate the complaint it is necessary to have a sales contract that provides possibility for the reclamation, recognized methods of semen quality control and trustful business relationship between all interested parties.

Key words: boars, reproduction, semen, complaints

Introduction

Pig production is an important branch of animal husbandry as there is an annual 2.2% increase of pork meat consumption in the world. Intensification of pig production aims to achieve greater proportion of meat in the carcass with the

reduction of fat at the same time. Contemporary selection approach has resulted in realization of carcasses which contain more than 60% meat, with the share of fat up to 20%. Whoever wishes to compete successfully in this increasingly tough competitive market strives for the necessary improvements in performance, whilst at the same time maintaining economic cost and investment levels *(Wahner and Huhn, 2003)*. Countries with developed pig production nowadays make significant incomes from the export of breeding materials, technologies, equipment and finished products. For developing countries, import of sire boars is the main way to increase the profitability of production, by means of increased fertility and meat quality in order to meet the needs of growing population and narrow standards of economical operation in food production sector.

In order to increase the share of meat in carcass, our country has continuously imported breeding boars and gilts. However, despite the decades of constant import of superior breeding material, there was no expected improvement in pig meat production which would come closer to the level of developed countries.

Exploitation of imported breeding material is often limited by the reproductive capacity of imported animals. Imported boars give more quality ejaculates of native sperm, but are sensitive to non-specific infection that can reduce sperm viability, induce acrosome damage, disrupt sperm maturation, and thus, the fertile ability of diluted semen *(Jovičin et al., 2003)*.

Breeding material enters the exploitation with certain exterior and genetic characteristics, along with controlled health status, but without control of their fertilizing ability. In boars of high genetic potential, it can be expected that up to 10% of animals manifest signs of reduced fertility or complete infertility *(Robinson and Buhr, 2005)*. Animals are imported in period of puberty, when their fertilizing ability cannot be determined. After the quarantine test procedures, boars are entering exploitation, and only then can their fertilizing ability be assessed.

The experiences of breeding centers which approached improvement of genetic lines by purchasing boars from abroad are different. Large commercial farms often don't have a clear vision of the importance and the necessary measures of ensuring quality of semen production. The boars are usually intraduced to exploitation without previous sperm quality control, therefore, it is only after repeatedly failed fertilization and small number of piglets per litter over a long time, that the boars are excluded from further reproduction, even though they were imported as elite breeding animals, with a fairly high cost.

Contrary to this, progressive farms set long-term plans and program objectives which are over the nominally required regulations of our country. It is exactly those kind of farms that can improve business through better control of semen quality provided by modern laboratory diagnostics, food quality control, housing conditions, health care, general hygiene, etc.

The progress of computer technology and its introduction in andrology allows automaticity in working with semen, the analysis of a large number of sperm

samples in a short period of time, high repeatability and analyticity, and excludes subjectivity of method. Computer analysis of the mobility of semen evaluates the kinetics of spermatozoa in a very sophisticated way. At the same time, it is possible to receive reports in Serbian and English, suitable for electronic delivery, along with a picture of mobility, as well as a short film, available for electronic data exchange.

Some semen defects can not be routinely detected in farm control conditions. Flow cytometry allows analysis of the integrity of spermatozoa and acrosome membranes, sperm chromatin structure and status of phospholipid membrane, mitochondria polarization, oxidation of cell membrane, etc. It is believed that the membrane integrity test and acrosome status are reliable methods of semen quality control *(Tsamakidis et al., 2010; Boe-Hansen et al., 2008)*.

Chromatin structure test provides information about the status of DNA and its impact on fertility. In the last decade data has been collected on the results of semen fertility of humans and animals, which indicates that fragmentation-degenaration of sperm DNA has a negative impact on fertility and number of offspring in pluriparous animals *(Evenson et al., 2002)*, where this indicator is not associated with sperm motility. Sperm DNA is highly condensed and stable, however SCSA test detects sperm with chromatin sensitive to heat or acid denaturation. *Boe-Hansen et al. (2008)* state that boars with damaged sperm chromosomes, even in small percent such as 2.1% of spermatozoa, have reduced number of piglets per litter up to 0.5-0.9 depending on the breed. SCSA is a standardized test and is performed in accordance with strict protocol, determined by special software (SCSA-Soft) for analyzing and processing the data from the flow cytometer. Regardless of the limited laboratory variation, SCSA is accurate regarding the comparison of results between different laboratories *(Bungum, 2012)*.

The aim of this paper is to highlight the advantages and disadvantages of control of breeding animals during the importation and before the introduction into intensive exploitation (use), and the possibility of action claims for boars of inadequate breeding characteristics.

Materials and Methods

The experiment was conducted on 40 boars (10 Landrace, 15 Yorkshire and 15 Duroc), imported from Denmark, which arrived on 15th of May 2011. Quarantine ended on 15th of June 2011, when boars were moved to the reproduction center. At the age of 6 to 8 months acclimatization to the phantom and training of semen collection started. After several successive semen collections, semen samples were diluted in commercial solvents and were sent to the test. The semen was used exclusively in their own nucleus farm and 5 commercial farms, with a total of 5000 sows for breeding. The first three analyses were performed in the average intervals of 30 days. Only the best quality characteristics of 3 consecutive analyses were

included in the processing. Third analysis was mostly used, because it followed the boar sperm maturation.

Flow cytometry (Guava Milipore-IMV, USA) was used to perform acrosome membrane and sperm membrane integrity test (combination of fluorometric colors PNA-FITC and propidium iodide) and chromatin structure test (SCSA-Sperm Chromatin Structure Assay, with acridine orange).

Concentration, total and progressive motility and velocity parameters of sperm movement were determined by the computer analysis of semen (ISAS-Integrated system for the analysis of semen, Proiser, Spain). Ratio of live/dead spermatozoa, a finding of intact acrosomes, protoplasmic droplets and total forms of pathological spermatozoa were determined by cyto-morphological examination of supravital smears stained according to Bloom (direct microscopy was done using Olympus BX 40 with phase-contrast microscopy at 1000x magnification, under immersion, with 100x lense). On the basis of cyto-morphological classification according to *Jovičin et al. (1997)*, chromosome damage by *Evenson et al. (2002)* and internal classification by number of progressively motile sperm in a dose, the semen was grouped into I, II, III or "out of class."

Results and Discussion

Forty analysed boars were of average age of 11.3 months (339.3 days), when three times consecutive analysis was finished. It was considered that there is sufficient data for the relevant assessment of breeding values of boars and monitoring of the maturation process. Out of the three consecutive analyses for each boar, most favourable result was taken for the further processing, as a sign of progression of spermatogenesis. The average volume of ejaculate was 167.8 mL, of which were produced 16.8 doses, on the average (Table 1).

Table 1. Age of boars during the execution of the analysis (days and months), ejaculate volume and number of produced doses

N = 40	*Age at analysis*		*Ejaculate Volume (ml)*	*No. of doses produced*
	in days	i*n months*		
Mean	339.3	11.3	167.8	16.8
St. Dev.	84.6	2.8	70.7	7.7
Max.	502	16.7	362	36
Min.	233	7.8	58	5

Table 2 presents the results on the number and motility of spermatozoa obtained using CASA. Recommendation for a farm is to use a minimum of 1.5×10^9 progressively motile sperm in the dose, i.e., a total of 3×10^9 sperm per dose. There are substantial variations in the percentage share of mobile and progressively

motile sperm, which indicates the uneven quality of semen and possibility of its stratification by mobility.

Table 2. CASA (Computer Assisted Sperm Analysis) results of young boars semen

N = 40	*No. of sperm.* (per ml)	*Total No.of sperm.* (per *dose)*	*No. of motile sperm.* (per ml)	*Total No. of motile sperm.* (per dose)	*% of motile sperm.*	*% mobile progressive spermat.*	*No. of mobile progressive* (per ml)	*Total No. of mob. progr.* (per dose)
Mean	46.8	4680.4	35.3	3527.4	74.8	36.5	18.1	1723.9
St. Dev.	13.2	1321.5	12.6	1259.4	15.0	10.6	9	707
Max.	94.5	9454.1	64.8	6476.5	92.5	56.5	52.5	4290.7
Min.	26.1	2611.6	4.3	428.3	12.7	2.4	0.8	80.8

By cyto-morphological examination of supra-vital stained semen according to Bloom, relatively high percentage of total live spermatozoa (79.6%) (79,6±16,1) was established (table 3). However, the percentage of sperm with damaged acrosomes (∑DA=10.1), protoplasmic droplets (∑PPD=11.4), and total forms of pathological spermatozoa (∑TA=15.2) is relatively high, with high standard deviations, indicating a lack of stable sperm production, i.e., the layering of young boars in terms of semen quality and varying degrees of their readiness for full exploitation. If the classification of semen is performed using the presence of living sperm with intact acrosome *(Jovičin et al., 1997)*, then 21 boars (52.5%) can be classified as the first class (LIA=≥60%), 10 boars (25%) can be classified as the second class (LIA=46-59%), 2 boars (5%) as the third class (LIA=40-45%), while as much as 7 boars (17.5%, LIA≤39%) can be classified outside the class.

Table 3. Different sperm subpopulations in esoine-nigrosine supravital stained semen samples (semen citology)

Number of samples	*Detection of specific subpopulations of spermatozoa (%)*							
	Total	*Normal sperm morphology*				*Abnormal sperm morphology*		
N = 40	*Live spermatozoa*			*Total, live and death*				
	∑ L	LIA	LDA	∑ DA	∑ PPD	I PA	II SA	∑ TA
	1.	2.	3.	4.	5.	6.	7.	8.
Mean	79.6	57.9	1.8	10.1	11.4	12.9	2.3	15.2
St.dev.	16.1	18	2.5	7.4	8.6	13.1	3.2	15.5
Max.	95	87	11	34	33	61	16	73
Min.	14	6	-	2	1	2	-	2

Legend: *Sperm subpopulations:* % L-*Total live*; LIA–*live with intact acrosome*; LDA-*live with damaged acrosome*; PPD-*protoplasmic droplet*; I PA-*primary abnormalities*; II SA-*secondary abnormalities*; % TA-*total abnormalities*

Fluorescein isothiocyanate, conjugated with the bean agglutinin (FITC-PNA, in the final concentration of 3 mg/mL) and propidium iodide (PI; final concentration of 2.5 mg/mL) was added to the 4μL of diluted semen for testing of the membrane integrity of spermatozoa and acrosome. This test is an excellent indicator of the stability of membarane, especially in sensitive, immature acrosomes, or the existence of genetic defects of acrosomes (such as knobbed acrosome).

Table 4. Sperm membrane and acrosome integrity assay of young boars by flow citometry

N = 40	%LIA	%DIA	% LDA	%DDA	∑ L	∑ DA
Mean	67.9	9.4	13.6	8.9	81.5	22.2
St.dev.	13.6	8.7	9.8	4.6	10.1	13.1
Max.	86.8	45	50	19.7	94.7	68.9
Min.	28.6	1	0.26	0.6	46.4	0.2

Legend: *Sperm subpopulations:* % LIA-*live sperm. with intact acrosome*; % DIA-*dead sperm. with intact acrosome*; % LDA-*live sperm. with damaged acrosome)*; % DDA-*dead sperm. with damaged acrosome*; % L-*total live*; DA-*total damaged acrosome*

SCSA test indicates incomplete maturation of sperm, which may be due to the existence of a series of physiological and environmental factors *(Martinez, 2005)*. Mean SCSA values for the 40 analysed young boars indicates a relatively high degree of chromatin damage (9.4%) with 8 boars (20%) having more than 15% of spermatozoa with damaged chromatin. *Waberski et al. (2011)* analysed 692 samples of semen from 79 boars on the day of sampling. The mean chromatin damage was 2.6%, with fluctuations from 0.2 to 48.8%. *Boe-Hansen (2008)* argues that damage to chromosomes which is over 2.1% already exerts a negative effect on the number of piglets born alive, while damages over 20% result in litters of 6.4 piglets.

Table 5. Results of Sperm Chromatin Structure Assay (SCSA) of young boars

N = 40	*Chromatin structure (%)* SCSA test	
	Intact	*Damaged*
Mean	90.3	9.4
St.dev.	9	9
Max.	99.4	39.6
Min.	60.5	0.6

Table 6. Distribution of sperm chromatin damage in young boars semen

% of damaged chromatin structure	*Chromatin structure (%)*			
	No. of boars	*(%)*	$\bar{x}$	SD
0-3	11	27.5	1.6	0.8
4-15	21	52.5	7.9	3.4
15-30	6	15	20.04	1.9
>30%	2	5	37.3	3.2

Analysis of boars with poor quality semen: During the testing of semen of 40 young boars, significant difference was observed in the quality of semen in 4 young boars, which do not meet the technical criteria of regular production, even after the third consecutive control. These are semen samples of Landrace boars (marked as L1, L2 and L3) and the Duroc breed (D1).

The diluted semen of boars L1, L2 and L3, showed the absence or reduced total or progressive motility (CASA analysis results, Table 7). Progressive motility ranged from 1.3% to 13.1%.

Boar D1 showed complete absence of spermatozoa in the smear and CASA analysis (azoospermia).

Boar L3, despite the slightly better progressive motility, detected by CASA system, had an extremely low average curvlinear speed for moving spermatozoa (VCL=41μm/s, video documentation of L3, (Pictue 3). Average VCL for the 40 boars was 62.8 μm/s. In the analysis of 308 semen samples Didon (2008) recorded VCL of 128.88 μm/s.

In boar L1, there was agglutination of 60% of spermatozoa (Picture 1), and of 30% in the boar L2 (Picture 2).

Table 7. CASA results of reclaimed boars (Computer Assisted Sperm Analysis)

Boar No.	*No. of sperm.* (per ml)	*Total No.of sperm.* (per *dose)*	*No. of motile sperm.* (per ml)	*Total No. of motile sperm.* (per dose)	*% of motile sperm.*	*% mobile progressive*	*No. of mobile progressive* (per ml)	*Total No. of mob. progr.* (per ml)
1. L1	18.4	1837.0	3.5	347.5	18.9	7.0	1.3	129.3
2. L2	16.5	1646.1	9.8	981.2	59.6	40.8	6.7	671.8
3. L3	23.9	2394.5	19	1901.6	79.4	54.7	13.1	1309
4. D1	a z o o s p e r m i a							

According to the cyto-morphological analysis, 52% of L1 boar spermatozoa had bent or broken connecting part, with or without protoplasmic droplets, a total of 78% of pathological forms of different shape (photo-documentation, (Picture 4).

L2 boar was ranked outside the class (24% of live sperm with intact acrosome). Acrosome damage was dominant (∑DA=27%, of which 12% belonged to the so-called knobbed acrosoms) (micro-photographs, Picture 5). *Thundathil et al. (2000)* suggest that the occurrence of these acrosome defects is highly correlated with reduced fertility. Sperm with a knobbed acrosome has no possibility of binding to the zona pellucida of the egg cell, but has preserved mobility. It is also hereditary defect (autosomal recessive sex-linked gene on chromosome 15, *Kopp et al., 2008*). The flow-cytometry PNA-FITC/PJ test detects these damages (Table 9).

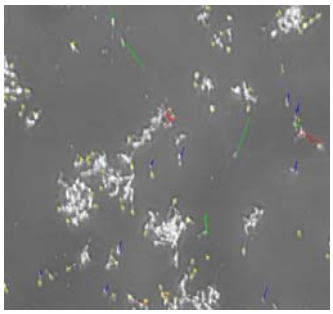
Based on the analysis of cyto-morphological smear, boar L3 was also classified as outside of the class because of a small number of live sperm with intact acrosome (LIA = 29%).

Table 8. Spermatozoa subpopulations in esoine-nigrosine supravital stained semen samples of reclaimed young boars (semen citology)

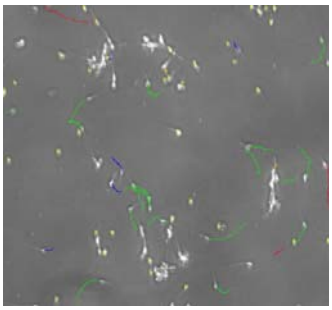
Boar No.	*(Detection of specific subpopulations of spermatozoa (%)*								*Semen class*
	Total	*Normal sperm morphology*				*Abnormal sperm morphology*			
	Live spermatozoa				*Total, live and dead*				
	∑ L	LIA	LDA	∑ DA	∑ PPD	I PA	II SA	∑ TA	
	1.	2.	3.	4.	5.	6.	7.	8.	
1. L1	71	13	2	5	4	58	20	78	out of class
2. L2	28	24	1	27	2	8	-	8	out of class
3. L3	44	29	2	25	10	8	-	8	out of class
4. D1	a z o o s p e r m i a								out of class

Table 9. Results of Sperm Chromatin Structure Assa, sperm membrane and acrosome integrity of reclaimed boars′ semen

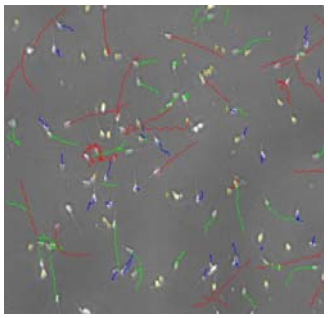
	1. *Chromatin structure* (%)		2. *Sperm membrane and acrosome integrity* (%)					
Boar No.	*Intact*	*Damaged*	%LIA	%DIA	% LDA	%DDA	∑ L	∑ DA
1. L1	62.9	37.1	31.7	2.3	27.0	39	58.7	66
2. L2	91.6	8.4	49.2	1.8	24.3	24.7	73.5	49
3. L3	66.9	33.2	61.7	12.4	12.6	13.3	74.3	25.9
4. D1	a z o o s p e r m i a							



Picture 1: Boar L1 (60% agglutinations)



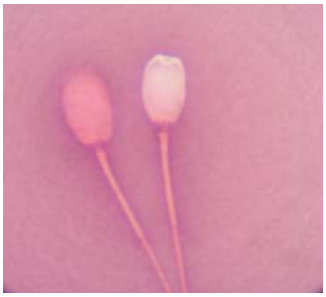
Picture 2: Boar L2 (30% agglutinations)



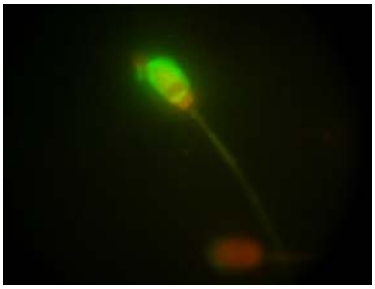
Picture 3: Boar L3 slow and medium speed

Figures 1-3. CASA recording and processing of boars semen samples (red path-rapid sperm, green-medium, blue-slow and the yellow pat-static)

Picture 4: Bent principal piece, boar L1



Picture 5: Knobbed acrosome, boar L2



Picture 6: PNA-FITC/PJ, boar L3

Figures 4-6. Spermatozoa subpopulations in esoine-nigrosine supravital stained semen samples of reclaimed young boars and the flow-cytometry PNA-FITC/PJ test

Conclusion

This paper describes the import of 40 breeding boars on which the semen quality control was performed by computer analysis and flow cytometry. Based on these analyses, satisfactory semen quality was found in 33 boars, 3 boars have poorer quality, which can be compensated by increasing the number of spermatozoa in a dose and 4 boars did not meet the technical standards for semen production. Recommendation for the Reproduction center would be not to use sperm of those 4 boars for artificial insemination before the quality of semen is improved, since it does not meet the technical standards. As a contribution, written reports are submitted, reports from CASA, microphotographs with specific findings of spermatozoa and CASA sperm motility film. Given that this is also a combination of genetic defects, the recovery is uncertain.

Today, there are modern methods, assisted with electronic technology, for assessing the fertilizing capacity of semen. Repeated and cross-check control and use of several methods, allow the possibility of reliable determination of fertilizing ability of boars.

For successful import of breeding animals it is necessary to make a quality purchase agreement, with a clause which provides and defines the possibility of claims in the case when parameters of the fertilizing capacity of semen do not provide regular exploitation of boar, which cannot be determined or guaranteed in the moment of export.

For the exploitation of breeding animals it is necessary to early determine semen quality and evaluate its fertilizing ability, to document it with the original reports, both in Serbian and in English (with an attached photo documentation). Reactions of importers should not be delayed, non-valid, or that claim becomes unused.

In this case, the importer, i.e., the client who ordered the testing, based on the analyses results and documentation, has submitted a claim for boar semen which did not meet technical standards. The exporter has, based on the submitted documentation of performed tests, accepted the complaint and delivered new breeding boars.

Uvoz nerastova – kontrola semena i mogućnost reklamacije

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Rezime

Srbija se svrstava u red zemalja sa kontinuiranim uvozom priplodnih nazimica i nerastova. Nerastovi se uglavnom uvoze u dobi od 4 do 6 meseci, u periodu kada se kvalitet priplodnjaka ne može pouzdano utvrditi usled polne nezrelosti (prepubertetsko ili pubertetska dob nerastića).

U ovom radu opisan je postupak i rezultati kontrole kvaliteta semena kod 40 mladih nerastova iz uvoza, kao i način dokumentovanja razloga za pokretanje postupka reklamacije. Kod sumnjivog kvaliteta semena potrebno je izvršiti najmanje 3 uzastopne kontrole u razmaku od po mesec dana, kako bi se donela konačna ocena o kvalitetu semena i upotrebljivosti nerasta za priplod. Od 40 uvezenih nerastova, 4 su reklamirana (10%) usled: azoospermije (1 nerast), odsustva ili smanjenje ukupne ili progresivne pokretljivosti, uz prisustvo aglutinacija spermatozoida (2 nerasta), i povećanog broja patoloških formi spermatozoida (78%; 1 nerast). Kod dva nerasta zabeležen je povećan udeo spermatozoida sa nestabilnom strukturom hromatina (SCSA test - 33,2% i 37,1%).

Za pokretanje reklamacije neophodno je imati kupoprodajni ugovor koji predviđa mogućnost reklamacije, priznate metode kontrole semena kao i izgrađen poslovni odnos poverenja zainteresovanih strana.

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