

Andrological examination of Hungarian Large White and Landrace boars

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SUMMARY

The Hungarian Large White and Hungarian Landrace pig breeds have outstanding lifetime performance, production parameters and cross-breeding ability. Nevertheless during recent decades these breeds could not compete with the West European hybrid pigs concerning on production results. In this study we assessed and andrologically examined boars in Hungarian nucleus breeding stocks. After taking blood and semen samples, performing GnRH challenge, ultrasonic and thermographic examinations were done. Laboratory tests were accomplished in reproduction labs of NARIC ABNMS. Our goals were to determine reproductive performances of boars and collecting samples for future genetic examinations.

Keywords: Hungarian Large White, Hungarian Landrace, reproduction

ÖSSZEFOGLALÁS

A magyar nagyfehér és a magyar lapály sertések nagyon jó életteljesítménnyel, termelési paraméterekkel bírnak, ezen kívül keresztezési vonalként kiemelkedő szerepet játszanak. Az elmúlt pár évtizedben a magasabb termelésre képes nyugat-európai hibridek túlszárnyalták teljesítményben e két hazai sertésfajtát. Kísérletünk célja az volt, hogy törzstenyészetekben felmérjünk és andrológiai szempontból megvizsgáljunk magyarországi nagyfehér és lapály kanokat. A spermavétel, vérvétel, ultrahang vizsgálat, hőkamerás vizsgálat, GnRH teszt után a NAIK ÁTHK laboratóriumában vizsgáltuk meg a mintákat és értékeltük eredményüket. Célunk az volt, hogy a kanok szaporodásbiológiai teljesítményét értékeljük és a későbbi genetikai vizsgálatok céljára mintákat gyűjtsünk.

Kulcsszavak: Magyar nagyfehér, Magyar lapály, szaporodásbiológia

INTRODUCTION

Swine plays important role regarding in food supply as well as in economical point of view. During recent decade cc 40% of world's meat consumption has been the pork (Xiaodong et al. 2012). The pig went through a strong selection in the course of domestication. Worldwide there are several swine races resistant to different environmental factors e.g. cold and hot temperature, many pathogens and diseases. Therefore those animals can adapt easily to the extreme conditions where they live (Chen et al. 2007). For all breeders it is very important to achieve the best results of production. One of the most prominent features is the reproductive performance. The economic activity can be significantly influenced by the reproductive success. It was detected that cc 30% of total loss was originated from reproductive problems (Stalder et al. 2004). The Hungarian Large White and Hungarian Landrace reportedly have very good life span performance, production parameters and cross-breeding ability. Nevertheless during recent decades these breeds could not compete with the West European crossbreeding pigs concerning on production results.

Development of the Hungarian Large White was beginning in the early 1900s when the predecessors were the English Large White, the German Large White, the English Middle White and the Swedish Yorkshire. The herd-book was established about 100 years ago. This breed belongs to the Breedgroup 1 and the main goal is to increase reproductive performance, the growth potential, and the feed conversion rate, maintaining robustness, good adaptability, increasing stress-tolerance and outstanding meat quality (Horn 1976, Horn et al. 2011).

The Hungarian Landrace is registered from the second half of the 20th century. It has good adaptability, stress tolerance and sensitive to the housing method. Outspoken breeding goal is to improve reproductive outcome and lean meat yield. Maternal line is either pure bred or crossed by Large White. (Horn 1976).

The reproductive analysis of boar

Sperm quality assessment

For successful artificial insemination all quantity and quality parameters of the semen and sperm cells must be known. Xu et al. (1998) found that the litter size can be influenced by sperm number per

ejaculate. Moreover number of normal sperms in the ejaculate would have influence on litter size. Alm et al. (2006) demonstrated that the farrowing rate is strongly affected by sperm number per ejaculate. Both studies declared the fertility was lower if the semen dose contained lower sperm number in the course of insemination. Wekerle et al. (1985) investigated the morphology of 30 boars' semen at different parts of the testis and epididymis after castration. Following dilution, and making smear they studied the ratio of semen abnormalities. The results of the study showed that hair pin tail had largest number in the epididymis while in the testis the number of this abnormality was lower. The migration of the cytoplasmic droplets went off in the head of the epididymis and the selective resorption took place in the tale section of the epididymis. By microscopic examination of semen we can get clear information of abnormalities, the cell membrane integrity, and acrosome status. These three parameters are indispensable to assess the fertility ability (Donadeu 2004). Actually the most practical method to assess the semen motility is using light microscopy or phase contrast microscopy. This method is highly depending on the personal experience of researcher running the analysis (Vyt et al. 2004). For the mentioned reason beside the visual evaluation automated evaluation systems (CASA – Computer assisted semen analysis) have been also developed for proper evaluation. The system provides detailed information on the movements of the spermatozoa, shows the progressive motility, hyperactivity and various sub-populations of sperm within an ejaculate (Verstegen et al. 2002, Rijsselaere et al. 2003).

GnRH test

Reduced fertility or infertility can be estimated by examination of boar semen. The individual testosterone production in each animal significantly influences their reproductive performance even within a day so single sampling does not provide accurate results. However the testosterone response to GnRH treatment reflects for hormone production of boars (Sarlós et al. 2006). GnRH has a high influence on the reproductive function of animals. Post et al. (1987) figured out that response of testosterone has a significantly positive correlation with the fertilizing ability of individuals. Einarsson and Larsson (1980) tested Large White and Landrace boars for blood testosterone levels and they found the testosterone peak 2-4th hour post GnRH. Sarlós et al. (2006) studied Mangalica boars and found that testosterone concentration after GnRH did not reach the same high peak than other breeds. Wekerle et al. (1989) applied a new GnRH which resulted an increased testosterone concentration in peripheral blood. The relationship between blood testosterone content and the sizes of the testis in boars has already been described by Schinckel et al. (1984) and Schnaider et al. (1988).

Ultrasonic examination

Ultrasonography is a non-invasive imaging technology based on ultrasound effect giving the chance to visualise the internal structures of the body e.g. tendons, muscles, vessels, organs. In this way experienced professionals can locate abnormalities. Introducing of this technology to human and veterinary practice started in early 80's (Goldberg and Kimmelman 1988). There are several imaging mode (A-mode (Amplitude mode), C-mode, M-mode (Motion mode), Doppler mode, Pulse inversion mode, Harmonic mode) but the most generally imaging mode is the B-mode (Brightness-mode). It is also called 2D mode but already 3D ultrasound is available for veterinarians and just recently started introduction of 4D to the human health institutes (Cobbold 2007). Coulter and Bailey (1988) found ultrasonography a non-invasive tool for checking the testicular and epididymal function of beef bulls. Ultrasound did not affect the percentage of progressively motile spermatozoa, primary sperm defects, secondary sperm defects or normal acrosomes, scrotal circumference, testicular consistency, paired epididymal weight.

Thermographic examination

Infrared thermography is detecting infrared energy emitted from an object, converting it to apparent temperature, and displaying the result as an infrared image. It is a non-invasive technology that has been used to indicate thermal biometric changes in animal metabolism resulting from increased body temperature and changes in blood flow in response to environmental or physiological conditions. This method can be a useful device and general stress indicator as well as indicate inflammatory processes, pain and disease (McManus et al. 2016).

MATERIAL AND METHODS

In the present study andrological and genetical features of Hungarian Large White and Hungarian Landrace pigs are investigated. After it genomic analyses is planned, as well. On the basis of the expected results our aim is to improve the breeding potential of the pigs and to promote the further breeding activity of Hungarian breeders throughout modern genetic and reproductive examinations. 18 blood and sperm samples of 4 nucleus breeding farms were collected. The collection procedure was made by the veterinarian and the inseminator of the farm. Following blood collection each boar was administered by Gonadotropin-releasing hormone (GnRH) (in vein, 50 microgram Fertagyl iv. Intervet

International B.V., Boxmeer, Netherlands) injection. 90 minutes after GnRH application blood was repeatedly collected to determine the production of testosterone of testis and the relationship between the testosterone serum concentration and fertility (*Table 1*).

Table 1

Farms	Collected samples											
	Blood samples (n)		Ejaculates (n)		US		GnRH		CASA		Thermography	
	HLW*	HL**	HLW	HL	HLW	HL	HLW	HL	HLW	HL	HLW	HL
A	3	4	3	4	3	4	3	4	3	4	3	4
B	3	1	3	1	-	-	-	-	3	1	-	-
C	-	-	3	2	5	-	-	-	3	2	5	-
D (other breeds)	2	2	2	2	2	2	2	2	-	-	2	2
Total	13		18		14		9		16		14	

* Hungarian Large White

** Hungarian Landrace

The table includes 18 samples of animals which were taken on the nucleus breeding farms. We had not possibility to complete every test because of the preceding checks before visiting the breeding farms. At some farms neither GnRH injection nor blood sampling I allowed nor to use the ultrasound machine and the thermographic camera. The “D” farm is marked with red colour because we only practiced and acquired the basic method there (on other breeds).

During semen collection ultrasonic photographs were taken to estimate the morphology of testis (*Figure 1*) and thermographic photographs (*Figure 2*) for assessing the heat balance of the testis. The infrared camera can show the temperature with different colours of the whole body and refers to inflammatory areas. The white colour shows the warmest areas and the blue one the coldest.

Figure 1: Ultrasonic tissue pattern

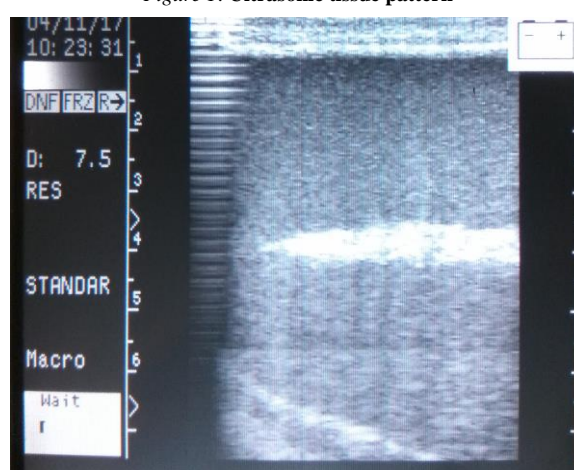
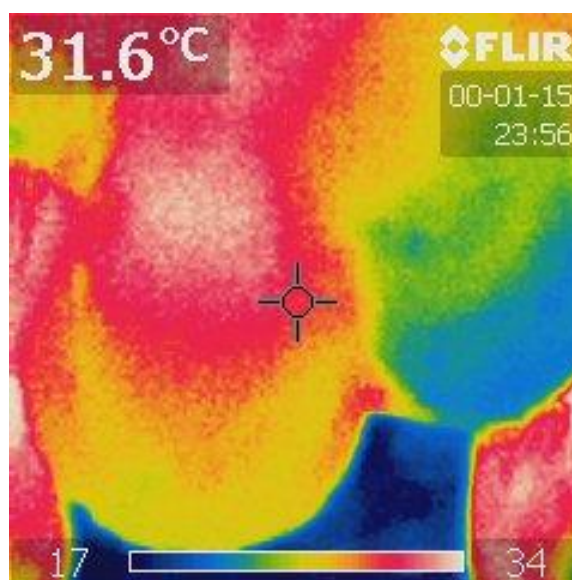


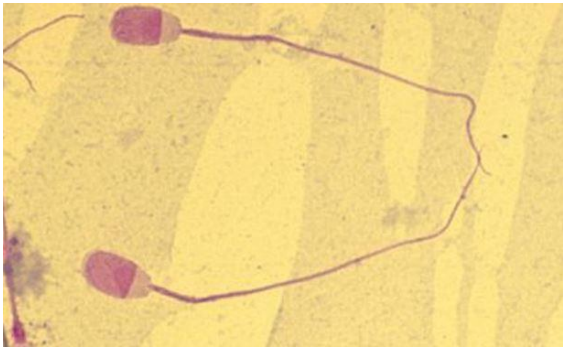
Figure 2: Assessing the heat balance of testis



The laboratory work i.e. analysis of spermatozoa morphology, evaluation of the live/dead and acrosome status on stained smear (Kovacs-Foote staining method), investigation of semen motility by CASA (Computer Assisted Semen Analysis) program was performed at Breeding and Reproduction Research group, Research Institute for Animal Breeding, Nutrition and Meat Science, National Agricultural Research and Innovation Centre. Using CASA was right after the samples transport from the farms to the institute. The transport temperature was 16 °C. After dilution we measured the motility of the sperms (*Figure 5, Figure 6*). We used regular microscope slide to count about 100 cells. The cells was categorized in three category (Non-motile: sperm are not moving. Non-progressive motile: sperm are moving but not going anywhere. Progressive motile: sperm are moving and are getting somewhere).

Kovacs-Foote staining method was made right after the blood samples transporting to the institute. This method can show the live and dead spermatozoa, the acrosome status and abnormalities of the cells. It can examine on stained smear of the semen (*Figure 3*).

Figure 3: Kovacs-Foote staining method



RESULTS AND DISCUSSION

The analysis of the thermographic images was performed using FLIR thermal imaging program. The *Figure 4* shows the minimum and the maximum temperature the whole area and the AR01 which is the assigned area (with pink line) goes to show that the warmest area of the testis is 33 °C and the coldest is 27,1 °C. The mean temperature (the testis) is 30,1 °C.

Figure 4: An evaluation with FLIR Infrared Camera Software

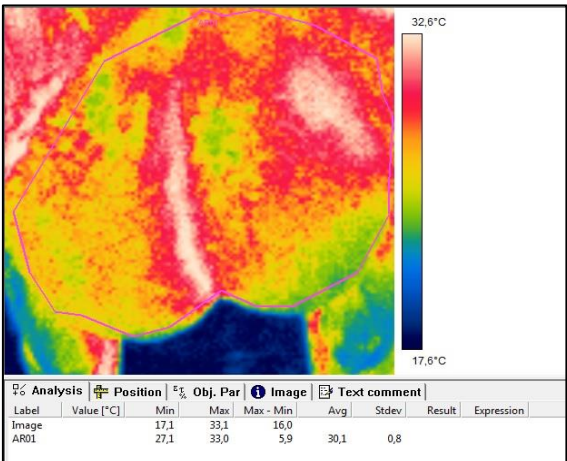


Table 2 shows minimum, maximum and mean temperatures of the testis in each animal at farm “A”. Between the items there are not raised differences. The identity number 2 Hungarian Large White’s testis heat balance evaluation can be seen on the Figure 3.

Table 2

Evaluation with FLIR Infrared Camera at the farm "A"

ID number of animals	breed	Temperature (°C)		
		min.	max.	mean
1	HLW*	27,7	33,6	30,5
2	HLW	27,1	33	30,1
3	HLW	24,3	33,8	29,9
201	HL**	27,2	36,2	31,2
204	HL	27,1	33,1	29,5
205	HL	25,7	33,8	28,8
206	HL	25	32,4	28,9

*Hungarian Large White

**Hungarian Landrace

The evaluation of ultrasonic pictures was done with GIMP (GNU Image Manipulation Program) pixel graphic image editing program. The echotexture was checked for morphological inhomogeneity. Figure 5 shows normal ultrasonic image of the testis with regular echo pattern including mediastinum testis (M). The GIMP pixel graphic image editing program creates a histogram (Figure 5 on the right) what is useful for evaluation of the average grey level of the ultrasound image. Due to the 256 grey levels of the ultrasonic images not just the average grey levels are interesting for image evaluation but also the different white (dense tissue) and/or black (fluid accumulation) spots as well. Zero represents black while 255 refers for the whitest level. As the Figure 5 shows the mean value is 181 on the measured area. Table 3 shows mean, dispersion and median values of the Farm “A” animals.

Figure 5: An Evaluation with GIMP pixel graphic image edition program

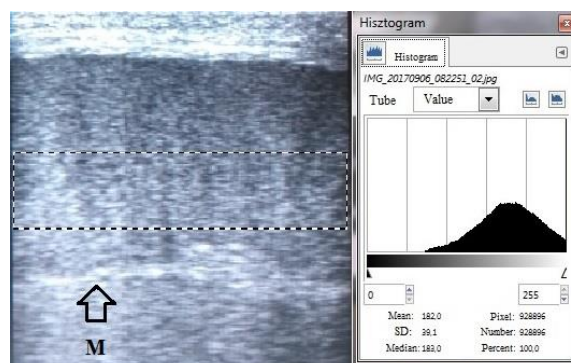


Table 3

Evaluation with GIMP pixel graphic image edition program at the Farm “A”

ID number of animals	breed	Grey level (GIMP)		
		mean	SD	median
1	HLW*	188,5	38,8	191
2	HLW	182	39,1	183
3	HLW	187,4	32,9	196
201	HL**	205,1	33,4	207
204	HL	210,3	18,9	211
205	HL	194	19,4	194
206	HL	197,6	32,6	200

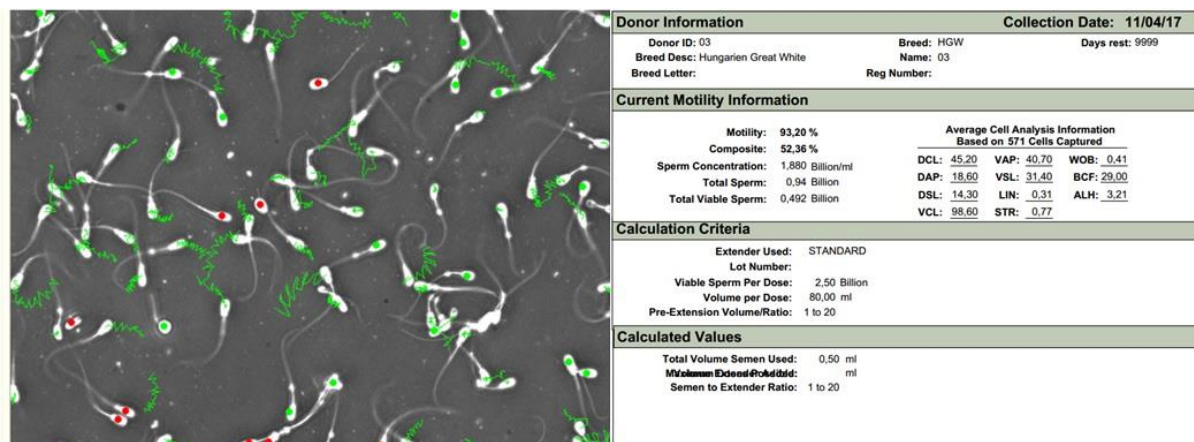
* Hungarian Large White

** Hungarian Landrace

All ejaculates of the animals were analysed for motility by CASA software (AndroVision, Minitüb, Germany). The software automatically sign the cells by different colours: green means motile cells while red refers for non-motile (Figure 6 on the left). The software generates a report about the measure parameters (motility, composite, sperm concentration, total sperm, total viable sperm, Velocity Average

Path (VAP), Distance of Average Path (DAP), Straight Line Distance (DSL), Curvilinear Distance (DCL), Wobble (WOB), Velocity curvilinear (VCL), Velocity straight line (VSL), Linearity (LIN), Beat cross frequency (BCF), Straightness (STR), Amplitude of lateral head (ALH)) (Figure 6 on the right).

Figure 6: Evaluation of spermatozoa motility



During the evaluation of sperm cells with Kovacs-Foote staining method nine parameters were noticed (live, intact acrosome; live, intact and proximal droplet; live, intact and distal droplet; live, intact and abnormal tails; live, damaged acrosome; dead head and live tail; live head and dead tail; dead, damaged and exploded acrosome; detached head or tail). Table 4 shows the absolutely live, dead cells and the other parameters per farms.

Table 4

Kovacs-Foote staining method results							
	Hungarian Large White (3 samples/farms) (%)			Hungarian Landrace (different samples/farm) (%)			
Farm "A"	a	b	c	a	b	c	d
live	37	40	66	60	69	41	65
dead	42	35	19	21	16	45	25
other abnormalities	21	25	15	19	15	14	10
Farm "B"	a	b	c	a	b	c	d
live	15	54	15	6			
dead	9	17	0	45			
other abnormalities	76	29	85	49			
Farm "C"	a	b	c	a	b	c	d
live	54	30	49	52	83		
dead	12	17	38	14	11		
other abnormalities	34	53	13	34	6		

With the Kovacs-Foote staining method dead cells, sperm cell abnormalities were found but there were also good samples with low cell mortality. The abnormalities can come among others from the time of the transporting, the weather, and the dilution. There were some samples which contained many abnormal cells so in the future samples should be repeatedly taken.

Unfortunately there was no adequate amount of samples with Gonadotropin-releasing hormone (GnRH) for the correct evaluation.

CONCLUSION

Following examination of the reproductive organs of boars and evaluation of samples no serious abnormalities were found by ultrasound in the testicular tissue or scrotal thermoregulation by thermography even in ejaculates by CASA. With the Kovacs-Foote staining method the quantity of cells was examined. There were also dead and live spermatozoa but were not extreme abnormalities. Blood and semen samples are stored on -20°C until genetic SNP chip analysis. The samples collection was inhomogeneous because there were some farms where there was no opportunity to make the complete method. To the correct comparative evaluation

between farms more places should be found where every necessary data can be collected for a completely homogeneous database.

For complete the present study more boars and collected samples should be included for further genetic examination in order to demonstrate scientific evidence of association between reproduction performance and genetic markers in Hungarian Large White and Hungarian Landrace boars.

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