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PRESERVATION OF NATIVE SHEEP AND TURKEY BREEDS WITH MOLECULAR GENETIC METHODS

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Abstract

Molecular genetic lab of the University Debrecen Centre of Agricultural Sciences was established in 2002, and it moved to the Centre of Life Sciences in 1 Sept. 2005. The main aim of our Institute is the gene preservation in autochthonous Hungarian breeds. We have experience in estimation of genetic difference with sheep, cattle and goose. However important studies are performed to improve milk-, meat production of different breeds, species (sheep, turkey, cattle, goat), QTL mapping in sheep and cattle, seasonality in sheep, semen quality in sheep and disease resistance in goat. Selection of positive gene carrier individuals could be easier, faster and more efficient because of rapid development of biotechnology, molecular genetics methods and techniques. Continuously we extend our knowledge and experiments on different breeds, subjects, methods.
In this paper we shortly summarize our gene preservation study in sheep and turkey breeds from the last 2 years.

Keywords: breeding , molecular genetic methods, sheep, turkey

Gene Preservation and Maintaining of Native Bronze Turkey

In 2007, 10 Native Bronze Turkey lines were kept at our experimental station. Number of examined individuals are presented in Table 1.

Table 1.

Number of examined animals in the 10 lines and in the control, Wide Breath Bronze Turkey populations

Native Bronze Turkey lines/breeds	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	Wide Breath Bronze
N	23	18	30	23	17	21	20	10	12	17	13

Altogether 191 individuals were studied from 10 lines. As control, 13 Wide Breath Bronze Turkies were examined. Genomic DNAs were extracted from blood for the molecular genetic study.

For genetic diversity study 16 microsatellite markers were choosen (Table 2). They are in different chromosome, or far from each other in the same chromosome.

Table 2

Main characters of the selected microsatellite markers

Name	Chromosome	Repeats	Sequence of Primers ('5-'3)	Primer dye
MNT162	M6	(TG)12	AAAAACCTGAAAATGTAAATCCA TTCAGATCTTTTATTTTTCGAAGC	FAM
ADL0292	M5	-	CCAAATCAGGCAAAACTTCT AAATGGCCTAAGGATGAGGA	FAM
MNT106	M18	(CA)14	TGCAGTGTGAATATTGGCTTG AAATAATGAAGACACCGACATTTTC	FAM
MNT174	M1	(TG)14	AAAATTCAGTCCCCCAGAGG CTCAGGATGCAAGCCTTCTC	FAM
MNT197	M8	(CT)22	GCTTACGGAGATAAGAGCTTTGG CCACATTGCAGAGGGTCAC	VIC
MNT387	M14	(GT)19	AAGCGTTCCATCTGTTTTGG TTCCTAGCCTCTCATCTGTGC	VIC
ADL0272	M16	-	TATGGTAAGGTGAGCAAACC GGGAAAGCTATGAAAGATTT	VIC
ADL0149	M12	-	ATAGCATACACCCAGCCACC GAATAAGAATGTTNCCCTGC	VIC
MCW0080	M13	-	CCGTGCATTCTTAATTGACAG GAAATGGTACAGTGCAGTTGG	VIC
ADL0293	Gga17-M12	-	GTAATCTAGAAACCCCATCT ACATACCGCAGTCTTTGTTC	PET
MNT199	M3	(CT)13	AGCTTCCTATTCAAGAGTTTTGG AGTCCAAGACCAGCCACCAG	PET
ADL0266	M4	-	GTGGCATTCAGGCAGAGCAG AATGCATTGCAGGATGTATG	NED
MNT332	M15	(CA)24	TTGGTCAACATTTGGAAGACC ATTAGCTAACAGCTGCAAAATGG	NED
MNT192	M26	(CA)15	ATTGGTCAGGGTGCCAATAG AGCACATTGCAGTTGTTTGC	NED
MNT214	M4	(TG)26	GCCATGAATGTCAAAAGGAAC GGGTGAGCCTGGGTAGAATG	PET
MNT327	M24	(CA)12	TTGTGTTATGCAAGTAAAAGCATC GGCTAACCAGAGCTTCATGC	VIC

PCR conditions were determined by gradient PCRs. On the most efficient annealing temperature PCR reactions were done, and its amplicons were runned on ABI 310 genetic analyzator in order to check its quality. Two multiplex groups were created to save time and decrease expenses.
After the optimalization of each microsatellites, genotypes were determined of individuals.

In case of 4 markers (ADL0266, ADL0293, MCW0080, MNT174) optimalization were not successful, but they were considered in statistical study. Genetic structure, inbreeding, genetic distance among Native Bronze Turkey lines were estimated using allele and genotype frequency data. PopGene32 program was used for statistical evaluation.

One of the main character of genetic diversity of population, breed is the rate of polymorphic loci. It shows how many locus have more than one allele among the examined locus. This value is 100 % in examined 10 Na-

tive Bronze Turkey lines, and Wide Breath Turkey population. It means that both 15 microsatellites were polymorphic. Altogether 53 allele were determined on 15 locus in the examined lines.

The most polymorphic locus was MNT162 with 6 alleles (Table 3), however the less polymorphic were (2 allele): ADL0266, ADL0293, ADL0149, MCW0080. Beside the detected number of allele per locus, the effective number of alleles was determined as well. This data considers that alleles are presented in different probability in mating because of their different frequencies. The effective number of alleles is closer to the detected number of allele when the allele frequencies are similar to each other on the same locus.

Table 3.

Detected and effective allele number per locus

Locus	Sample Size	na*	ne*	I*
ADL0266	192	2	1.7413	0.6169
ADL0292	412	5	1.1968	0.3992
ADL0293	132	2	1.0624	0.1358
MNT106	412	3	2.2934	0.9513
MNT162	414	6	3.6376	1.3687
MNT199	378	3	2.4439	0.9613
ADL0149	398	2	1.3953	0.4572
MCW0080	196	2	1.0960	0.1863
MNT174	278	3	2.7718	1.0560
MNT192	408	5	2.8190	1.1393
MNT197	414	4	1.6589	0.7825
MNT214	396	4	1.8082	0.7009
MNT327	412	4	1.3595	0.4988
MNT332	412	5	1.3691	0.5899
MNT387	412	3	1.8777	0.6730
Mean	351	3.5333	1.9021	0.7011
St. Dev		1.3020	0.7482	0.3482

* na = detected number of allele
* ne = effective number of allele [Kimura and Crow (1964)]
* I = Shannon's Information Index [Lewontin (1972)]

Allele frequenciy values differentiated very much. 4 and 5-5 alleles were detected in MNT327, MNT332 and ADL0292 locus, respectively. Among these alleles, one allele was presented with high frequency (84-91%), while the left ones frequencies were very low. Among microsatellites with two alleles (MCW0080, ADL0149 and ADL0293) our results were the same. One of allele frequency was significantly higher than the other allele frequency. This result could be very important in breeding strategy, because breeders have to keep in breeding those individuals whose have rare alleles.

The expected heterozygosity value (H_{exp}) is calculated from alelle frequency value in a population which is in Hardy-Weinberg equilibrium. The observed heterozygosity value (H_{obs}) is the real rate of heterozygosite individuals in the examined populations. The ratio between the two values is able to show the value of difference from genetic balance of examined population (Table 4.).

Table 4.

Heterozygosity values per locus

Locus	Sample Size	Obs_Het	Exp_Het*	Nei**	Ave_Het
ADL0266	192	0.4479	0.4280	0.4257	0.3456
ADL0292	412	0.1553	0.1649	0.1645	0.1680
ADL0293	132	0.0000	**0.0592**	0.0588	0.0330
MNT106	412	0.5291	0.5653	0.5640	0.4791
MNT162	414	0.7053	**0.7268**	0.7251	0.6452
MNT199	378	0.6931	0.5924	0.5908	0.5655
ADL0149	398	0.3116	0.2840	0.2833	0.2814
MCW0080	196	0.0918	0.0881	0.0876	0.0809
MNT174	278	0.6187	0.6415	0.6392	0.5804
MNT192	408	0.5686	0.6469	0.6453	0.5952
MNT197	414	0.3816	0.3982	0.3972	0.3841
MNT214	396	0.3737	0.4481	0.4470	0.4121
MNT327	412	0.2379	0.2651	0.2644	0.2321
MNT332	412	0.2427	0.2702	0.2696	0.2485
MNT387	412	0.3107	0.4686	0.4674	0.3691
Mean	351	0.3779	0.4031	0.4020	0.3613
St. Dev		0.2154	0.2096	0.2091	0.1893

* Expected homozygosty and heterozygosity were computed using Levene (1949)
** Nei's (1973) heterozygosity value

Mean expected heterozygosity was 0.4031 on the 15 locus. The lowest value was 0.0592 on the ADL0293 loci, while the highest was 0.7268 on the MNT162 loci. The expected heterozygosity value was higher than expected in all markers except on ADL0266, MNT199, ADL0149 and MCW0080 markers.

Mean observed heterozygosity was 0.3779 on the all examined locus. The lowest value was 0.000 on the ADL0293 loci, while the highest was 0.7053 on MNT162 loci. Important note, that 30 % of the total individuals were successfully genotyped with ADL0293 marker!

Mean observed heterozygosity values for lines variated between 0.3198-0.4263, while mean expected heterozygosity values were between 0.3234 and 0.4406. Line 3, 5, 6 (Native bronze Turkey) and 11 (Wide Breath Bronze Turkey) were less heterozygosity than it was expected. The most homogenous line was the line 5, the less homogenous was the line 9. The difference between the two values was very low in all locus, and it could mean that effects (drift, selection etc.) which have influence on rate of heterozygosity were already in the populations.

Genetic distance matrix were calculated using Nei standard genetic distance values (Table 6).

Table 6.

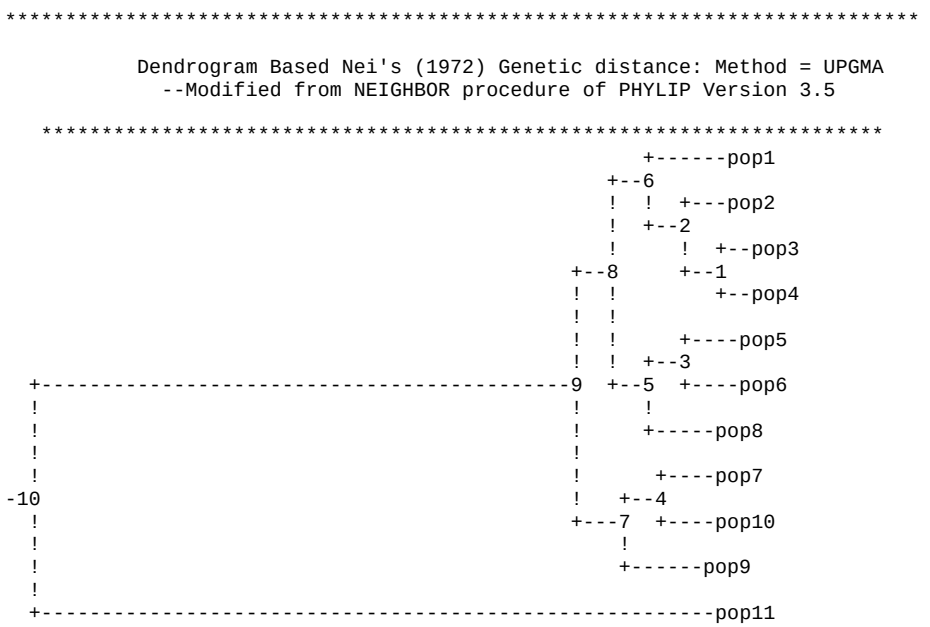
Nei’s genetic distance among examined lines, populations

pop ID	1	2	3	4	5	6	7	8	9	10	11
1	****	0.9500	0.9578	0.9779	0.9572	0.9478	0.9344	0.9667	0.9068	0.9267	0.7146
2	0.0513	****	0.9732	0.9791	0.9356	0.9415	0.9304	0.9482	0.9368	0.9332	0.6679
3	0.0431	0.0272	****	0.9802	0.9382	0.9571	0.9437	0.9578	0.9532	0.9593	0.7275
4	0.0224	0.0211	0.0200	****	0.9422	0.9572	0.9371	0.9661	0.9426	0.9543	0.7041
5	0.0438	0.0666	0.0638	0.0595	****	0.9722	0.9372	0.9667	0.8912	0.9150	0.7046
6	0.0536	0.0603	0.0438	0.0437	0.0282	****	0.9343	0.9702	0.9327	0.9462	0.7071
7	0.0678	0.0721	0.0580	0.0649	0.0649	0.0680	****	0.9623	0.9560	0.9693	0.7748
8	0.0338	0.0532	0.0432	0.0344	0.0339	0.0303	0.0384	****	0.9440	0.9510	0.7350
9	**0.0978**	0.0653	0.0480	0.0591	**0.1152**	0.0697	0.0450	0.0576	****	0.9656	0.7366
10	0.0761	0.0692	0.0416	0.0468	**0.0888**	0.0553	0.0312	0.0503	0.0350	****	0.7668
11	0.3360	0.4036	0.3181	0.3508	0.3501	0.3466	0.2551	0.3079	0.3057	0.2656	****

*Nei’s genetic identity (above diagonal) and genetic distance (below diagonal)

All examined lines were separated from control, Wide Breath Bronze Turkey populations. The line 5 and 9 (0.1152) seemed to have the largest genetical differences from each other. Compare to the other lines, Line 1 and 9 (0.0978), and Line 5 and 10 (0.0888) were also genetically far from each other. This relations are presented on the Figure 1.

Figure 1.: Nei's genetic distance dendogramm among examined lines and the distance



Distance between two points

10	9	12.92424
9	8	0.77472
8	6	0.55181
6	pop1	1.94694
6	2	0.73807
2	pop2	1.20886
2	1	0.20679
1	pop3	1.00207
1	pop4	1.00207
8	5	0.89347
5	3	0.19460
3	pop5	1.41067
3	pop6	1.41067
5	pop8	1.60527
9	7	1.27343
7	4	0.43923
4	pop7	1.56080
4	pop10	1.56080
7	pop9	2.00003
10	pop11	16.19770

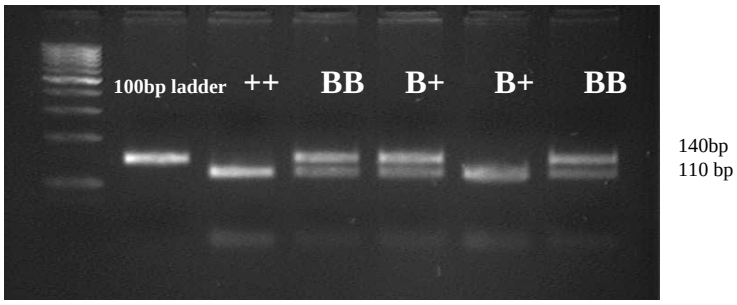
Aim of our work was to know the genetic relation, background of Native Bronze Turkey lines, populations in order to prevent alleles with low frequencies, or distribute rare alleles.

Study on Debrecen Fecundity Sheep breed

Altogether 52 Fecundity Merino were genotyped for Booroola (FecB) gene using PCR-RFLP method.

FecB genotype means detection of point mutation (SNP) in BMPR-1B receptor. To detect this mutation –adenine/guanine- Wilson et al (2001)'s protocol was used. 140 bp long PCR product was amplified what contained AvaII restriction enzyme site. After digestion with AvaII enzyme, homozygote carriers gave 110bp long, heterozygote 110 bp and 140 bp long while homozygote non-carriers gave 140 bp long products. These fragments were separated with agarose gelelectrophoresis (Figure 2).

Figure 2: PCR-RFLP with FecB genotypes



Genotype distribution of examined 52 individuals were the following:
11 ind. non carriers, ++ genotype;
28 ind. heterozygosite, B+ genotype;
13 ind. homozygosite, BB genotype.

These results were used in selection of lambs.

Study of Cokanski Tsigai Population

32 Cokanski Tsigai were examined in this study. 2.5-3 ml blood samples were taken from vena jugularis and they were stored in tubes with EDTA at -20°C until the start of experiment. Genomical DNAs were extracted from blood using Zsolnai and Orbán (1999) method. Following microsatellites were used in the study (Table 7.).

Table 7.

Used microsatellites, and their main characters

Chromo-some	Name	Length (bp)	Label	Forward sequence of primer (5'-3')	Reverse sequence of primer (5'-3')	Reference
1	BM6506	184-212	JOE	GCACGTGGTAAA-GAGATGGC	AGCAACTTGAG-CATGGCAC	Bishop et al, 1994
2	OarFCB20	92-118	NED	AAATGTGTTTAA-GATTCCATA-CAGTG	GGAAAACCCCCAT ATATACCTATAC	Buchanan and Crawford, 1992
4	MAF70	128-175	FAM	GCAG-GACTCTACGGGGC CTTTGC	CACGGAGTCA-CAAAGAGTCA-GACC	Crawford et al, 1992
5	MCM527	150-185	FAM	GTCCATTGCCTCA AATCAATTC	AAACCACTTGAC-TACTCCCCAA	Hulme et al, 1994
8	INRA127	181-215	JOE	CTA-CAGCTCTGATGA-GAACC	CGTTTTCTCAAACT TCATTGCC	Vaiman et al, 1994
9	ILSTS11	180-296	JOE	GCTTGCTA-CATGGAAAGTGC	CTAAAATGCA-GAGCCCTACC	Brezinksky et al, 1993
12	TGLA53	114-143	FAM	CAGCA-GACAGCTGCAA-GAGTTAGC	CTTTCA-GAAATAGTTTGCA TTCATGCAG	Crawford et al, 1995
14	TGLA357	113-154	FAM	GCA-GAGTCTGAGTTTA AACTTCTCTAACA CC	GAGGGCAAAAAGG TTTGGGGTGTATG G	Georges and Massey, 1992
15	MAF65	116-140	FAM	AAAGGCCAGAG-TATGCAATTAG-GAG	CCACTCCTCCTGA GAATATAACATG	Buchanan et al, 1992a
17	OarCP49	88-140	NED	CA-GACACGGCTTAG-CAACTAAACGC	GTGGGGAT-GAATATTCCTTCAT AAGG	Ede et al, 1995
19	OarAE119	98-160	FAM	CTCAG-CAAATGGTTCCTG GGCACC	TTTTA-TAGTGAGGTGAC-CACTTGATG	Penty et al, 1993
21	OarCP20	88-195	NED	GATCCCCTGGAG-GAGGAAACGG	GGCATTTCATGGC TTTAGCAGG	Ede et al, 1995
22	BM1314	136-176	FAM	TTCCTCCTCTTCTC TCCAAAC	ATCTCAAACGCCA GTGTGG	Bishop et al, 1994
23	MAF35	104-122	NED	AGTTA-CAAATGCAAG-CATCATACCTG	TCAA-GAATTTTGGAG-CACAATTCTGG	Swarbrick et al, 1991
25	MCMA7	228-270	JOE	ATCAGTCCTTCA-CAAGGTTG	CCTGTTGCTATGTC ATGTTG	Beh et al, 2000
26	CSSM43	237-273	JOE	AAAACTCTGGGAA CTTGAAAACTA	GTTA-CAAATTTAAGA-GACAGAGTT	Moore et al, 1994

ABI 9700 and MJ Research Thermocycler PCRs were used for amplifications. ABI PRISM 310 Genetic Analyzer was used for allele detection. GeneMapper 3.7 software (Applied Biosystems) was used for data analysis.

On the 16 examined locus altogether 142 allele were detected. The less allele (3) was detected on MAF 35 loci, while the most was detected (16) on ILSTS11 loci (Figure 3.).

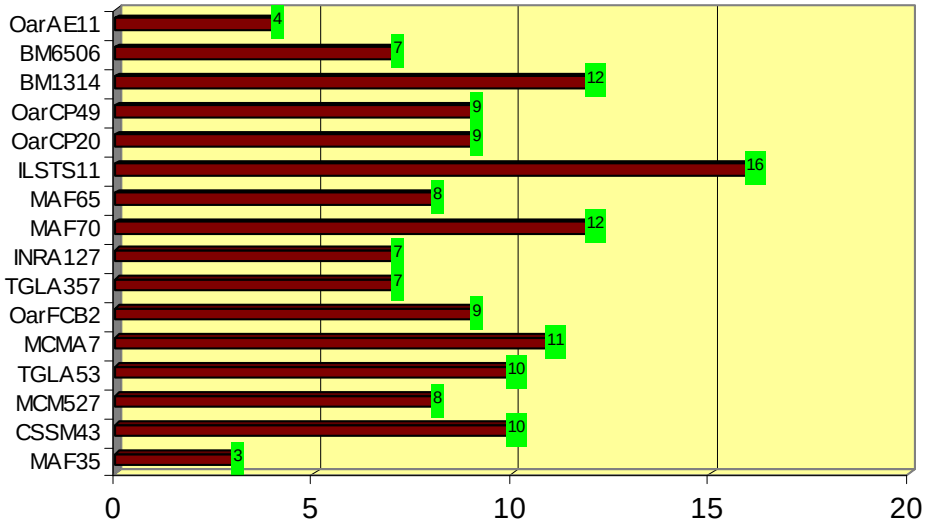


Figure 3.: Number of detected allele on the examined locus

Allele frequencies on the MAF35 and ILSTS11 locus were presented on the Figure 4.,5.

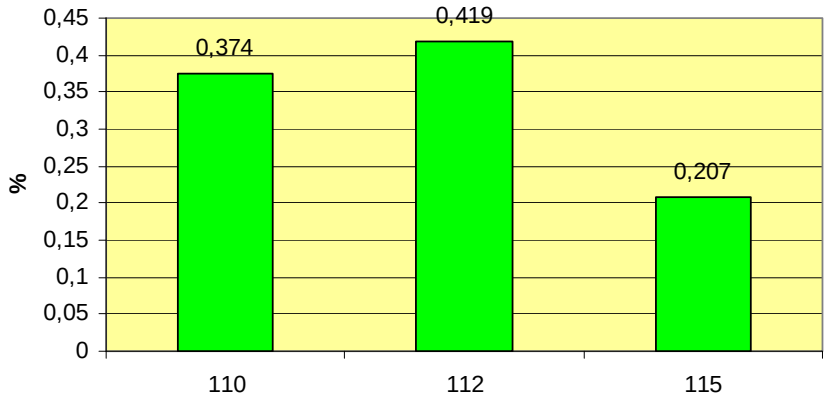


Figure 4.: Allele frequency on MAF35 loci

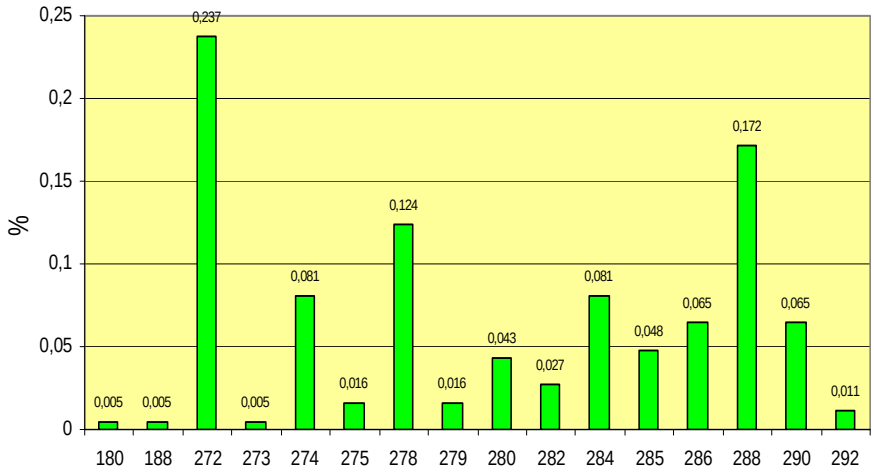


Figure 5.: Allele frequency on ILSTS11 loci

Mean expected heterozygosity value on the 16 locus was 0.746. The lowest value was 0.561 on the BM6506, the highest value was 0.882 on the ILSTS11 locus. The expected heterozygosity value was higher than observed heterozygosity value in all locus, except on MAF35.

Mean observed heterozygosity value on the 16 locus was 0.518. The lowest value was 0.226 on the MAF65, the highest value was 0.677 on the ILSTS11 locus.

3 population specific alleles were detected on the examined locus. They are on two locus: ILSTS11-180 and 188 alleles; OarCP49- 97 allele. One of this allele was detected in 5 individuals from the examined 32. Preservation of these alleles would be very important, because they can specify populations.

Study of Debrecen Fecundity Merino population was performed fast and efficient with this microsatellite set. We have to avoid inbreeding, to loose genes and to maintain the genetic variability.

Study of Rams Semen Continously in Every Season with Kovács-Foote Dying Method

In this study semen from 6 Debrecen Fecundity Merino and 9 Tsigai were examined.

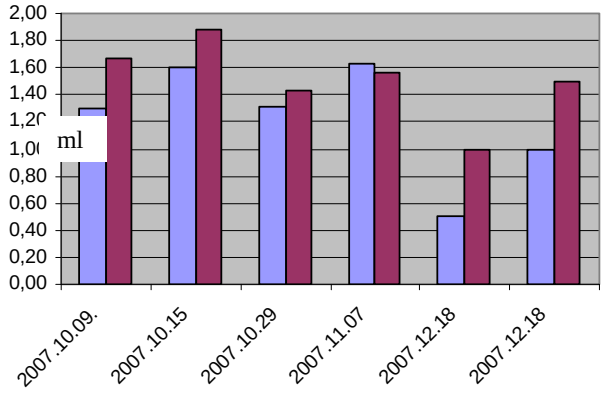
Taking and frozen semen was from 09. October 2007 til 07. November 2007. This is the main breeding season. During the proseason, one time (18. December, 2007.) semen was taken two times, after each other.

In our Institute's Laboratory quantity, density, moving, living percentage and pH of fresh semen were measured

Kovács and Foote (1992) method was used for dying living/died spermium and acrosome. After dying OLYMPUS BX61 microscope and DP71 camera was used for analysis. Nagy et al. (1999) method was used for estimation of samples.

According to our results quantity of ejaculatum from Tsigai rams during the main breeding season was much more than from Debrecen Fecundity Merino (Figure 6.).

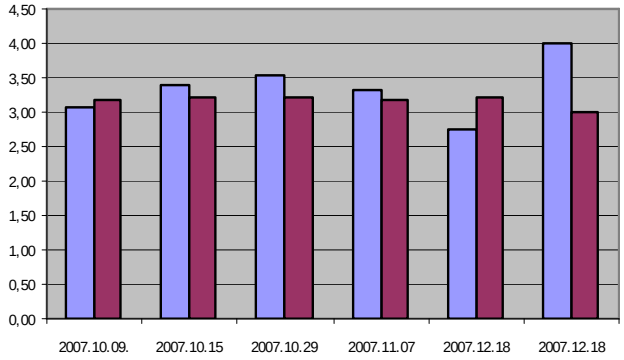
Figure 6.
Quantity of ejaculatum in different time in the Debrecen Fecundity Merino and Tsigai breeds



Blue: Debrecen Fecundity Merino; Purple: Tsigai

Density of fresh ejaculatum were more homogenious in Tsigai than in Debrecen Fecundity Merino (Figure 7.).

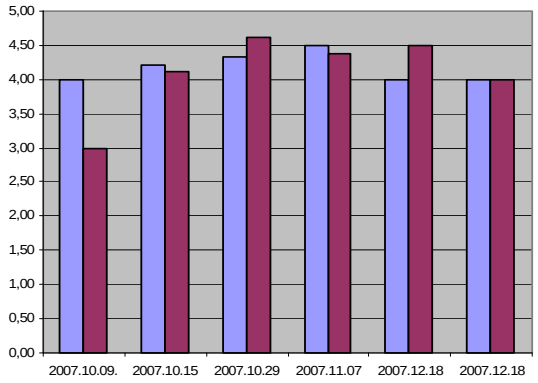
Figure 7.
Density of ejaculatum in different time in the Debrecen Fecundity Merino and Tsigai breeds



Blue: Debrecen Fecundity Merino; Purple: Tsigai

In fresh ejaculatum there was no difference between two breeds in moving of semen (Figure 8.).

Figure 8.
Moving of semen in fresh ejaculatum in different time in the Debrecen Fecundity Merino and Tsigai breeds

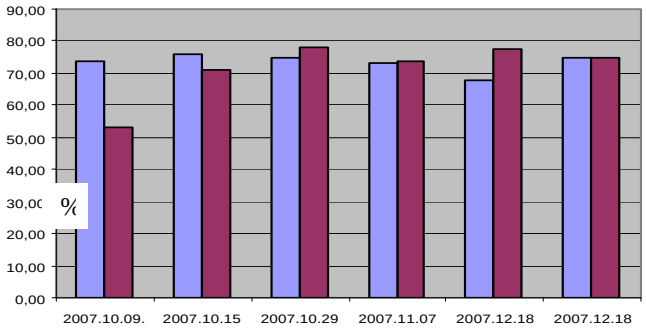


Blue: Debrecen Fecundity Merino; Purple: Tsigai

Analysis of samples were performed with 1000fold immersion objective.

There was a high difference in ratio of living and dead cell in semen from rams from different breeds and individuals. The amount of living cells in Tsigai rams semen were acceptable amount only from the end of October (70%), while living cells in Debrecen Fecundity Merino rams semen were 70-75% all the examined time (Figure 9.).

Figure 9.
Ratio of living cell in semen in different time in the Debrecen Fecundity Merino and Tsigai breeds



Blue: Debrecen Fecundity Merino; Purple: Tsigai

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