

Thesis of doctoral (Ph.D.) dissertation

REPRODUCTIVE SEASONALITY IN VARIOUS GENOTYPES OF FEMALE SHEEP

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1. Premises and objectives of the thesis

The successful management of the Hungarian sheep industry can be done by having an accelerated domestic production and presenting the Hungarians' interest at the professional boards of the European Union effectively in the future.

Presently the most important product is lamb for slaughter in Hungary, the quality and also quantity of producing meat depends on the reproductive pattern of female sheep. There are studies worldwide to evaluate more influencing factors on the ovarian physiology of sheep, and also to find proper breeds for improving the indexes of productivity.

The features of reproductive biology are influenced by genetical factors, nutrition, housing system and the daily sunlight hours. The animal husbandry is using several methods as light programs, hormone treatments, which are criticised from some aspects of human food safety and also animal welfare. Using appropriate breeds of sheep can result in production and the timed lambing corresponding to the market demands. Nowadays the hormone treatments have spread all over the world in the animal husbandry for controlling the reproductive pattern. While the artificial methods for influencing the natural way of ovarian cycles as extending the breeding season artificially are open to criticism, the farmers' fundamental interest is not using any of those methods.

There are advanced examinations and procedures available for evaluating the ovarian activity in various breeds of sheep. We all should use these techniques in order to find the appropriate breeds we are able to produce more economic with.

Using the proper breeds and crossing them with the Hungarian traditional ones could help to accelerate the Hungarian sheep industry serving the domestic and the export demand anytime all over the year.

The examinations of my dissertation consist three studies for answering the declared hypothesis under the control of a sophisticated professional board.

1. Firstly I wanted to study the hairy and shedding wool sheep population of the University of Debrecen regarding to conception and lambing data from January of 2005 to June of 2010. It was essential to categorize the results of the reproductive performance of the hairy sheep based on the ovarian seasons of the other sheep's population, then I planned to compare the data with the results of Prolific Merino

and Tsigai breeds. There was an other purpose to evaluate some details according to the birth and weaning rates of the various genotypes as well as to determine the distribution of the seasonal birth and weaning results. I intended to get the data from 2008 and 2009 focusing to have details from similar periods of the year.

2. The cyclicity of ovarian performance was planned to follow by endocrinological investigations in order to evaluate the reproductive physiology of the recently imported breeds and their crossbred progeny.
 - a. Following the professional board's advice, I had protocol to collect blood samples from certain groups of sheep breeds and genotypes. Determination of the very first ovulation and also monitoring the ovarian activity were planned to be answered by analysing the individual blood plasma progesteron level.
 - b. Ultrasonographic method was given as possibility to measure exact details of the development of fatty layers at different anatomical points. Correlations were required to have looking for between the ovarian performance and the body conditions.
 - c. The meteorological parameters as the daily avarage temperature and the daily avarage humidity were headed to monitor permanently during the duration of examination at the certain group of the experimental animals.

2. Methods

The examination of the seasonal/aseasonal ovarian performance of hairy and shedding wool sheep, and the analysis of the reproductive indexes of woolly, hairy and crossbred genotypes

I have done my experiments at the University of Debrecen, Centre of Agricultural and Applied Economic Sciences, Faculty of Agricultural and Food Sciences and Environmental Science, Institute for Animal Husbandry. I have collected data regarding to lambings from January of 2005 to the end of June of 2010 analyzing the performance of two hair sheep, the Somali and Barbados Blackbelly, and a shedding wool sheep the Dorper. Nearby the hairy and shedding wool genotypes there are Prolific Merino and Tsigai breeds at the Experimental Farm of the UD which were set up as experimental materials for getting data from 2008 and 2009 to evaluate the weaning losses. I collected the numbers of born and weaned lambs of the hairy, woolly and crossbred breeds, the data according twins were evaluated as lambs pro mother index. The ewes got lambled by the same labourers, they were kept and fed at similar housing and nutrition conditions. Getting more exact results of the hairy and shedding wool sheep I evaluated their reproductive indexes seasonally deviding the year into three periods as mainseason, trailerseason, and out of season based on the shepherds' experiences in the reproductive seasonality of female sheep in Hungary.

The main season lasts from 20th of August to 31st of December, the trailer season is from 1st of March to 30th of April, and finally the period of out of season is the rest of the time of the year. The date of fertilisation had been determined individually from the dates of lambing calculating with the gestation period of 150 days. None of the ewes were treated for induction or synchronization of ovulation. Lambs were weaned at the age between 4-5 weeks and 3,5 months, when they achieved the min. weight of 14 kg.

The rams were exposed on ewes all over the duration of examination at the fullbred hair sheep flocks. Therefore the evaluation of the born / weaned indexes as well as weaning rates were essential in the different periods of the year. In the case of woolly x hairy crossbred and also the woolly breeds the results were summarized and evaluated yearly.

All of the details were led onto an excel document, in which the genotype of mother, the genotype of lamb(s), the lambing date, the number of lambs, and the weaning rates of the lambs were typed. Then the summarized weaning rates were calculated yearly in each group and also seasonally at hairy and shedding wool sheep.

Getting a simple method for presenting the results of the figures and tables I classified the genotypes into groups, „prolific merino” as Prolific Merino mothers and their progeny, „tsigai” as Tsigai mothers and their progeny, the Prolific Merino and Tsigai ewes crossing with hair rams and their progeny as „(woolly x hairy) F₁” divided into two more groups of „(prol. mer. x hairy)F₁” and „(tsigai x hairy) F₁”. Among the hairy and shedding wool ewes and their progeny I ranked two groups of „hair sheep concepted in breeding season” and „hair sheep concepted out of season”. Getting more details regarding to the number of twins of the groups, the hairy groups were evaluated without the mostly single-lambing Somali breed.

Reproductive performance of (Barbados Blackbelly x Dorper) F₁ crossbred ewes in the mirror of their body condition

This study was carried out to evaluate the age of puberty and also to monitor the ovarian cycles of (Barbados Blackbelly x Dorper) F₁ ewes born in July of 2008. Correlations were suspected in the body weight gains of ewes and the layers of lipid deposits and some meteorological factors versus their ovarian activity. Present study started in November 2008 and ended in June of 2009. The mothers of above mentioned ewes were fertilised and got lambled in out of season according to the terminology of the Hungarian sheep breeding. The average date of birth of the experiment ewes was 07.07.2008. ± 2.75 days. It was easy to decide whether the ewes have active ovarian cycles attended ovulation or not by taking blood samples regularly and analysing them. Above 3 nmol/L of progesterone hormone level of the blood samples showed the presence of corpus luteum.

I collected the blood samples from the 18th of November 2008 until the 8th of June 2009, initially two times a week, afterly once a week. Same genotype ram was exposed on ewes on May, one month before the end of experiment. Technically I took the blood by puncture on vena jugularis and inserting it. The progesterone profiles were presented in figures

consisting the P₄ concentrations and dates on each ewes. By analysing the luteal phasis of ovarian cycles on the diagrams it can be decided whether the ewes were in regular ovarian cycle having ovulations or they were in anoestrus.

The measurable body weight growth could be determined by caclulating the daily avarage weight gains. Ultrasonographic technique was applied to get more details about the body condition measuring the certain lipid depos of the ewes.

During the whole period of the study the Observatory for Agricultural Meteorology of the University of Debrecen served the temperature and humidity data.

SPSS for Windows software was used for evaluating the correlations between the body condition parameters and the ovarian performance of the experimental animals. The software was also used to calculate any correlations between the length of the biological breeding season and meteorological parameters.

The time of puberty of (Merino x Somali) F₁, (Merino x Barbados Blackbelly) F₁ and fullbred Hungarian Merino ewes

Present study was similar to the previous one. I wanted to examine further genotypes and get more details about the starting of the ovarian activity of ewes. The goal of this study was to find any significant differences between the puberty of the examined three genotypes.

In this experiment it was not possible to use the ultrasonographic techniques for measuring the body condition parameters. At the Farm of the Szűcs Juh Inc. located in a rural field of Mikepércs, Hungary, 27 ewes born in March- May of 2009 were involved in this study. The experimental animals were weaned and housed to the same place of the farm. (Merino x Somali) F₁ and fullbred Merino ewes had gained the weaning weight in June of 2008, however the (Merino x Barbados Blackbelly) F₁ ewes were able to be weaned in the middle of July as they were born later than the rest of the experiment ewes. At the time of making the experiment protocoll I intended at least 7 animals of each genotype to be involved. Therefore 10 (Merino x Somali) F₁, 7 (Merino x Barbados Blackbelly) F₁ and 10 fullbred Hungarian Merino ewes were involved in this experiment. Similarly to the previous study's method I took the blood samples once and twice a week from the animals, although only

one sample pro ewe from each week was analysed to determine the age of puberty of ewes. The animals were fed and housed at the same conditions. The P₄ profiles were not analysed because the certain phases of the ovarian cycle might have been missed by having results only once a week. The samples of each ewes had been examined until reaching the concentration of 3 nmol / L of plasma progesteron indicating the first ovulation of ewes.

Collection and storage the blood samples

I took the blood samples making punction on vena jugularis, and getting the blood into EDTA tubes. The taken blood samples were centrifuged for 8 minutes at 5100 G within 3 hours after taking. I got 1.5 mL of blood plasma tubes. I put the tubes into small plastic bags and indicated the eartag of ewes on it, and the bags were put to the deepfrost freezer (-20 °C).

Analysis of the progesteron concentration of blood plasma samples

The blood plasma samples were analysed at Szent Istvan University, Faculty of Veterinary Sciences, Department of Endocrinology.

The progesteron concentration was measured by MEIA method of Axsym analyzer. The machine is able to receive 150 frozen-thawed samples at the same time. The technicians and myself put the samples in date order. Before getting the automata hormon analyzer started I had to make sure the whole machine is washed and the controll samples are measured to get the exact results simultaneously. The Axsym machine displays the results of the examined sample on its monitor automatically. Then the results were typed to an exercise book for making evaluation and also creating graphs.

Examination of body tissues at different anatomical points

For getting ultrasonographic pictures, Falco 100 Pie Medical real time machine was used. The attachments of the machine were enclosed as ASP-type 18 cm diameters, 3.5 MHZ frequence ultrasonographic examination head. The pictures of different anatomical points

were recorded onto the hard-drive of a notebook computer, the evaluation was made by applying Ultrasond Engineer 3.0 software. The thickness of various lipid layers and the muscles can be determined from records with help of experts.

The interactions of meteorological factors and the biological breeding season of the ewes

The Observatory for Agricultural Meteorology of the University of Debrecen served all of the parameters as the daily average humidity and temperature. The observation station is located very close (50 meters distance) to the Experimental Farm of the UD. Its coordinates are north latitude of + 47° 34' 35.81 and east longitude of + 21° 34' 53.65.

3. Main results of the thesis

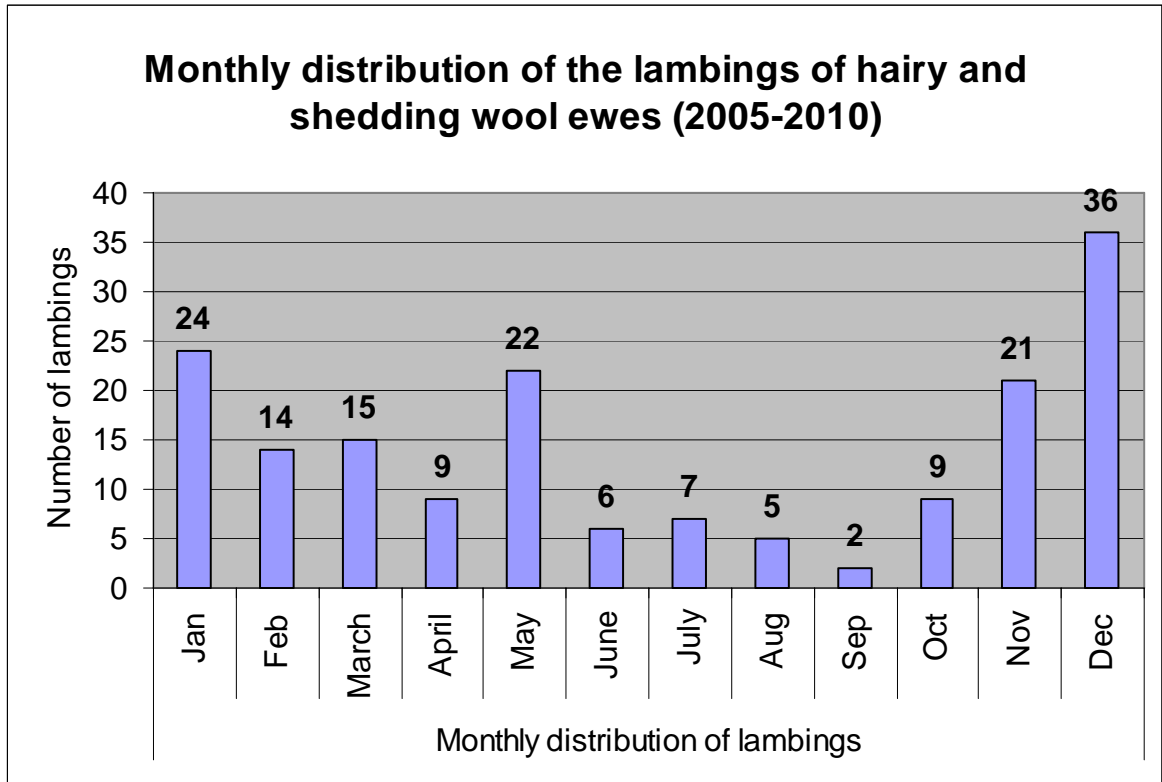
The results of the conception and lambings on Barbados Blackbelly, Somali, Dorper and their crossbreds ewes

The calculated statistical data of conceptions are showed in *Table. 1* categorically in different seasons, it summarizes all the conceptions of the hairy and shedding wool sheep kept at the Experimental Farm of the UD in the past semi-decade. Lambings from January of 2005 to the end of June is presented on *Fig. 1*.

Table 1.

The numbers of conceptions (N) estimating from the lambing dates (2005-2010), the average dates of conceptions (x), and the average square differences of deviation in days (s) devided in breeding-seasons		
Periods of estimated conceptions	Statistical data	
From 1 st of January to 28 th of February	N	13
	x	22 nd of January
	s	21.54
From 1 st of March to 30 th of April	N	7
	x	31 st of March
	s	6.60
From 1 st of May to 19 th of August	N	69
	x	16 th of July
	s	27.09
From 20 th of August to 31 st of December	N	81
	x	28 th of September
	s	44.86

Figure 1.



The weaning rates of Prolific Merino lambs

There were 147 lambings in the breed at the farm. The number of born lamb pro ewe index was 2.05, the number of the weaned lamb pro ewe was 1.07, the weaning rate was calculated as 52.49 % (*Table 2., Fig. 2.*).

The weaning rates of Tsigai lambs

186 lambings were registered over the two years of experiment. The number of born lamb pro ewe was 1.43, then the number of weaned lamb pro ewe was 1.16, the weaning rate of 81.20 % was computed (*Table 2., Fig. 2.*). The rams were not exposed out of season,

although 5 ewes got pregnant in that period of time accidentally, so ability of getting pregnant in March-April of Tsigai breed had been confirmed.

The weaning rates of crossbred woolly x hairy and woolly x shedding wool sheep

Crossbred lambs belonged to this group. The Prolific Merino and Tsigai ewes were mated by hair and shedding wool rams. As the rams were on ewes only in the main breeding season, I had details only from that period of time. Total of 80 lambings of Prolific Merino and Tsigai ewes were counted. The total average number of born and weaned lamb pro ewe were 1.60 and 1.35 respectively. The lambs of 84.38 % were weaned. The lambs born from Tsigai ewes had got much better weaning rate as 87.50 percent than the lambs born and mothered from Prolific Merino where the lambs of 81.94 % were weaned.

The weaning rates of fullbred and crossbred lambs mothered hairy and shedding wool ewes concepting in season and out of season

There were a total of 77 lambings registered at Dorper, Somali and Barbados Blackbelly ewes. The distribution of 38:39 conceptions were checked in the main season and in the out of season (**Fig. 2.**). In the trailer season no pregnancies were found in this group. The number of born and weaned lamb pro ewe were 1.26 and 1.14 respectively in average, it means 91.56 percent weaning rate in the hairy and sheeding wool genotypes. Suprisely, in the out of season higher birth and weaning numbers were found compared to the same parameters of the main breeding season. As the Somali ewes lamb mostly singles, weaning rates were calculated separately in this group.

The statistical analysis of the reproductive indexes of the examined groups

The weaning rates of hairy, wooly and crossbred lambs were analysed in SPSS for Windows ver. 15 statistically.

One sample T probe was applied. The weaning rates were analysed comparing to the theoretical value of 100 % which is equivalent of the number of born lambs. The results can be seen on the *Table 2*.

„Explore” menu of SPSS was run to analyse the standard error, the variance and the statistical significance of the data (*Table 2., Fig. 2.*).

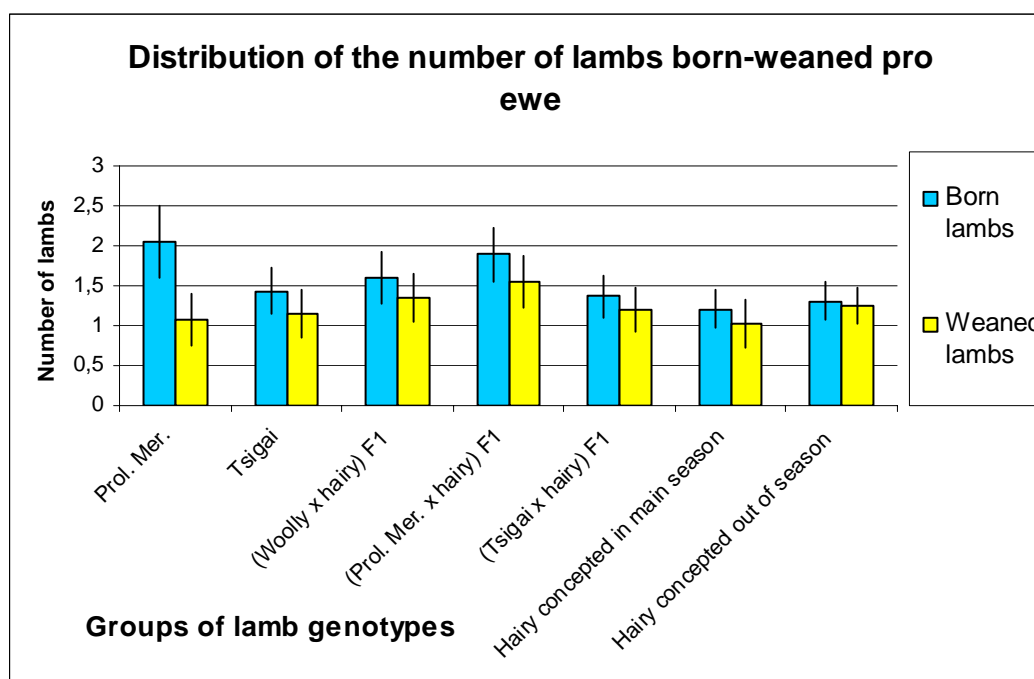
„Crosstabs” menu was run to calculate the single, and multi-twin lambings on each groups. At the group of Prolific Merino 43 single lambs were born, 103 lambings resulted twins from which 66 were twins, 27 were triplets, 10 were quadruplets, on the top of that, a lambing with quintuplet was experienced. 114 single lambings were summarised at the group of Tsigai breed, 72 lambings resulted twins, from which 64 were 2-twins and 3-twins were born eight times. The group of woolly and hairsheep, woolly and shedding wool sheep crossbred genotypes lambled singles 39 times and twins were counted 41 times. Separately, the Prolific Merino ewes lambled single lambs 10 times, twins 23 times, triplets 4 times and once quadruplet, at the same time Tsigai ewes lambled single lambs 29 times, twins 12 times, and once triplets born.

According the groups of hair sheep, the lambings of Dorper and Barbados Blackbelly ewes were analysed together, and the results of Somalis were handled an other file because they lamb mostly singles. In the main season, twins were born 7 times, in the period of the out of season 12 times.

Table 2.

The average weaning rates of live born and weaned lambs							
Groups of lamb genotypes	Prolific Merino	Tsigai	(Woolly x hairy) F ₁	(Prol. Mer. x hairy) F ₁	(Tsigai x hairy) F ₁	Hairsheep conceived in main season	Hairsheep conceived out of season
Lambs born alive	301	266	128	72	56	46	51
Lambs weaned	158	216	108	59	49	39	49
Weaning rates (%)	52.49	81.20	84.38	81.94	87.50	84.78	96.08
Statistics of weaning rates							
Standard error (SE%)	3.11	2.36	3.03	4.12	4.46	5.62	1.79
Standard deviation (SD%)	37.66	32.23	27.17	25.42	28.9	34.69	11.17
Significance (P)	<0.05	<0.05	<0.05			<0.05	<0.05

Figure 2.



The results of the reproductive performance of (Barbados Blackbelly x Dorper) F₁ ewes in the mirror of their body condition and in relation with meteorological parameters

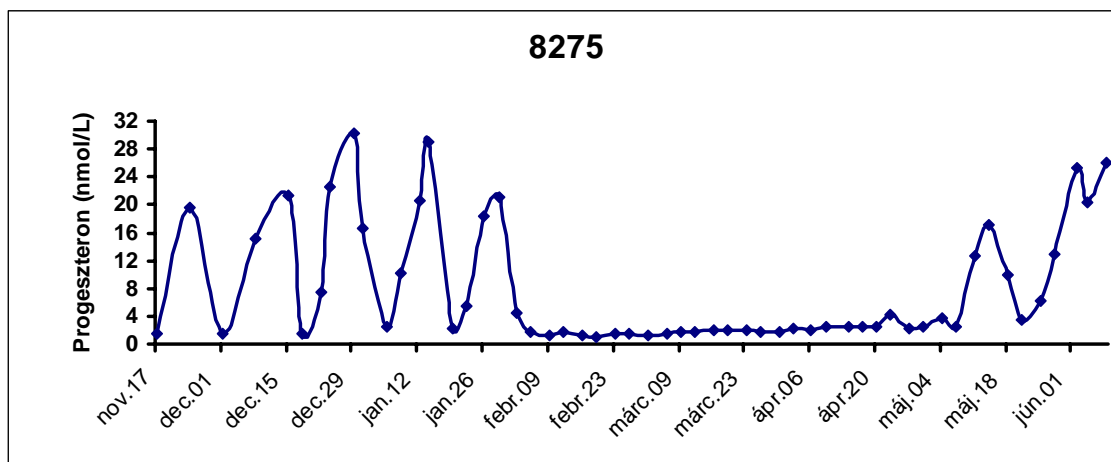
All of the ewes had in oestrus in the duration of the study. Two of them – eartaged 8275 and 8278 - showed impressive long biological breeding season (**Fig. 3.**, **Fig. 4.**), however there were ewes with long-time anoestrus, too. I did not get answer for the first question of the study as the puberty of the ewes had been gone when I began to collect the blood samples and every ewes were in ovarian cycle attended by ovulation. It reflects to the fact that the experiment ewes have their puberty at their age of 3.5-4 months. The days of breeding seasons were able to be declared by checking the oestrus cycles on the progesteron profiles of the ewes.

The most regular ovarian cycle was showed by the eartaged 8275 ewe, where the active ovarian performance was found by the end of January, then it was in anoestrus for 2.5 months, afterthen it began to show oestrus in the beginning of May. After releasing the ram it got pregnant from its second ovulation (**Fig. 3.**). The ewe eartaged 8278 also had very regular ovarian cycles followed by one of the other, then it was in anoestrus, endly it was able to be mated in May (**Fig. 4.**). The eartaged 8273 ewe had irregular cycles. At the end of December only 1 short luteal phase was experienced, then it had acyclia, then some short luteal phases were seen its P₄ profile, subclinical endometritis was supposed. The body condition parameters of this animal were below the average (**Fig. 5.**). Two normal and regular oestrus cycles can be seen on the P₄ profile of the ewe eartaged 8274, where acyclia was experienced from the middle of December. CL presence was recognized with very low progesteron level at the end of April, followed by a regular cycle, then it was acyclia again. At the ewe eartaged 8277 had only one regular cycle in the duration of the monitoring time, although it showed P₄ level reflecting an ovulation had been happend. Certainly, the results of the ultrasonographic parameters intercorrelate with the reproductive performance. The eartaged 8280 animal was the third one among them who got pregnant in the middle of June. Until the end of December the ewe had very emphasized ovarian cycles, from January it experienced anovulation period for a while, then two cylcles were showed in May when the second one resulted in pregnancy. The seventh experimental animal eartaged 8281 showed one of the worst oestrus cycles, correlated with thin fatty layers on the back,

moreover the muscle of gluteus medius was behind the expectation. Only one regular ovarian cycle was monitored in May, and an other ovulation may have been happend in the beginning of June based on progesteron level.

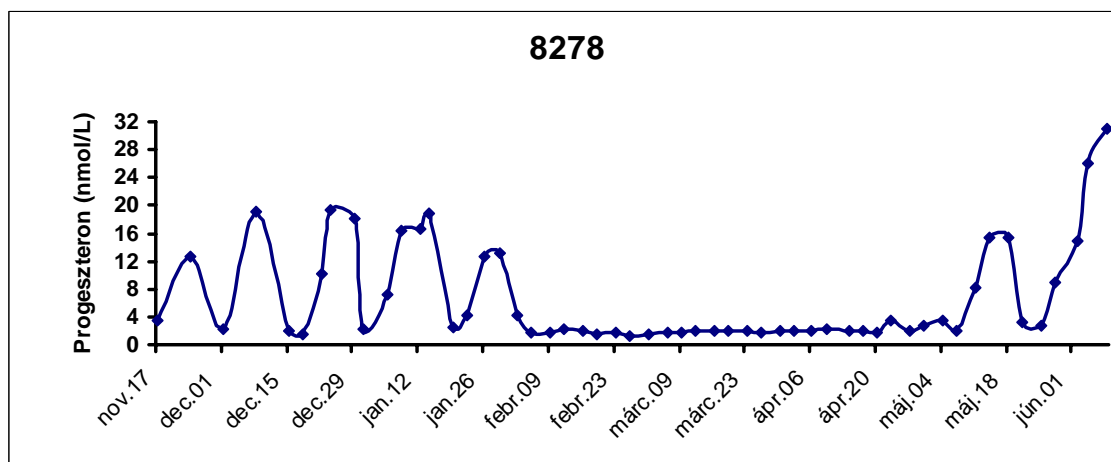
The progesteron profile of the ewe eartaged 8275

Figure 3.



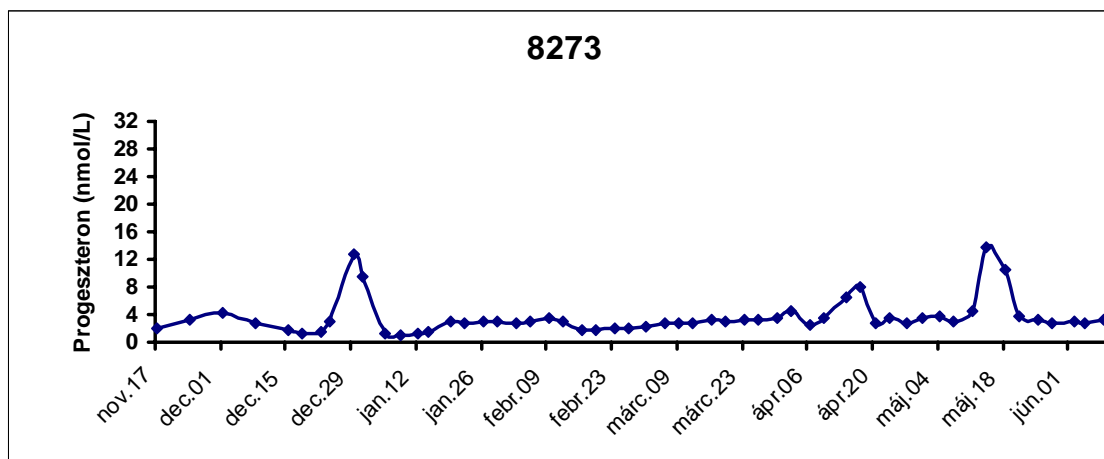
The progesteron profile of the ewe eartaged 8278

Figure 4.



The progesteron profile of the ewe eartaged 8273

Figure 5.



On the first day of the experimental procedure 15.80 ± 2.45 kg of body weight was measured in average before the planned blood taking. At the end of the weighting procedure 25.21 ± 3.49 kg was measured in average. At the beginning of collecting the blood samples 25.48 ± 3.49 kg was an average weight of the animals, at the end of the study 41.17 ± 2.80 kg was measured.

Non-linear regression and correlation of SPSS program showed a significant correlation ($r=0.955$; $P=0.01$) between the body weight gains and the biological breeding season of Barbados Blackbelly ewes. Based on the ultrasonographic examinations, the thickness of backfat layer, the rumpfat layer as well as the diameter of M. gluteus medius showed a medium correlation with the biological breeding season on the level of $P=0.254$; $P=0.135$ and $P=0.149$ (*Table 3.*)

Table 3.

Correlation-table

		Activity of ovarian performance	Body weight gain	Thickness of back fat layer	Thickness of rump fat layer	Diameter of M.gluteus medius
Activity of ovarian performance	Pearson Correlation	1	,955(**)	,499	,623	,607
	Sig.	-	,001	,254	,135	,149
	N	7	7	7	7	7
Body weight gain	Pearson Correlation	,955(**)	1	,786(*)	,806	,577
	Sig.	,001	-	,044	,028	,175
	N	7	7	7	7	7
Thickness of back fat layer	Pearson Correlation	,499	,768(*)	1	,854(*)	,912(**)
	Sig.	,254	,044	-	,014	,004
	N	7	7	7	7	7
Thickness of rump fat layer	Pearson Correlation	,623	,806(*)	,854(*)	1	,884(**)
	Sig.	,135	,028	,014	-	,008
	N	7	7	7	7	7
Diameter of (M.gluteus medius)	Pearson Correlation	,607	,577	,912(**)	,884(**)	1
	Sig.	,149	,175	,004	,008	-
	N	7	7	7	7	7

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level

The meteorological parameters measured during the monitoring of ovarian cyclicity were set in a colored calendar-like datatable in order to get the certain periods clarified when active ovarian performances were found in ewes. The average values and variances of parameters were summarized to make differences between the certain periods of time and to describe the effect the temperature and humidity may exist on reproductive performance. Finally there was no significant correlation proved between the wheater conditions and the length of breeding seasons of ewes. Although Pearson correlation was run to declare the statistical connection about the above mentioned factors more exactly, and a medium correlation $r=0.327$ was evaluated between the temperature and breeding season, and a negative low correlation $r= - 0.099$ was calculated between the humidity and the length of breeding season.

The exposing of ram was succesfull as three of the ewes got conceived by the beginning of May based on the P₄ profiles and further pregnancy checking. The rest of the ewes got pregnant by the beginning of August proved later with the presence of the new-born lambs.

The age at puberty of fullbred Merino and crossbred Merino and hairsheep F₁ ewes

The first ovarian cycles of the ewes were analysed by monitoring the presence of luteal phase indicating 3.00 nmol/L progesteron of blood plasma. The ages of ewes were evaluated at puberty. Statistical deviation and standard error were calculated. The results showed the (Hungarian Merino x Somali) F₁, and also the (Hungarian Merino x Barbados Blackbelly) F₁ crossbred genotypes had their puberty much earlier as compared to the fullbred Hungarian Merino ewes. (*Table 4., Fig. 6.*) Due to the differences of standard deviations of the groups, the significance level was evaluated as the value of P=0.181 using the Tamhane-test of the ANOVA by SPSS statistical program.

Figure 6.

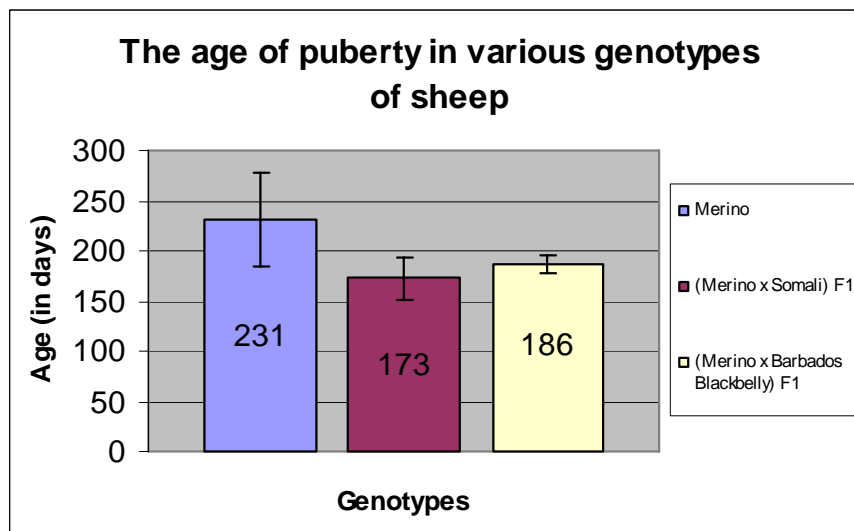


Table 4.

The median, standard deviation and standard error of the age of puberty (days)			
Genotype	Avarage age at puberty	Standard deviation	Standard error
Merino	231	95	36
(Merino x Somali) F ₁	173	43	14
(Merino x Barbados Blackbelly) F ₁	186	19	8

4. New scientific results

1. Conceptions were found in Dorper, Barbados Blackbelly and Somali ewes in Hungarian weather conditions all over the year. There were no environmental effects evaluated on the weaning rates of the out of season period, when hairy and shedding wool sheep also can be bred. The reproductive patterns of hairy and shedding wool genotypes have significantly better parameters in Hungary than the traditional breeds.

2. A significant strong correlation was showed between the ovarian activity of the (Barbados Blackbelly and Dorper) F₁ ewes and the daily average weight gains. Medium correlation was calculated between the ovarian cyclicity and the back- and rumpfat layers measured ultrasonographically. The active ovarian performance of ewes was able to start in May reflecting to the exposition of ram. No significant results were showed on the relation of the oestrus behaviour and temperature, as well as humidity.

3. The age at puberty of (Merino x Somali) F₁ and (Merino x Barbados Blackbelly) F₁ ewes was earlier than the time of first ovulation of Merinos under the same housing conditions. The variance of the age at puberty of the crossbred genotypes are less, than in the fullbred Merino.

5. Practical utilization of results

Shepherds can find some essential information in my dissertation how to use hair and shedding wool sheep in Hungary.

1., The breeds of Dorper, Barbados Blackbelly and Somali and their crossbred genotypes can be bred and mated succesfully in Hungarian weather conditions round the year.

2. Better birth and weaning rates – compared to the fullbred Hungarian Merino and the fullbred Tsigai - can be achieved by using hairsheep, and crossing them with the conventional breeds.

3. Slaughter lambs can be sold at Christmas time by crossing the hairsheep with traditional sheep. Optimal condition is essential for getting the chances of mating all year round.

4. The mating time can be started at younger age of woolly x hairy crossbred ewes. Consequently, the live-performance of ewes will be more produceable.

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