

# Interreg - IPA CBC



Greece - Republic of North Macedonia

PAPESHE

## Deliverable 3.2.3 Reproductive performance of Pelagonia sheep

Project acronym: **PAPESHE**

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## Giannitsa, 2021

### Key information

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<b>Date</b>	15/03/2021

## Abstract

This report is the Deliverable 3.2.3 is drafted by RIAS. In order to evaluate reproductive characteristics of Pelagonia sheep breeds, various studies were implemented in the purebred Pelagonia flock kept in Research Institute of Animal Science (RIAS) of ELGO-DIMITRA. The first study concerned the estimation of the age of puberty onset of Pelagonia ewe lambs. Blood samples were collected weekly from the age of 5 months to 10 months, in which progesterone concentration was measured. Consecutive high progesterone levels indicated ovarian activity, thus attainment of puberty. According to results the mean age of puberty onset of Pelagonia ewe lambs is  $296.58 \pm 21.83$  days or  $9.7 \pm 0.71$  months. Most of ewe lambs attain puberty at October and late season born ewe lambs reached puberty earlier than early season born ones. The second study concerned the estimation of onset of breeding season in adult females. Blood samples were collected weekly from late of May to early of August, in which progesterone concentration was measured. Consecutive high progesterone levels indicated presence of a corpus luteum and therefore the onset of ovarian activity, thus the onset of breeding season. Data revealed that the breeding season of Pelagonia sheep is around late of June to early of July. The third study concerned the percentage of embryonic loss. For this reason, in ewes after oestrus synchronization and ram introduction, blood samples were collected in which progesterone concentration was measured for pregnancy confirmation and pregnancy-associated glycoprotein (PAG) for pregnancy establishment or early embryonic loss incidence confirmation. Results revealed that only 6.25% of the pregnant ewes present embryonic loss and 64.00% of the total ewes studied became pregnant at the first oestrus cycle after synchronization. Moreover, previous reproductive data kept in RIAS Pelagonia flock were collected and processed. Results showed that Pelagonia ewes have high fertility rate (76.01% to 92.15%) and satisfactory for the breed litter size (1.46 to 1.50), depending of the year studied. Neonatal mortality is rather low (3.06%) and according to mothers' lactation period the proportion of male and female lambs born is almost the same (53.26% male lambs born and 46.74% female lambs born). Mean birth weight of male lambs is  $3.21 \pm 0.71$ kg and for female lambs  $3.06 \pm 0.56$ kg.

## Table of Contents

1. Introduction.....	5
2. Aim of the deliverable .....	5
3. Estimation of puberty onset of Pelagonia female lambs .....	6
3.1 Material and Methods .....	6
3.1.1 Animals management .....	6
3.1.2 Blood progesterone concentration measurement .....	6
3.1.3 Statistical analysis .....	7
3.2 Results.....	7
4. Estimation of onset of breeding season of Pelagonia female adult sheep .....	15
4.1. Materials and Methods .....	15
4.1.1 Animals management .....	15
4.1.2 Blood progesterone concentration measurement.....	15
4.1.3 Data analysis.....	16
4.2. Results.....	16
5. Evaluation of embryonic loss of Pelagonia adult ewes .....	21
5.1 Materials and Methods .....	21
5.1.1 Animals management .....	21
5.1.2 Blood progesterone and PAG concentration measurement .....	22
5.1.3 Data analysis .....	
5.3 Results.....	23
6. Other productive characteristics of Pelagonia breed.....	26
6.1 Materials and methods.....	26
6.2 Data analysis.....	26
6.3 Results.....	26
7. Discussion .....	28
8. Conclusions.....	29
References .....	30

## 1. Introduction

Greek sheep breeds are mainly farmed for their milk production, which is always combined with lamb meat production, while wool production is third in importance. The lambing period usually occurs from October through February. Due to uncontrolled breeding, a number of sheep breeds in Greece have disappeared or are disappearing. The Pelagonia breed, which used to be farmed in the mountainous less favored areas of Pelagonia region, is now legally listed as endangered, since it presents low percentage of total sheep population. It is known for its meat quality, high survival rate and resistant to harsh mountainous conditions.

Reproduction is a crucial process for livestock breeding. If animals fail to reproduce they can even face the threat of extinction. So, reproduction allows more effective animal improvement activities and selection. However, each breed has its unique reproductive efficiency qualities.

Sheep breeding is one of the most important agricultural sectors in Greece. Production characteristics of indigenous sheep breeds are relatively low, especially reproductive efficiency. The most important factor determining the success of sheep production is reproductive efficiency, which is the net biological accomplishment of all reproductive activities i.e. puberty, estrus, ovulation, fertilization, implantation, gestation and successful lambing, as well as survival and growth after birth. Thus, knowledge of the main reproductive characteristics of Pelagonia breed is crucial for the appropriate breeding management and productive efficiency of a Pelagonia flock.

## 2. Aim of the deliverable

The aim of this deliverable is to study and evaluate the main reproductive characteristics of Pelagonia female sheep, so as Pelagonia farmers establish an efficient breeding management.

### 3. Estimation of puberty onset of Pelagonia female lambs

#### 3.1 Material and methods

##### 3.1.1 Animals management

The present study was implemented at the Research Institute of Animal Science (RIAS) of ELGO-DIMITRA in Giannitsa, where a purebred flock of the Pelagonia breed is bred. Fifty (n=50) Pelagonia female lambs were selected from the flock and blood samples were collected weekly from 5 months to 10 months of age, approximately, in order to determine the age of puberty onset. The lambs were born from November to February. The lambs were grouped fed daily, with a balanced diet consisting of concentrated mixture (Table 1) and alfalfa hay.

**Table 1.** Composition of growing female lambs' concentrate mixture

Ingredients composition (g/kg, as mixed)	
Corn grain, ground	508
Wheat grain, ground	80
Barley grain, ground	200
Soybean meal (483 g CP/kg DM)	80
Sunflower	80
Limestone	14
Monocalcium phosphate	10
Salt	3
Vitamin-trace mineral premix	25

##### 3.1.2 Blood progesterone concentration measurement

Collected blood samples were centrifuged for 15' at 2800 rpm, serum was collected and stored at -40°C. Blood progesterone concentration was measured in serum by radioimmunoassay (Radio Immune Assay - RIA). The measurement was performed at the Laboratory of Anatomy and Physiology of Farm Animals of the Agricultural University of Athens. The method used was the competitive radioimmunoassay, based on the presence of a fixed amount of 125 I-labeled progesterone, which competes with the serum progesterone to be measured for a fixed amount of antibody sites

immobilized in the tube walls. A  $\gamma$ -radiation meter and the DIASource PROG-RIA-CT kit (Belgium) were used to measure progesterone. The coefficient of variation for the same assay was 4.0% CV and for different assays was 6.5% CV respectively.

When the concentration of progesterone was greater than 2ng/ml, we confirm the presence of a corpus luteum and therefore the onset of ovarian activity, thus the onset of puberty of female lambs.

### 3.1.3 Statistical analysis

Data were analyzed using a Mixed model, with a stable effect the date of birth (early or late animals). Results are presented as averages of least squares  $\pm$  standard error and significance level was set at  $P < 0.05$ . All analyzes were performed with the statistical package Sas/Stat (2005).

## 3.2. Results

Table 1 presents the results of the age of puberty onset of each female lamb, in days and months and the mean age.

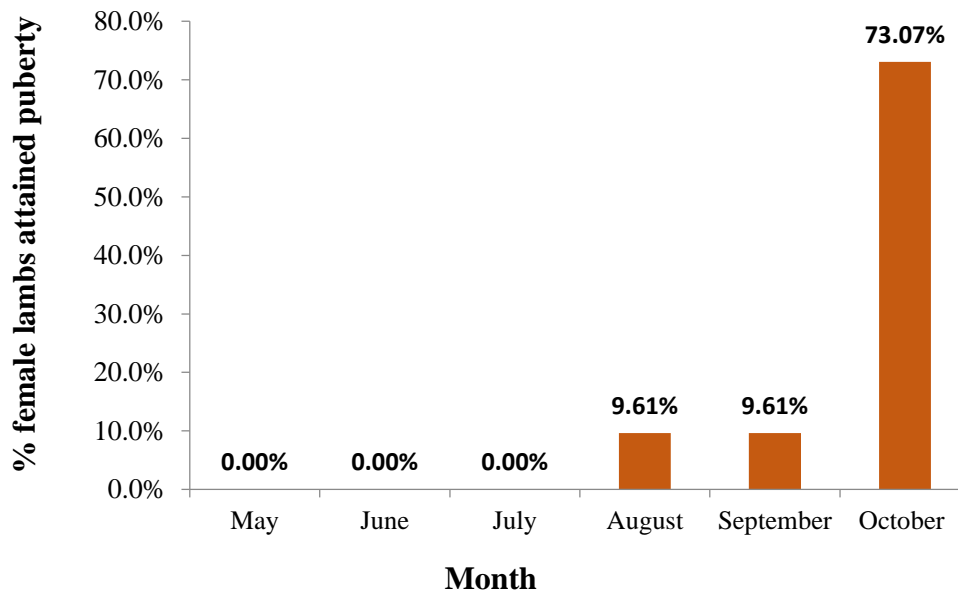
**Table 1.** Age of puberty onset of female lambs

Code number	Birth date	Puberty onset	Age of puberty onset (days)	Age of puberty onset (months)
8582	4/12/2018	1/8/2019	240	7.9
8577	28/11/2018	27/8/2019	272	9.0
8585	10/12/2018	27/8/2019	260	8.6
8621	13/12/2018	27/8/2019	257	8.5
8590	18/12/2018	17/9/2019	273	9.0
8581	20/11/2018	24/9/2019	308	10.1
8595	2/12/2018	24/9/2019	296	9.7
8605	7/12/2018	24/9/2019	291	9.6
8623	19/12/2018	24/9/2019	279	9.2
8619	17/11/2018	1/10/2019	318	10.5
8584	2/12/2018	1/10/2019	303	9.9

8613	9/12/2018	1/10/2019	296	9.7
8598	11/12/2018	1/10/2019	294	9.7
8600	11/12/2018	1/10/2019	294	9.7
8627	13/12/2018	1/10/2019	292	9.6
8618	16/12/2018	1/10/2019	289	9.5
8586	27/12/2018	1/10/2019	278	9.1
8610	27/12/2018	1/10/2019	278	9.1
8629	4/12/2018	8/10/2019	308	10.1
8597	24/12/2018	8/10/2019	288	9.5
8594	26/12/2018	8/10/2019	286	9.4
8616	3/12/2018	15/10/2019	316	10.4
8615	4/12/2018	15/10/2019	315	10.4
8612	5/12/2018	15/10/2019	314	10.3
8579	6/12/2018	15/10/2019	313	10.3
8599	9/12/2018	15/10/2019	310	10.2
8603	9/12/2018	15/10/2019	310	10.2
8593	10/12/2018	15/10/2019	309	10.2
8601	10/12/2018	15/10/2019	309	10.2
8614	16/12/2018	15/10/2019	303	9.9
8604	26/12/2018	15/10/2019	293	9.6
8611	8/2/2019	15/10/2019	249	8.2
8568	18/11/2018	22/10/2019	338	11.1
8608	4/12/2018	22/10/2019	322	10.6
8578	6/12/2018	22/10/2019	320	10.5
8596	6/12/2018	22/10/2019	320	10.5
8592	9/12/2018	22/10/2019	317	10.4
8607	9/12/2018	22/10/2019	317	10.4
8588	11/12/2018	22/10/2019	315	10.3
8576	27/12/2018	22/10/2019	299	9.8
8589	5/1/2019	22/10/2019	290	9.5
8626	5/2/2019	22/10/2019	259	8.5
8622	9/12/2018	29/10/2019	324	10.6
8591	17/12/2018	29/10/2019	316	10.4
8625	17/12/2018	29/10/2019	316	10.4
8602	7/1/2019	29/10/2019	295	9.7

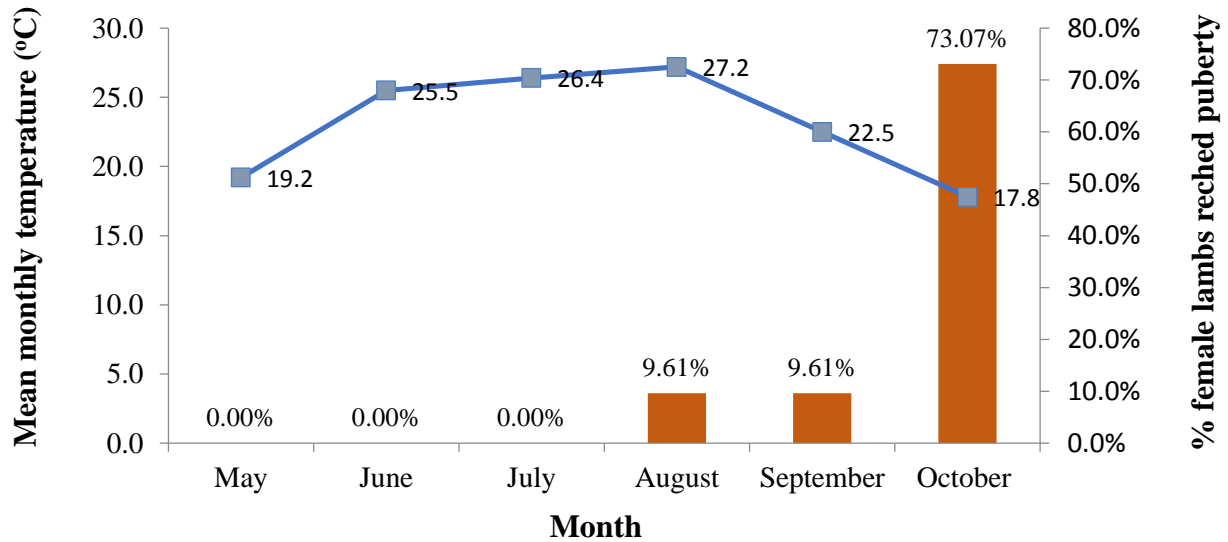
8624	11/1/2019	29/10/2019	291	9.6
8609	14/12/2018			
8620	26/1/2019			
8580	2/2/2019			
8617	3/2/2019			
<b>Mean age of puberty onset</b>			<b>296.58 ± 21.83</b>	<b>9.7 ± 0.71</b>

Results revealed that the mean age of onset of puberty of the female lambs was  $296.58 \pm 21.83$  days, or  $9.7 \pm 0.71$  months. Of the 52 female lambs, the 48, i.e. 92.30%, attained puberty at the first year of their age. The four of them, i.e. 7.69% did not attain puberty during this period. A proportion of 9.61% of the total female lambs examined and 10.41% of those attained puberty the first year of age, reached puberty at August. The respective percentages were 9.61% and 10.41% for September, and 73.7% and 79.16% at October, as shown in figure 1.



**Figure 1.** Percentage of female lambs that reaches puberty at each month

In figure 2, it is presented the percentage of female lambs that attained puberty at each month, in relation to mean air temperatures occurred the period studied.



**Figure 2.** Percentage of female lambs that attained puberty and mean temperatures, at each month.

Female lambs were divided in two categories according to their birth date: early season born (born up to 21 of December) and late season born (born after 21 of December), as shown in table 2 and 3, respectively.

**Table 2.** Age of puberty onset of early season born female lambs (up to 21<sup>st</sup> of December)

Code number	Birth date	Puberty onset	Age of puberty onset (days)	Age of puberty onset (months)
8619	17/11/2018	1/10/2019	318	10.5
8568	18/11/2018	22/10/2019	338	11.1
8587	18/11/2018	1/8/2019	256	8.5
8581	20/11/2018	24/9/2019	308	10.1
8577	28/11/2018	27/8/2019	272	9
8584	2/12/2018	1/10/2019	303	9.9
8595	2/12/2018	24/9/2019	296	9.7
8616	3/12/2018	15/10/2019	316	10.4
8582	4/12/2018	1/8/2019	240	7.9
8608	4/12/2018	22/10/2019	322	10.6
8615	4/12/2018	15/10/2019	315	10.4
8629	4/12/2018	8/10/2019	308	10.1
8612	5/12/2018	15/10/2019	314	10.3
8578	6/12/2018	22/10/2019	320	10.5
8579	6/12/2018	15/10/2019	313	10.3
8596	6/12/2018	22/10/2019	320	10.5
8605	7/12/2018	24/9/2019	291	9.6
8592	9/12/2018	22/10/2019	317	10.4
8599	9/12/2018	15/10/2019	310	10.2
8603	9/12/2018	15/10/2019	310	10.2
8607	9/12/2018	22/10/2019	317	10.4
8613	9/12/2018	1/10/2019	296	9.7
8622	9/12/2018	29/10/2019	324	10.6
8585	10/12/2018	27/8/2019	260	8.6
8593	10/12/2018	15/10/2019	309	10.2
8601	10/12/2018	15/10/2019	309	10.2
8588	11/12/2018	22/10/2019	315	10.3

8598	11/12/2018	1/10/2019	294	9.7
8600	11/12/2018	1/10/2019	294	9.7
8621	13/12/2018	27/8/2019	257	8.5
8627	13/12/2018	1/10/2019	292	9.6
8614	16/12/2018	15/10/2019	303	9.9
8618	16/12/2018	1/10/2019	289	9.5
8591	17/12/2018	29/10/2019	316	10.4
8625	17/12/2018	29/10/2019	316	10.4
8590	18/12/2018	17/9/2019	273	9
8623	19/12/2018	24/9/2019	279	9.2
8609	14/12/2018	--	-	-
<b>Mean age of puberty onset</b>			<b>300.81 ± 22.10</b>	<b>9.89 ± 0.71</b>

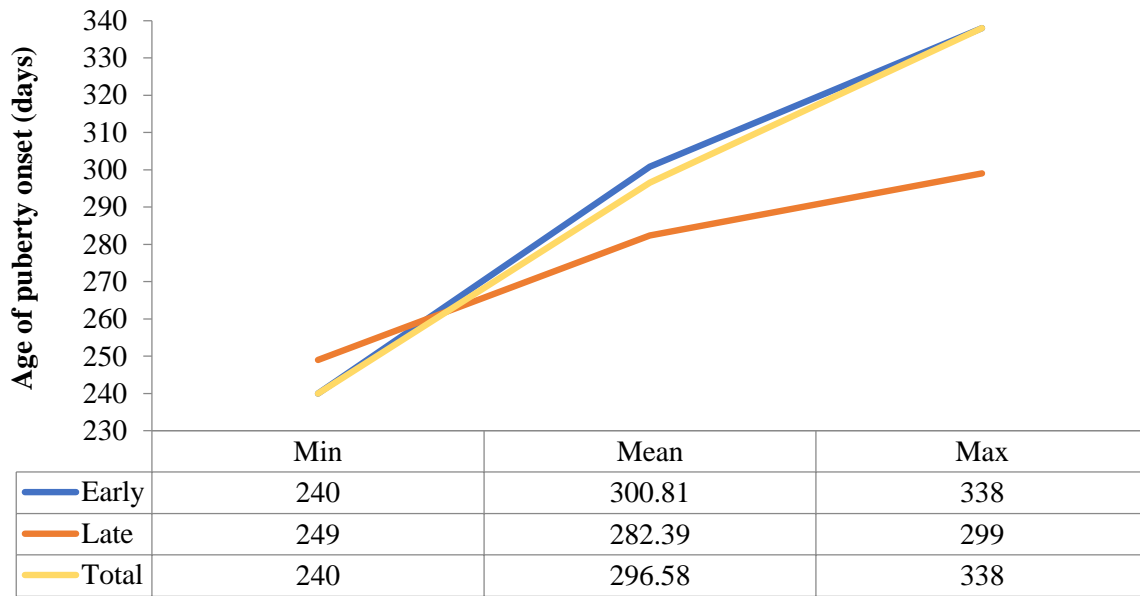
**Table 3.** Age of puberty onset of late season born female lambs (after 21<sup>st</sup> of December)

Code number	Birth date	Puberty onset	Age of puberty onset (days)	Age of puberty onset (months)
8597	24/12/2018	8/10/2019	288	9.5
8594	26/12/2018	8/10/2019	286	9.4
8604	26/12/2018	15/10/2019	293	9.6
8586	27/12/2018	1/10/2019	278	9.1
8610	27/12/2018	1/10/2019	278	9.1
8589	5/1/2019	22/10/2019	290	9.5
8602	7/1/2019	29/10/2019	295	9.7
8624	11/1/2019	29/10/2019	291	9.6
8626	5/2/2019	22/10/2019	259	8.5
8611	8/2/2019	15/10/2019	249	8.2
8620	26/1/2019			
8580	2/2/2019			
8617	3/2/2019			
<b>Mean age of puberty onset</b>			<b>282.36 ± 15.58</b>	<b>9.27 ± 0.51</b>

**Table 4.** Percentage of early season born and late season born female lambs that attained puberty

Season of birth	Number of female lambs	Percentage of female lambs attained ovarian activity (%)	Percentage of female lambs attained ovarian activity per category (%)	Age of puberty onset (days)	Age of puberty onset (month)
<b>Early born</b>	38	71.15% (38/52)	97.36% (37/38)	300.81 ± 21.79	9.89 ± 0.71
<b>Late born</b>	14	21.15% (14/52)	78.57% (11/14)	282.86 ± 14.84	9.27 ± 0.51
<b>All females</b>	52	92.30% (48/52)		296.58 ± 21.83	9.70 ± 0.71

The percentage of early season born lambs, born up to 21 of December, was 71.15% of the total female lambs sampled, while the late ones 21.15%. Of the early season born lambs, 37/38, a rate of 97.36% reached puberty and only 2.63% did not show ovarian activity. Of the late season born ones 11/14, a rate of 78.57% reached puberty, while 21.43% did not. Concerning early season lambs the mean age that onset of puberty occurred was 300.81 ± 22.10 days or 9.89 ± 0.71 months, whereas mean age of onset of puberty of late season female lambs occurred earlier at 282.36 ± 15.57 days or 9.27 ± 0.51 months (tables 2 & 3) ( $P < 0.013$ ). Thus, it is concluded from the above data that the late season born female lambs attained puberty at age earlier than the early season born female lambs (table 4). The following figure presents the mean, the maximum and the average age at puberty of early and late season born female lambs.



**Figure 3.** Minimum, maximum and mean age of puberty (days) of early born, late born and all female lambs.

The results of the experiment to determine the age of puberty onset of Pelagonia female lambs are comparable with data from previous studies. In a corresponding study of 65 lambs of the breed, the incidence rate in August was 9.23%, in September 45.15% and in October 41.54%, with an average age of puberty onset of  $254.47 \pm 36.92$  days (Triantafillidis et al., 1993).

## 4. Estimation of onset of breeding season of Pelagonia female adult sheep

### 4.1 Material and methods

#### 4.1.1 Animals management

The present study was implemented at the Research Institute of Animal Science (RIAS) of ELGO-DIMITRA in Giannitsa, where a purebred flock of the Pelagonia breed is bred. Fifty (n=50) Pelagonia adult ewes were randomly selected from the flock and blood samples were collected weekly from 23<sup>rd</sup> of May to 1<sup>st</sup> of August, in order to determine the beginning of breeding season. The ewes were of various lactating periods. The ewes were grouped fed daily, with a balanced diet consisting of concentrated mixture (Table 5) and alfalfa hay.

**Table 5.** Composition of growing female lambs' concentrate mixture

Ingredients composition (g/kg, as mixed)	
Corn grain, ground	480
Wheat grain, ground	60
Barley grain, ground	150
Soybean meal (483 g CP/kg DM)	120
Sunflower	150
Limestone	5
Monocalcium phosphate	5
Salt	5
Vitamin-trace mineral premix	25

#### 4.1.2 Blood progesterone concentration measurement

Weekly collected blood samples were centrifuged for 15' at 2800 rpm, serum was collected and stored at -40°C. Blood progesterone concentration was measured in serum by radioimmunoassay (Radio Immune Assay - RIA). The measurement was performed at the Laboratory of Anatomy and Physiology of Farm Animals of the Agricultural University of Athens. The method used was the competitive radioimmunoassay, based on the presence of a fixed amount of 125 I-labeled progesterone, which competes with the serum progesterone to be measured for a fixed amount of antibody sites

immobilized in the tube walls. A  $\gamma$ -radiation meter and the DIASource PROG-RIA-CT kit (Belgium) were used to measure progesterone. The coefficient of variation for the same assay was 4.0% CV and for different assays was 6.5% CV respectively.

When the concentration of progesterone was greater than 2ng/ml, we confirm the presence of a corpus luteum and therefore the onset of ovarian activity, thus the onset of breeding season.

#### 4.1.3 Data analysis

Data were analyzed using a Mixed model, with a stable effect the date of birth (early or late animals). Results are presented as averages of least squares  $\pm$  standard error and significance level was set at  $P < 0.05$ . All analyzes were performed with the statistical package Sas/Stat (2005).

## 4.2 Results

According to the results obtained, when the concentration of progesterone was greater than 2ng/ml, we confirm the presence of a corpus luteum and therefore the onset of breeding season.

Table 6 presents the date of the beginning of the breeding season according to progesterone concentration ( $>2\text{ng/ml}$ ) and the lactation period of each Pelagonia ewes studied. As shown in this table from the fifty ewes samples the 49 attained ovarian activity from 23 of May until 4<sup>th</sup> of July and only one did not presented ovarian activity until 1<sup>st</sup> of August, neither gave birth.

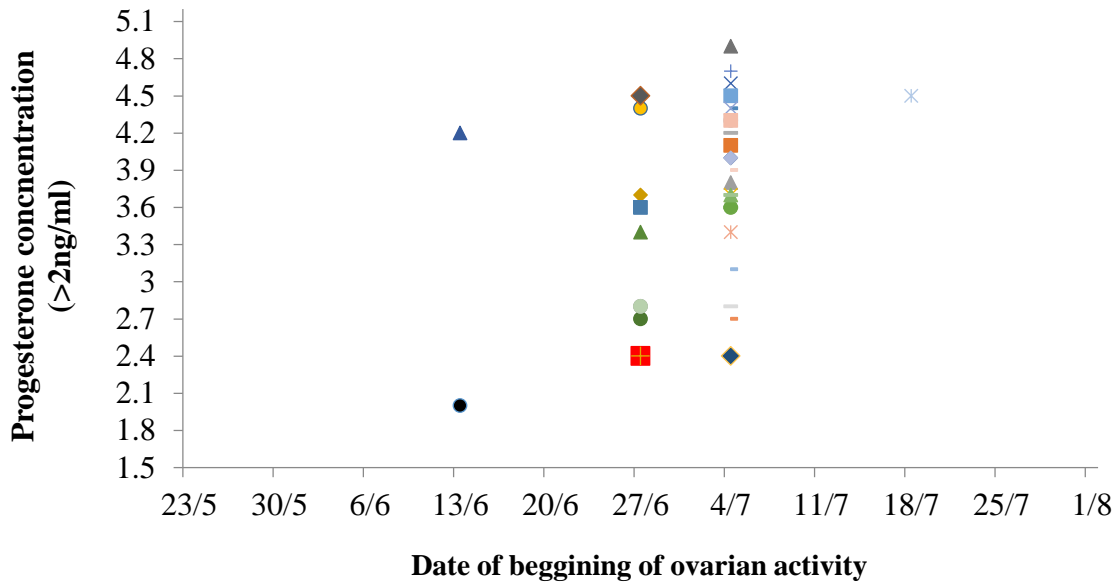
**Table 6.** Individual onset of ovarian activity of Pelagonia adult ewes

<b>Code Number</b>	<b>Lactation Period</b>	<b>Onset of ovarian activity</b>
6226	3	23/5/2019
6656	2	6/6/2019
5917	5	13/6/2019
6665	3	13/6/2019
5216	6	20/6/2019
5155	6	27/6/2019
5196	5	27/6/2019
5968	4	27/6/2019
6187	4	27/6/2019
6239	4	27/6/2019
6627	3	27/6/2019
6662	3	27/6/2019
6664	3	27/6/2019
7288	2	27/6/2019
7295	2	27/6/2019
7438	1	27/6/2019
4711	6	4/7/2019
4737	6	4/7/2019
5181	6	4/7/2019
5188	6	4/7/2019
5160	5	4/7/2019
5163	5	4/7/2019
5912	5	4/7/2019
5963	5	4/7/2019
5974	5	4/7/2019
6196	5	4/7/2019
6204	5	4/7/2019
6223	5	4/7/2019
5971	4	4/7/2019
6219	4	4/7/2019
6228	4	4/7/2019

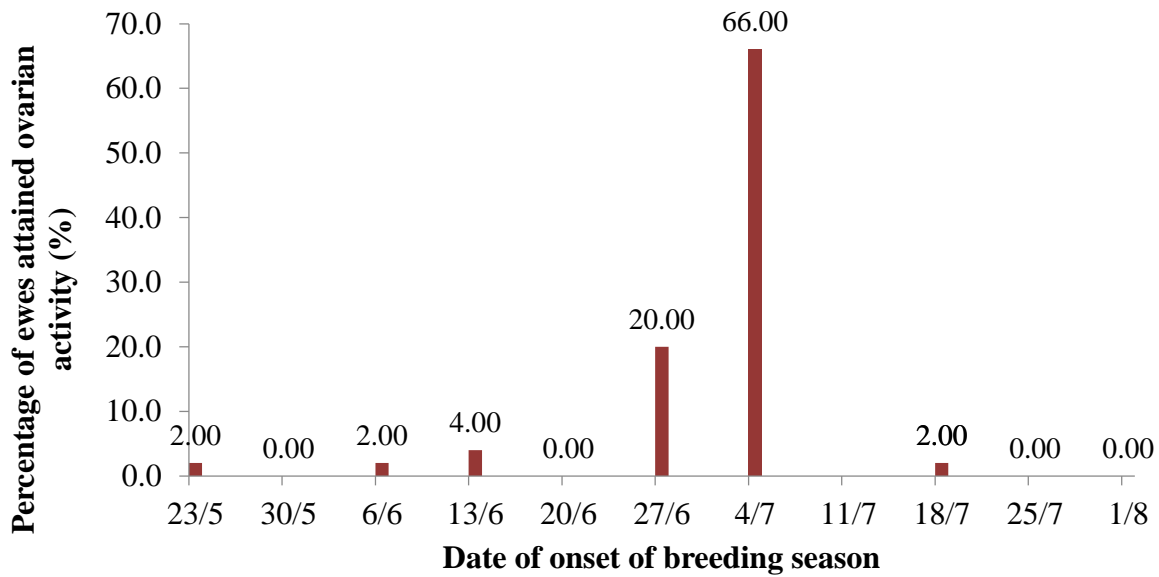
6243	4	4/7/2019
6191	3	4/7/2019
6267	3	4/7/2019
6636	3	4/7/2019
6638	3	4/7/2019
6647	3	4/7/2019
6653	3	4/7/2019
6667	3	4/7/2019
6652	2	4/7/2019
6677	2	4/7/2019
6685	2	4/7/2019
7296	2	4/7/2019
7303	2	4/7/2019
7322	2	4/7/2019
7328	2	4/7/2019
7366	2	4/7/2019
6679	1	4/7/2019
5949	5	11/7/2019
6200	3	Progesterone < 1 ng/ml)

**Table 7.** Time and number of ewes attained onset of ovarian activity and percentage at each time

Date	23/5	30/5	6/6	13/6	20/6	27/6	4/7	11/7	18/7	25/7	1/8
No of ewes attained onset of ovarian activity	2	0	1	2	1	11	31	1	0	0	0
% to total ewes (50)	4.00	0.00	2.00	4.00	2.00	22.00	62.00	2.00	0.00	0.00	0.00
% to ewes attained ovarian activity (49)	4.08	0.00	2.04	4.08	2.04	22.45	63.27	2.04	0.00	0.00	0.00



**Figure 4.** Distribution and progesterone levels of ewes at the time that attained ovarian activity (n=49).



**Figure 5.** Percentage of ewes at each time that attained ovarian activity (n=50).

**Table 8.** Time and number of ewes attained onset of ovarian activity and percentage at each time according to lactation period.

Number of ewes according to lactation period	23/5	30/5	6/6	13/6	20/6	27/6	4/7	11/7	18/7	25/7	1/8
Lactation period 6 (number)					1	1	4				
Lactation period 6 (%)	0.00	0.00	0.00	0.00	16.67	16.67	66.67	0.00	0.00	0.00	0.00
Lactation period 5 (number)				1	1	1	7	1			
Lactation period 5 (%)	0.00	0.00	0.00	9.09	9.09	9.09	63.64	9.09	0.00	0.00	0.00
Lactation period 4 (number)						3	4				
Lactation period 4 (%)	0.00	0.00	0.00	0.00	0.00	42.86	57.14	0.00	0.00	0.00	0.00
Lactation period 3 (number)	2			1		3	7				
Lactation period 3 (%)	15.38	0.00	0.00	7.69	0.00	23.08	53.85	0.00	0.00	0.00	0.00
Lactation period 2 (number)			1			2	8				
Lactation period 2 (%)	0.00	0.00	9.09	0.00	0.00	18.18	72.73	0.00	0.00	0.00	0.00
Lactation period 1 (number)						1	1				
Lactation period 1 (%)	0.00	0.00	0.00	0.00	0.00	50.00	50.00	0.00	0.00	0.00	0.00

As it is illustrated from tables 7 and 8 and figures 4 and 5, most of the ewes sampled attained ovarian activity i.e. onset of their breeding season at 4<sup>th</sup> of July (62.00% of the total ewes or 63.27% of those presented ovarian activity), whereas a week earlier at 27<sup>th</sup> of June the respective percentages were 22.00% and 22.45%. A small percentage of the total ewes presented ovarian activity at earlier time from late May to early June (4.00% at 23<sup>rd</sup> of May, 2.00% at 6<sup>th</sup> of June, 4.00% at 13<sup>teen</sup> of June and 2.00% at 20<sup>th</sup> of June. All ewes had been started their breeding season until 4<sup>th</sup> of July, except one.

When ewes were evaluated according to their lactation period (i.e from 1 to 6 lactation period), it was also revealed that from all ewes, independently from their lactation period, the higher percentage of them attained ovarian activity, thus the beginning of breeding season at 4<sup>th</sup> of July and secondly at 27<sup>th</sup> of June.

## 5. Evaluation of embryonic loss of Pelagonia adult ewes

### 5.1 Materials and methods

#### 5.1.1 Animals management

The present study was implemented at the Research Institute of Animal Science (RIAS) of ELGO-DIMITRA in Giannitsa, where a purebred flock of the Pelagonia breed is bred. Fifty (n=50) Pelagonia adult ewes were randomly selected from the flock at breeding season. The ewes were of various lactating periods. They were allocated into groups of five animals (n=5/group) and they were grouped fed daily, with a balanced diet consisting of concentrated mixture (Table 9) and alfalfa hay.

**Table 9.** Composition of growing female lambs' concentrate mixture

Ingredients composition (g/kg, as mixed)	
Corn grain, ground	480
Wheat grain, ground	60
Barley grain, ground	150
Soybean meal (483 g CP/kg DM)	120
Sunflower	150
Limestone	5
Monocalcium phosphate	5
Salt	5
Vitamin-trace mineral premix	25

Pelagonia ewes (isolated from rams) were treated with an intravaginal sponge (30 mg fluorogestone acetate; Chronogest, Intervet) for 14 days. At sponge withdrawal all ewes received 300 IU equine chorionic gonadotropin (eCG) and rams were introduced to ewes at a portion 1 ram / 5 ewes (1 ram/group). At 18<sup>th</sup> and 21<sup>st</sup> day after sponge removal blood samples were taken from the ewes in order to confirm pregnancy, measuring serum progesterone levels, as consecutive progesterone levels > 2ng/ml confirms ewes pregnancy. Moreover, at 28<sup>th</sup> day after sponge removal blood samples were taken from the ewes in order to measure plasma pregnancy-associated glycoprotein (PAG) levels and detect ewes pregnancy or embryonic loss.

PAG is a glycoprotein secreted by binucleate cells for detection of pregnancy. The PAGs, comprise a large group of placental proteins that are expressed in pre-placental trophoblast and after implantation in the trophoctoderm-the chorionic epithelium of eutherian mammals (Placentalia) with different placenta types. These proteins are detectable in maternal blood and were reported to be useful as prenatal markers for pregnancy diagnoses in various domestic species, included sheep (Szafranska et al. 2006).

Early embryonic loss (first month of pregnancy) was confirmed in ewes in which progesterone levels confirmed pregnancy ( $>2\text{ng/ml}$ ) but PAGs level were low, whereas confirmation pregnancy establishment was detected when both progesterone and PAGs levels were high.

### **5.1.2 Blood progesterone concentration measurement**

Blood samples were centrifuged for 15' at 2800 rpm, and serum and plasma were collected and stored at  $-40^{\circ}\text{C}$ . Blood progesterone concentration was measured in serum by radioimmunoassay (Radio Immune Assay - RIA). The measurement was performed at the Laboratory of Anatomy and Physiology of Farm Animals of the Agricultural University of Athens. The method used was the competitive radioimmunoassay, based on the presence of a fixed amount of  $^{125}\text{I}$ -labeled progesterone, which competes with the serum progesterone to be measured for a fixed amount of antibody sites immobilized in the tube walls. A  $\gamma$ -radiation meter and the DIAsource PROG-RIA-CT kit (Belgium) were used to measure progesterone. The coefficient of variation for the same assay was 4.0% CV and for different assays was 6.5% CV respectively. Plasma PAG levels measurement was performed at the Clinic of Obstetrics & Reproduction of Veterinary Faculty, University of Thessaly.

When the concentration of progesterone was greater than  $2\text{ng/ml}$  at the two consecutive sampling times (18<sup>th</sup> and 21<sup>st</sup> after sponge removal) we confirm the presence of a persisted corpus luteum and therefore ewes pregnancy. When of the

pregnant ewes PAG levels were higher than (>>>), we confirmed pregnancy establishment, otherwise early embryonic loss.

### 5.3 Results

In the following table (table 10), it is presented each studied ewe pregnancy confirmation, taken together high Pg and PAG blood levels, embryonic loss confirmation when high Pg blood levels occurred until 21 days after sponge removal and ram introduction, but low levels of PAG blood levels found at 28 day after ewes mating and no pregnancy after estrus synchronization and ram insertion, when low Pg and PAG levels were found in ewes blood serum and plasma, respectively at each sampling time.

**Table 10.** Pregnancy, embryonic loss and non pregnancy confirmation of Pelagonia ewes with Pg and PAG concentration, parturition date and twinning.

Code number	Pg (18 day after sponge removal)	Pg (21 day after sponge removal)	PAG (28 day after sponge removal)	Parturition date	Twinning
4745	8.2	8.6	Positive	22/12/2020	2
5181	7.8	6.1	Positive	17/12/2020	1
5224	9.9	9.4	Positive	18/12/2020	1
5949	7.5	8.0	Positive	21/12/2020	1
5959	11.0	9.1	Positive	18/12/2020	1
6187	12.0	7.6	Positive	15/12/2020	1
6194	8.0	6.0	Positive	19/12/2021	2
6247	8.5	8.8	Positive	20/12/2020	1
6249	8.2	7.8	Positive	18/12/2020	2
6286	5.7	5.8	Positive	19/12/2020	1
6636	6.0	7.6	Positive	18/12/2020	1
6637	6.9	8.3	Positive	17/12/2020	2
6651	10.0	10.0	Positive	19/12/2020	1
6666	9.3	8.7	Positive	19/12/2020	2

6672	6.2	5.9	Positive	20/12/2020	1
6681	7.7	6.8	Positive	19/12/2020	1
7263	10.3	10.5	Positive	20/12/2020	1
7361	9.8	8.2	Positive	17/12/2020	2
7362	9.5	8.1	Positive	17/12/2020	2
7438	9.2	7.0	Positive	21/12/2020	1
8052	6.4	5.3	Positive	17/12/2020	1
8053	8.0	6.2	Positive	16/12/2020	1
8068	7.5	7.6	Positive	18/12/2020	2
8078	8.0	7.0	Positive	18/12/2020	2
8110	6.7	5.1	Positive	17/12/2020	2
8125	6.8	5.3	Positive	16/12/2020	2
8141	12.0	11.0	Positive	17/12/2020	1
8143	8.2	7.1	Positive	18/12/2020	2
8145	8.5	8	Positive	17/12/2020	1
6639	9.0	6.5	Positive	-	-
5220	2.5	2.4	<b>Embryonic loss</b>	5/1/2021	2
6253	5.5	6.2	<b>Embryonic loss</b>	11/1/2021	1
4761	3.1	1.4	Negative	6/1/2021	1
5528	2.4	0.9	Negative	3/1/2021	2
5963	6.1	0.9	Negative	5/1/2021	1
5974	2.0	0.9	Negative	4/1/2021	1
6204	7.6	1.3	Negative	5/1/2021	1
6655	4.1	1.1	Negative	3/1/2021	1
6679	5.0	1.1	Negative	6/1/2021	1
7322	6.8	0.6	Negative	22/1/2021	1
7349	6.3	1.1	Negative	6/1/2021	1
8067	1.5	1.3	Negative	3/1/2021	2
8076	8.1	1.1	Negative	7/1/2021	1
8098	5.7	0.7	Negative	6/1/2021	1

8127	6.6	0.8	Negative	5/1/2021	2
6210	6.1	1.1	Negative	-	
6676	7.1	1.1	Negative	-	
7288	8.4	0.8	Negative	-	
8074	0.7	0.7	Negative	-	

Table 11 summarizes the results of ewes found pregnant or non pregnant after estrus synchronization and rams introduction after sponge removal.

**Table 11.** Percentage of ewes

Pregnant after estrus synchronization	Early embryonic loss	Late embryonic loss	Non pregnant after estrus synchronization	Later pregnancy	No pregnancy
32/50 (64.00%)	2/32 (6.25%)	1/32 (3.13%)	18/50 (36.00%)	13/18 (72.22%)	5/18 (27.78%)
	2/50 (4.00%)	1/50 (2.00%)		13/50 (26.00%)	5/50 (10.00%)

According to results, from the fifty (50) ewes studied 32 (64.00%) were found pregnant with high levels of blood progesterone. Among them 30 (93.75%) were confirmed pregnant with high PAG blood levels, whereas 2 (6.25%) found with low PAG level, which confirmed early embryonic loss. Among the thirty (30) positively pregnant ewes, one ewe (3.13%) did not give birth which means late embryonic loss. Moreover results revealed that from the fifty (50) ewes studied 18 (36.00%) were found non pregnant after estrus synchronization, as the low levels of blood progesterone at 18<sup>th</sup> and 21<sup>st</sup> day after sponge removal and ram introduction were confirmed by low blood PAG levels. Among them, 13 (72.22%) gave birth at later dates than the first pregnant ewes and five (5) ewes (27.78%) did not give birth at all.

## 6. Other productive characteristics of Pelagonia breed

## 6.1 Material and methods

In order to evaluate further reproductive characteristics of Pelagonia female sheep, data from RIAS, that are systematically recorded, were collected and processed. The data concerned the proportion of Pelagonia ewes that gave birth to ewes that were mated (fertility), the proportion of newborn to the number of ewes mated (lambing rate) or to the number of ewes that gave birth (litter size), as indicators of Pelagonia reproductive performance. Data collected concerned reproductive years 2017-2018, 2018-2019 and 2019-2020.

## 6.2 Data analysis

Data collected were analysed using SPSS statistical software and means are expressed as mean  $\pm$  standard deviation.

## 6.3 Results

As shown in table 12, data collected the last three years revealed that Pelagonia ewes' fertility (ewes lambing per ewe exposed) ranges from 76.01% to 90.15%, lambing rate (lambs born per ewe exposed to mating) ranges from 1.32 to 1.34 and litter size (lambs born per ewe lambing) ranges from 1.46 to 1.50.

**Table 12.** Reproductive performance of Pelagonia ewes from 2017 to 2020.

Parameter	2019-2020	2018-2019	2017-2018
Fertility (ewes lambing per ewe exposed)	178/200 (89.00%)	183/203 (90.15%)	206/271 (76.01%)
Lambing rate (lambs born per ewe exposed)	267/200 (1.34)	267/203 (1.32)	307/271 (1.32)
Litter size (lambs born per ewe lambing)	267/178 (1.50)	267/183 (1.46)	307/206 (1.49)

When ewes were evaluated according to their lactation period (table 13) it was found that 100% of mated ewes, of lactation period 6, 5, 4 and 1, were lambing, whereas that of ewes of 3 and 2 lactation period and ewes that was first year mated, the proportion were 84.38%, 77.78% and 76.00%, respectively. The mean number of lambs per ewes was higher at older ewes than the youngest ones. Mean birth weight of all lambs born was  $3.14 \pm 0.65$  kg. The percentage of newborns that survived at lambing was 96.94%, thus only neonatal lamb mortality was only 3.06%.

**Table 13.** Reproductive performance of Pelagonia ewes per lactation period.

Ewes lactation period	Fertility (ewes lambing per ewe exposed)	Mean number of lambs born	Mean birth weight (kg)
Lactation period 6	100.00%	$2.00 \pm 0.82$	$3.13 \pm 0.35$
Lactation period 5	100.00%	$1.64 \pm 0.63$	$3.19 \pm 0.60$
Lactation period 4	100.00%	$1.48 \pm 0.68$	$3.14 \pm 0.71$
Lactation period 3	84.38%	$1.56 \pm 0.51$	$2.94 \pm 0.52$
Lactation period 2	77.78%	$1.37 \pm 0.55$	$3.14 \pm 0.67$
Lactation period 1	100.00%	$1.45 \pm 0.60$	$3.30 \pm 0.73$
First year mating	76.00%	$1.35 \pm 0.48$	$3.15 \pm 0.63$
<b>In total</b>		<b><math>1.45 \pm 0.57</math> (96.94% live newborn)</b>	<b><math>3.14 \pm 0.65</math></b>

Table 14 presents the percentage of male and female lambs and their birth weight according to their mothers' lactation period. According to results the proportion of male and female lambs was almost the same (53.26% male lambs born and 46.74% female lambs born). Mean birth weight of male lambs was  $3.21 \pm 0.71$  kg and for female lambs  $3.06 \pm 0.56$  kg.

**Table 14.** Mean birth weights of Pelagonia male and female lambs according to their mother lactation period.

Ewes lactation period	Male lambs born (%)	Mean birth weight of male lambs (kg)	Female lambs born (%)	Mean birth weight of female lambs (kg)
Lactation period 6	37.50%	$3.33 \pm 0.58$	62.50%	$3.00 \pm 0.15$
Lactation period 5	38.09%	$3.38 \pm 0.52$	61.91%	$3.08 \pm 0.64$
Lactation period 4	71.88%	$3.20 \pm 0.78$	28.12%	$3.00 \pm 0.50$
Lactation period 3	48.80%	$3.04 \pm 0.55$	51.20%	$2.84 \pm 0.47$
Lactation period 2	54.00%	$3.15 \pm 0.72$	46.00%	$3.13 \pm 0.63$
Lactation period 1	54.39%	$3.35 \pm 0.84$	45.61%	$3.23 \pm 0.59$
First year mating	54.72%	$3.21 \pm 0.68$	45.28%	$3.08 \pm 0.58$
<b>Mean</b>	<b>53.26%</b>	<b><math>3.21 \pm 0.71</math></b>	<b>46.74%</b>	<b><math>3.06 \pm 0.56</math></b>

## 7. Discussion

Considering the results of this deliverable, concerning reproductive characteristics, it was revealed that Pelagonia sheep is a premature sheep breed, as ewe lambs attain puberty onset the first year of their life. This finding is of high importance since it gives the opportunity to the farmer to use ewe lambs for breeding even from the first year of age and extend their reproductive and productive life. Moreover, according to results, most of ewe lambs attain puberty at October and some of them at August and September. Furthermore, ewe lambs born at late December to February attain puberty at an earlier age than ewe lambs born from middle of November to late December. The

findings are in accordance with those of Triantafilidis et al. (1993), who found that breeding in Pelagonia ewe lambs starts at the first year of age, most of ewe lambs attained puberty at September and October. Moreover, Pappa et al. (2005) found that Pelagonia ewe lambs attained puberty at  $259.37 \pm 27.20$  days of age, earlier than Chios and Karagkouniko ewe lambs, whereas late season born ewe lambs attained puberty at earlier age than early season ewes lambs.

Breeding season of Pelagonia ewes started at the end of June to early of July, whereas a low number of ewes showed ovarian activity during June. This finding suggests that Pelagonia breed presents an anoestrus period during late May to late of June. This fact could be a significant tool for farmers to get knowledge of the exact time that breeding season of Pelagonia sheep starts and plan efficiently their breeding management.

Regarding early embryonic loss, it was found that it was rather low, 2 of the 32 pregnant ewes (4.00%) or 2 of the 50 ewes mated (i.e. 4.00%). The percentage of Pelagonia ewes that were mated after estrus synchronization was 64.00%, whereas 26.00% became pregnant the next estrus cycles and 10.00% did not give birth at all. Data collected from breeding seasons (i.e. 2017-2020) revealed that Pelagonia ewes presents high fertility rates, from 76.01% up to 90.15%, lambing rate 1.32 to 1.34, litter size from 1.46 to 1.50 and rather low neonatal mortality of 3.06%. Fertility is higher in older ewes relative to younger ones. Mean birth weight is  $3.14 \pm 0.65$  kg,  $3.21 \pm 0.71$  kg for male lambs and  $3.06 \pm 0.56$  for female lambs.

## 8. Conclusions

Considering all the data together of this deliverable, it is concluded that Pelagonia sheep breed is a premature breed, thus attain puberty the first year of age, a rather significant characteristic that influences the total reproductive and productive life of female animals and therefore the profit of a sheep farm. Growth performance of young animals kept for breeding is the period when they are not fruitful and are costly. Shorter this period, lower the costs for the establishment and increase the profitability of the establishments, as a result of increased efficiency from the animals.

Moreover, the low early embryonic loss, the high fertility rate and the satisfactory litter size are strong indicators that highlight the importance of Pelagonia sheep breed in its area of origin, since it is well known that it has been well adopted to the harsh mountain conditions and shows resistance to diseases.

The satisfactory reproductive characteristics of the breed in combination with the peculiarities it presents, the durability and the very good quality of the carcasses, make it necessary to ensure its genetic material and its preservation for the next generations. With information and state support, the population of the breed can increase and under proper management to improve the most of the productive capacities of the Pelagonia sheep.

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