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Summary of a Doctoral (PhD) Thesis

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ENDOCRINOLOGICAL EXAMINATION IN EWE REFLECTING ON RESULT OF ARTIFICIAL INSEMINATION AND PROCESS OF PREGNANCY

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I. I N R U D U C T I O N

However, reproduction in sheep is seasonal, many breeds of sheep are able to mate not only in autumn, but out-of-season as well. The main factor determining seasonality is the photoperiod, but other factors can influence reproductive pattern, such as genetics, management practices and social cues. The fertility of spring and early summer breeding is usually lower; this imposes the need for alternative methods, e.g.: hormonal treatments, biotechnological practice, to increase the conception rate.

The manipulation of the oestrus cyclus means a schedule created by breeders It's enable the timed artificial insemination and synchronization of lambing, comply with market requirements. Nowadays modern biotechnological and endocrinological methods are available for following up the assisted reproduction.

II. P U R P O S E S

There are four main topics of my study:

1. **Examination the out-of season hormonal function of the ovary and the fertility of Prolific Merino sheep breed.**
   - **1.1.** what is the rate of the *Prolific Merino* ewes with cyclical ovarian function in out-of –season (spring-time) reproductive period
   - **1.2.** is it possible to determine the hormonal status of the ovary and to detect the pregnancy from the hormonal parameter's of faces;
   - **1.3.** which hormones are suitable to value the energetic status of the flock.
2. **Examination the effects of the synthetical gestagens on the adrenocortical function**
3. **Examination the endocrine characteristics of late pregnant hypercetonaemic spring-lambing ewes and their reproductive performance following the induction of ovarian cyclicity 3 months later**
4. **Examination if the pregnancy stage and number of foetuses may influence maternal plasma leptin in ewes**

III. M A T E R I A L A N D M E T H O D E S

Animals, oestrus synchronization, insemination

I carried out 3 experiments on the *Prolific Merino* farm of Agricultural Faculty of Debrecen University in Kismacs; one experiment was done in a *Merino* flock.

I used ***Chrono-gest*** sponges (30 or 40 mg FGA content, Intervet, Boxmeer, Holland) and 300 mg progesterone content ***Easi-breed*** (CIDR-G^{TM}, InterAG, Hamilton, New-Zealand), respectively.

The progesterone treatments were supplemented with follicle stimulation (PMSG) to induce follicular growth and ovulation. I used 500 NE/ewe ***Folligon*** inj. i.m. (Intervet, Boxmeer, Holland) as a PMSG source at same time of sponge removal.

The artificial insemination was done by laparoscope in three experiments and once cervical insemination was applied.

Examination of adrenocortical function

To examine the adrenocortical function by stimulation tests I took blood to heparinized tube (basal sample t_o). 60µg $_{1\text{-}24}$ACTH i.v. (***Cortosin inj.Organon)*** was injected at the same time (t_o) and collected blood 60 (t_{60}) and 120 (t_{120}) min. later. The cortical level were determined from the blood plasma by RIA.

Collect of samples

Blood samples were collected into heparinized tubes by puncturing the vena jugularis. After centrifugation at 2000 G for 15 minutes 1,5 ml of plasma was taken to plastic tubes and was stored at -20 C° until assayed.

Faecal samples were taken from rectum and the samples were stored in polypropylene sacs at –20C° until assayed.

Preparation of the samples; statistical valuation

The hormonal examinations were done in Endocrinological Laboratory of Szent István University Faculty of Veterinary, Budapest. We determined the progesterone level of the plasma and the gestagen-metabolit concentration of faces by ELISA method, (progesteron: Nagy et.al 1998, gestagen metabolites: Kulcsár et. al, 1999)

The results were evaluated with chi-square test completed with Yates-correction, with Student t-test and ANOVA analysis respectively

Other kit-based assay systems used in the study

Hormone	Technique	Kit
Thyroxine (T_4)	^{125}I-RIA	^{125}I-T_4 RIA MIS kit (Institute of Isotopes Co., Ltd. Budapest, Hungary)
3,3',5-tri-iodothyronine (T_3)	^{125}I-RIA	^{125}I-T_3 RIA MIS kit (Institute of Isotopes Co., Ltd. Budapest, Hungary)
Insulin	^{125}I-RIA	^{125}I-Insulin RIA CT kit (CIS Bio International Ltd, Gif-Sur-Yvette, France)
Aspartate amino-transferase AST)	IFCC, determination in UV range	AST Kit, Kat. # 7249, Reanal RT, Budapest
Glucose	Enzimatic (GOD-POD) reaction	Glucose kit, Kat. # 40841, Diagnosztikum RT, Budapest
βOH-butirat (BHB)	βOH-butirat-dehidrogenaz reaction	D-3-Hydroxybutyrate kit, Kat. # RB 1007, Randox Laboratories Ltd, Ardmore, UK
Non-esterified fatty acids (NEFA)	Enzimatic	NEFA kit, Kat. # FA 115, Randox Laboratories Ltd, Ardmore, UK
Total cholesterol (TCH)	Enzimatic (CHOD-PAP) reaction	Cholesterol-PAP kit, Kat. # 40121, Diagnosztikum RT, Budapest
Cortisol	Direkt ^{3}H-RIA	After Csernus (1982) own develpoement (ÁoTK, Budapest)
Insulin-like growth factor-I (IGF-I)	Heterolog ^{125}I-RIA	Own developement (A. Nikolic, Zemun, az ÁoTK Budapest)
Leptin	Homolog ^{125}I-RIA	Adaptation of Delavaud et al. (2000) methode's

IV. R E S U L T S

1. Examination the out-of season hormonal function of the ovary and the fertility of prolific merino sheep breed

The examination was done in spring-early summer period of the year. The animals were *Prolific Merino* ewes in different ages and condition. I detected the ovarian function by plasma progesterone analysis and faecal gestagen metabolite analysis as well.

The physiological basis of this method is, that some product of disintegration of the progesterone (i.e. gestagen metabolites) are present in faecal. The ELISA-antibodies had cross-reaction with some metabolites such as 5α-pregnon-3-20-dion.

The advantage of this method is to collect the samples is easy; the disadvantage is the more time and chemical requirement.

On the basic of the results of blood plasma (ppl) and faecal (gml) samples 48% of the Prolific Merino ewes had cyclical ovarian function in out-of-season period.

There is close connection between plasma progesterone level (ppl) and gestagen metabolite level (gml) of faeces by the examination of lineal-regression method ($r=0,816$; $P<0.01$).

After the progesterone+PMSG treatment the laparoscopical ovulation rate (OR) examination showed, only 3 animals had no corpus luteum (CL) on the ovary. The flushing had increasing effect on the body weight almost for every animal. Unfortunately the lambing rate was very low (20%).

Comparing the dates of the ewes with acyclic and cyclical ovarian activities, the ewes which had cyclical ovarian activity had higher leptin level and significantly higher IGF-1 and insulin concentration than acyclic ones ***(Table 1.).***

Compare the dates of the pregnant and non pregnant ewes, under the flushing period the ewes, became pregnant later, had higher increase in body weight than the non fertilized ones and the end of the flushing their had higher IGF-1 level.

At the advanced stage of the pregnancy (44 days after AI) at the end of the ovary-dependent period we realized the decreased of pp level; after the fulfill of placental

steroid production (100 days after AI) we realized the increase of the pp.level, while the g.m level of fecal permanently increased ***(Figure 1).***

Summary of the results:

- ***in out-of –season (April-May) breeding period most of the examined Prolific Merino ewes had cyclical ovarian function***
- ***The acyclic ones give good reaction for cyclus-induction, however, the precondition of this reaction is to satisfy the energy-requirement of the ewes.***
- ***The determination of faecal gestagen metabolites is a useful technique in taking care of sheep reproduction,***
- ***We can get valuable information about energy-supply from determination of some metabolic hormones as IGF-1, insulin, leptin.***

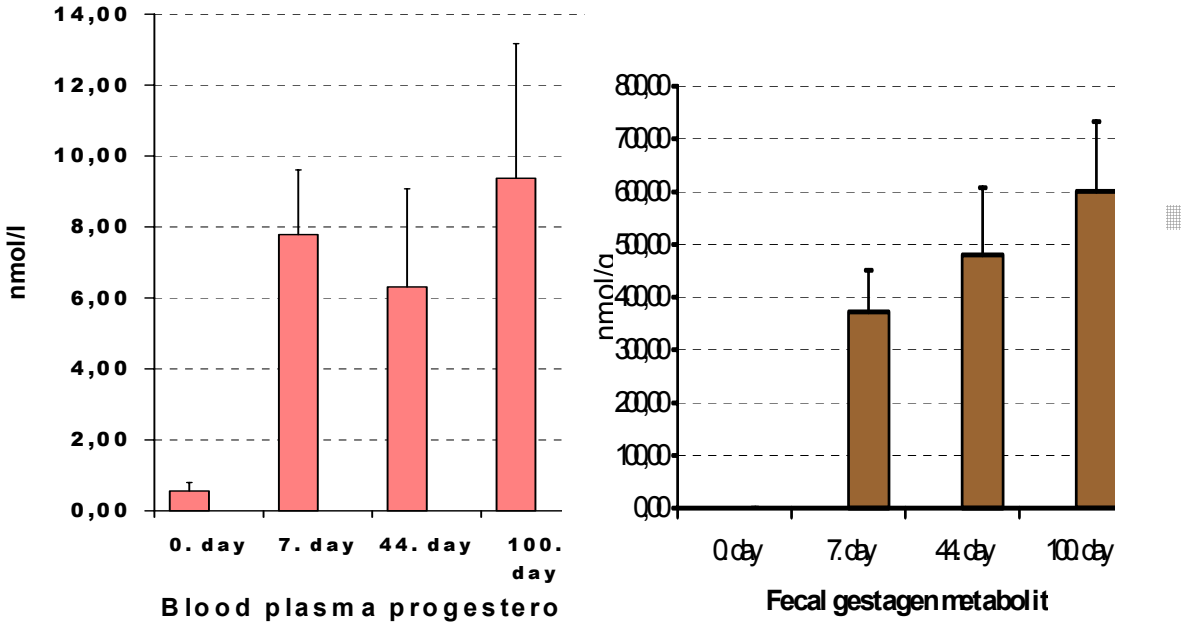

Figure 1.: The plasma progesterone level and the fecal gestagen metabolit level in the pregnant ewes (n=8) on the day of insemination (O. day) and the 7th, 44th and 100th day after. (Note: there were no fecal samples collected on the day of insemination)

***Table 1*:** The plasma level values of some metabolic hormones on the begin of the experiment in acyclic and cyclical ewes

		Ovarian activity		P=
		Acyclic (n=21; 57%)	**Cyclical (n=16; 43%)**	
Leptin, ng/ml HE				
On the 12th day of the flushing	$\bar{x}$	**2,05**	**2,19**	***0,184***
	± sd	0,30	0,35	
On the 23rd day of the flushing	$\bar{x}$	**1,94**	**2,11**	***0,154***
After 48 hour starvation	**± sd**	0,32	0,36	
IGF-1, nmol/l				
On the 12th day of the flushing	$\bar{x}$	**19,95**	**22,57**	***0,076***
	± sd	4,38	4,26	
On the 23rd day of the flushing	**x**	**12,45**	**16,24**	***0,019***
After 48 hour starvation	**± sd**	3,50	5,21	
Inzulin, μIU/L				
On the 12th day of the flushing	$\bar{x}$	**20,98**	**24,44**	***0,016***
	± sd	4,74	3,56	
On the 23rd day of the flushing	$\bar{x}$	**14,34**	**17,64**	***0,046***
After 45 hour starvation	**± sd**	4,07	5,22	
T3 (nmol/l)				
On the 12th day of the flushing	$\bar{x}$	**1,21**	**1,26**	***0,161***
	± sd	0,12	0,11	
On the 23rd day of the flushing	$\bar{x}$	**0,91**	**0,92**	***0,794***
After 48 hour starvation	**± sd**	0,13	0,10	
T4 (nmol/l)				
On the 12th day of the flushing	$\bar{x}$	**104,98**	**111,36**	***0,226***
	± sd	16,47	14,88	
On the 23rd day of the flushing	$\bar{x}$	**76,76**	**85,19**	***0,172***
After 48 hour starvation	**± sd**	17,09	18,94	

2. The effects on the adrenocortical function of the synthetical gestagens used for oestrus synchronization/induction in sheep

To examine of effect on the adrenocortical function of the synthetical gestagens used for oestrus synchronization/induction in sheep I carried out two experiments. First *Prolific Merino* ewes were treated with fluorogeston acetate (FGA)-contained (Chrono-gest) sponges; in the second experiment Prolific Merino ewes were treated with FGA-contained and nature progesterone (P4)-contained (CIDR) sponges for 14 days, respectively.

To investigate the changes of the adrenocortical function the cortisol responses exogenous adrenocorticotropic hormone (60 μg $_{1\text{-}24}$ACTH-t Cortrosyn inj., Organon) was injected. The cortisol concentration of the plasma was detected (1) before the FGA and progesterone treatment (2) on the day of sponge removal (3) 14 days after the sponge removal by radioimmunoassay (RIA).

The results of the first treatment are shown on the ***Figure 2.*** Lower basal cortisol concentrations and the suppression of the cortisol responses after ACTH administration were most pronounced during flurogeston treatment. Adrenal suppression did not exist two weeks later when flurogeston dosage ceased. There were no significant differences between 40 and 80 mg FGA source treated animals, respectively.

Based on result of the second experiment, there was no negative effect on adrenocortical function of nature progesterone-source sponge ***(Figure 3).***

Between 10-14th day of treatment the plasma *tiroxin* level of the FGA-treated animals was significantly lower (P<0,1) than P4-treated ones ***(Table 2)***. Reason of this increase, probably is, the body realize FGA as cortisol and speed up the metabolism of T4.

The *insulin* level increased significantly (P<0,03) in FGA-treated animals. FGA's cortisol-like effect decrease the insulin-resistance of the body, therefore more insulin secretion necessary for unit effect.

There were no significant differences in *leptin* level of FGA and P4 –treated animals, respectively. However on 14th day FGA-treated ones have slightly leptin level increasing, probably the FGA cortisol-like effect sped up the adypocyte's leptin gene-expression. This action is reversible; some days after FGA-treatment disappeared.

Summary of results:

- ***the synthetical gestagen effect not directly on adrenocortex, but on a higher level of the hypothalamus-pituitary-adrenocortex axis, probably on the hypothalamus' corticoliberin neurons has suppressive effect***
- ***FGA suppresses the adrenocortical function in sheep, but the adrenocortical response to ACTH stimulation returns to the pre-treatment level within two weeks, so this effect is reversible. The nature progesterone-source sponge has no suppressive effect on adrenocortical function.***

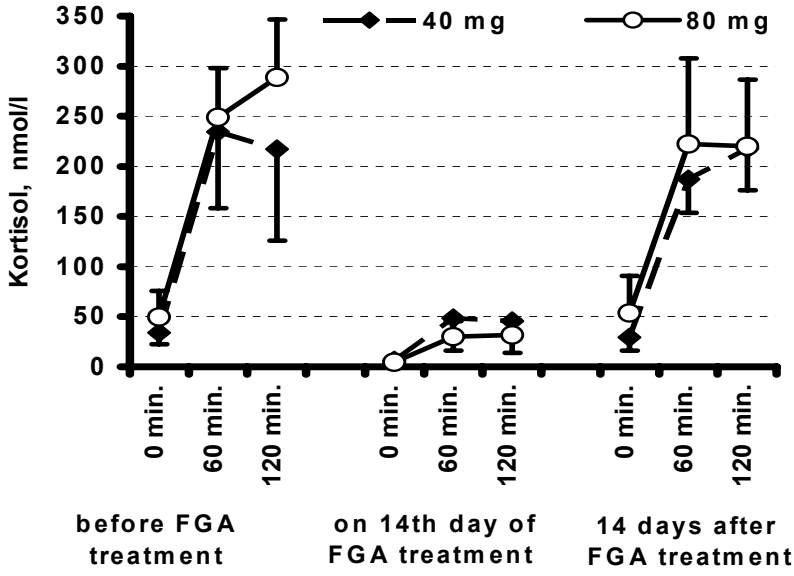

Figure 2.: The plasma cortizol levels before and after FGA and treatment (after ACTH stimulation)

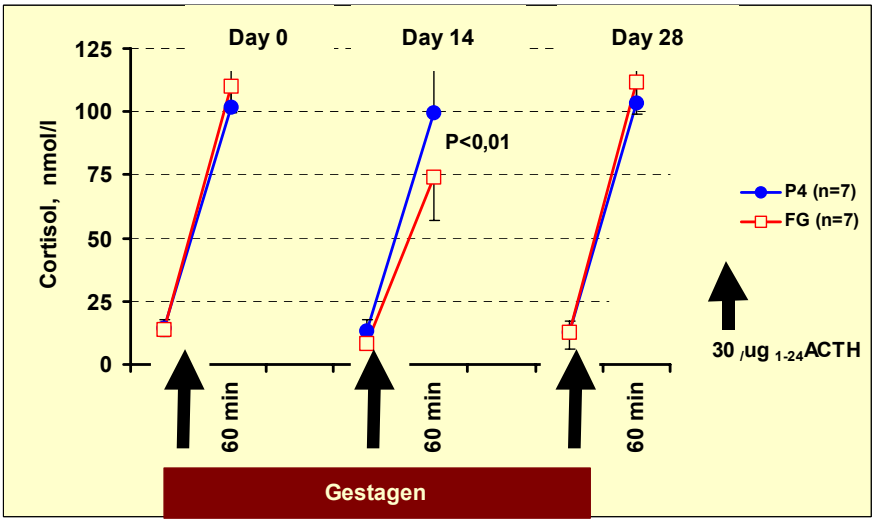

Figure 3.: The plasma cortisol levels before and after FGA and progesterone treatment (after ACTH-stimulation)

Table 2.: The T_4, insulin and leptin level of the animals treated with nature progesterone (P4) source *CIDR*-sponge and fluorogeston (FGA) contained *Chrono-gest* sponge, respectively

T_4 (nmol/l)									
Treat-ment /Day	0	3	7	10	14	17	21	24	28
P4 $\bar{x}$	94,7	93,7	93,6	91,2	93,1	95,6	96,3	98,1	97,2
Sd	15,4	14,2	17,5	15,0	15,2	15,5	15,4	16,3	17,6
FG $\bar{x}$	94,2	87,4	80,3	76,6	76,4	93,6	94,5	95,5	97,1
Sd	14,8	14,4	13,7	12,8	12,6	11,6	12,6	15,1	16,6
P=	0,954	0,440	0,141	0,074	0,045	0,790	0,810	0,765	0,991
Inzulin (µIU/ml)									
Treat-ment /Day	0	3	7	10	14	17	21	24	28
P4 $\bar{x}$	16,7	16,8	16,8	17,6	16,7	17,0		16,9	16,8
Sd	3,6	3,1	2,9	3,2	3,2	2,6	2,8	3,3	3,1
FG $\bar{x}$	17,4	18,1	19,9	21,2	21,7	17,3	17,0	17,3	17,2
Sd	4,1	3,6	3,8	2,9	3,1	4,0	3,6	4,2	3,4
P=	0,760	0,477	0,115	0,022	0,031	0,735	0,997	0,837	0,815
Leptin (ng/ml)									
Treat-ment /Day	0	3	7	10	14	17	21	24	28
P4 $\bar{x}$	8,54	8,53	8,45	8,50	8,55	8,59	8,61	8,62	8,71
Sd	0,86	0,89	0,92	0,91	0,88	0,93	0,93	0,93	0,89
FG $\bar{x}$	8,61	8,64	8,84	8,96	9,05	8,63	8,63	8,63	8,71
Sd	0,65	0,61	0,67	0,68	0,68	0,63	0,63	0,73	0,75
P=	0,865	0,787	0,379	0,314	0,257	0,914	0,961	0,973	0,995

3. Endocrine characteristics of late pregnant hyperketonaemic spring-lambing ewes and their reproductive performance following the induction of ovarian cyclicity 3 months later

Ketosis (pregnancy toxicosis) was diagnosed in a flock of Merino ewes conceived from synchronised oestruses in the early autumn period. On day 140 of gestation significantly lower βOH-butyrate (BHB) levels were detected in the ewes with single (n=41) than with twin (n=57) pregnancies.

Hyperketonaemia (BHB: >1.60 mmol/l) was detected only in case of twin pregnancies: almost half of these ewes (n=27) were affected by this metabolic disorder. These symptomless ketotic animals showed more elevated non-esterified fatty acid (NEFA) and cortisol, and lower total cholesterol, insulin, IGF-I and T_3 levels than the normoketonaemic twins and those with single pregnancy ***(Table 3)***. There were, however, only slight or no differences in the blood glucose content, as well as in the circulating leptin concentrations. The formerly hyperketonaemic individuals were characterised by lower leptin level 3 months after lambing ***(Figure 4)***.

At that time the formerly hyperketonaemic ewes gave a significantly poorer response to a standard (fluorogestone plus eCG) cycle induction procedure than those with normoketonaemia and/or single pregnancy ***(Table 4)***. The non-responders had lower IGF-I and leptin levels than the responders. These data gave the evidence that the subclinical form of ovine ketosis is characterised by simultaneous endocrine alterations and if after lambing the cycle is attempted to induce soon out of the breeding season, it may depress also the ovarian response and fertility.

Summary of the results:

Formerly hyperketonaemic ewes had

- ***at the time of disease, increased BHB level***
    - ***decreased plasma glucose, total cholesterol, insulin, T3 and IGF-I concentration***
    - ***increased NEFA and cortisol level and AST activity, respectively.***
- ***90 days after lambing the formerly hyperkaetonemic animals had***
    - ***lower plasma IGF-I and leptin level***
    - ***and worse reaction for cyclus- induction than in normoketonaemic ewes.***

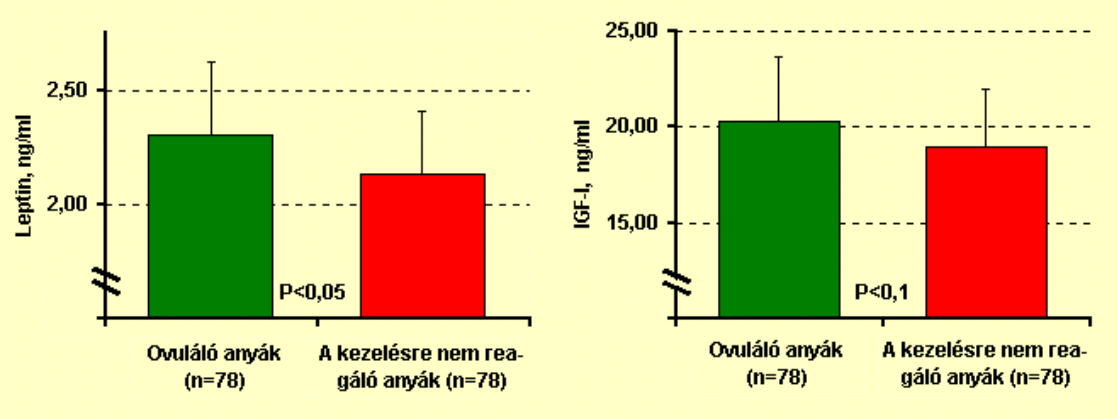

Figure 4: The plasma leptin and IGF-I level of the ewes which has ovulation (green column) or not (red column), respectively after cyclus induction (on day 92-94.after lambing)

Table 3: The metabolic and endocrine characteristics of ewes with single and twin pregnancies on day 140 of gestation, and 92-94 days after lambing.

	Ewes with single pregnancy **(n=41)**	*Ewes with twin pregnancy* *BHB on day 140 of gestation:* **<1.60 mmol/l (n=30)**	*Ewes with twin pregnancy* *BHB on day 140 of gestation:* **≥1.60 mmol/l (n=27)**	**F**	**$LSD_{P<0,05}$**
	x ± sd				
On day 140 of pregnancy:					
Glucose, mmol/l	3.38 ± 0.65	3.19 ± 0.77	2.88 ± 1.63	1.87	ns (P<0,1)
NEFA, mmol/l	0.27 ± 0.12	0.31 ± 0.15	0.44 ± 0.18	11.15	0.08
AST, NE/l	87 ± 27	95 ± 31	131 ± 74	7.95	25
TCH, mmol/l	2.18 ± 0.45	2.21 ± 0.65	1.78 ± 0.71	4.75	0.32
Insulin, µNE/l	15.54 ± 7.55	12.74 ± 9.33	6.78 ± 5.,55	10.67	4.14
T_4, nmol/l	75.1 ± 28.5	69.3 ± 31.1	61.1 ± 31.2	1.76	ns
T_3, nmol/l	1.14 ± 0.27	1.07 ± 0.35	0.87 ± 0.38	5.65	0.18
Cortisol, nmol/l	35.1 ± 25.5	37.3 ± 27.0	59.4 ± 39,2	5.88	16.3
IGF-I, ng/ml	17.38 ± 4,35	15.44 ± 4,32	9.10 ± 7,23	19.84	2.91
At the time of insemination (on day 92-94 after lambing), following induction of cyclicity:					
BHB, mmol/l	0.36 ± 0.27	0.44 ± 0.25	0.38 ± 0.29	0.79	ns
Glucose, mmol/l	3.61 ± 0.36	3.65 ± 0.34	3.59 ± 0.,33	0.23	ns
NEFA, mmol/l	0.19 ± 0.04	0.20 ± 0.04	0.19 ± 0.03	0.74	ns
AST, NE/l	75 ± 25	77 ± 23	73 ± 28	0,18	ns
TCH, mmol/l	3.15 ± 0.33	3.19 ± 0.35	3.21 ± 0.38	0.26	ns
Insulin, µNE/l	21.28 ± 3.17	21.75 ± 3.82	21.52 ± 4.72	0.13	ns
T_4, nmol/l	105.0 ± 21.3	103.1 ± 27.4	109.4 ± 25.1	0.49	ns
T_3, nmol/l	1.33 ± 0.18	1.38 ± 0.21	1.35 ± 0.27	0.46	ns
Cortisol, nmol/l	25.8 ± 10.3	27.7 ± 10.4	26.4 ± 9.3	0.31	ns
IGF-I, ng/ml	20.00 ± 3.21	20.48 ± 3.35	19.44 ± 5,83	0.68	ns

Table 4.: The ovarian response and reproductive performance of ewes following a standard fluorogestone + eCG cycle inducing protocol started on day 78-80 after lambing

	Ewes with single pregnancy **(n=41)**	*Ewes with twin pregnancy*		*P<*
		BHB on day 140 of gestation:		
		<1.60 mmol/l (n=30)	*≥1.60 mmol/l (n=27)*	
	n (%)			
Following the induction of cyclicity:				
Ewes ovulated	37 (90,2 %)	25 (83,3 %)	16 (59,3 %)	0,01
Ewes re-conceived	35 (85,4%)	24 (80,0 %)	15 (55,6 %)	0,05
of them: twin pregnancies	21 (60,0 % of pregnancies)	10 (58,3 % of pregnancies)	4 (26,7 % of pregnancies)	(0,1)

4. Pregnancy stage and number of foetuses may influence maternal plasma leptin in ewes

Leptin is a 16 kDa protein secreted from white adipocytes, was discovered in 1994. It could play a central role in the regulation of body energy homeostasis and important regulator of reproductive function and provides a signalig link between nutritional status and reproduction.

I studied whether there is any relation between number of foetuses and maternal plasma leptin measured by an ovine-specific RIA in *Prolific Merino* ewes (n=58). In late January the ovarian cycle was induced/synchronized with a gestagen+eCG treatment. Blood and faecal samples were collected at the time of insemination and again 41, 81 and 101 day later.

The plasma levels of leptin and progesterone (P_4) and the faecal P_4 metabolite (P_4-met) content were determined. The pregnancy-related changes of these hormones were evaluated by repeated measures ANOVA using time (d 41, d 81, and d 101) as the within subject factor, the number of foetuses (1, 2, and ≥3) as a between subject factor and the initial (d 0) measurement as a covariant.

The d 0 level of L was higher in pregnants (n=24) than in non-pregnants (n=34), suggesting that fat ewes became pregnant more easily. According to EHRHARDT (2001) results by d 41 the plasma leptin of pregnant had doubled, it showed a moderate further increase on d 81, and decreased slightly thereafter.

During pregnancy the P_4 and P_4-met rose continuously and were positively correlated at all stages. The mean levels of leptin, P_4 and P_4-met were lower in ewes bearing single (n=12) than 2 (n=6) or 3-5 foetuses (n=6). According to the number of foetuses the ANOVA proved significant differences in leptin and P4, but not in P4-met (p=0.042, 0.044, and 0.051, respectively). On day 41 and 81 the leptin was in mild positive correlation with P_4 and P_4-met, which reduced slightly on day 101 ***(Figure 5)***.

Summary of the results:

- ***In ewes during pregnancy the leptin levels rose continuously till the mid-pregnancy and decrease slightly thereafter***
- ***The degree of increase has mild positive correlation with plasma progesterone and faecal gestagen metabolite level***
- ***There is positive correlation between number of foetuses and plasma leptin and progesterone level***
- ***In ewes both the stage of pregnancy and the number of foetuses influence the degree of maternal leptin level increase, respectively.***

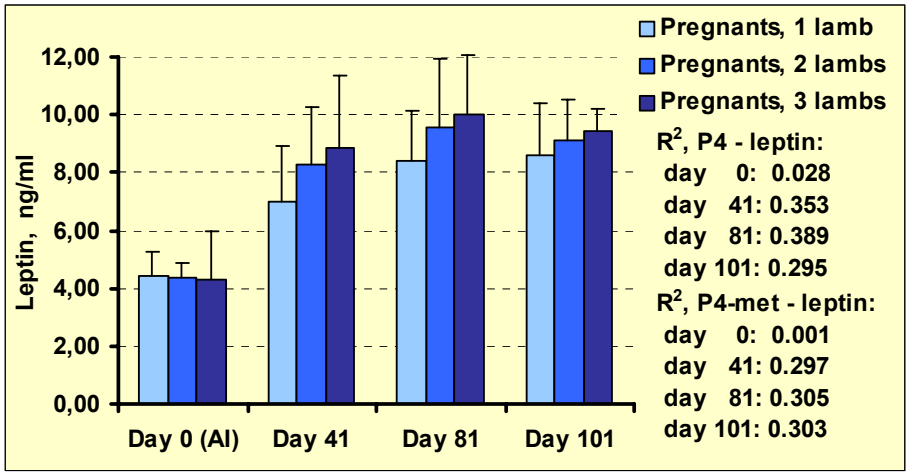

Figure 5: The plasma leptin levels in pregnant ewes with 1, 2, 3 lambs, respectively on day 0. 41, 81 and 101 day after AI, respectively; and the R^2 values between P_4- leptin and P_4-met- leptin.

V. SUMMARY

During my PhD study I did my examinations in topic of sheep reproduction on four different fields. I did endocrinological examinations in ewe reflecting on result of artificial insemination and process of pregnancy. The experimental animals were Prolific Merino ewes of University of Debrecen Faculty of Agronomy. The RIA and ELISA hormonal analyses were done in Endocrine Lab of Szent István University, Faculty of Veterinary, Budapest.

Examined the ovarian function of Prolific Merino ewes in the out-of –season (springtime) reproductive period I established, that most of the examined Prolific Merino ewes had cyclical ovarian function. The acyclic ones give good reaction for cyclus-induction, based on the laparascopic ovulation rate examination dates however, the precondition of this reaction is to satisfy the energy-requirement of the ewes.

The determination of faecal gestagen metabolites is a useful technique in taking care of sheep reproduction. The plasma progesterone level and the faecal gestagen metabolite level give nearly the same results and suitable to determine of the cyclical or acyclic status of the ewe. We can get valuable information about energy-supply from determination of some metabolic hormones as IGF-1, insulin, leptin.

It is concluded that FGA suppresses the adrenocortical function, but the adrenocortical response to ACTH stimulation returns to the pre-treatment level within two weeks, so this effect is reversible. The nature progesterone-source sponge has no suppressive effect on adrenocortical function.

Concerning the plasma glucose, BHB, TCH, insulin and cortisol levels, as well as the AST activity found in late pregnant normo- and hyperketonaemic ewes our results are in accordance with the few data available in the literature.

In this study fewer formerly hyperketonaemic than normoketonaemic ewes responded with ovulation to the standard (synthetic gestagen plus eCG based) cycle-inducing treatment regime, and those ovulated after this treatment were characterised by somewhat higher leptin and IGF-I levels than the non-responding individuals. The same tendency with the advantage of normoketonaemic dams was observed in the rate of re-conceived animals.

The hyperketonaemia-related impairment of follicular development is supposed to be a more likely reason of the weaker ovarian response.

Leptin is a hormone -like protein secreted from white adipocytes has been implicated in the regulation of food intake, energy expenditure and whole body energy balance. It plays role in the turning into acyclic to cyclical function of the ovary and helps the maturation of the follicle and even the development of the embryo in early embryonic stage.

To examine the plasma leptin level of the pregnant ewe I determined, that the plasma leptin level increase till mid-pregnancy (day 81), and decreased slightly thereafter. During pregnancy the plasma progesterone (pP_4) and faecal gestagen-metabolite (P_4-met) rose continuously and were positively correlated at all stages. The mean levels of L, pP_4 and P_4-met were lower in ewes bearing single than 2 or 3-5 foetuses.

I concluded new scientific results and result for the practice as follow:

NEW SCIENTIFIC RESULTS

1. The synthetical gestagens (like FGA) used for oestrus- synchronization, induction in ewes has a reversible suppressive action on adrenocortical function. The nature progesterone-source sponge has no suppressive effect on adrenocortical function.
2. In ewes both the pregnancy stage and the number of foetuses may regulate leptinaemia.

RESULTS FOR THE PRACTICE

1. The plasma progesterone level and the faecal gestagen metabolit level give nearly the same results and suitable to determine of the cyclical or acyclic status of the ewe.
2. To value energetically status of a flock, determination of some metabolic hormones as IGF-1, insulin, leptin can give valuable information.
3. It can be demonstrated by endocrinologist methods that the success of cyclus induction in ewe depends on energy supply of the animal.
4. The week reproductive performance of formerly hyperketonaemic ewes is due mainly to an analogue follicular malfunction.

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