

**Summary of a Doctoral (Ph.D.) Thesis**

**FACTORS AFFECTING THE EFFICIENCY OF SHEEP EMBRYO TRANSFER**

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## 1. Introduction

Demand towards products of animal origin is continuously rising, because of population growth and changes in consumers' habits. According to the forecasts, the phenomenon mentioned above is going to exist in the future as well. The ever-growing pretention will be satisfied through improving the production ability of livestock animals, not through rising the number of animals in production. According to a survey's result: amount of meat produced by livestock animals will rise three times and milk produced of livestock animals will rise two times faster than the number of animals set in production (CUNNINGHAM, 1996; GEROSA and SKOET, 2012).

This trend is representative in sheep industry as well. Market and consumers' interest is rising towards good quality and special sheep meat and milk products all over the world.

Sheep products fit in today's health-centric, health-conscious life, because of these animals' feeding and keeping. Sheep breeds are usually kept extensively, walking on pastures all the time, grazing herbals- allowing sheep meat and milk to be one of the healthiest food in the world.

Compared to the leading sheep-breeder countries, Hungary has a huge discommodity in keeping technology, processing, merchantry, consumption of sheep products, and in applying assisted reproductive techniques (ART) and reproductive innovations in breeding programmes. This problem could be easily solved with coherent professional work and applying innovations. It is hard to imagine modern animal breeding in the 21st century without using assisted reproduction and biotechnological innovations (even if sheep breeding has a typically conventional technology). Quality and quantity of hungarian animal breeding needs urgent improving and assisted reproduction and biotechnology could help as the catalyser of development (DOHY, 2000).

Out of AR techniques, artificial insemination (with fresh, chilled or frozen sperm), embryo transfer (ET) and connected methods (superovulation, embryo flushing, in vitro cultivation, embryo freezing, in vitro embryo production, in vitro fertilization) could be easily used in farm conditions in the sheep sector.

Similar to other species, superovulation and success of the embryo transfer program is very unpredictable in sheep. Several known and unknown factors have an impact on the efficiency (number and quality of flushed embryos, implantation ratio, success of ET, etc.). According to

scientific results keeping, feeding, breed, age, condition of the donor and the recipient ewes', season, treatment protocol, number of embryos transferred..etc. have impact on the outcome of the ET program (CSEH and SOLTI, 2001; BARI et al, 2002; GONZALES- BULNES, et al, 2004). Success of the ET program in sheep is mainly affected by the reaction of donor ewes' on the superovulation treatment (number of useful embryos). Unfortunately, gaining of our knowledge in connection with the success of superovulation is very little in the last few decades.

The aim of our research has been to investigate the impact on ET program of: **metabolic hormone** (insulin-like growth factor 1 /IGF-1/, triiodothyronine /T3/, tiroxine /T4/ and insulin) and **energy-status dependent metabolite** (non esterified fatty acids /NEFA/, beta-hidroxy-butirate /BHB/, total-protein /TP/, urea and cholesterol) levels in the donor and recipient ewes' peripheral blood. Discovering these connections embryo flushing and transfer in sheep will be more safe and predictable, and the variability between individuals will be more understandable.

## **2. Objectives of the thesis**

Metabolic hormones and metabolic parameters have a huge impact on ruminants' reproduction. It is well known, that these factors are nutrient dependent. We have mentioned above, that the success of the sheep embryo transfer is affected by nutrition, nutritional status, and condition of the donors and recipients. However, nothing is known about the impact of metabolic hormones and metabolic parameters on sheep ET programs. Next to these it has to be mentioned, that before any laparoscopic or surgical action, the animals has to be starved at least 36 hours before the operation (avoiding regurgitation and to help the operator to see clear in the abdominal cavity). The question is whether this starvation affects the outcome of the program or not?

### **Objectives of the experiment can be summarized in the followings:**

1. Performing two ET programs in and two ET programs out of season with Hungarian Merino breed sheep in farm conditions, where we examine:
  - (a) the ovulation ability by the superovulation hormone treatment in donor ewes (ovulation rate, number of corpora lutea (CL), number and quality of the embryos flushed)

(b) in recipient ewes after oestrus synchronisation: number of corpora lutea, progesteron (P4) production of the corpora lutea, and pregnancy rates after ET.

2. Determining the peripheral blood level of **energy-status dependent metabolites** (non esterified fatty acids /NEFA/, beta-hidroxy-butirate /BHB/, total-protein /TP/, urea and cholesterol), **metabolic hormones** (insulin-like growth factor 1 /IGF-1/, triiodothyronine /T3/, tiroxine /T4/ and insulin) and **progesterone (P4)**-informing about the presence and luteal activity of corpora lutea) in donor and recipient ewes in ET programs.

3. Investigating the connection of metabolites, metabolic hormones and P4 with

(a) the ovulation ability by the superovulation hormone treatment in donor ewes (ovulation rate, number of corpora lutea, number and quality of the embryos flushed)

(b) in recipient ewes after oestrus synchronisation: number of corpora lutea, progesteron (P4) production of the corpora lutea, and pregnancy rates after ET.

4. Are these connections mentioned above different in and out of breeding season?

5. We try to find the answer to the question: has the 36 hour long starvation (metabolic stress) before surgical embryo flushing or transfer any impact on the ewes'? Is the number and quality of flushed embryos/success of ET dependent of the metabolic and endocrine answer of the animals?

6. Describing the diagnostic and prognostic role of metabolites and metabolic hormones.

### 3. Materials and methods

Our research has been carried out in Mikepércs, in the farm of Szűcs-Juh Ltd., in the frame of a NIO (National Innovation Office) project ("Breeding and improving sheep corresponding to the market demands and climate"- *NKFP07A3-NOGYAPJU*).

More than 4000 Hungarian Merino, Charollais and British Milk Sheep are bred in the modern farm.

Assisted reproductive and embriological work (selecting donor and recipient ewes for the project, heat synchronisation, superovulation and insemination, embryo flushing, classification and transfer, embryo freezing, transferring frozen-thawed embryos) in the project was done

by the leadership of Prof. Dr. Sandor Cseh (Szent Istvan University, Faculty of Veterinary Sciences, Laboratory of Andrology and Assisted Reproduction). Endocrinological surveys were done by the leadership of Prof. Dr. Gyula Huszenicza† (Szent Istvan University, Faculty of Veterinary Sciences, Laboratory of Endocrinology). Researches mentioned in the dissertation were performed in the farm of Szűcs-Juh Ltd., while examinations of the blood samples were done in Budapest, Szent Istvan University, Faculty of Veterinary Sciences, Laboratory of Endocrinology.

Donor and recipient ewes chosen for the experiment had to suit for the following criteria: clinically healthy, aged between 2-5 years, lambed in the previous year, has a medium or good condition. Because of the separated treatments, feeding and examinations these animals had been chosen 6 months before the experiment started in 2009. February. We sorted the donor and recipient animals before the ET programs from a group of 100 ewes. Vasectomy (surgical ligation of the ductus deferens) has been done on four charollais breed rams aged between 1-2 years, to use them as teaser rams in the experiment.

Keeping and feeding of the animals represented the Hungarian farm conditions. Sheep were kept separated in a closed pen (with a runner).

We carried out 2 experiments per year (all together 4 ET programs during the 2 years): in 2009. February (in season), 2009. April (out of season), 2010. February (in season), 2010. April (out of season). 16 (7 donor and 9 recipient) ewes took part in the experiment in 2009. February, also 16 (6 donor and 10 recipient) in 2009. April. In 2010 February, 6 donor and 14 recipient, while in 2010. April 6 donor and 13 recipient animals participated in the essay. During the 2 years, all together 71 animals took part in the trial.

Preparation of the donor and recipient animals happened the same way during the ET programs, cycle/ovulation in biological breeding season/acyclic spring time has been induced/synchronised according to the same method, as generating superovulation and follow-up of ovarian response (Table 1.). Blood samples were taken from Merino ewes in and out of season 3 times for measuring the peripheral blood level of hormones and metabolites:

Donor ewes: at the time of artificial insemination (D0 sample), at the beginning of 36 hour long fasting before surgery (D2 sample), at the time of embryo flushing (D3 sample).

Recipient ewes: at the time of heat (D0 sample), at the beginning of 36 hour long fasting before surgery (D2 sample), at the time of ET (D3 sample).

Table 1.: Treatment and blood sampling protocol of the donor and recipient ewes

Treatment day	Time	Treatment	
		Donors	Recipients
1	8 h	Inserting intravaginal sponge <sup>1</sup>	
12	8 h	FSH <sup>2</sup>	---
	20 h	FSH <sup>2</sup>	---
13	8 h	FSH <sup>2</sup>	---
	20 h	FSH <sup>2</sup>	---
14	8 h	FSH <sup>2</sup>	Intravaginal sponge withdrawal+ eCG <sup>3</sup>
	20 h	FSH <sup>2</sup> + Intravaginal sponge withdrawal	---
15-16-17	a.m./p.m.	36h fasting before A.I.	Heat observing <sup>4</sup>
16	8 h	Laparoscopic A.I.+ GnRH <sup>5</sup> Followed by natural mating <sup>6</sup>	Heat observing <sup>4</sup>
		Blood sample collecting for metabolic and hormonal surveys (sample: D0)	
19	8 h	Blood sample collecting for metabolic and hormonal surveys (sample: D2), Followed by 36h fasting	
21	8 h	Embryo flushing (surgical) <sup>7</sup>	Embryo transfer (surgical) <sup>7</sup>
		(a) Blood sample collecting for metabolic and hormonal surveys (sample: D3)	
		(b) number and morphological features (size and colour) of CL (c) Determining body mass and nutritional status	

<sup>1</sup> 20 mg Chronogest CR intravaginal sponge, MSD Animal Health, France).

<sup>2</sup> FSH: Ovagen® inj. A.U.V., Immuno Chemical Products Ltd, New Zealand

<sup>3</sup> 500 IU Folligon (PMSG, MSD Animal Health, France.)

<sup>4</sup> Vasectomised rams.

<sup>5</sup> 50 µg buserelin- acetate (Receptal, Intervet, Angers, France)

<sup>6</sup> Placing rams into the group of donor ewes

<sup>7</sup> After 36 h fasting

Determining the peripheral blood level of **energy-status dependent metabolites** (non esterified fatty acids /NEFA/, beta-hydroxy-butirate /BHB/, total-protein /TP/, urea and cholesterol), **metabolic hormones** (insulin-like growth factor 1 /IGF-1/, triiodothyronine /T3/, tiroxine /T4/ and insulin) and **progesterone (P4)**-informing about the presence and luteal activity of corpora lutea) in donor and recipient ewes in ET programs was done in Szent Istvan University, Faculty of Veterinary Sciences, Laboratory of Endocrinology.

### **Artificial insemination (AI)**

Donor animals were inseminated artificially by using a laparoscope. Sperm of a white dorper breed ram (from the Experimental Farm of the University of Debrecen, Debrecen Kismacs) was used for the insemination. Freshly collected sperm was right evaluated (volume, consistency, motility, ratio of motile sperm cells). Animals has been prepared before all the surgical interventions (AI, embryo flushing, ET) according to the same protocol.

### **Embryo flushing and embryo transfer (ET)**

In the case of donor ewes, surgical embryo flushing, in the case of recipients, surgical embryo transfer was used (CSEH et al, 1986).

### **Statistical analysis**

Descriptive statistical analyses were performed by Microsoft Office Excel 2003 and SPSS for Windows 13.0 (SPSS Inc. Chicago, IL.) program. The results of the metabolic hormones and metabolites has been estimated by using correlation test, comparing the results of the single years. Comparison of the season and out of season period has been done by using T-probe (SPSS for Windows 13.0 /SPSS Inc. Chicago, IL./ program).

## **4. Results**

### **Results of the embryo transfer programs**

1. Comparing the ET programs 2009. and 2010. in season with the 2010. out of season program, we did not find any significant difference in the number of flushed, transferable embryos and the number of CL per donor ewe (Table 2.). We can conclude that in this case (donors) season did not affect the success of superovulation treatment and the outcome of the ET program. Next to the number of flushed and transferable embryos and CL of donors, results of recipient ewes can be seen in the next table.

Table 2: Summary of the average results of donor and recipient animals per ET program

	Number of flushed embryos/ donor	Number of transferable embryos/ donor	Number of CL/ donor	Recipients/ lambbed recipients (%)	Implantation ratio (%)*
<b>2009. February</b>	8	7.57	9	8/5 (62.5)	48
<b>2009. April</b>	2.3	0	4.5	6/0 (0)	0
<b>2010. February</b>	7.8	6.4	8.2	12/8 (66.6)	34
<b>2010. April</b>	11	7.3	13.16	12/7 (54)	27

\*lambs born/number of embryos transferred into recipients

In our research the average number of CL per ewe (considering the animals reacted to the superovulation treatment) was between 1 and 20. Average number of CL per recipient was between 4.5 and 13.16 (considering season and out of season as well).

2. In the case of recipients there was no significant difference between the programs in season nor in lambing rate neither in lambs born/number of embryos transferred into recipients ratio. The out of season program (2010. April) failed in the case of the parameters mentioned above from the season programs (Table 2.).

The explanation for this could be the out of season period, which influenced the pregnancy rate of Hungarian Merino recipients and the peripheral blood level of certain metabolic hormones. In the case of donors, the success of the superovulatory treatment was not influenced by the season, but in the same time pregnancy rate was affected by that in the case of recipient ewes (although significance  $/P \leq 0.1$  or  $P \leq 0.05/$  could not be shown with the statistical methods we used- we can mention these results as a tendency).

## **Results of the metabolic parameters in blood samples of the donor animals' in 2009. and 2010. April**

The influence of the blood level of metabolic parameters on the success of the ET programs was also evaluated in our research. It was analysed, how the blood level of some nutrient dependent metabolite (BHB, NEFA, AST, urea, cholesterol, TP) affects the blood level of P4, the answer of donor animals on the superovulatory treatment, the number of CL, flushed and transferable embryos per donor, and all together the success of the ET program.

The other approach is, that what is the nutritional background of the donor ewes with completely different superovulation reaction?

16% of the ewes (2/12) did not react to the superovulatory treatment at all, 33% (4/12) had low embryo number. Also 33% had good embryo production (5-10 embryo), while 16% of the ewes had record embryo production (>10 embryo). One of the donors produced 5 two-celled embryos, which has been transferred into recipients, but certainly we did not expect implantation (pregnancy).

According to the facts mentioned above, we can conclude, that breed, age, keeping and feeding conditions, out of season period, treatment protocol was the same in the case of all the donor animals. Despite these we found major differences in their reaction to the superovulatory treatment, which can be in connection with the level of nutrient-dependent metabolites in their peripheral blood.

The nutrient dependent metabolites (BHB, NEFA, urea, cholesterol, TP) we investigated are in close connection with the nutritive status of the animals. AST is an enzyme, which reflects to the damage rate of the liver.

1. Although we expected, that higher BHB levels will be in connection with the number of CL and embryos (SENOSY et al, 2012; MAILLO et al, 2012) there was no significant connection between these parameters. The explanation could be, that this low and short time change of ketone bodies in the peripheral blood had no influence on the ET program so far. Peripheral blood level of BHB on D0 had significant positive connection with the level of P4 on D3.

2. We found significant positive connection between the level of NEFA (on D0, D2 and D3) with the number of flushed embryos, also between the level of NEFA on D3 and the number of transferable embryos. In this case we expected exactly the opposite result, as some scientific article shows: higher levels of NEFA has a negative impact on the number of CL

and embryos (VAN HOECK et al, 2011; SENOSY et al, 2012; OBA et al, 2013). For the explanation, further investigations are needed with higher number of experimental animals.

3. Cholesterol values of the donors (on D0 and D3) show significant positive connection with the number of CL. We found significant positive connection with the number of flushed embryos and the cholesterol levels on D0, D2 and D3, and also significant positive connection with the number of transferable embryos and the level of cholesterol on D0 and D2 (Figure 2.). So we can conclude that the peripheral blood level of cholesterol influences the number of CL and embryos in an ET program.

Figure 1.

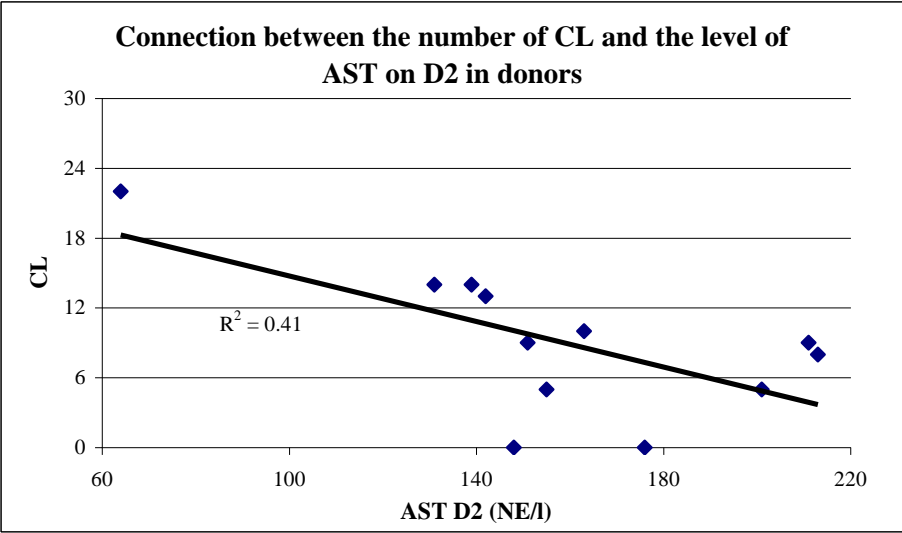
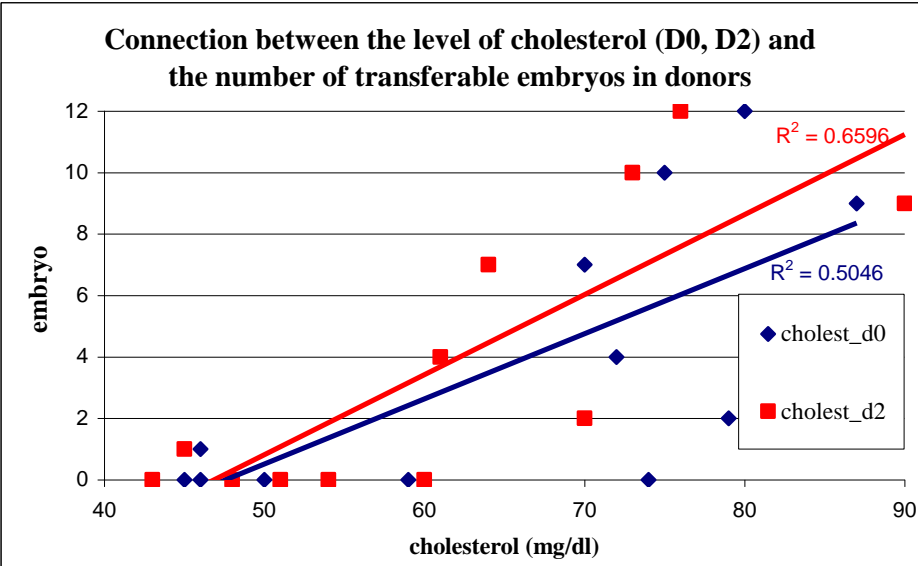


Figure 2.



4. According to our prior expectations D2 level of AST (days after AI) show significant negative connection with the number of CL (Figure 1.), flushed embryos and the blood level of progesterone. It means, that elevated level of AST in sheep has a strong negative effect on the reproduction and the ET program. The blood level of this enzyme influences the number of CL and embryos, and also reduces the blood level of P4. This phenomenon has not been published in small ruminants yet.
5. We proved, that D0 level of urea has a significant negative connection with the number of CL, flushed and transferable embryos in sheep, so has a great role in the success of the ET program.
6. We found no connections between the total protein levels and the outcome of the program (reproductive performance), although such connections has already been published (WALSH et al, 2012).

We concluded by the results and connections shown above, that peripheral blood level of certain metabolic parameters has a relationship with the success of the ET programs in sheep. Even if these metabolic parameters were in the normal, physiological range (except for AST), their level effected the donors' reaction to the superovulatory treatment. The so called "relative energy defficiency" has not been manifested in clinical signs, but influenced that sensitive hormonal and reproductive balance necessary for good/high embryo production. We also demonstrated, that these metabolites, mentioned above have a great impact on number of corpora lutea, flushed and transferable embryo number.

### **Peripheral blood level of metabolic hormones and it's connection with the outcome of the ET program**

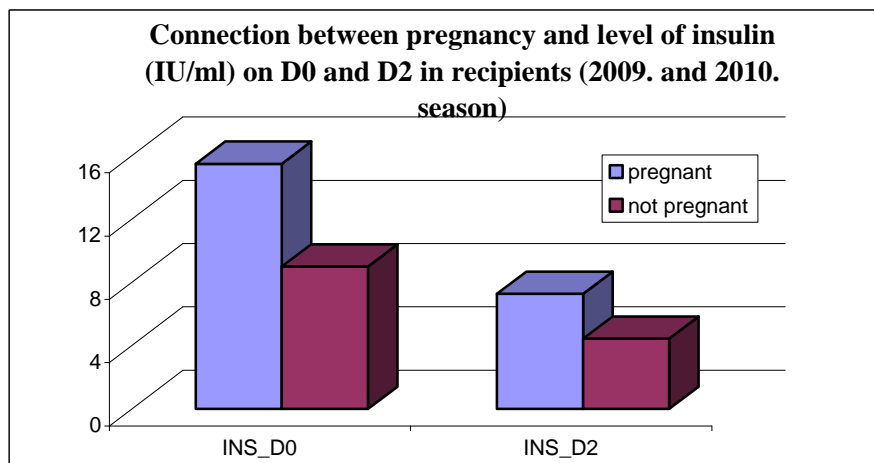
Blood level of metabolic hormones are also important factors in the success of the ET program.

1. Recipients: there is a significant connection between the blood level of T4 and pregnancy of the ewes in 2009. Tiroxine concentration was lower in pregnant ewes' blood on D0. We found significant positive connection between the peripheral blood level of insulin (D0 and D2) and the pregnancy (Figure 3.). Level of insulin in the blood changes paralelly with the level of IGF-1 (VELAZQUEZ et al, 2005), and the role of IGF-1 is unquestionable in reproduction: these can explain the positive effect of insulin on the implantation of embryos.

2. Donors: there is a significant negative connection between the level of T4 (D2) and the number of flushed embryos and CL. Number of CL and flushed embryos has significant positive connection with the level of P4 on D0 and D3.

Peripheral blood level of IGF-1 has a significant positive connection with the number of flushed embryos (2009.). Further investigations did not confirm these results: peripheral blood level of IGF-1 showed significant negative connection with the number of flushed embryos (in 2010.) and CL (in 2010). In this case, BLOCK's (2007) statement is relevant, who published, that the impact of IGF-1 on the development of the embryo can be positive or negative as well- in abeyance of the stimulus of the endogenous environment.

Figure 3.



Changing of the IGF-1 level from D0-D2, and D0-D3 has a significant positive connection with the number of flushed embryos and CL. More the blood level of IGF-1 reduces between the blood samplings, more CL and collected embryos could be found per donor. This confirms the results of 2010. (where the negative effect of IGF-1 was shown in connection with the embryo survival and development).

Comparing **the level of metabolic hormones in and out of season**, we found seasonal variance. Our investigation shows, that basic level of IGF-1 in donors and IGF-1 and insulin level in recipients is different in February (season) and April (out of season).

In donors basic level of IGF-1 was higher in April, while in recipients levels of IGF-1 and insulin was higher in February. T3 (D0 and D3) level of donors show significant difference between February and April (higher in April), and T4 level is also higher in April (tendency).

In recipients peripheral blood level of T3 on D3 is significantly higher in April. In the case of P4: basic level of P4 is higher out of season, while D2 and D3 level is higher in season. Seasonal variance is in connection with the results of the programs in every case.

In the comparison of the results of the ET programs (2 season and 2 out of season in 2009. and 2010.) and the levels of metabolic hormones and metabolites, we can conclude, that these factors affecting the success of an ET program in sheep.

The results, described above help scientists and breeders to understand how this complex system of superovulation and embryo production could be influenced to make ET programs more successful and predictable.

## **5. New scientific results**

1. Merino breed sheep for superovulation and as embryo donor can be successfully applied in and out of season, while using the breed as recipient failed in pregnancy and lambing rate as well (and greatly influenced by season). That's why using the breed in ET programs as donor is recommended, but not as a recipient.

2. We assessed, that even if the metabolic parameters are in the normal, physiological range, their level can effect that sensitive hormonal and reproductive balance necessary for average/good/high embryo production in sheep.

3. We found out that elevated levels of AST has a huge negative impact on reproductive performance in sheep. It affects (reduces) the number of corpora lutea, transferable embryos, and also reduces the peripheral blood level of P4.

4. Certain metabolic hormones (IGF-1, insulin, T3, T4) show seasonal variance when using Merino breed in ET programs, which affects the embryo production of donors, and the pregnancy rate of recipients. Blood level of T4 has a negative impact on the number of flushed embryos and corpora lutea. Peripheral blood level of tiroxine has a negative, while level if insulin has a positive effect on the pregnancy rate of the recipient ewes.

## 6. Useability of the results in practice

Animal breeding in the 21st century is inconceivable without using assisted reproductive techniques and reproductive innovations. Unfortunately not the simplest artificial insemination, nor the embryo transfer could not disperse among sheep breeders in Hungary. The reason why is the additional prices, ignorance, etc., but the most important is: the unpredictability of the superovulation and the ET programs. Although these programs are quite expensive, we can not forecast how a donor or recipient ewe will perform in a program. Several known and unknown factors have an impact on the efficiency. These "known" factors are the followings: keeping, feeding, breed, age, condition of the donor and the recipient ewes', season, treatment protocol, number of embryos transferred..etc. Unfortunately, gaining of our knowledge in connection with the success of superovulation is very little in the last few decades.

Several new, unpublished scientific results has been described in the dissertation. These help the better understanding of sheep reproduction and phisiology of superovulation:

1. We performed 2-2 embryo transfer programs in (February) and out (April) of breeding season, with the most current breed in Hungary, the Hungarian Merino. There was no significant difference between the number of corpora lutea nor the number and quality of flushed and good quality embryos/donor in the programs of 2009. and 2010. All together we can conclude, that season had no effect on the effectiveness of superovulation, so using the breed as embryo donor is recommended.

In the case of recipients in season, there has been no difference in lambing rate or lambs born/embryos transferred ratio. In 2010. out of season lambing rate and lambs born/embryos transferred ratio is failed compared to season programs. In conclusion, Hungarian merino breed is a very good donor in ET programs, but we suggest to use another breed (out of season) as recipient.

2. Role of optimal nutrition is well known in ruminant reproduction, but it was obscure yet, that how this sensitive hormonal and reproductive balance necessary for average/good/high embryo production is affected by the level of some metabolic parameters. Peripheral blood level of certain metabolic parameters (BHB, urea, NEFA, TP and cholesterol) affect the number of corpora lutea and the number and quality of flushed and transferable embryos in donor animals after the superovulation treatment even if their level "stand" in the normal, physiological range. In small ruminants, elevated levels of AST has a negative impact on the

reproductive performance, lowers the number of embryos and CL, and reduces the level of P4 in the peripheral blood.

Before planning an embryo transfer program in farm/experimental conditions, it is worth to take a blood sample from the donor animals, because its result could forecast the ewes' performance in the program. After a simple blood sampling it can be decided whether the energetic and animal health status of the chosen donor animal is suitable for the ET program or it has to be improved. With the help of these metabolic parameters we can avoid to choose ewes' whose energetic/animal health status does not support medium/good/record embryo production.

3. Metabolic hormone levels in the peripheral blood is a less known area in connection with sheep ET programs. IGF-1 and tiroxine levels have a negative impact on embryo development (number and quality of flushed and transferable embryos) in donors. Pregnancy of the recipients is affected by the level of tiroxine (negatively) and insulin (positive correlation). When we are talking about the practical useability, the role of insulin on recipients is the most important, because it's basic, D0 level already forecasts the outcome of the pregnancy. Elevated levels of insulin in the recipients' blood serum during heat indicates higher pregnancy rates after ET.

In the same time, we have to underline, that further investigations (with higher number of experimental animals) are needed to lay down the details of prognostic and diagnostic role of metabolic parameters and hormones.

4. Certain metabolic hormones (IGF-1, insulin, T3, T4, P4) show seasonal variance when using Merino breed in ET programs, which affects mainly the pregnancy rate of recipients. Elevated levels of IGF-1 and insulin, and reduced level of T3 in season is favourable for higher pregnancy rates in recipients. These data are very important when planning an ET program in a flock in Hungary. Choosing Hungarian Merino for recipient is not a good decision when planning the embryo transfer program out of season in Hungary.

The role of metabolic hormones and parameters in connection with the sheep ET program was discussed in the Ph.D. dissertation. These factors could have diagnostic role, and able to predict, whether a donor or recipient ewe is suitable for the program or not.

## 7. Publications

### Scientific papers in reviewed journals in Hungarian:

**VASS N.** – JÁVOR A. – SOLTI L. – CSEH S. (2013): A juhtenyésztésben alkalmazott asszisztált reprodukciós technikák. Magyar Állatorvosok Lapja. 135. 400- 409. (IF: 0.146)

NOVOTNINÉ DANKÓ G.- GYIMÓTHY G.- OLÁH J.- KULCSÁR M.- MAGYAR K.-  
**VASS N.**- POSTA J.- BALOGH P.- JÁVOR A. (2012): Tavaszi- nyári reprodukciós folyamatok nyomon követése és takarmánykiegészítő hatásának vizsgálata juhnál. Magyar Állatorvosok Lapja. 134. 83-88. (IF: 0.146)

OLÁH J. – FAZEKAS G. – PÉCSI A. – **VASS N.** – KOVÁCS A. – JÁVOR A. (2009): Tárolási hőmérséklet hatása különböző fajtájú kosok ondójának minőségére. Acta Agraria Debreceniensis. 37. 75-79.

**VASS N.** – KLAMBAUER P. – JÁVOR A. – KERESZTES ZS. – CSEH S. (2009): Embrió mélyhűtés a vitroloop vitrifikációs technikával. Acta Agraria Debreceniensis 37. 81-83.

FAZEKAS G. – HARANGI S. – **VASS N.** – OLÁH J. (2009): A réti csík (*Misgurnus fossilis*) kora és növekedése. Acta Agraria Debreceniensis. 37. 37-44.

**VASS N.** – JÁVOR A. – BALOGH P. – CSEH S. (2010): A szuperovuláció és az embrióátültetés eredményességét befolyásoló tényezők. Szezon és hormonális háttér. Acta Agraria Debreceniensis. 40. 73- 76.

OLÁH J. – **VASS N.** – KUSZA SZ. – POSTA J. – HARCZA A. – PÉCSI A. – KOVÁCS A. – JÁVOR A. (2010): Tenyészkosok ugrási sorrendjének vizsgálata. Acta Agraria Debreceniensis. 40. 59-62.

### **Scientific papers in Hungarian reviewed journals in foreign language:**

KLAMBAUER P. – KERESZTES ZS. – KANYO K. – VARGA E. – KRISTON R. – VASS N. – JÁVOR A. – KONC J. – SOLTI L. – CSEH S. (2009): Vitrification of cleavage mouse embryos by cryoloop procedure. *Acta Veterinaria Hungarica*. 57. 3. 399-410. (IF: 0.642)

FAIGL V. – VASS N. – JÁVOR A. – KULCSÁR M. – SOLTI L. – CSEH S. (2012): Artificial insemination of small ruminants (Review). *Acta Veterinaria Hungarica*. 60. 1. 115-129. (IF: 1.173)

### **Scientific papers in reviewed journals in foreign language:**

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