**Szent István University**
**Postgraduate School of Veterinary Science**

# Examination of risk factors influencing health status, reproductive performance and milk yield in the peripartal period in dairy cows

(Tejhasznú tehenek egészségi állapotát, szaporodási teljesítményét és tejtermelését befolyásoló kockázati tényezők vizsgálata az ellés körüli időszakban)

**PhD thesis (brief summary)**

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# Abbreviations

AcA: acetoacetic acid
AI: artificial insemination
AST: aspartat-aminotransferase
BCS: body condition score
CEM: clinical endometritis
CL: corpus luteum
NABE: net acid-base excretion
NEB: negative energy balance
NEFA: non-esterified fatty acids
NS: not significant
OR: odds ratio
P: significance
$P_4$: progesterone
PM: puerperal metritis
pp.: postpartum
prep.: prepartum
PUD: postpartum uterine diseases
RP: retained placenta
TPM: toxic puerperal metritis
vs.: versus

# 1. Introduction

The "Strategy for Animal Health" of the European Union for the period between 2007-2013 years subtitled as: **"Prevention is better than therapy".** This fact indicates the importance of the preventive approach the animal health problems.

Intensively producing dairy herds are characterised by increasing milk production accompanied by declining reproductive performance largely due to nutrition- and management-related production diseases developing in the peripartal period. Involution problems, retained placenta (RP), postpartum uterine diseases (PUD) and ovarian malfunction are important disorders of the dairy cows.

The role of metabolic status, more specifically of the disturbances of energy metabolism and acid-base status, as risk factors preceding the development of the above production diseases is not completely clarified. The negative energy balance (NEB) characterising the peripartal period in dairy cows is a well-known phenomenon. The adaptation process of cows to NEB involves a series of physiological, metabolic and endocrine changes. This adaptation process can be monitored with the help of carefully selected parameters. Deviations of these parameters from the physiological range indicate disturbances of this adaptation and can, therefore, be defined as risk indicators. The knowledge of these indicators makes possible assessing the risk of disease development and to elaborate measures for the prevention.

Limited information is available about strategies for the prevention of production diseases, although continuous development of such strategies is needed. Early diagnosis and prevention of the production diseases should be based on a thorough knowledge of the risk factors involved in their development.

The objective of the work was to obtain a better understanding of risk factors predisposing cows to production diseases. For this purpose we studied the correlations between uterine involution, uterine diseases, ovarian function and parameters that characterise the energy balance and acid-base status of dairy cows as well as certain animal- and environment-dependent factors. The consequences of RP and PUD for subsequent metabolic status, reproductive performance and milk production were also analysed.

## 2. Materials and methods

The present thesis has been based on two experiments performed in dairy cows.

### 2.1. First experiment

### 2.1.1. The aim of the experiment

The aim of the first experiment was to evaluate the effect of negative energy balance (NEB) - manifested in elevated plasma NEFA concentration - on the anatomical involution of the uterus, on the reactivation of the ovarian function, and on the incidence of retained placenta (RP) and postpartum uterine diseases (PUD).

### 2.1.2. The design of the experiment

Clinically healthy Holstein-Friesian dairy cows (n=28) were involved in the survey. The investigation started 10 days before their expectedtime of parturition. The studies comprised the following elements.

*Metabolic and endocrine tests*

To study the *energy balance* blood samples were taken for laboratory tests 3–5 hours after the morning feeding on day 10 prepartum, on the $3^{rd}$ day postpartum (pp.) and on every $10^{th}$ day onwards until day 60 postpartum,. Blood samples were taken from the *vena epigastrica superficialis* into test tubes containing 50 µl sodium heparin as anticoagulant. The samples were kept at + 4 °C and transported to the laboratory within 4 hours. The energy metabolism of the cows was evaluated by determination of the body condition score (BCS), the plasma concentrations of non-esterified fatty acids (NEFA), acetoacetic acid (AcA), glucose and activity of the aspartat-aminotransferase (AST) enzyme.

Onset of cyclic *ovarian activity* was monitored by analysing the plasma progesterone ($P_4$) concentration on every $3^{rd}$ day from days 20 to 21 until days 59 to 60 postpartum.

The *uterine health* was clinically examined on days 5, 20 and 40, respectively. Transrectal ultrasonography was applyied on days 30 and 60 to check the *uterine involution* and follow up the structural changes of the ovaries. To characterise the anatomical involution of the uterus a scoring system ($U_1$-$U_4$ score) was applied.

Data were collected on the incidence of retained placenta and postpartum uterine diseases, and the onset of oestrus.

Cows were divided into two groups on basis of their plasma non-esterified fatty acid (NEFA) concentration. Cows (n=12) that had ≥0.2 mmol/l plasma NEFA concentration at least two subsequent samplings were collected into a group designated H-NEFA. The other group (Normal group, n=16) had either lower plasma NEFA concentration than the aforementioned threshold or had surpassed it only at one occasion.

### 2.1.3. Results of the first experiment

In the H-NEFA group, on days 10 and 20 postpartum (pp.) the AST activity ($P<0.05$), on day 10 the plasma acetoacetic acid (AcA) concentration ($P<0.05$), and prepartum (prep.) body condition score ($P<0.05$) were higher than in the Normal group. The plasma glucose concentration of the H-NEFA cows on day 3 was lower ($P<0.05$) than that of the Normal cows. In the H-NEFA group, uterine score was higher (2.8 vs. 2.2; $P<0.05$) on day 30. pp. It follows: the energetic status of the groups has shown remarkable difference.

Corpus luteum was less frequently present in H-NEFA cows until days 30 and 60 pp. (8.3% vs. 18.8%; not significant (NS) and 33.3% vs. 75.0%; NS, respectively). The first progesterone ($P_4$) phases were detected in lower percentage of H-NEFA cows until days 28 (8.3% vs. 31.3%; NS) and 35 (25.0% vs. 43.8%; NS) postpartum. Proportion of oestrus cows was less in the H-NEFA group until day 60 pp. (6.3% vs. 25.0%). Until day 30 pp. the incidence of RP was higher in the H-NEFA cows (41.7% vs. 18.8%; NS). The proportion of clinical PUD was also higher in the H-NEFA cows (until days 30 and 60 pp.: 100.0% vs. 68.8%; NS and 66.7% vs. 37.5%; NS, respectively).

## 2.2. Second experiment

### 2.2.1. The aim of the experiment

The objective of the second experiment was to study the relevance of certain metabolic parameters characterising the energy balance and acid-base status of dairy cows as well as certain environmental and animal-dependent predisposing factors in the risk assessment of manifestation of RP and PUD. Other goal was to study the effects of RP and PUD including puerperal metritis (PM) on the metabolic status, reproduction and milk yield.

### 2.2.2. The design of the experiment

A total of 105 clinically healthy Holstein-Friesian cows in two weeks before the expected calving were included in the study which comprised the following elements.

*Metabolic tests*

For measuring the parameters of the *energy balance and acid-base status*, blood and urine samples were taken 3–5 hours after the morning feeding on day <14 prepartum and on days 4, 10–14, 28–35 and 56–63 postpartum. Blood samples were withdawn from the *vena epigastrica superficialis* into test tubes containing 50 µl sodium heparin as anticoagulant. Urine samples were obtained by catheterisation of the urinary bladder. The samples were kept at + 4 °C and transported to the laboratory within 4 hours.

The energy metabolism of the cows was evaluated by determination of the BCS (1-5 score), the plasma NEFA, AcA, and glucose concentrations and AST enzyme activity. The grade of ketonuria (+/++/+++) was measured by semi-quantitative determination of the urine AcA concentration.

The acid-base status of the cows was evaluated by determination of the urine pH and net acid-base excretion (NABE).

*Clinical examinations and data collection related to calving and health status*

The data of parity, date of calving and the use of calving assistance, and the viability and sex of the calf were recorded. During the first 14 days postpartum, the rectal temperature and the clinical diseases were recorded daily. Retained placenta was diagnosed if spontaneous expulsion of the foetal membranes did not occur within 24 hours after calving.

*Monitoring uterine health and involution*

To diagnose different clinical manifestations of PUD - as puerperal metritis (PM), toxic puerperal metritis (TPM), clinical endometritis (CEM) and pyometra - and to monitor uterine involution, rectal and clinical examinations were performed on postpartum days 4, 10–14 and 28–35.

To characterise the anatomical involution of the uterus a scoring system ($U_1$-$U_4$ score) was applied at 28-35 days postpartum.

*Study of the ovaries and the oestrous cycle*

In order to characterise ovarian function and the structures present on the ovaries, on postpartum days 28–35, 56–63 and 84–91 rectal examination of the ovaries was performed using an ultrasound scanner. The presence of follicles, corpus luteum (CL), follicular and luteal cysts was studied and recorded. Ovarian function was considered cyclic if a CL was present.

*Data on reproduction and milk production*

The dates and results of artificial inseminations (AI), the calving to conception interval, and the serial number of the successful AI were recorded. The quantity of milk produced on day 4 (kg), the quantity of milk, butterfat and milk protein (kg) and the butterfat and milk protein percentage produced during the first 100 days of lactation and, in the case of the multiparous cows, the milk production in the previous 305-day lactation (kg) were also recorded.

### 2.2.3. Results of the second experiment

On days <14 prepartum, the plasma NEFA concentration showed positive [Odds Ratio (OR):102.1; P<0.05], while urinary net acid-base excretion (NABE) exhibited negative correlation (OR:0.99; P<0.05) with the odds of RP, while NEFA and NABE were negatively correlated (P<0.05). Ketonuria of grade at least 2+ increased the probability of RP (OR:Infinite; P<0.05). The odds of RP were not influenced by parity, the sex and viability of the calf, and the calving assistance. RP increased the risk of PM (OR:27.3; P<0.001). The RP alone did not influence the metabolic status, reproductive performance or milk production of cows. When at least 1+ grade ketonuria was present on day 4 pp. PUD including PM had higher odds on days 10–14 pp. (OR:2.64; P<0.05 and OR:2.65; P<0.05, respectively). Plasma NEFA concentrations >0.2 mmol/l in days <14 prep. indicated higher risk of PUD (OR:3.44; P<0.05). The odds of PUD increased, depending on whether a BCS loss of at least 1.0 occurred between days <14 prep. and 28–35. pp. (OR: 2.82; P<0.05], between days <14 prep. and 10–14. pp. (OR:4.79; P<0.01) or between pp. days 10–14 and 28–35. (OR:10.81; P<0.01). The risk of PUD was lower in multiparous than in primiparous cows (OR:0.29; P<0.01). PM increased the risk of the presence of morphologically inactive ovaries between pp. days 28-35 (OR:2.83; P<0.05). Cows affected with PM (PM+ cows) had lower milk production on day 4 (kg; P<0.05) and produced less milk (P<0.05), milk fat and milk protein (kg; P<0.01 and P<0.01, respectively) in the first 100 days of lactation than PM– cows.

The information obtained by the present experiments contributes to predicting, monitoring and preventing peripartum adaptive health and reproductive disorders of dairy cows.

## 3. New scientific results

(1) Data of the present investigations suggest the existence of

- positive correlation between probability of placenta retention and increased plasma concentration of NEFA and ketonuria characterised by higher than 2+ grade and
- negative correlation with urinary NEBA concentration.

Therefore, in the last 14 days prepartum, the increased plasma NEFA concentration, the decreased urinary NABE concentration and the at least 2+ grade ketonuria have been identified as risk indicators of the development of retained placenta in dairy cows.

(2) Significant positive correlation was found between development of postpartum uterine diseases and the at least 0.2 mmol/l plasma NEFA concentration measured in the period of 14 days prior to parturition. This correlation may identify the higher than 0.2 mmol/l prepartum NEFA concentration as early indicator of risk for postpartum uterine diseases.

(3) In cows that had at least 0.2 mmol/l plasma NEFA concentration detected within 7-10 days difference in the first 30 days of lactation, the anatomical involution of the uterus lagged behind those that had either lower plasma NEFA concentration than the threshold mentioned above or the concentration had elevated over the threshold value only one occasion. Therefore, the increased plasma NEFA concentration has been identified as risk indicator of the development of the delayed anatomical involution of the uterus in dairy cows.

(4) Odds for the development of postpartum uterine diseases increased in dependence of time and degree of declining body condition.

# 4. The author's subject related publications

**Full papers in peer reviewed scientific journals**

Könyves, L., Szenci, O., Jurkovich, V., Tegzes, L., Tirián, A., Solymosi, N., Gyulay, Gy. and Brydl, E. (2008): Periparturient risk assessment for retained placenta in dairy cows. Acta Veterinaria Brno (accepted for publication). (IF: 0.41)

Könyves, L., Szenci, O., Jurkovich, V., Tegzes, L., Tirián, A., Solymosi, N., Gyulay, Gy. and Brydl, E. (2008): Risk assessment of metritis and consequences of puerperal metritis for subsequent metabolic status, reproduction and milk yield in dairy cows. Acta Veterinaria Hungarica (accepted for publication). (IF: 0.54)

Könyves L., Szenci O., Jurkovich V., Tegzes L., Beckers, J. F., Brydl E. (2008): Examination of some reproductive indices of peripartal period in relation with energy metabolism in dairy cows. Magyar Állatorvosok Lapja (accepted for publication) (IF: 0.114)

**Abstracts of the presentations on scientific congresses**

Könyves, L., Szenci, O., Jurkovich, V., Tegzes, L., Tirián, A., Solymosi, N., Gyulay, Gy. and Brydl, E. (2008): Periparturient risk assessment for retained placenta in dairy cows. Abstract. XXV Jublee World Buiatrics Congress, 6-11 July, 2008. Budapest, Hungary. Magyar Állatorvosok Lapja 130. Suppl. II. 10.

Könyves, L., Brydl, E., Szenci, O., Jurkovich, V., Tegzes, L.: The effect of the increased fat mobilisation on the uterus involution and ovarian cycle in dairy cows. Abstract. In: Proceedings of the 23rd World Buiatrics Congress, 11-16 July, 2004 Québec City, Kanada. Le Médecine Vétérinaire du Québec 34. Suppl. 1-2. 144.