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Growth hormone genotype (*AluI* polymorphism), metabolic and endocrine changes, and the resumption of ovarian cyclicity in postpartum dairy cows

Theses of Ph.D. dissertation

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dr. Balogh Orsolya

INTRODUCTION

The onset of lactation involves an orchestrated system of various metabolic and endocrine changes in Holstein-Friesian (HF) cows where growth hormone (GH) have a crucial role. GH usually increases in concentration from the dry to the early lactation period and even further during ketonemia *(Chagas et al., 2006)* accelerating lipolysis in adipose tissue and gluconeogenesis in the liver *(Bell, 1995; Lucy et al., 2001)*. In ruminants growth hormone is responsible for galactopoiesis and for the persistency of lactation *(Svennersten-Sjaunja and Olsson, 2005)*. Dairy cattle lines selected for high milk production release larger amounts of endogenous GH than lines with average (lower) milk production *(Løvendahl et al., 1991; Zinn et al., 1994)*, although recent reports were not able to prove this association (*Baumgard et al., 2002; Weber et al., 2005)*. *Taylor et al. (2006)* could neither demonstrate a relationship between prepubertal GH, insulin like growth factor-I (IGF-I), insulin and glucose measures in female HF calves with their subsequent 305-day lactation and peak yields.

A polymorphic site of the GH gene (*Alu*I polymorphism) that results in an amino acid change at position 127 of the polypeptide chain (leucine, L to valine, V; *Lucy et al., 1991*) has been linked to milk production traits. Some authors favored the leucine *(Shariflou et al., 2000; Dybus, 2002)* or the valine allele *(Grochowska et al., 2001; Kovács et al., 2006)* in regards to milk yield, while others could not prove an association *(Yao et al., 1996, Lechniak et al., 2002a)*. Endocrine features and a few reproductive characteristics related to different *Alu*I genotypes have also been tested, but results remained inconsistent and further investigations are lacking.

AIMS OF STUDIES

- to investigate (i) the role of *Alu*I polymorphism in the resumption of ovarian activity post partum (PP) and (ii) whether GH genotype may directly or indirectly influence milk production and the degree of body condition change shortly after calving in HF cows.
- to determine if there is an interrelationship between *Alu*I genotype, incidence of hyperketonemia status and plasma concentrations of certain metabolic hormones in the first two weeks after calving in HF cows.
- to study (i) whether *Alu*I polymorphism of the GH gene is involved in the development of insulin resistance (IR) at the onset of lactation and (ii) whether *Alu*I genotype is related to milk yield and reproductive characteristics during the first 200 d PP in HF cows.

EXPERIMENTS

Experiment 1

Study conditions

HF cows from four large-scale herds in Hungary were involved in this study (n=356, November 2000-January 2001 calvings, average 305-d yield: 7402 kg). Inflammatory conditions with extensive endotoxin and cytokine release may delay the first PP ovulation, therefore cows with systemic signs of toxic puerperal metritis (n=14), mastitis (n=25) or both (n=10) 6-10 d PP were excluded and 307 cows participated in the experiment. Due to the expected low frequency of the valine allele and the VV genotype, cows were classified into groups of leucine homozygous (LL) and heterozygous cows, which also included small numbers of the valine homozygous animals (LV + VV). Individual body condition scores (BCS) were monthly obtained and estimates of losses between Day 1 and Day 30 PP ($BCSL_{30}$) were used in the statistical model after logarithmical transformation. Total (cumulative) fat and protein corrected milk yield in the first 30 d PP (TMY_{30}) was provided from monthly recordings. Resumption of cyclic ovarian activity was monitored by individual milk progesterone (P_4) profiles.

Results and discussion

The leucine allele was more frequent than the valine (0.896 vs. 0.104), so 80.13 % of cows were LL, 18.89 % were LV and only 0.98 % were VV. These allele and genotype frequencies are similar to previous reports in the HF breed.

All animals became cyclic during the study period (27.6 ± 0.69 d PP on average); all LV cows ovulated by 64 d PP and all LL cows resumed cyclicity by 98 PP. However, there was no significant association between *Alu*I genotype and the interval from calving to first ovulation. Earlier studies could not find a difference between *Alu*I genotypes in breeding values of dairy bulls and in oocyte and embryo characteristics, however, PP reproductive traits were not investigated. We found that multiparous cows ovulated sooner than primiparous cows possibly due to the higher energy demand imposed on first parity animals by simultaneous growth and lactation. Cows with more $BCSL_{30}$ took longer to restore ovarian cyclicity. A negative association between fertility traits and severe BCS loss after calving was previously demonstrated so that cows with higher BCS at calving and during early lactation or with less BCS loss PP ovulated sooner than their herdmates. The degree of BCS is an indirect measure of energy balance (EB) and the extent of negative EB and its nadir are inversely related to commencement of PP ovulatory cycles. Because of the positive association between early resumption of cyclicity and conception rates, it is of great importance that cows ovulate soon after calving. In accordance with previous results of our research group, the presence of puerperal metritis without systemic signs did not delay re-establishment of ovarian activity in the current study. *Benzaquen et al. (2007)* and *Shrestha et al. (2004)* did not find a difference in the interval from calving to first estrus and to conception or in the percentage of anovulation between healthy cows and cows with uterine infection. On the other hand, irregular cycles and prolonged days to first ovulation were associated with abnormal uterine content and (endo)metritis before. This inconsistency may partly result from discrepancies in the description and determination of uterine abnormalities occurring at various stages of the periparturient period.

Cumulative milk yield of LL cows was similar to LV, which might be due to differences in management and nutrition practices between herds, and to the fact these cows were average producers and the effect of *Alu*I genotype might only be expressed at higher production levels. Multiparous cows yielded significantly more milk than primiparous cows. *Alu*I polymorphism was not associated with the degree of BCS loss, but cows with puerperal metritis experienced severe losses compared to healthy animals. The catabolic state that characterizes the onset of lactation can be further aggravated by inflammatory conditions resulting in reduced dry matter intake and fatty acid mobilization from adipose tissue. Decreasing fat deposits are reflected in an increase in BCS loss.

In conclusion, under field conditions, *Alu*I polymorphism of the GH gene has no effect on the interval from calving to first ovulation and can not be directly related to differences in milk yield and to BCS loss during the first month PP in HF cows. Severe BCS loss shortly after calving translates to delayed resumption of ovarian cyclicity. Multiparous cows ovulate sooner and produce more milk compared to primiparous cows. Puerperal metritis without systemic clinical signs is associated with more BCS loss.

Experiment 2

Study conditions

Group-fed HF cows (n=379; =2nd lactation, BCS at calving: 3.00-3.75, 305-d previous yield: 7764 kg) calving from February to mid-June, 2004 in seven herds were included in this study. Cows with clinical signs of metabolic disorders, toxic puerperal metritis, mastitis, laminitis, chronic gastro-intestinal illnesses and those that received glucocorticoids, non-steroidal anti-inflammatory drugs, any glucoplastic agents in the previous two weeks were excluded (n=122) and a total of 257 cows participated in the experiment. Blood samples were taken 4-13 d PP for *Alu*I genotyping and plasma ß-hydroxibutyrate (BHB), insulin, IGF-I and leptin determination. Threshold of hyperketonemia was set at plasma BHB > 1.2 mmol/L.

Results and discussion

Allele ($p_{Leucine}$=0.920 and q_{Valine}=0.080) and genotype frequencies in this study were in accordance with previous reports and with *Exp. 1.* Distribution of LV cows, however, was unequal among herds, as 72 % belonged to Herd 4 and 6 (n=100).

*Alu*I genotype was not associated with the incidence of hyperketonemia or any metabolic hormone measurements either in all 7 herds or in Herd 4 and 6. Similarly, *Ge et al. (2003)* could not relate IGF-I levels to *Alu*I polymorphism in Angus calves, whereas there was a tendency to higher IGF-I concentrations in LV Simmental bulls. Conversely, LL calves showed the highest insulin and IGF-I levels and VV animals had significantly higher leptin and triglyceride concentrations Direct comparison of these data to our findings is not possible due to differences in breed (dairy or meat type), age, allele frequency and physiological state.

Plasma BHB ranged between 0.11 and 5.18 mmol/L (1.25 ± 0.95 mmol/L), and a total of 100 animals had BHB above the threshold of 1.2 mmol/L and 157 cows were normoketonemic.Cows with plasma BHB over the threshold had significantly lower insulin, IGF-I and leptin levels than normoketonemic animals. There were negative correlations between BHB and IGF-I, insulin and leptin levels, while all hormones were significantly and positively related to each other. At the onset of lactation, cows enter a state of negative energy balance (NEB) that lasts for several weeks due to nutrient incongruency between demand of the mammary gland and intake. As a consequence they mobilize adipose fat and skeletal muscle protein stores, and according to the magnitude of NEB decreased plasma insulin, IGF-I, thyroid hormones (T_4, T_3), leptin and increased BHB and non esterified faatty acids (NEFA) concentrations are found. Therefore, hormonal profiles of our hyperketonemic cows may suggest that they were in a deeper point of NEB compared to normoketonemic cows.

In our study plasma insulin was influenced by the number of days elapsed since calving and BHB was related to previous 305-day lactation yield. Insulin levels usually fall around calving but recover by Day 30 PP and are less associated to EB, than IGF-I. Highest individual milk yield was weakly, but significantly correlated with highest milk acetone level, however, an inverse relationship between actual milk yield and plasma BHB has also been demonstrated.

In conclusion, *Alu*I polymorphism did not affect plasma concentrations of ß-hydroxybutyrate and metabolic hormones in the first two weeks after calving. Hyperketonemia, on the other hand, was associated with a significant decrease in insulin, IGF-I and leptin blood levels. We infer that heterozygous cows and leucine homozygous animals may have similar endocrine and metabolic responses to the challenge of increased nutrient demand in the early postpartum period and that hyperketonemia is closely linked to hormonal and metabolic changes occurring at the onset of lactation.

Experiment 3

Study conditions

Winter-calving (January-February, 2002) multiparous HF cows (n=32, parity: 2-4) from one herd were involved. On Day 9-14 PP n=10 cows were excluded based on the same criteria as in *Exp. 1*. On Day 10-15 PP healthy animals (n=22) were subjected to an intravenous glucose tolerance test (ivGTT). During ivGTT plasma glucose, insulin and leptin were assayed at all time points, while BHB, NEFA, total cholesterol (TCh), aspartate-aminotransferase activity (AST), thyroid hormones and cortisol were measured at 0. min. Parameters of ivGTT in the statistical evaluation were either actual concentrations or calculated by exponencial curve fitting. The Revised Quantitative Insulin Sensitivity Check Index (RQUICKI), which can be applied for the quick estimation of insulin sensitivity was modified to include basal concentration of BHB into the equation ($RQUICKI_{BHB}$). Average milk yield of the current lactation (4-45 d PP) for each cow was obtained and resumption of ovarian cyclicity was monitored by individual milk P_4 profiles. Several reproductive traits within the first 200 d PP were also recorded.

Results and discussion

The frequency of the L and V allele ($p_{Leucine}$ = 0.909, q_{Valine} = 0.091) was as in previous reports. Average milk production 4-45 d

PP was not different between *Alu*I genotypes similarly to short-term cumulative yields in *Exp. 1*. Nevertheless, LV cows tended to have higher 305-day lactation yields. Despite conflicting reports in the literature a recent study carried out in six large-scale Holstein herds also showed the advantage of LV dams over LL cows in 305-day lactation and test-day milk yields *(Kovács et al., 2006)*.

In lactating dairy cows IR develops concomittantly with depressed pancreatic insulin secretory capacity, and ivGTT is a good method to measure the degree of insulin resistance under field conditions. Glucose, insulin and leptin responses to ivGTT in this study were comparable to those described elsewhere. Resting glucose concentration, peak and clearance rate during ivGTT were similar in LV and LL cows despite higher average insulin levels, longer half-life and larger area under the curve (AUC) in LV animals, reflecting a state of IR. In Japanese Balck calves higher insulin levels were reported in LL compared to LV animals despite similar glucose concentrations. In our study, glucose uptake by insulin-dependant peripheral tissues should have been similar in both GH genotypes due to similar glucose clearance rate. However, LV cows needed more insulin to trigger the same glucose response without provoking hypoglycemia which often accompanies IR conditions. Accordingly, both RQUICKI and $RQUICKI_{BHB}$ were reduced in LV cows further pointing to lower insulin sensitivity, although due to their small numbers (n=4) these results should be interpreted with caution.

RQUICKI and $RQUICKI_{BHB}$ were positively related to insulin clearance rate and showed negative correlations with many glucose parameters and with basal and mean plasma insulin of ivGTT. We infer that both indexes may be useful for the rapid estimation of insulin sensitivity in dairy cows.

NEFA predominantly compromised glucose response to ivGTT, while high BHB levels mostly accounted for decreased insulin response and clearance rate. NEFA was negatively related to insulin AUC and peak, but did not influence glucose parameters in a study of *Bossaert et al. (2008)*. Earlier reports showed that hyperketonemic cows have markedly reduced insulin secretory capacity and decreased insulin responsiveness. In dairy cows impaired pancreatic islet function and islet regression may occur as a result of feed depression and its hormonal and metabolic consequences (e.g. increased NEFA and BHB levels) shortly before and after calving.

BCS and body weight had negative relationships with $RQUICKI_{BHB}$ indicating that cows in better condition have diminished insulin sensitivity. Overconditioned periparturient cows are more likely to develop IR and glucose intolerance and *Holtenius and Holtenius (2007)* showed a significant negative linear relationship between BCS and RQUICKI.

There were no differences in the commencement of luteal activity and in the time of first observed estrus by *Alu*I genotype similarly to results of *Exp. 1.* even though numbers of participating cows were much smaller here which needs to be taken into consideration when interpreting results.

We conclude that Holstein-Friesian cows heterozygous for *Alu*I polymorphism of the GH gene seem more likely to develop insulin resistance during early lactation than leucine homozygous cows. Decreased insulin sensitivity could be part of a homeorhetic adaptation process that supports nutrient partioning for the use of the mammary gland and may allow heterozygous cows to reach higher yields throughout lactation. *Alu*I genotype does not seem to be involved in the onset of postpartum ovarian activity and in the time of first observed estrus. The Revised Quantitative Insulin Sensitivity Check Index (RQUICKI) and its modified variant ($RQUICKI_{BHB}$) appear equally able to estimate changes in insulin sensitivity.

NEW SCIENTIFIC RESULTS

- In Holstein-Friesian cows *Alu*I polymorphism of the GH gene is not related to the onset of postpartum ovarian activity and to first observed estrus
- *Alu*I genotype is not associated with the extent of body condition loss shortly after parturition
- Changes in plasma ß-hydroxybutyrate, insulin, IGF-I and leptin concentrations occur irrespectively of *Alu*I genotype in the first two weeks after calving
- Hyperketonemia triggers a significant decrease in insulin, IGF-I and leptin blood levels
- *Alu*I heterozygous Holstein-Friesian cows seem more likely to develop insulin resistance during early lactation
- *Alu*I heterozygous Holstein-Friesian cows may reach higher yields throughout lactation even though it is not apparent in short-term milk production
- The Revised Quantitative Insulin Sensitivity Check Index and its modified variant ($RQUICKI_{BHB}$) both seem useful for the detection of changes in peripheral insulin sensitivity

PUBLICATIONS RELATED TO THE DISSERTATION

1. Full-text papers published in peer-reviewed journals in English

1.1. **O. Balogh**, K. Kovács, M. Kulcsár, A. Gáspárdy, A. Zsolnai, L. Kátai, A. Pécsi, L. Fésüs, W. R. Butler, Gy. Huszenicza: *Alu*I polymorphism of the bovine growth hormone (GH) gene, resumption of ovarian cyclicity, milk production and loss of body condition at the onset of lactation in dairy cows. *Theriogenology*, accepted for publication **(IF**: 1.911)

1.2. **O. Balogh**, K. Kovács, M. Kulcsár, A. Gáspárdy, H. Fébel, A. Zsolnai, L. Fésüs, C. Delavaud, Y. Chilliard, R.O. Gilbert, Gy. Huszenicza: Interrelationship of growth hormone *AluI* polymorphism and hyperketonemia with plasma hormones and metabolites in the beginning of lactation in dairy cows. *Livest. Sci.*, accepted for publication **(IF**: 1.083)

1.3. **O. Balogh**, O. Szepes, K. Kovács, M. Kulcsár, J. Reiczigel, J. A. Alcazar, M. Keresztes, H. Fébel, J. Bartyik, S. Gy. Fekete, L. Fésüs, Gy. Huszenicza: Interrelationships of growth hormone *Alu*I polymorphism, insulin resistance, milk production and reproductive performance in Holstein-Friesian cows. *Vet. Med. Czech,* submitted for publication **(IF**: 0.624)

1.4. Gy. Huszenicza, M. Keresztes, **O. Balogh**, V. Faigl, L. Kátai, J. Földi, K. Lemoniati, M. Kulcsár: Peri-parturient changes of metabolic hormones and their clinical and reproductive relevance in dairy cows. *Magy. Állatorv. Lapja*, 2008. 130. Suppl. 1, 45-51. (**IF**: 0.104)

2. Full-text papers published in peer-reviewed journals in Hungarian

2.1. **O. Balogh**, K. Kovács, M. Kulcsár, A. Zsolnai, A. Gáspárdy, J. Reiczigel, L. Kátai, L. Fésüs, Gy. Huszenicza: A növekedési hormon genotípus (*Alu*-I polimorfizmus) hatása az ellés utáni elso ovuláció idejére holstein-fríz tehenekben.

Állattenyésztés és Takarmányozás, 2005. 3. 237-245. (**IF**: 0.000)

2.2. Gy. Huszenicza, M. Kulcsár, Kátai L., **O. Balogh**: A nagy tejtermelésu tehén takarmányozásának, tejtermelésének és szaporodóképességének kapcsolata. Irodalmi áttekintés. 2. A petefészek muködése az ellés utáni idoszakban. *Magyar Állatorv. Lapja*, 2003. 125 (58). 75-82. (**IF**: 0.089)

2.3. Gy. Huszenicza, M. Kulcsár, G. Dankó, **O. Balogh**, T. Gaál: A nagy tejtermelésu tehén takarmányozásának, tejtermelésének és szaporodóképességének kapcsolata. Irodalmi áttekintés. 4. A ketonanyag-képzodés fokozódása és annak klinikai következményei. *Magyar Állatorv. Lapja*, 2003. 125 (58). 203-208. (**IF**: 0.089)

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