Szent István University
Postgraduate School of Veterinary Science

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

Ph.D. dissertation

Written by:

Dr. Orsolya Balogh

Supervisor: Prof. Gyula Huszenicza

2008

Szent István Egyetem
Állatorvos-tudományi Doktori Iskola

Témavezeto:

.....
Prof. Dr. Huszenicza Gyula
Szent István Egyetem Állatorvostudományi Kar
Szülészeti és Szaporodásbiológiai Tanszék és Klinika

Témabizottsági tagok:

…….
✝ Prof. Dr. Rudas Péter
Szent István Egyetem Állatorvostudományi Kar
Élettani és Biokémiai Tanszék

……
Prof. Dr. Fésüs László
Állattenyésztési és Takarmányozási Kutatóintézet Herceghalom

Az értekezés a Prof. Huszenicza Gyula elnökletével Budapesten 2008. október 7-én tartott munkahelyi vita nyomán nyerte el végleges formáját.

Készült 8 példányban. Ez a ___. sz. példány.

…….
dr. Balogh Orsolya

Content

Abbreviations and acronyms 4
Summary 6
Összefoglalás 7
1. Introduction 9
2. Aims of studies 10
3. Review of literature 11
3.1. The physiology of energy metabolism in postpartum dairy cows 11
3.2. *Alu*I polymorphism of the growth hormone gene. The function of the somatotropic axis and its position in the secretory capacity of insulin during early lactation. 19
3.3. The onset of cyclic ovarian function in high-producing dairy cows 30
4. Materials and Methods 38
4.1. Animal housing and management 38
4.3. Laboratory procedures 39
4.4. Statistical evaluation 42
5. Study descriptions and results 44
5.1. *Alu*I polymorphism of the bovine GH gene, resumption of ovarian cyclicity, milk production and loss of body condition at the onset of lactation in dairy cows (Exp. 1) 44
5.1.1. Description of study conditions 44
5.1.2. Results 46
5.1.3. Discussion 49
5.2. Interrelationship of growth hormone *AluI* polymorphism and hyperketonemia with plasma hormones and metabolites in the beginning of lactation in dairy cows (Exp. 2) 52
5.2.1. Description of study conditions 52
5.2.2. Results 54
5.2.3. Discussion 58
5.3. Interrelationships of growth hormone *Alu*I polymorphism, insulin resistance, milk production and reproductive performance in Holstein-Friesian cows (Exp. 3) 60
5.3.1. Description of study conditions 60
5.3.2. Results 65
5.3.3. Discussion 70
7. References 74
8. The candidate's publications related to the present dissertation 90
Further publications not related to the current thesis 91
Acknowledgement 92

Abbreviations and acronyms

AI	artificial insemination
*Alu*I	*Arthrobacter luteus* I restriction endonuclease
AST	aspartate-aminotransferase
AUC	area under the curve
BCS	body condition score
$BCSL_{30}$	body condition score loss in 30 days after calving
BHB	β-hydroxybutyrate
bp	basepair
bST	bovine somatotrop hormone
BW	body weight
C	cytosine
CHOD-PAP	cholesterol oxidase-phenol aminophenazone
COD	cystic ovarian disease
CR	clearance rate
CV	coefficient of variation
d	day(s)
DF	dominant follicle
DMI	dry matter intake
E_2	17β-estradiol
EB	energy balance
E. coli	Escherichia coli
ELISA	enzyme-linked immuno-sorbent assay
FFA	free fatty acid
FSH	follicle-stimulating hormone
FSTOV	first ovulation postpartum
G	guanine
GH	growth hormone (syn. somatotrop hormone, STH)
GHR	growth hormone receptor
GHRH	growth hormone releasing hormone (syn. growth hormone releasing factor, GRF)
GLUT	glucose transporter
GnRH	gonadotropin-releasing hormone
GOD-POD	glucose oxidase-peroxidase
GTT	glucose tolerance test
HF	Holstein-Friesian
HPO axis	hypothalamus-anterior pituitary-ovary axis
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IGFs	insulin-like growth factors
IGF-I	insulin-like growth factor-I
IGF-II	insulin-like growth factor-II
IGFBP	IGF-I binding proteins (IGFBP-1 to 5)
IR	insulin resistance
IRMA	immunoradiometric assay (^{125}I-IRMA: ^{125}I-labelled version of this assay
ISBGR	insulin-stimulated blood glucose response
ITT	insulin tolerance test
IU	international unit
iv	intravenous
ivGTT	intravenous glucose tolerance test
IVM	in vitro maturation
kDa	kiloDalton
L	liter
L allele	leucine allele
LDA	left displaced abomasum
LH	luteinizing hormone

LL	leucine homozygous for *Alu*I polymorphism of the bovine growth hormone gene
LPS	lipopolysaccharides, e.g. cell wall component of Gram negative bacteria (endotoxin)
LSM	least square of the mean
LV	heterozygous for *Alu*I polymorphism of the bovine growth hormone gene
NEB	negative energy balance
NEFA	non-esterified fatty acids
NE_G	net energy gain
NE_L	net energy lactation
NE_M	net energy maintenance
NPY	neuropeptid Y
P_4	progesterone
PCR-RFLP	polymerase chain reaction, restriction fragment length polymorphism
PMNL	polymorphonuclear leukocyte
POU1F1	POU domain, class 1 transcription factor 1
PP	postpartum
RIA	radioimmuno assay (^{3}H-RIA, ^{125}I-RIA: ^{3}H- or ^{125}I-labelled version of this assay)
RQUICKI	Revised Quantitative Insulin Sensitivity Check Index
$RQUICKI_{BHB}$	Revised Quantitative Insulin Sensitivity Check Index modified with β-hydroxybutyrate
SCK	subclinical ketosis
SD	standard deviation
SEM	standard error of the mean
SRIF	somatotrophin release-inhibiting factor
T_3	triiodothyronine (3,3',5-triiodothyronine)
T_4	thyroxine
TCh	total cholesterol
TG	triglyceride
TNF-α	tumor necrosis factor-α
TMR	total mixed ration
TMY_{30}	total (cumulative) fat and protein corrected milk yield in 30 days postpartum
TRH	thyrotropin releasing hormone
TSH	thyroid stimulating hormone
UV	ultraviolet
V allele	valine allele
VLDL	very low density lipoprotein
vs.	versus
VV	valine homozygous for *Alu*I polymorphism of the bovine growth hormone gene

Summary

An orchestrated system of various metabolic and endocrine changes characterizes the transition from the dry period to lactation in dairy cows. Growth hormone, through its crucial role in galactopoiesis and thus in the persistency of lactation as well as an important part of the GH-IGF-I axis is involved in the homeorhetic adaptation to increased mammary demand for nutrients. 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 to valine; LL, LV and VV genotypes) has been linked to milk production traits with various outcome. To a limited extent, endocrine features (basal and GHRH-induced release of growth hormone and plasma levels of certain metabolic hormones) and reproductive characteristics related to different *Alu*I genotypes have also been tested, but results were inconsistent.

The purpose of our study was to investigate the association of *Alu*I polymorphism with reproductive traits, milk yield, body condition change, and whether GH genotype could be related to the occurrence of hyperketonemia, plasma levels of metabolic hormones and peripheral insulin sensitivity in early postpartum Holstein-Friesian cows.

A total of 586 cows participated in the experiments. Our results show that *Alu*I genotype is not related to the interval from calving to first ovulation and to short-term milk production and is not associated with the extent of body condition loss shortly after calving (n=307; *Exp. 1.*). Furthermore, changes in plasma ß-hydroxybutyrate, insulin, IGF-I and leptin concentrations post partum happen irrespectively of GH genotype and that hyperketonemia is mainly linked to the hormonal and metabolic changes occurring at the onset of lactation causing a significant decrease in blood levels of insulin, IGF-I and leptin (n=257; *Exp. 2.*). Holstein-Friesian cows heterozygous for *Alu*I polymorphism of the GH gene are more likely to develop insulin resistance during early lactation and reach higher lactation yields than leucine homozygous cows. It appears that genotype is not associated with the onset of postpartum ovarian activity and first observed estrus. 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 (n=22; *Exp. 3.*).

The results of our experiments hopefully represent some contribution to the world of dairy science.

Összefoglalás

Nagyhozamú tejelo tehenekben számos élettani-biokémiai és endokrin folyamat egységessé hangolt rendszere teszi lehetové a szárazonállást követoen a laktáció megindulását. Szarvasmarhában a növekedési hormon (GH) szabályozza elsosorban a tejtermelés megindulását és fenntartását, és mint a GH-IGF-I tengely tagja dönto szerepet játszik a tejmirigy megnövekedett táplálóanyagszükségleteihez való homeorhetikus adaptációban. A növekedési hormon gén egy nukleotidjában bekövetkezett pontmutáció eredményeként a GH fehérjeláncának 127. aminosava a kódoló génszekvencia szerint leucinra vagy valinra változhat (*Alu*I polimorfizmus; LL, LV és VV genotípusok). A genotípusok tejtermelésének, valamint endokrin és szaporodásbiológiai funkcióinak (GH alap- és GHRH-stimulált szekréciója, egyes metabolikus hormonok plazmaszintje) vizsgálatakor eltéro, és sok esetben egymásnak ellentmondó eredmények születtek.

Célunk volt, hogy közvetlenül ellés után lévo Holstein-Fríz tehenekben vizsgáljuk a GH gén *Alu*I polimorfizmusának az egyes szaporodásbiológiai mutatókkal, a tejtermeléssel és az ellés utáni kondícióveszteséggel mutatott összefüggését, és felderítsük az egyes GH genotípusok közötti különbségeket a hiperketonémia elofordulásának, bizonyos metabolikus hormonok plazmaszintjének és a perifériás inzulinérzékenység változásának tükrében.

Kísérleteinket nagyszámú állaton (n=586) több magyarországi nagyüzemben végeztük. A GH genotípus (*Alu*I polimorfizmus) nem befolyásolta az ellés utáni elso ovuláció idopontját, a 30. napig megtermelt tej összmennyiségét és a kondíciópont-vesztés mértékét (n=307; *1. kísérlet*). Az ellést követo második héten a hiperketonémiás tehenekben jelentosen alacsony inzulin, IGF-I és leptin koncentrációk voltak mérhetok az egészséges állatokhoz képest. Nem voltak azonban kimutathatóak szignifikáns különbségek a leucin homozigóta és a heterozigóta állatok plazma ß-hidroxi-vajsav, inzulin, IGF-I és leptin szintjei között (n=257; *2. kísérlet*). Az ellés utáni elso két hétben a heterozigóta állatokat a leucin homozigótákénál alacsonyabb inzulinérzékenység jellemezte, de ezek az egyedek valószínuleg magasabb laktációs tejtermelésre is képesek. Nem volt megfigyelheto *Alu*I genotípusok szerinti különbség a postpartum elso ovuláció és elso ivarzás idejében. A perifériás inzulinérzékenység gyors és egyszeru mérésére javasolt Revised Quantitative Insulin Sensitivity Check Index és annak általunk módosított változata ($RQUICKI_{BHB}$) egyaránt alkalmasnak látszanak

teljes test inzulinérzékenység mértékének a becslésére (n=22; *3. kísérlet*). Eredményeink reményeink szerint hozzájárulnak az ellés körüli metabolikus és hormonális változások jobb megértéséhez.

1. 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. In ruminants growth hormone is responsible for galactopoiesis, thus for the persistency of lactation *(Bell, 1995; Svennersten-Sjaunja and Olsson, 2005)*. Total lactation yield was positively correlated with GH and negatively related to insulin levels in high producing dairy cows *(Sorensen and Knight, 2002)*. In milking goats, however, prolactin seems equally important in maintaining lactation yield *(Flint and Knight, 1997)*. Dairy cattle lines selected for high milk production release larger amounts of endogenous GH than lines with average (lower) milk production *(Lukes et al., 1989; Beerepoot et al., 1991; Zinn et al., 1994)*. These endocrine characteristics could be tested early in life and may serve as excellent selection tools for increasing milk production in the future. *Løvendahl et al. (1991)* were able to show that 4-month-old calves selected for higher milk yield responded better to GH secretagoges than average control lines. On the other hand, *Baumgard et al. (2002)* and *Weber et al. (2005*) failed to prove the advantage of selected line Holstein calves in growth hormone releasing hormone (GHRH)-induced GH response studies. *Taylor et al. (2006)* could not demonstrate a relationship of prepubertal GH, insulin like growth factor-I (IGF-I), insulin and glucose measures in Holstein-Friesian female calves with their subsequent lactation and peak milk 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 with various outcome. Some authors favored the leucine *(Lucy et al., 1993; Lee et al., 1996; Shariflou et al., 2000; Dybus, 2002)* or the valine allele *(van der Werf, 1996; Sabour et al., 1997; Grochowska et al., 2001; Zwierzchowski et al., 2002; Kovács et al., 2006)*, while others could not prove an association *(Yao et al., 1996, Lechniak et al., 2002a)*. Endocrine features *(Schlee et al., 1994b; Grochowska et al., 2001; Sørensen et al., 2002; Ge et al., 2003; Katoh et al., 2008)* and reproductive characteristics *(Lechniak et al., 1999 and 2002b)* related to different genotypes have also been tested, but results were inconsistent.

2. Aims of studies

The association between *Alu*I polymorphism of the GH gene and metabolic, endocrine and reproductive characteristics of the early postpartum (PP) period have not been addressed in dairy cows, yet. The objectives of the current studies were:

1. to investigate (i) the role of *Alu*I polymorphism in the resumption of ovarian activity 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.
2. 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.
3. 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 days (d) after calving in HF cows.

3. Review of literature

3.1. The physiology of energy metabolism in postpartum dairy cows

Negative energy balance and its metabolic consequences

Milk production of the high-yielding dairy cow has increased dramatically in recent decades. Milk yield usually peaks at 4–7 weeks after calving, while dry matter intake (DMI) starts to decrease already during the final three weeks before calving with the majority (89%) of that decline occurring in the last week of gestation. Feed intake then improves gradually by each week and reaches maximum level only between 8–20 weeks PP *(Butler et al., 1981; Beam and Butler, 1997; Hayirli et al., 2002; Svennersten-Sjaunja and Olsson, 2005)*. Thus, at the onset of lactation, cows enter a state of negative energy balance (NEB) that lasts for several weeks as nutrient demand of the mammary gland and the energy required for maintenance exceed the energy available from dietary sources *(Goff and Horst, 1997; Block et al., 2001)*. NEB nadir occurred between 1-3 weeks PP and returned to zero by week 8 *(Block et al., 2001)*, however, in a study of *Jorritsma et al. (2005)* cows reached nadir only by week 5 and returned to balance later. Energy balance (EB) was more negative in high yielding cows than in low producers *(Kornalijnslijper et al., 2003)* and in cows overfed during the dry period compared to feed restricted cows *(Jorritsma et al., 2005)*. Approximately 80% of the net energy intake is needed for milk synthesis at peak lactation and 80% of the total glucose turnover is utilized by the mammary tissues *(Bauman and Currie, 1980; Svennersten-Sjaunja and Olsson, 2005)*. A homeorhetic regulation takes control and coordinates changes in metabolism of body tissues and in nutrient partioning to support the priorities of the new physiological state *(Bauman and Currie, 1980)*. These homeorhetic changes are summarized in *Table 3.1.1*.

Table 3.1.1. Metabolic processes in the high-producing dairy cow in transition associated with the onset and development of lactation (after *Ingvartsen, 2006)*

Process or metabolism	Response	Tissue involved
Milk synthesis	↑ Number of secretory cells	*Mammary gland*
	↑ Blood flow	
	↑ Nutrient consumption	
Fat metabolism	↓ *de novo* fat synthesis	*Adipose tissue*
	↓ Absorption of fatty acids	
	↓ Estherification of fatty acids	
	↑ Lipolysis	
	↑ Use of lipids, fatty acids and ketones as energy	*Adipose and muscular tissue*
	↑ Ketogenesis	*Liver*
Glucose metabolism	↑ Liver size	*Liver*
	↑ Blood flow	
	↑ Rate of gluconeogenesis	
	↓ Use of glucose as energy	*Other body tissues*
Protein metabolism	↓ Protein synthesis	*Muscular tissue*
	↑ Proteolysis	
	↑ Protein synthesis	*Other body tissues*
Mineral metabolism	↑ Absorption	*Gut*
	↑ Mobilization	*Bones*
Feed intake	↑ Feed intake	*Central nervous system*
Digestion	↑ Hypertrophy	*Digestive tract*
	↑ Absorption rate and capacity	
	↑ Metabolical activity	
Blood flow	↑ Output of blood from the heart	*Heart*
	↑ Partioning to mammary gland	
	↑ Partioning to gastrointestinal tract and liver	

Metabolism shifts from an anabolic seen during pregnancy to a catabolic state after parturition while adipose tissue fat and skeletal muscle protein stores are mobilized in response to the negative nutrient balance caused by copious milk production in early lactation *(Bauman and Currie, 1980; Chilliard, 1999; Ingvartsen, 2006)*. Proteolysis is only temporarily increased (as determined from plasma 3-methylhistidine levels) in the first 1–2 weeks PP and mobilized amino acids contribute to liver gluconeogenesis and mammary milk protein synthesis *(Blum et al., 1985; Doepel et al., 2002)*. The most important signal for the initiation of lipolysis seems to be more a drop in DMI rather than reaching a threshold level of NEB *(Grummer et al., 2004)*. Circulating plasma non-esterified fatty acids (NEFA) levels increase markedly according to the magnitude of adipose tissue fat mobilization *(Pullen et al., 1989; Bell, 1995; Rukkwamsuk et al., 1999; Chagas et al., 2006)*. This rise already starts before calving with peak concentration occurring approximately 3 weeks PP and declines afterwards *(Grummer, 1993; Whates et al., 2007)*. In the liver NEFA becomes predominantly oxidized (incompletely) into ketone bodies, stored as triglyceride (TG), or some TG exported in

the form of very low density lipoprotein (VLDL; *Bobe et al., 2004; Grummer et al., 2004)*. NEFA (and VLDL to a much smaller extent) taken up by the mammary gland is utilized for milk fat synthesis *(Pullen et al., 1989; Bell, 1995)*. When increased NEFA uptake and lipid synthesis in the liver overrides TG hydrolysis and output as VLDL, hepatic lipidosis becomes significant. A four- to fivefold increase in lipid content occurs during the last 2-3 weeks prior to parturition with peak at or near calving that parallels increasing blood NEFA concentrations and liver uptake *(Grummer, 1993)*. In ruminants periods of increased liver free fatty acid (FFA) uptake does not translate to an equally high VLDL export capacity compared to other mammalian species *(Kleppe et al., 1988; Pullen et al., 1990)* and hepatic lipid accumulation also decreases TG secretion *(Pullen et al., 1988)*. Therefore, dairy cows in the beginning of lactation are predisposed to fatty liver and various forms of ketosis *(Grummer et al., 2004; Ingvartsen, 2006)*. Gluconeogenic activity of the liver during periods of excessive lipid accumulation is also compromised *(Cadorniga et al., 1992; Grummer et al., 1993)*. Hepatic lipidosis probably precedes ketosis, so the ratio between liver TG and glycogen may be a useful indicator for susceptibility to ketosis *(Grummer, 1993)*. Some degree of NEB is expected after calving in the majority of healthy postparturient animals, thus a moderate increase in the concentration of ketone bodies (e.g. acetoacetate, acetone and ß-hydroxybutyrate [BHB]) in various body fluids is detectable and can be used as energy source by peripheral tissues when carbohydrates are limited *(Baird, 1982; Vazquez-Anon et al., 1994; Leslie et al., 2000)*. When EB becomes more negative and adipose tissue mobilization further increases, ketosis, which is caracterized by hyperketonemia (elevation in circulating blood concentration of ketone bodies) may develop and may be accompanied by hypoglycemia and hypoinsulinemia *(Baird, 1982; de Boer et al., 1985; Huszenicza et al., 2003; Ingvartsen, 2006)*.

Predisposing factors to ketosis

Ketosis exists in subclinical and clinical forms; the subclinical form of ketosis (SCK, hyperketonemia without clinical signs) is most prevalent during the first 65 d of lactation with peak prevalence within 2-4 weeks PP and disappears after Day 85 *(Andersson and Emanuelson, 1985; Andersson, 1988; Duffield et al., 1997; Wood et al., 2004)*. Primiparous cows are less likely to experience hyperketonemia compared to multiparous cows and the prevalence of SCK increases with increasing parity *(Baird, 1982; Dohoo and Martin, 1984; Andersson and Emanuelson, 1985; Duffield et al.,*

1997; Enjalbert et al., 2001; Wood et al., 2004). In a study of *Whates et al. (2007)* plasma BHB started to rise one week prior to parturition both in primiparous and multiparous cows and despite a sharper rise from significantly lower prepartum values in first lactation cows it never reached PP concentrations of those found in multiparous animals. It seems that selection for increased milk yield may also be positively correlated with the risk of ketosis and foot/leg disorders *(Ingvartsen et al., 2003; Oetzel, 2004). Whates et al. (2007)* found a weak positive correlation between plasma BHB levels and actual milk yield 4 weeks PP in primiparous cows. However, in multiparous cows the relationship between BHB and yield was negative, while NEFA was positively correlated with milk production at 7 weeks PP. Highest individual milk yield was positively, although weakly related to highest milk acetone in a study of *Andersson and Emanuelson (1985).*

The influence of hyperketonemia on disease prevalence

Increased ketone body status soon after parturition probably predisposes cows to subsequent health problems and disorders. Cows with hyperketolactia or hyperketonemia in the early PP period are more likely to show signs of clinical ketosis thereafter *(Dohoo and Martin, 1984; Duffield et al., 1997).* Hyperketolactia in the first 2 weeks PP was also associated with a higher risk for later developing left displaced abomasum (LDA; *Geishauser et al., 1997).* Cows had higher BHB, NEFA, and aspartate-aminotransferase (AST) activity and lower insulin, calcium and glucose levels within 10 days prior to a diagnosis of LDA than healthy controls. *LeBlanc et al. (2005)* found that serum NEFA concentrations above 0.5 mEq/L 4-10 d prepartum or serum BHB, NEFA and milk BHB above a certain cut-off level (1.2 mmol/L, 1.0 mEq/L and 0.2 mmol/L, respectively) PP can each alone increase the risk of an LDA about fourfold. Most studies could associate subclinical ketosis with reduced milk yield and reassure that peak production would not be fulfilled *(Baird, 1982; Andersson and Emanuelson, 1985).* Production loss was 1.0 to 1.4 kg/day in a study of *Dohoo and Martin (1984).* During an induced ketonemic period initial mean daily milk yields in early lactation dropped from 33.0 to 26.9 kg by the end of feed restriction and did not return to original levels *(Boer et al., 1985)*. Various fertility traits are also impaired in cows with SCK *(Baird, 1982; Dohoo and Martin, 1984)* possibly due to the detrimental carry-over effect of ketone bodies on reproductive functions. Cows conceiving earlier than 80 d after calving had significantly lower ketone levels during the first 6 weeks PP than those

concieved later, although the interval from calving to first ovulation was not different *(Koller et al., 2003)*. On the contrary, *Walsh et al. (2007a)* and *Huszenicza et al. (2006)* found that SCK predisposed cows for prolonged acyclic period. A higher percentage of clinically ketotic cows experienced delayed cyclicity PP than healthy animals *(Opsomer et al., 2000)*. Pregnancy rates to first artificial insemination (AI) were decreased in cows above a threshold of 1.0 or 1.4 mmol/L serum BHB in week 1 or in week 2, respectively *(Walsh et al., 2007b)*. Long-lasting hyperketonemia (up to 4-5 weeks PP) was characterized by decreased pregnancy rates, while in lactational ketosis (hyperketonemia in the 5^{th} or $4\text{-}5^{th}$ weeks) cessation of ovarian cyclicity was the most prominent ovarian malfunction. The interval from calving to first visible estrus, to first AI and to re-conception was prolonged in both types of ketosis *(Huszenicza et al., 2006)*. Various in vitro studies intended to reveal the association between reproductive disturbances due to elevated ketone bodies seen in vivo. Theca cell function was not influenced by BHB, but granulosa cell proliferation increased and progesterone (P_4) and 17ß-estradiol (E_2) production decreased in a study of *Vanholder et al. (2006)*. *Leroy et al. (2006)* showed an additive toxic effect of increased BHB concentration (1.8 mmol/L) on oocyte and embryo development in vitro that was present only when glucose levels were moderately low. A much higher BHB concentration (4.0 mmol/L) did not aggravate the effect of extremely low (1.375 mmol/L) glucose levels, although oocyte maturation and embryo development were further compromised. It seems that during periods of hyperketonemia it is the associated hypoglycemia that is probably responsible for disturbed oocyte and embryonic development and perhaps decreased conception and pregnancy rates in vivo. The immune defense mechanisms of early lactation cows are reduced *(Leslie et al., 2000)* and higher BHB concentrations in vitro or in vivo can further compromise leukocyte functions and predisposing cows to (sub)clinical metritis *(Dohoo and Martin, 1984)* or mastitis *(Suriyasathaporn et al., 2000)*. *Hammon et al. (2006)* found that cows with puerperal metritis or subclinical endometritis had significantly higher pre- and postpartum NEFA and postpartum BHB levels than healthy animals and also presented a decrease in polymorphonuclear leukocyte (PMNL) functions. Cows with serum BHB over 1.0 mmol/L immediately PP are 5 times more likely to suffer from Gram negativ mastitis in the following 4 weeks than normoketonemic animals *(Jánosi et al., 2003)*. Plasma BHB >1.4 mmol/L predisposed cows to more severe clinical signs of experimentally induced Escherichia coli (E. coli) mastitis *(Kornalijnslijper et al., 2003)*. The severity of E. coli mastitis was

not related to the chemotactic capacity or peripheral numbers of PMNL in hyperketonemic cows, but there was a significant negative correlation between the chemotactic response and circulating PMNL numbers with the severity of the disease in nonketotic animals *(Kremer et al., 1993)*. Chemotactic differential of leukocytes from hyperketonemic cows was lower compared to leukocytes from cows with BHB <0.8 mmol/L. When leukocytes coming from cows with BHB <0.8 mmol/L were subject to acetoacetate and acetone at SCK and clinical ketotic levels in the media, their chemotactic capacity was significantly reduced at clinical ketotic compared to SCK level. Interestingly, leukocytes from cows above 0.8 mmol/L blood BHB did not show any difference in the same setup *(Suriyasathaporn et al., 1999)*. Leukocytes in an environment with high and long-standing ketone and low glucose levels may switch their energy source (to some degree) from glucose, which they normally use *(Weisdorf et al., 1982)* to ketone bodies that are less metabolizable *(Lavau et al., 1978),* but highly diffusible *(Lean et al., 1992)*. Therefore their glucose-metabolizing enzymes might be limited enabling them to be less sensitive to low glucose concentrations and use BHB as an alternate source of energy. Leukocytes from normoketonemic cows with intact glucose-metabolizing systems are not prepared to efficiently use ketone bodies, so their capacity to fight infection becomes impaired depending on the level of hyperketonemia as shown in the studies of *Kremer et al. (1993)* and *Suriyasathaporn et al. (1999)*. In vitro, acetoacetate, acetate and acetone seem to compromise leukocyte function more than BHB *(Suriyasathaporn et al., 1999)*.

Homeorhetic and homeostatic mechanisms of the early postpartm period

There is a strong interaction between homeorhetic and homeostatic (maintenance of a physiological equilibrium in the internal environment) control mechanisms at the onset of lactation *(Bauman and Curie, 1980)*. Adaptations that occur at this time include changes in circulating hormone levels and their production as well as in periferic tissue sensitivity and response to insulin. Some endocrine changes are listed in *Table 3.1.2*.

Table 3.1.2. Homeorhetic and homeostatic hormones, their relationships and involvement in tissue sensitivity and response during pregnancy and early lactation in high-producing dairy cows (after *Ingvartsen, 2006*)

	Late pregnancy	Early lactation
Homeorhetic hormones		
Progesterone	(↓)	↓
Placental lactogen	↑	↓
Estrogen	↑	↓
Prolactin	(↑)	↑
Somatotropin	(↑)	↑
Glucocorticoids (cortisol)	-/↑	↑
Leptin	↑↓	↓
Homeostatic hormones		
Insulin	↑↓	↓
Glucagon	-	↑?
Parathyroid hormone	-/↑	↑
1,25-dihydroxy vitamin D_3	-/↑	↑
Calcitonin	-/↓	↓
Tissue sensitivity (except mammary gland)		
Insulin	↓	↓
Catecholamines	↑	↑
Tissue response (except mammary gland)		
Insulin	↓	↓
Catecholamines	↑	↑

Plasma glucose, insulin, insulin-like growth factor-I (IGF-I) and leptin levels fall below prepartum concentrations, the ratio of GH and insulin increases as well as GH level peaks at parturition and stays elevated throughout lactation *(Block et al., 2001; Accorsi et al., 2005; Ingvartsen, 2006; Bossaert et al., 2008)*. Insulin levels started falling over parturition, reached nadir 1-3 weeks after calving, increased thereafter consistent with improving EB and fully recovered by Day 30 PP *(Meikle et al., 2004; Andersen et al., 2005; Chagas et al., 2006)*. Also, some degree of insulin resistance in early lactation (2-4 weeks PP) develops in adipose tissue and in the muscle to promote lipolysis and mobilization of amino acids *(Bell, 1995)*. The phenomenon of IR will be discussed further in details in the next chapter. Leptin and IGF-I concentrations started to decline before parturition and reached a minimum level by 2-3 weeks PP. Although IGF-I started to slowly recover thereafter, leptin remained low, at approximately 50% of prepartum values *(Block et al., 2001; Whates et al., 2007)*. A possible explanation to falling leptin concentrations in the periparturient period may be the disappearance of body lipid stores which shows in loss of body condition score (BCS*; Rukkwamsuk et al., 1999; Whates et al., 2007)* and a ~42% drop in the abundance of leptin mRNA expression in the adipose tissue *(Block et al., 2001)*. Insulin but not GH may interact

with plasma leptin levels, because insulin administration to late pregnant and early lactating cows increased peripheral leptin concentrations whereas GH treatment did not reduce leptin values *(Block et al., 2003; Leury et al. 2003)*. Growth hormone usually increases in concentration from the dry to the early lactation period and even further during ketonemia *(de Boer et al., 1985; Chagas et al., 2006),* accelerates lipolysis in the adipose tissue and gluconeogenesis in the liver *(Bell, 1995; Lucy et al., 2001)*. An enhanced responsiveness to lipolytic stimuli (e.g. cathecolamines) as part of the homeorhetic coordination to enhance lipid mobilization occurs in cows during the periparturient period *(Bernal Santos, 1982; Mc Namara, 1988; Ingvartsen, 2006)* and adipocytes become more sensitive to ß-adrenergic stimulation increasing the number of ß-receptors *(Jaster and Wegner, 1981)*. The thyrotropin releasing hormone (TRH) induced thyroxine (T_4) response is only slightly altered except for severe forms of ketosis *(Huszenicza et al., 2006)*, but decreased T_4 and 3,3',5-triiodothyronine (T_3) levels and increased concentrations of the inactive reverse-triiodothyronine are usually found in the peripheral circulation *(Huszenicza et al., 2002; Meikle et al., 2004)*.

3.2. *Alu*I polymorphism of the growth hormone gene. The function of the somatotropic axis and its position in the secretory capacity of insulin during early lactation.

Growth hormone and AluI polymorphism of the growth hormone gene

The somatotropic axis (GH – IGF-I axis) consist of growth hormone, insulin-like growth factor-I, their associated binding proteins and receptors and plays a critical role in the regulation of metabolism and various physiological processes *(Breier, 1999; Renaville et al., 2002)*. GH is a single chain polypeptide hormone of approximately 22 kiloDalton (kDa) molecular weight and joined by two disulphide bridges forming a dimer *(Wallis, 1992)*. Two variants at the NH_2 terminus (alanin, 191-amino acid sequence or phenilalanine, 190-amino acid sequence) result from removal of the signal peptide of the precursor molecule during secretion *(Wood et al., 1989)*. There are inter-species differences in the structure of mammalian growth hormone. Bovine and ovine GH differ only at a single position in the amino acid sequence. Bovine and porcine GH share a high degree (~90%) of similarity and both of them vary in ~35% from the human growth hormone, so that their binding affinity to the human growth hormone receptor is several orders of magnitude lower and neither of them affects human growth *(Bauman and Vernon, 1993; Etherton and Bauman, 1998)*. GH is synthesized in the anterior pituitary by the somatotroph cells and its expression is activated mainly by the POU domain class 1 transcription factor 1 (POU1F1). Pulsatile release of GH is regulated directly by two antagonistic hypothalamic neuropeptide hormones, the stimulatory GHRH which also increases GH synthesis and the inhibitory somatostatin (SRIF, somatotrophin release-inhibiting factor). GH itself also participates in its own control through a negative feed-back mechanism on GHRH and SRIF *(Giustina and Veldhuis, 1998; Anderson et al., 2005)*. A fall from pubertal maximum values in GH secretion is apparent with aging in humans and sexual dimorphism in secretion pattern is present in all species *(Gatford et al., 1998; Giustina and Veldhuis, 1998)*. In cattle, GH pulse frequency, pulse amplitude, interpulse plasma GH and mean GH concentrations are greater in male than in the female. Sexual differences in GH profile and growth rate are apparent before puberty in sheep, while in humans and rats they become dimorphic in the peripubertal period *(Gatford et al., 1998)*. Furthermore, a complex network of neuropeptides and other neurotransmitters, hormones and metabolic substrates from the brain, gut and other tissues as well as nutritional and

environmental signals modulate GH secretion *(Giustina and Veldhuis, 1998; Anderson et al., 2005)*. Many of GH actions on tissue growth and metabolism are mediated via insulin-like growth factors (IGFs) that are released from target tissues as a response to GH binding *(Sjogren et al., 1999; Renaville et al., 2002)*. IGF-I and insulin-like growth factor-II (IGF-II) are approximately 7.5 kDa molecular weight single chain polypeptides with 70 and 67 amino acids, respectively, and are also known as somatomedins. Their structure is similar to proinsulin. The IGF-I gene has two distinct promoters and their transcript, the IGF-I mRNA with exons 1 or 2 are the fetal and postnatal forms, respectively. IGF-II has critical role in fetal development, but its involvement in postnatal functions is still unclear *(Clark, 1997)*. Most cells produce and secret IGF binding proteins (IGFBP) from which there are at least 6 variants (IGFBP 1-6) and another 9 IGFBP–related proteins. The affinity of IGFBPs to their ligands is 10-times stronger than that of IGFBP–related proteins *(Jones and Clemmons, 1995; Rajaram et al., 1997)*. IGFBPs are supposed to enhance the biological half life of IGFs *(Rajaram et al., 1997)*, but at the same time they also inhibit IGF actions by competing with their receptors for the ligand given their stronger affinity to IGFs than IGF-receptors. On the other hand, IGFBPs can also concentrate IGFs on the cell surface close to the IGF-receptors and thus increasing their activity. Most IGF-I in the blood (75- 85%) is bound to IGFBP-3 and the acid-labile subunit in a trinary complex (150-200 kDa) which increases the half life of IGF-I in the circulation *(Thissen et al., 1994; Rajaram et al., 1997)*. Less than 1% of IGF-I is present in free form in the circulation and the rest (15-25%) is bound to low molecular weight IGFBP in complexes that are capable of crossing the capillary epithelium and thus facilitate specific IGF-I actions in tissues *(Jones and Clemmons, 1995)*. Sexual dimorphism exists in IGF-I and IGFBP-3 levels in the species studied and the direction of these sexual differences is generally consistent with that of GH *(Gatford et al., 1998)*.

Three different polymorphic sites within the 5th exon of the bovine GH gene have been identified *(Lucy et al., 1991a; Chikuni et al., 1994; Yao et al., 1996)*. A point mutation in position 2141 of the nucleotide sequence of the GH gene (cytosine [C] to guanine [G] transversion) could be detected by *Arthrobacter luteus* I (*Alu*I) restriction endonuclease and gives rise to a change from leucine to valine in the amino acid sequence at position 127 of the bovine GH *(Lucy et al. 1993)*. Allele and genotype frequencies differ greatly among breeds. *Lucy et al. (1993)* found that dairy breeds with the largest mature size (Brown Swiss, HF) had the highest frequency of the L allele

(1.00 and 0.93, respectively), whereas smaller breeds (Jersey, Ayrshire) had the highest frequency of the V allele (0.44 and 0.21, respectively). In Polish Black-and-White cattle the L allele was also less frequent ($p_{leucine}$=0.64-0.87) than in HF cows *(Grochowska et al., 2001; Zwierzchowski et al., 2002)*.

*Alu*I polymorphism has been related to milk production traits, although results are contradictory. Estimated transmitting ability for milk production tended to be greater in leucine homozygous (LL) HF cows (but not in sires), while in Jersey cows valine homozygous (VV) animals showed better predicted transmitting ability for milk production *(Lucy et al., 1993). Lee et al. (1996)* found that in lines of animals selected for high milk production V allele had a negative effect on genetic merit for milk production, but not in average-producing cows. Polish Black-and-White cattle of LL genotype produced more milk, fat, and protein yield in the first 305-days lactation, but not in 2nd and 3rd lactation compared to heterozygous (LV) cows *(Dybus, 2002)*. In a study of *Shariflou et al. (2000)* on Australian Holstein cattle the L allele was associated with higher milk, fat and protein yields (95 liter, 7 kg and 3 kg of gene substitution effect, respectively). When dairy cattle were injected with recombinant forms of bovine somatotrop hormone (bST), a greater increase in milk yield occured in cows treated with valine-substituted bST compared to leucine-substituted bST *(Eppard et al., 1992).* Presence of the V allele positively affected milk production traits in a study of *van der Werf et al. (1996),* while *Sabour et al. (1997)* found no direct influence of genotype on sires` breeding values for yield characteristics, although LV animals were more frequent among top HF bulls. Heterozygous HF cows had higher 305-day lactation and test-day milk yields and LL cows produced higher 305-day fat and protein percentages in a study of *Kovács et al. (2006).* Milk and protein yields were the highest in LV cows, whereas LL cows produced higher fat yields compared to other genotypes *(Grochowska et al., 2001). Zwierzchowski et al. (2002)* showed that in Polish Black-and-White cows VV animals excelled in daily milk yield and daily yield of most milk constituents, although the contribution of *Alu*I polymorphism to milk production traits was the smallest among all factors (e.g. cow's parity, stage of lactation) studied. *Yao et al. (1996)* did not observe any associations between *Alu*I polymorphism of the GH gene and estimated breeding values for milk production traits in HF bulls, although in Bavarian Simmental bulls genotype effect approached significance in case of milk fat and protein content *(Schlee et al., 1994a)*. Similarly, no significant relationship existed between GH-*Alu*I locus and breeding values for milk, milk fat and protein yields and fat and protein

content in dairy bulls *(Lechniak et al., 2002a)*. When *Alu*I genotype was associated with reproductive parameters in beef and dairy bulls *(Lechniak et al., 1999)*, a non-significant tendency was observed in LL bulls to have lower ejaculate volumes and in VV animals to have higher non-return rates. GH genotype did not influence significantly the number of oocytes collected from donor ovaries suitable for in vitro maturation (IVM), the number of matured oocytes, mean oocyte diameter and embryos produced *(Lechniak et al., 2002b)*. Endocrine characteristics of *Alu*I genotypes have also been investigated. Valine homozygous calves reached the highest blood GH peak following a TRH challenge and leucine homozygous calves had the highest IGF-I concentrations *(Grochowska et al., 2001)*, while in Danish Jersey calves the leucine allele was favorable for a higher GHRH-induced GH response *(Sørensen et al., 2002)*. *Schlee et al. (1994b)* found significantly higher GH levels in LL German Black and White bulls and a tendency for higher IGF-I concentrations in LV Simmental bulls. *Alu*I polymorphism of the GH gene was not associated with serum IGF-I levels and growth traits in Angus cattle divergently selected for high or low IGF-I concentration *(Ge et al., 2003)*. Japanese Black calves homozygous for the leucine allele had the highest insulin, IGF-I, basal GH and GHRH-induced GH plasma concentrations, while valine homozygous animals had higher leptin and triglyceride levels *(Katoh et al., 2008)*.

The somatotropic axis in the periparturient period

The majority (approximately 75%) of serum IGF-I concentration is derived from the liver *(Sjogren et al., 1999)* following GH stimulation and consequently liver IGF-I mRNA expression *(Vicini et al., 1991; Vanderkooi et al., 1995)*. A similar increase in serum IGFBP-3 and a decrease in IGFBP-2 were noticed. In turn, IGF-I controls GH synthesis and release via a negative feed-back loop on the hypothalamus and pituitary *(LeRoith et al., 2001; Veldhuis et al., 2001)*. The physiological actions of GH are initiated through transmembrane GH receptors (GHR) that are part of the cytokine-hematopoietin receptor superfamily and can be found in various organs of the body *(Bazan, 1990)*. Several forms of the bovine GHR mRNA exist based on differences in the 5'-untranslated region of exon 1 *(Edens and Talamantes, 1998)*. This variability arises from the presence of multiple promoters that control the start of the transcription site on the GHR gene *(Jiang et al., 1999, 2000; Jiang and Lucy, 2001)*. The sequence of the GH receptor protein itself is the same, because the coding region of the mRNA runs from exon 2 to 10 *(Lucy et al., 2001)*. The three major variants of GHR mRNAs are

GHR 1A, 1B and 1C due to a significantly higher expression level (>90 %) in several tissues compared to the other variants *(Jiang et al., 1999; Jiang and Lucy, 2001)*. In cattle the highest GHR and GHR mRNA expression is found in the liver *(Hauser et al., 1990; Lucy et al., 1998; Butler et al., 2003)* and GHR 1A, which is only present in the liver of adult cattle has also the highest abundance corresponding to ~50% of the total hepatic GHR mRNA followed by ~40% of 1B and ~10% of the 1C variant. In skeletal muscle the majority (approximately 70%) belonged to the 1B variant and 20% were 1C *(Jiang et al., 1999, Jiang and Lucy, 2001)*. Therefore, GHR 1B and 1C are important for local GH effect and promote local production and autocrine/paracrine actions of IGF-I *(Jing et al., 2000)*. Translational efficiencies of the various mRNA products are different: GHR 1A is translated to a much higher degree than 1B and 1C, so more GHR proteins are present in the liver, where 1A is the dominant mRNA template than in the muscle and in other tissues despite their high mRNA content *(Jiang and Lucy, 2001)*. Accordingly, the amount of secreted IGF-I from the liver greatly depends on the abundance of hepatic GHR 1A mRNA and its translational capacity.

In the dairy cow during early lactation concentrations of IGF-I in the peripheral circulation are low despite consistently high GH levels *(Block et al., 2001; Accorsi et al., 2005; Ingvartsen, 2006)*. Fasting and other states of undernourishment induce a GH hypersecretory state *(Rigamonti et al., 1998)* due to GH refractoriness to SRIF. NEB possibly triggers the uncoupling of the IGF - GH axis, so decreased levels of IGF-I exert a reduced negative feed-back effect on GH synthesis and secretion which further elevates peripheral GH concentration *(Veldhuis et al., 2001; Jiang et al., 2005)*. The periparturient liver seems to be in a state of refractoriness to the actions of GH. This is mediated via decreased hepatic GHR 1A mRNA expression as well as reduced specific GH binding from the prepartum period to calving, followed by an increase to prepartum levels during the first 2-3 weeks PP *(Kobayashi et al., 1999; Radcliff et al., 2003; Jiang et al., 2005)*. Concentration of non-1A mRNA variants *(Jiang et al., 2005)* or 1B mRNA *(Kobayashi et al., 1999)* remained unchanged during the transition period. Total GHR mRNA tended to be diminished at calving as compared to the pre- and postpartum periods *(Kobayashi et al., 1999; Jiang et al., 2005)* or did not change in a study of *Radcliff et al. (2003)*. The numbers and affinity of GHRs were estimated to be highest prepartum, decreased immediately PP then rose again, and similarly, GH binding sites decreased to only 5% of prepartum levels by Day 3 PP and increased by Day 17 PP *(Radcliff et al., 2003)*. IGF-I mRNA concentration in the liver was also reduced at

parturition compared to before or two weeks after calving parallelling alterations seen in serum IGF-I levels and in liver GHR 1A mRNA expression *(Kobayashi et al., 1999; Radcliff et al., 2003; Jiang et al., 2005)*. Decreased feed intake on the day and immediately before parturition together with the accompanying endocrine events might be the signal for decreased liver GHR 1A mRNA expression that consecutively leads to the above mentioned steps in the uncoupling mechanism of the GH-IGF-I cascade *(Lucy et al., 2001)*. In a study of *Radcliff et al. (2006)* liver GHR 1A mRNA expression was similar in feed-restricted and control cows before and at parturition, but PP increase rate was slower and concentration on Day 21 PP was lower in feed-restricted animals. No differences were detected in total GHR mRNA. Plasma GH tended to be lower in control cows during feed restriction (first 2 weeks PP), but there was no difference by Day 21 PP. On the other hand, despite similar trends in plasma IGF-I levels pre- and post-calving, control cows had significantly higher PP concentrations compared to feed-restricted cows. Interestingly, no differences in hepatic IGF-I mRNA concentrations were noticed. *Butler et al (2003)* also found that feed intake was positively related to liver IGF-I and GHR 1A mRNA in early PP cows.

It appears that as EB improves concomittantly with increasing plasma insulin concentrations PP, so does liver glycogen/TG ratio *(Andersen et al., 2002)* and responsiveness to GH in lactating dairy cows. Insulin seems to play a crucial role in the recoupling of the GH-IGF-I axis. In humans, reduced hepatic IGF-I mRNA in diabetic patients could be restored by insulin injections *(Rajaram et al., 1997)*. *Butler et al (2003)* recently demonstrated that long term (96 hours) administration of iv insulin in the second week PP increased plasma IGF-I, IGFBP-3, liver GHR 1A and IGF-I mRNA concentrations, while plasma GH, NEFA and IGFBP-2 declined and liver total GHR mRNA remained unchanged. There was a high positive correlation between hepatic GHR mRNA and IGF-I mRNA in insulin treated but not in control cows. Conversely, insulin administration diminished total GHR and IGF-I mRNA abundance in adipose tissue by 1.8 and 3.4-fold, respectively.

The phenomenon of insulin resistance

In general, insulin resistance refers to a condition in which the biological response to physiological levels of insulin is diminished below what normally would be expected *(Kahn, 1978)*. The term *insulin resistance* refers to insulin responsiveness (insulin response to glucose) or insulin sensitivity (tissue responsiveness to exogenous insulin

which estimates glucose utilization by peripheral tissues) or both *(Kahn, 1978; Sano et al., 1991)*. Impairment of insulin action can be localized to pre-receptor, at receptor or to post-receptor levels *(Hayirli, 2006)*. Pre-receptor impairment occurs prior to insulin interaction with its receptor and includes reduced insulin production, increased insulin degradation or both. At receptor level, the alteration includes decreased number of receptors and/or binding affinity. Impairment at post-receptor level consists of disturbed intracellular signalling mechanism and failure of translocation of glucose transporters (GLUT). There are several isoforms of GLUT in various tissues. In peak- and late-lactating dairy cows the mammary gland expresses a non-insulin dependant transporter, GLUT 1 in approximately three times greater magnitude than in dry cows to facilitate entry of glucose into the udder *(Komatsu et al., 2005)*. GLUT 4 is the only transporter that requires insulin for glucose uptake and it is present in skeletal muscle, in the heart muscle and in adipose tissue, thus in the insulin-sensitive tissues *(DeFronzo et al., 1992; Zhou et al., 1999)*. The abundance of GLUT 4 in skeletal muscle and in fat stores did not change as lactation progressed and was not different during the dry period, either *(Komatsu et al., 2005)*. The most accurate experimental methods to diagnose insulin resistance are the hyperinsulinemic-euglycemic and hyperglycemic clamp tests *(Lomax et al., 1979; Rose et al., 1996; Mason et al., 1999)*, but they are difficult to implement under field conditions because of their labor-intensity. Therefore, alternative methods have been found and extensively used in cattle *(Hollenbeck et al., 1984; McCann and Reimers, 1985; Denbow et al., 1986; Sakai et al., 1996; Subiyatno et al., 1996; Holtenius et al., 2003; Bossaert et al., 2008)* such as various challenge tests to stimulate pancreatic insulin secretion and assess insulin responsiveness and tissue sensitivity to insulin e.g. the intravenous/oral glucose tolerance tests (GTT) or propionate/xylitol challenge tests and the intravenous insulin tolerance test (ITT). Recently, a new model have been introduced by *Holtenius and Holtenius (2007)* to use in cattle for a rapid and easy estimation of insulin sensitivity (Revised Quantitative Insulin Sentitivity Check Index, RQUICKI). It was adapted from human medicine *(Perseghin et al., 2001)*, but its accuracy in animal models have not been widely investigated, yet.

In lactating dairy cows IR develops as part of a complex homeorhetic adaptation process to keep up with the metabolic challenge of increased milk production *(Holtenius and Traven, 1990; Bell, 1995)*. Insulin response to glucose challenge was depressed, while glucose clearance rate increased PP compared to prepartum values possibly due to greater glucose utilization by the lactating mammary gland *(Holtenius et al., 2003;*

Bossaert et al., 2008). However, PP increase in glucose disappearance rate was smaller in cows fed high energy diet before calving due to IR in fat cows *(Holtenius et al., 2003)*. Cows' BCS was also significantly and negatively correlated with RQUICKI in the same data set *(Holtenius and Holtenius, 2007)*. Obese heifers developed insulin resistance that was reflected in higher basal insulin concentrations with euglycemia and higher insulin responsiveness to glucose meanwhile glucose fractional removal rates were similar to lean animals *(Mc Cann and Reimers, 1986)*. *Sano et al. (1993)* found that insulin responsiveness to glucose was reduced, but peripheral tissue sensitivity to insulin was unchanged despite higher metabolic clearance rate of insulin in late lactating (~150 d PP) compared to nonlactating cows. Lactating dairy cows had lower pancreatic insulin output and consequently lower hepatic insulin uptake than non-lactating animals *(Lomax et al., 1979)*. They also showed reduced insulin responsiveness to iv glucose and propionate challenges and a decrease in hepatic glucose output equal to the rate of glucose infusion. In turn, *Denbow et al. (1986) and Blum et al. (1999)* did not find a difference in insulin responses to intravenous glucose challenge, insulin metabolic clearance rate and insulin-dependant glucose utilization among early or mid- and late-lactating cows. Similarly, *Holtenius and Holtenius (2007)* did not find a change in insulin sensitivity by lactation weeks using the RQUICKI. Conversely, *Mashek et al. (2001)* concluded from a hyperinsulinemic-euglycemic study that mid-lactation cows had reduced tissue sensitivity to insulin compared to early-lactation cows. Insulin response was reduced PP compared to the dry period, but increased from Day 14 to 42 after calving *(Bossaert et al., 2008)*. Chronic malnutrition decreased pancreatic islet numbers and islet size in a study of *Tse et al. (1998)* that lead to reduced insulin secretion in rats. Cows on restricted pasture feeding prepartum tended to have lower insulin responses to glucose 2 weeks PP than their herdmates that were fed ad libitum *(Chagas et al., 2006)*. From these previous reports it seems that low plasma insulin levels found in dairy cattle after calving could be the consequence of impaired pancreatic islet function and islet regression, which is the result of feed depression commonly encountered shortly before and following parturition and its hormonal and metabolic consequences *(Hayirli, 2006)*.

Several endocrine and metabolic factors might be implicated in the initiation of an insulin resistant state during early lactation. Growth hormone seems to be one of these endocrine signals, as GH concentration is usually increased PP and its metabolic effects are antagonistic to insulin by enhancing lipolysis in adipose tissue and gluconeogenesis

in the liver *(Bell, 1995; Block et al., 2001; Ingvartsen, 2006)*. After chronic GH treatment blood glucose level was increased *(Putnam et al., 1999)* and insulin resistance occurred within a few hours in insulin-sensitive tissues, but GLUT 4 translocation was not disturbed *(Yokota et al., 1998)*. Treatment with GHRH (followed by an increase in plasma GH) diminished glucose turnover rate in a hyperinsulinemic-euglycemic clamp test and thus provoked IR in late lactation cows, but interstingly, it had no effect during early lactation *(Rose et al., 1996)*. High NEFA levels can also create IR by impairing insulin actions at various levels. Systemic administration of FFA inhibited glucose uptake by muscle in a dose-dependent manner and increased hepatic glucose production (reviewed by *Ruan and Lodish, 2003)*. Short-term hyperlipidemia following an intravenous fat (tallow) infusion in nonlactating, non-pregnant dairy cows increased basal glucose and insulin levels as well as decreased glucose clearance rate during both GTT and ITT *(Pires et al., 2007a)*. Insulin sensitivity could be reinstituted by lowering FFA and thus enhancing glucose clearance rate despite lower insulin secretion *(Pires et al., 2007b)*. Plasma NEFA levels were negatively related to glucose-induced insulin secretion in a recent study of *Bossaert et al. (2008)*. The inhibition of glucose uptake by NEFA in insulin-sensitive tissues involves intracellular signalling pathways in the liver and in peripheral tissues (e.g. abnormality in GLUT 4 translocation, receptor downregulation, decreased coupling between stimulated receptors and glucose transport) and the suppression of GLUT 4 abundance (reviewed by *Hayirli, 2006)*. Rat adipocytes exposed to high levels of FFA in vitro showed insulin resistance within 4 hours (especially with palmitate and even at lower concentrations) and the mechanism by which IR developed was through the inhibition of GLUT 4 activation affecting insulin-mediated glucose transport, but not interfering with glycogenesis *(van Epps-Fung et al., 1997)*. Free fatty acids can acutely enhance glucose-induced insulin secretion *(Stein et al., 1996; Pires et al., 2007a)*, but chronically increased levels desensitize insulin secretory capacity of pancreatic ß-cells and provoke IR that was evident by higher basal insulin and glucose concentrations and decreased glucose infusion rates *(Mason et al., 1999)*. Insulin is known to stimulate leptin production and output by adipocytes and in turn, leptin can act on insulin secretion (indirectly or directly via its receptors in the pancreas). Leptin has stimulatory effect during underfeeding, but inhibiting further insulin output after refeeding *(Houseknecht et al., 2000; Chilliard et al., 2001; Amstalden et al., 2002; Block et al., 2003; Zieba et al., 2005)*. Leptin is known to enhance insulin sensitivity, glucose utilization and energy

expenditure in skeletal muscle, stimulates fatty acid oxidation in the muscle and liver and lipolysis in adipose tissue, inhibits lipogenesis in hepatocytes and in fat stores. Therefore, through its stimulatory effects on fatty acid oxidation leptin has a crucial role in lowering lipid content and enhancing insulin sensitivity in peripheral tissues *(Ahima et al., 1996; Havel, 2004)*.

Diseased states and insulin resistance

Hyperketonemia initiates a state of IR in lactating dairy cows. Spontaneously ketotic and fasted cows had markedly reduced insulin secretory capacity after an ivGTT than healthy animals, and glucose clearance was also significantly lower in fasted cows than in the other two groups *(Hove, 1978)*. Similar results of *Sakai et al. (1996)* showed that ketotic cows had depressed pancreatic ß–cell function and decreased glucose and insulin disappearance rates after glucose and xylitol challenge. In agreement with reports mentioned above, several authors found lower than normal insulin responses to glucose, propionate or glucagon stimulation in ketotic cows *(Sakai et al., 1993; Samanc et al, 1996, Steen et al., 1997). Chagas et al. (2006)* found lower insulin secretion to a PP iv glucose challenge in cows with prepartum feed-restriction compared to ad libitum grazers, but glucose disappearance was unchanged among nutrition groups. Fatty liver was also directly related to IR by *Ohtsuka et al. (2001):* the severity of hepatic lipidosis was associated with the degree of decreased insulin responsiveness. Cows with fatty liver had increased plasma NEFA and BHB levels, decreased insulin concentrations and a compromised insulin-stimulated blood glucose response (ISBGR; *Oikawa and Oetzel, 2006)*. ISBGR was negatively related to NEFA, BHB and liver TG, and positively to insulin levels. In vitro cultures of bovine hepatocytes loaded with triglycerides had lower insulin clearance rates (CR) and impaired insulin- and/or glucagon-stimulated albumin synthesis than normal hepatocytes *(Strang et al., 1998)*.

Left displaced abomasum commonly occurs shortly after calving in dairy cows and has a complex etiology that still needs clarification *(*reviewed by *Doll et al., 2008)*. Cows with LDA showed impaired glucose tolerance and heterogeneity of insulin responses to glucagon stimulation *(Holtenius and Traven, 1990)*, increased basal insulin and glucose levels and low, slightly fluctuating myoelectric activity of the abomasoduodenum up to 7 days after surgical correction *(Pravettoni et al., 2004)*. Myoelectric patterns of non-insulin resistant patients were higher and improved progressively post-surgery. Peripheral insulin and glucose concentrations were

increased in cows with LDA despite concurrent metabolic disorders, e.g. ketosis and showed delayed abomasal emptying due to high insulin levels (*Doll et al., 2008)*. In conclusion, IR may play a role in the pathogenesis of LDA in dairy cows after calving.

Tumor necrosis factor-a (TNFa) is one of the inflammatory mediators that is released following experimental lipopolysaccharides (LPS) administration or inflammatory diseases e.g. mastitis *(Elsasser et al., 1994; McMahon et al., 1998; Hoeben et al., 2000)*. Endotoxin as well as TNFa are known to create a catabolic state depressing feed intake, milk and milk protein yield, transiently increasing plasma GH, NEFA and IGFBP-1 concentrations and decreasing IGF-I and T_3 plasma levels in lactating cows and in sheep *(Briard et al, 2000; Kushibiki et al., 2003)*. The simultaneous decrease in IGF-I contrasted the rise in GH suggesting a state of GH resistance and reduced bioavailability of IGF-I due to a moderate increase in IGFBP-1 *(Briard et al, 2000)*. TNFa also interferes with peripheral insulin sensitivity. Steers treated daily with TNFa had higher basal insulin levels, higher or normal basal glucose concentrations, less reduced glucose nadir and smaller glucose area under the curve (AUC) after ITT than in control animals reflecting a state of IR *(Kushibiki et al., 2001a and 2001b)*. Conversely, their insulin responses following an iv glucose challenge increased significantly compared to control steers.. Administration of LPS to steers resulted in a temporary hyperglycemia followed by decreased basal glucose and increased insulin levels *(McMahon et al, 1998)*. TNFa mRNA expression is increased in adipose tissue of obese rats and humans, thus may serve as a link between IR commonly found in fat subjects and obesity (reviewed by *Ruan and Lodish, 2003)*. This may, at least in part, explain why cows with high BCS have decreased tissue sensitivity to insulin as showed by *Holtenius et al. (2003),* and *Holtenius and Holtenius (2007)*. In case of hepatic lipidosis TNFa might be an important mediator of IR, as well. *Ohtsuka et al. (2001)* found 3 times higher TNFa values in cows with severe fatty liver than in cows with mild hepatic lipidosis.

Insulin resistance have been implicated in the development of polycystic ovary syndome in women *(Dunaif et al., 1989)*. However, *Opsomer et al. (1999)* could not prove a relationship between IR and cystic ovarian disease (COD) in high yielding dairy cows. Basal insulin and glucose levels, and glucose response to an ivGTT was not different between cystic cows and their matched controls, but insulin secretion was significantly reduced in COD.

3.3. The onset of cyclic ovarian function in high-producing dairy cows

Mechanism of the resumption of regular ovarian activity after calving

In dairy cows follicle-stimulating hormone (FSH) concentration in plasma increases 1-5 d after calving and initiates the development of the first follicular wave and the first dominant (>9 mm) follicle (DF) in the second week PP. Subsequent FSH waves follow every 7-10 days with continous turnover of new follicular cohorts and new DFs until ovulation despite an average -31.38 MJ/day NEB in the first 3 weeks PP *(Beam and Butler, 1997; Gong et al., 2002)*. During the early weeks of lactation it is luteinizing hormone (LH) and not FSH that appears to be deficient. Therefore regular onset of FSH dependent follicular growth seems to be insensitive to NEB *(Lamming et al., 1981; Rajamahandren and Taylor, 1990; Savio et al., 1990; Beam and Butler, 1997, 1999)*. The number of class 3 (10-15 mm in diameter) but not class 1 (3-5 mm) and 2 (6-9 mm) follicles increased with more positive EB before Day 25 PP *(Lucy et al., 1991b)* suggesting that as cows improve their EB the movement of smaller follicles into larger size is enhanced. However, cows on either a moderate or high fat diet had higher numbers of >15 mm follicles 14 d PP and it did not depend on the dietary groups' EB *(Beam and Butler, 1997)*, so DF development in PP dairy cows is tolerant to NEB. On the other hand, several studies demonstrated that the ultimate diameter and E_2 production of a DF are influenced by metabolic factors so that both DF size and plasma E_2 level increased after EB improved from its nadir *(Beam and Butler, 1997 and 1998)*. Interestingly, significantly more first and second-wave DFs got selected and ovulated on the ovary contralateral to the previously gravid uterine horn than on the ipsilateral ovary *(Sheldon et al., 2002)* suggesting that endocrine and/or inflammatory mechanisms associated with uterine involution might act locally on ovarian follicular growth.

Three patterns of PP follicular development based on the fate of the first DF have been described *(Rajamahandren and Taylor, 1990; Savio et al., 1990; Beam and Butler, 1997; 1998, 1999; Sheldon et al., 2002b)*: (1) ovulation; (2) development of one or more waves of non-ovulatory DFs before first ovulation; (3) cyst formation. In ovulatory cows, first wave DFs reached larger maximum diameter and had higher peak plasma E_2 than non-ovulatory DFs. In a recent study of *Butler et al. (2006)* non-ovulatory DFs were further subdivided by high or low E_2 production. Both groups underwent atresia after various days of dominance despite a preovulatory-like E_2 peak in high E_2 non-ovulatory DFs. Follicles that developed into cysts had high E_2 production

similar to ovulatory cows, but failed to regress after a period of dominance. In case of pattern 1, regular ovarian cycles follow ovulation of the first-wave DF. Patterns 2 and 3 may be repeated several times and can prolong the interval from calving to resumption of ovarian activity. Ovaries of cows with delayed onset of ciclicity were mainly considered inactive on palpation per rectum and only a few had cyst-like structures *(Opsomer et al., 1998)*. Cows with higher than average milk yield usually ovulate larger follicles despite lower circulating E_2 levels, which is possibly due to a remarkable rise in liver blood flow and thus hepatic steroid metabolism following increased feed consumption rate after calving *(Wiltbank et al., 2006)*.

The physiological signaling mechanisms informing the *hypothalamus - anterior pituitary - ovary* (HPO) axis about the current stage of EB are not revealed completely, yet. Restricted energy intake did not alter pituitary gonadotropin-releasing hormone (GnRH) receptor density in PP cows *(Moss et al., 1985)*, but dietary energy restrictions were followed by both decreased *(Rutter and Randel, 1984)* and increased *(Whisnant et al., 1985)* responsiveness to exogenous GnRH. Recovery of pituitary LH content and responsiveness to GnRH rapidly increases PP and is able to support ovulation by Day 8 after calving in dairy cows and available inter-species evidence suggests a predominantly hypothalamic locus for the primary effect of decreased energy intake *(Moss et al., 1985; Schillo, 1992)*. In the early weeks of lactation reduced activity of the GnRH pulse generator in dairy cows is expressed as reduced pulsatile LH support of follicular steroidogenesis that is necessary for triggering a preovulatory LH surge and subsequent ovulation. The loss of pulsatile LH secretion was shown to result from prolonged inadequacy of energy supply in both suckling beef cows and non-suckling dairy cows *(Lamming et al., 1981; Canfield and Butler, 1991; Perry et al., 1991)*. Moreover, an enhanced responsiveness of the hypothalamus to the negative feed-back effect of E_2 further compromises the already low pulsatility of GnRH (reviewed by *Beam and Butler, 1999; Wiltbank et al., 2002)*. Up to now it has been widely accepted that the re-establishment of a pulsatile LH secretion pattern is a key event in the return of ovarian cyclicity in PP dairy cows experiencing NEB *(Beam and Butler, 1999)*. LH pulse frequency increased (by 1.8 pulses/ 8 h) together with basal and mean LH concentrations, while LH amplitude remained unchanged or slightly declined from immediately before to following NEB nadir PP in lactating dairy cows *(Canfield and Butler, 1990, 1991)*. This increase in LH pulsatility from 2 to 5 weeks PP was significantly lower in cows with restricted access to pasture prepartum than in cows

with ad libitum grazing *(Chagas et al., 2006)*. However, *Jorritsma et al. (2005)* could not establish an association between days to first ovulation and LH pulsatility characteristics during the third week PP. LH pulsatility was not related to the extent of NEB nadir, either, but a longer period between calving and NEB nadir resulted in higher basal and mean blood LH concentrations. A seemingly low LH pulse frequency (2 pulses per 6 h) is apparently adequate to sustain the morphological development of a DF by the 2nd week PP. This observation is consistent with the growth and differentiation of competent DF-s during the mid-luteal phase of the bovine estrous cycle when LH pulse frequency is low *(Driancourt et al., 1991)*. Ovarian responsiveness to gonadotropins may also modulate the time of first PP ovulation. *Canfield and Butler (1991)* found no differences in LH pulsatility characteristics between lactating and nonlactating dairy cows until two weeks PP, but nonlactating cows ovulated sooner, reached NEB nadir earlier and had improved EB and metabolic profile compared to lactating cows. This might implicate the involvement of metabolites and metabolic hormones (e.g. insulin) signaling to the ovary and enhance follicular responsiveness to LH.

Consequences of NEB on the onset of cyclicity

In the past decades fertility of dairy cows has declined concurrently with genetic selection for improved milk production *(Royal et al., 2002; Berry et al., 2003)*. High genetic merit cows had longer calving to first ovulation, to first service and to conception intervals, decreased overall and first service conception rates and requiered more services per conception than low genetic merit cows in a study of *Gong et al. (2002)*. Cows over 40-50 kg milk yield per day also had higher double ovulation rate than average producers, which subsequently predisposes them to twinning *(Wiltbank et al., 2006)*. It is well known that conception rates improve with earlier restoration of ovarian cyclicity, thus with more cycles before AI *(Butler and Smith, 1989; Butler, 2001)*. Cows that resumed ovarian cyclicity >45 d PP had reduced conception and pregnancy rates and longer intervals to first AI and to conception *(Shrestha et al., 2004)*. Therefore, including commencement of luteal activity into selection programs of dairy cows could still be a useful tool to improve fertility even if its heritability is low (h^2=0.28; *Darwash et al., 1997)*.

In lactating dairy cows first PP ovulation takes place about 10 d after the nadir of NEB, when EB is already past its lowest value and is progressing towards balance *(Butler et al., 1981; Butler and Smith, 1989)*. Several studies demonstrated a positive

correlation between the interval from calving to NEB nadir and the interval from calving to first ovulation *(Canfield and Butler, 1990, 1991; Beam and Butler, 1997).* Conversely, *Jorritsma et al. (2005)* could not relate the onset of ovarian activity to days to NEB nadir, but a deeper nadir resulted in a longer interval to resumption of cyclicity. Cumulative NEB from calving to its nadir correlated positively with the onset of cyclicity *(Canfield and Butler, 1991)* and average EB in the first 20 d was inversely related to the interval from calving to first ovulation *(Butler et al., 1981)*. Similarly, *de Vries and Veerkamp (2000)* found that a larger energy deficit in early lactation, a deeper NEB nadir and a longer interval to reach positive EB corresponded to a later resumption of cyclicity and each 10 MJ of NE_L/day lower nadir resulted in a 1.25 d later first ovulation. None of NEB traits were associated with the time of first PP ovulation in a study of *Reist et al. (2003)*. A normal lactating dairy cow should regaine cyclic ovarian function by Day 50 PP and continue cycling regularly thereafter *(Lamming at al., 1981). Opsomer et al. (1998)* reported an average of 32 d for first ovulation and it was similar to results of 29.4 d by *Royal et al. (2002)* and 34 d by *Kadokawa et al. (2006)*.

Hormonal and metabolic signalling of NEB on PP ovarian activity

During the early weeks of lactation NEB is associated with reduced plasma concentrations of insulin, IGF-I, T_4, T_3 and leptin, and increased secretion of GH *(Beam and Butler, 1997, 1998 and 1999; Kadokawa et al., 2000; Block et al., 2001; Huszenicza et al., 2001 and 2002; Butler et al., 2003)*. These hormonal changes may, at least partially account for a link between reproduction and EB through exerting their effect on the hypothalamic, pituitary, and ovarian levels. *Huszenicza et al. (2006)* found a significant negative correlation between insulin and IGF-1 levels in the 1st week after calving and the duration of the PP acyclic period. Likewise, cows that ovulated within 35 d PP had higher IGF-I, glucose and insulin, and lower NEFA and BHB concentrations *(Huszenicza et al., 2001)*. Ovulatory cows (from the first follicular wave) showed higher insulin, IGF-I and lower NEFA levels from 5 to 25 d PP compared to non-ovulatory, low E_2 producing cows, while differences were not significant between ovulatory and non-ovulatory, high E_2 producing or cystic cows *(Butler et al., 2006).* Plasma IGF-I and insulin levels and insulin:GH ratio were higher and GH concentrations were lower in cows that ovulated the first wave DF compared to those that did not ovulate, although no differences were found in NEFA, glucose and TCh concentrations *(Beam and Butler, 1997; Kawashima et al., 2007)*. This relationship was

only present in the first week but not in the 2nd and 3rd week PP in the study of *Beam and Butler (1997). Gong et al (2002)* found that more cows fed a high insulin-inducing diet ovulated within 50 d PP and had shorter calving to first ovulation intervals compared to cows on a low insulin diet regardless of genetic merit. On the other hand, *Jorritsma et al (2005)* and *Canfield and Butler (1990)* could not demonstrate an association of blood glucose, insulin, NEFA, BHB and liver TG content with the time of first ovulation. *Reist et al. (2003)* found that among all blood parameters studied only thyroid hormones (T_3 and T_4) were positively associated with the onset of cyclicity.

In cows circulating IGF-I concentrations correlated with IGF-I levels found in the follicular fluid of large follicles *(Echternkamp et al., 1990)*. Insulin has been shown to stimulate follicular cells in vitro in cows and both insulin and IGF-I are known to stimulate steroidogenesis and proliferation of bovine theca and granulosa cell cultures *(Spicer et al., 1993; Spicer and Stewart, 1996; Spicer, 2001)*. In vitro IGF-I increased the number of LH-binding sites of bovine theca cells and enhanced LH-induced production of androstenedione and P_4 *(Spicer and Stewart, 1996)*. These in vitro findings could explain the in vivo observations of *Butler et al. (2004)* and *Beam and Butler (1998)* that long-term insulin administration to periparturient cows increased plasma E_2 and IGF-I levels and reduced testosterone:insulin ratio despite no differences in LH pulsatility between treated and control cows, and the close relationship between steroidogenic activity of first-wave DFs and circulating plasma levels of IGF-I.

Growth hormone can also exert direct or indirect (through the IGF-I system) effects on ovarian structures. GH influences the early stages of folliculogenesis both prenatally and postnatally *(Childs, 2000)* and promote early differentiation of preantral ovarian follicles directly via GH receptors on granulosa cells *(Kölle et al., 1998)* or coupled with IGF-I or activin. Activin is an ovarian produced protein known to stimulate pituitary FSH secretion, although its stimulatory action is tenfold less than that of GnRH *(deKretser and Robertson, 1989)*. In later follicular stages GH may enhance LH receptor expression and is also involved in the formation and differentiation of granulosa lutein cells *(Kölle et al., 1998)*.

Spicer et al. (2001) showed that T_3 and T_4 could directly stimulate theca cell steroidogenesis and their effect on androstenedione production was similar to that of LH. However, T_4 had a much weaker effect on theca cell P_4 production than LH (evident only at hyperthyroid levels), while T_3 did not affect granulosa and theca cell P_4 response at all.

Leptin was proposed to have a role in shifting ovarian function from anovulatory to ovulatory once a permissive, threshold concentration has been reached *(Williams et al., 2002; Zieba et al., 2005)* being an important biochemical messenger between fat stores, EB and the reproductive axis *(Ahima et al., 1996; Barb and Krealing, 2004)*. Indeed, *Kadokawa et al. (2000)* found a significant correlation between the time of the first PP ovulation and the interval from calving to leptin nadir, but there was no relationship with actual prepartum or pre- and postovulatory leptin levels. Leptin was not associated with PP first ovulation, but higher concentrations were related to shorter periods from calving to first estrus *(Liefers et al., 2003)*. In multiparous cows the frequency of LH pulses was positively correlated with both EB and plasma leptin concentrations, while LH pulse amplitude was only related to leptin (*Kadokawa et al., 2006)*. Low leptin markedly increased neuropeptid Y (NPY) production in the hypothalamus *(Ahima et al., 1996)* and high NPY levels inhibited the gonadal axis in ruminants *(Gazal et al., 1998)*. Intracerebroventricular administration of leptin increased LH secretion in the fasted cow and ewe, but not in control fed animals indicating that metabolic state is an important factor in modulating the response of HPO axis to leptin *(Morisson et al., 2001; Amstalden et al., 2002, 2003)*. Leptin receptors have also been found in the gonads. In vitro, supraphysiological levels of leptin increased insulin-induced proliferation of theca cells and had weak inhibitory effects on gonadotropin- and/or IGF-I-induced steroidogenesis of theca and granulosa cells *(Spicer et al., 2000; Spicer, 2001, 2003)*.

Diseased status of the puerperium on the resumption of ovarian activity

Disturbed metabolic status, e.g. hyperketonemia can impair reproductive functions of the high yielding dairy cow as it was discussed earlier in Chapter 3.1.

Inflammatory diseases with large amount of endotoxin and cytokine release may interfere with metabolic and endocrine changes (including plasma levels of leptin and hormones of the GH - IGF-I axis) of the periparturient period, thus affecting NEB and reproduction *(Huszenicza et al., 2004; Kulcsár et al., 2005a; Whitlock et al., 2008)*. Gram negative mastitis with severe clinical signs occurring between 15-28 d PP significantly delayed the onset of ovarian cyclicity and first visible estrus compared to healthy or early mastitis (from calving to 14 d PP) cows. In already cycling cows mastitis induced luteolysis or prolonged the follicular phase, but did not increase cyst formation *(Huszenicza et al., 1998, 2005)*.

Bacterial uterine complications after calving may cause sub-fertility and infertility in dairy cows *(Nakao et al., 1992; LeBlanc et al., 2002a; Reist et al., 2003)*. Abnormal P_4 profiles such as delayed first ovulation, prolonged or short luteal phases, cessation of cyclicity and ovarian cysts are often associated with PP uterine infection *(Opsomer et al., 2000; Royal et al., 2000; Sheldon et al., 2008).* Cows with puerperal metritis had a longer interval from calving to first ovulation and to first estrus, they were inseminated later and had longer days open than healthy animals *(Huszenicza et al., 1999; Reist et al., 2003)*. However, in a recent trial *Kulcsár et al. (2005a)* found that only toxic forms of puerperal metritis and not puerperal metritis without systemic signs accounted for significantly delayed first ovulation and decreased conception rates. Regardless of the presence or absence of systemic signs no difference in the interval from calving to first estrus and to conception was found between healthy cows and cows with puerperal metritis or clinical endometritis *(Benzaquen et al., 2007)*. On the contrary, cows with clinical endometritis had delayed first ovulation, decreased first service conception rates and therefore took longer to conceive in a study of *Nakao et al. (1992)*. Ovulation of the first DF was less likely in cows with uterine disease and they were also prone to have irregular P_4 profiles *(Sheldon at al., 2008)*. Interestingly, among cows that ovulated the first DF *Sheldon et al. (2002b)* did not find a difference in the interval from calving to first ovulation whether they had standard or high uterine bacterial growth scores on Day 7 and 14 PP. *Shrestha et al. (2004)* noticed a higher incidence of prolonged luteal phases in cows with delayed uterine involution, metritis or pyometra possibly due to impaired endometrial PGF_{2a} secretion and/or transportation to the ovary. No difference in the percentage of PP anovulation was noticed between healthy and diseased animals. Cows with abnormal vaginal discharge were 6.4 times more at risk for delayed cyclicity and 4 times more likely to have prolonged luteal phases (*Opsomer et al., 2000)*.

These inflammatory conditions of the postpartum uterus may disrupt endocrine and physiological pathways of the HPO axis at multiple levels. First DFs grew slower, reached smaller maximum size and produced less E_2 in cows with uterine infection *(Williams et al., 2007)*. Also, fewer first and second DFs were selected on the ovary ipsilateral to the previously gravid horn than on the contralateral ovary in cases of high uterine bacterial growth scores. The interval from calving to reach dominance as well as the fate of the first DF did not depend on bacterial growth scores *(Sheldon et al., 2002b; Williams et al., 2007)*. The regular rise of FSH waves followed by the emergence of a cohort of follicles does not seem to be affected by the presence of bacterial uterine

disease *(Sheldon et al., 2002b, 2008)*. It is more likely that endotoxin modulates hypothalamic and/or pituitary GnRH and LH pulsatility thus alters follicular growth and ovulation *(Battaglia et al., 1997; Williams et al., 2001). Battaglia et al. (2000)* found that a 26-hour intravenous endotoxin challenge in ewes also interrupted the preovulatory estradiol rise, completely blocked or delayed the preovulatory LH surge and estrus behaviour. Despite an adequate LH pulse frequency follicular E_2 secretion was markedly reduced showing the direct effects of LPS on the ovary. Cytokines like TNFa inhibited IGF-I–induced steroidogenesis in granulosa and theca cells and reduced IGF-I–mediated proliferation of theca cells in vitro *(Spicer, 2001)*.

4. Materials and Methods

4.1. Animal housing and management

All studies were carried out in Hungary and implemented according to the rules and under the permission and control of the Hungarian State Veterinary Service. Description of the common housing, management and nutrition systems of the three experiments are given here, specifications on the circumstances of the given study are detailed under the trial.

All cows were kept year-round in a loose-housing system without access to pasture or regular physical exercise. Groups of approximately 50 to 100 animals were created according to the stage of lactation and monthly milk yield records. Cows were milked either twice or three times daily depending on the management of each farm. Dry cows were housed separately. Animals calved year-round in continuously used maternity barns. Calves were weaned immediately after parturition. Diet on each farm consisted of corn silage, concentrate, alfalfa and grass hay, vitamin and mineral premix offered in a total mixed ration (TMR). Nutrient, mineral and vitamin requirements were calculated according to the *National Research Council (1989 or 2001)* recommendations, however, MJ was used instead of Mcal (1 Mcal=4.184 MJ). Quality of the TMR was somewhat different on each farm due to microclimate differences and feeding management practices. Drinking water was available *ad libitum*. The course of uterine involution was controlled on a regular basis by a general physical examination, palpation per rectum and vaginoscopy by the herd veterinarian, and bacterial complications (puerperal metritis, clinical metritis, pyometra, endometritis) were treated with commercially available antimicrobials, uterotonica and luteolytica. All cows were artificially inseminated = 50 d after calving when showing signs of heat. Pregnancy checks were carried out at 45-60 d post AI by palpation per rectum. Before milking udders were palpated and the first drops of milk from each quarter were examined for signs of clinical mastitis.

The day of calving was defined as Day 0. Scoring of body condition was done by the same persons on each farm 1 to 3, 28 to 35, and 55 to 65 d PP in *Exp. 1*. and 9-14 d (the day before the glucose tolerance test) and 24-28 d PP in *Exp. 3* using a 5-point scale with 0.25 unit increments *(Skidmore et al., 1997)*.

4.2. Sample collection, preparation and storage

All blood samples were collected by jugular or coccygeal venipuncture and before the morning feeding so that postprandial fluctuations of various plasma measurements could be avoided. It has been recently shown by *Wylie et al. (2008)* that peripheral BHB, urea and insulin increased, leptin and IGF-I remained unchanged and NEFA and glucose decreased post feeding. Samples were taken for the determination of GH genotype, hormones (insulin, IGF-I, leptin, cortisol, T_3, T_4), metabolites (glucose, BHB, NEFA, TCh) and AST into plastic tubes containing sodium fluoride (for glucose assay only), K_3EDTA (Vacuette®; Greiner Bio-One, Kremsmuenster, Austria; for GH genotyping) or heparin (for the determination of all other blood parameters). All sodium-fluoride and heparin containing samples were cooled and centrifuged within 60 min, plasma was harvested and stored at +4 °C if assayed within 48 h or at -20 °C if assayed later. Whole blood samples collected for *Alu*I polymorphism determination were frozen immediately after collection and stored frozen (-20 °C) until laboratory analysis. Three times weekly (prior to the morning milking) from 7 to 10 d PP until confirmation of the first luteal phase but no longer than 120 d PP milk samples (8 to 10 mL) were collected into plastic tubes containing 7.5 mg potassium-dicromate (Reanal Ltd., Budapest, Hungary). Samples were kept refrigerated at +4 °C until milk progesterone concentration was determined. All assays were carried out within 21 days of collection.

4.3. Laboratory procedures

GH genotype was determined by polymerase chain reaction, restriction fragment length polymorphism (PCR-RFLP) method *(Lucy et al., 1993)*. Total DNA (genomic and mitochondrial) was isolated from leukocytes. Whole blood samples (50 µL) were washed with 500 µL of solution (Tris-HCl 10mM, Na_2EDTA 1mM, pH 8), vortexed, and centrifuged at 12000 rpm for 2 min. This procedure was repeated three times and then the pellet was suspended in 100 µL lysis solution (10 mM Tris, pH 7.5; 50 mM KCl; 20 mM Tween; and 0.6 µg/µL proteinase K). After suspension, samples were incubated at 56 °C for 60 min, followed by incubation at 94 °C for 10 min (to inactivate proteinase enzymes). The extracted DNA was stored frozen at –20 °C pending further PCR analysis. Primers were designed to amplify a 428 basepair (bp) sequence of the bovine GH gene that included 55 bp of the 4th exon, the entire 4th intron, and 99 bp of the 5th exon (Genbank Accession Number J00008; *Woychik et al., 1982)*.

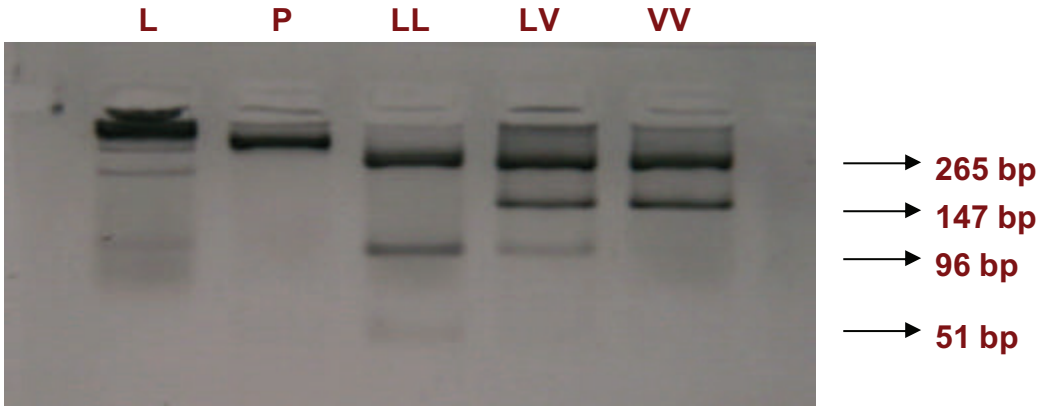
GH F 5’CGGACCGTGTCTATGAGAAGCTGAAG3’

GH R 5’GTTCTTGAGCAGCGCGTCGTCA3’

PCR reactions were performed in a total volume of 10 µL of 1 mM $MgCl_2$, 200 µM of each dNTP, 0.2 µM of primers, 10 x PCR buffer, 0.5 unit TaqI DNA polymerase (Promega U.S., Madison WI, USA) and 100 ng of total DNA. Pre-denaturation occurred at 94 °C for 1 min, followed by 32 cycles of denaturation at 94 °C (each for 30 sec), annealing at 61°C (for 1 min), extension at 72 °C (for 1 min), and final extension at 72 °C (for 10 min).

Digestion of PCR products with *Alu*I restriction endonuclease was conducted in a total volume of 13 µL, including all PCR products, 5 units of *Alu*I enzyme, and 10xB buffer (Promega) at 37 °C overnight. DNA fragments were run for 2 x 8 min. in 4% high-resolution agarose gels (Cambrex Biosciences, San Diego CA, USA) stained with ethidium bromide at 5 and 10 V/cm gradient and fragments typical of either leucine (265, 96, 51 and 16 bp) or valine (265, 147 and 16 bp) alleles *(Lucy et al., 1993)* were visualised under ultraviolet (UV) light *(Fig. 4.3.1)*.

Fig. 4.3.1. Electroforetogram of PCR products



L: 100 bp DNA ladder with fragments ranging from 100 bp to just above 1000 bp; P: “product”, the total length of amplified DNA fragments; LL: leucine homozygous - 265bp, 96bp and 51bp DNA fragments; LV: heterozygous - 265bp, 147bp, 96bp and 51bp DNA fragments; VV: valine homozygous - 265bp and 147bp DNA fragments

All metabolites and metabolic hormones were determined from plasma. Analysis of BHB (Exp. 2 and 3.) and NEFA (Exp. 3.) was carried out in the Research Institute for Animal Breeding and Nutrition in Herceghalom, Hungary by enzymatic methods (D-3-Hydroxybutyrate kit, Kat. # RB 1007 and NEFA kit, Kat. # FA 115, from Randox Laboratories Ltd, Ardmore, UK). Intra- and interassay coefficients of variation (CVs)

were ≤ 5.7 % and ≤ 7.5 % for BHB, respectively and ≤ 4.1% and ≤ 9.2 % for NEFA, respectively. Total cholesterol (TCh) was assayed by cholesterol oxidase–phenol aminophenazone (CHOD-PAP) reaction (Cholesterol-PAP kit, Kat. # 40121, Diagnosztikum RT, Budapest, Hungary), intra- and interassay CVs were ≤ 1.5 % and ≤ 3.8 %, respectively) in the Research Institute for Animal Breeding and Nutrition, as well. Plasma glucose was determined by glucose oxidase-peroxidase (GOD-POD) reaction (Glucose kit, Kat. # 40841, Diagnosztikum RT, Budapest, Hungary) with intra- and interassay CVs of ≤1.5% and ≤4.3 %, respectively. AST activity was determined by the IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) method in UV range with an AST Kit (Kat. # 7249, Reanal RT, Budapest, Hungary; intra- and interassay CVs ≤ 6.2 % and ≤ 7.8 %, respectively). In Exp. 2. plasma insulin was quantified as free insulin with a commercial ^{125}I-IRMA (immunoradiometric assay) kit developed for human samples (BI-Insulin IRMA kit; CIS Bio International Ltd – Subsidiary of Schering S.A., Gif-Sur-Yvette, France; sensitivity: 3.16 pmol/L; intra- and interassay CV: from 1.3 to 5.6 % and ≤8.5 %, respectively). The assay was validated for bovine plasma samples. The binding pattern of serially diluted bovine plasma samples was parallel to that of the standard curves. The recovery rates of added known quantity of insulin (35.9, 89.8 and 179.5 pmol/sample) to standard bovine plasma samples (n=3; all with pre-determined low insulin content) were 94 - 106 % *(Huszenicza et al., 2006)*. In Exp. 3. plasma insulin was assayed with a commercial ^{125}I-RIA (radioimmuno assay) kit (^{125}I-Insulin RIA CT kit; CIS Bio International Ltd, Gif-Sur-Yvette, France) developed for human and validated *(Huszenicza et al., 1998; Nikolic et al., 2003)* for bovine and ovine samples (sensitivity: 3.85 pmol/L, intra- and interassay CV: 5.5 - 8.4 % and ≤8.8 %). Plasma IGF-I concentration was analyzed with a commercial ^{125}I-IRMA kit developed for human samples (DSL-5600 Active IGF-I Coated-Tube IRMA Kit; Diagnostic Systems Laboratories Inc., Webster, Texas, USA; sensitivity: 0.11 nmol/L; intra- and interassay CV: from 3.4 to 6.6 % and ≤7.0 %, respectively). The assay procedure included a preceding extraction of IGF-I with an ethanolic HCl solution and a before-assay neutralization of extracts. The assay was run in accordance with the manufacturer's instructions with the exception of an overnight incubation of the neutralized extract at +4 ^{o}C (rather than a 3 h incubation at room temperature). The assay was validated for bovine plasma samples and the binding pattern of serially diluted bovine plasma samples was parallel to that of the standard

curves. The recovery rates of added known quantity of IGF-I (0.78, 2.60 and 7.80 nmol/sample, given either before or after the extraction) to standard bovine plasma samples (n=3; all with pre-determined low IGF-I content) were the following: 78 - 88 % (IGF-I given before extraction) and 89 - 107 % (IGF-I given after extraction; *Kulcsár, 2007).* T_4 and T_3 were determined by ^{125}I-T_4-Spec and ^{125}I-T_3 coated tube RIA kits (Institute of Isotopes Co., Ltd. Budapest, Hungary) previously developed for animal samples and for human use, respectively and both validated for bovine and ovine plasma *(Nikolic et al., 2003).* Assay sensitivities were 0.5 nmol/L for T_4 and 0.19 nmol/L for T_3. Intraassay CVs were 6.4 - 8.1 % (T_4) and 6.0 - 8.3 % (T_3) and interassay CVs were ≤5.8 % and ≤6.5 %, respectively. Leptin was determined by a ruminant-specific, homologous, double-antibody, non-equilibrium ^{125}I-RIA method *(Delavaud et al., 2002)* modified and validated for bovine plasma in our lab (assay sensitivity: 0.032 nmol/L; intra- and interassay coefficients of variation: 4.6 to 10.1 % and 5.6 to 12.2 %, respectively; *Kulcsár et al., 2006a)*. Plasma cortisol in Exp. 3. was determined by a direct ^{3}H-RIA method developed for human *(Csernus, 1982)* and equine *(Nagy et al., 1998)* samples and validated for bovine plasma without modification (sensitivity: 0.26 nmol/L, intra- and interassay CV: ≤ 5.6 and ≤ 9.5%; *Nikolic et al., 1998; Jánosi et al., 2003)*.

In order to detect resumption of ovarian cyclicity individual progesterone profiles were obtained from serial milk samples collected before the morning milkings in Exp. 1 and 3. and assayed by a microplate ELISA (enzyme-linked immunosorbent assay) method *(Nagy et al., 1998)* modified for whole milk *(Huszenicza et al., 2005; Taponen et al., 2002)*. Intra- and interassay CVs were <8 and 10 %, respectively, and sensitivity ranged from 0.17 to 0.25 nmol/L. Ovulation was assumed to have occurred when milk P_4 concentrations were $\geq$ 1.5 nmol/L for two or more consecutive samples.

4.4. Statistical evaluation

Distribution of *Alu*I genotypes was expected to be unequal in all three experiments due to the dominance of the leucine and the low frequency of the valine allele in the Holstein-Friesian breed *(Shariflou et al., 2000; Kovács et al., 2006).* Therefore, animals were classified into groups of leucine homozygous (LL) and heterozygous cows, which also included small numbers of the valine homozygous animals (LV + VV). Pearson's Chi-square test was applied to see whether Hardy-Weinberg genetic equilibrium was fulfilled in the population. When certain parameters did not follow a normal distribution

pattern they were logarithmically transformed in order to achieve better approximation to normality (see detailed descriptions in the given experiments). In general, Chi-square test was used for distribution analysis, Student's t-test for pairwise comparison of group means and Pearson's correlation coefficient to evaluate the strength of association between plasma parameters. A single trait linear model was used to evaluate the association of GH genotype with milk yield and BCS change (*Exp. 1*) or with certain plasma parameters (*Exp. 2*). For the description of results group least square means (LSM) ± standard error of the mean (SEM) or means ± standard deviations (SD) are given. Survival analysis with proportional hazards (Cox) regression was applied in *Exp. 1* to estimate the cumulative proportion of cows with regard to the time of first ovulation PP over time.

Level of significance was adjusted at $P<0.05$ in all experiments and in all models. Statistica 7.1 (StatSoft Inc. STATISTICA data analysis software system, Version 7.1, 2005), Excel (Version 5.0; Microsoft Corporation, Redmond, WA, USA) and R (Version 2.4.1, *R Development Core Team, 2006)* program packages were used for all data analysis.

5. Study descriptions and results

5.1. *Alu*I polymorphism of the bovine GH gene, resumption of ovarian cyclicity, milk production and loss of body condition at the onset of lactation in dairy cows (Exp. 1)

It is well known that in dairy cows EB becomes negative with the onset of lactation due to discrepancies between nutrient demand and expenditure. There are several reports on the unfavourable association between the extent of NEB and the time needed for the onset of PP ovarian activity *(Butler et al., 1981; Canfield and Butler, 1990, 1991; Jorritsma et al., 2005)*. Delayed resumption of cyclicity further compromises conception and pregnancy rates and the number of days open *(Butler and Smith, 1989; Butler, 2001; Shrestha et al., 2004)*. Selection for higher yields has changed the function of the GH-IGF-1 axis and conflicting results were obtained on the association of *Alu*I polymorphism and milk production traits. The current knowledge about the relationship of *Alu*I genotype with reproductive characteristics is also limited (please see Chapter 3.2 for details).

We infer that GH genotype may directly (through the GH-IGF-1 axis) or indirectly (e.g. milk yield) affect the interval from calving to resumption of cyclicity. This relationship has not been investigated yet, so the objectives of the current study were to determine whether *Alu*I polymorphism of the bovine GH gene is associated with the onset of ovarian cyclicity PP and whether genotype may influence milk yield and loss of body condition within the first month after calving in Holstein-Friesian cows.

5.1.1. Description of study conditions

Cows that calved between November 2000 and January 2001 in four commercial large-scale dairy herds in eastern Hungary were involved in this study (n=356). Cows in each herd were milked three times daily. Average 305-d milk yield of the previous lactation was 7401.6 ± 1995.2 kg (mean ± SEM). Vaginoscopy, examination of the reproductive tract by palpation per rectum and a general physical examination were performed between Day 6 – 10 PP for the detection of puerperal metritis. Puerperal metritis was characterized by a fetid, watery, red-brown or purulent uterine discharge and an enlarged, atonic, thin-walled (or occasionally edematous) uterus *(Földi et al., 2006; Sheldon et al., 2006)*. Because bacterial complications of the PP period may delay the first PP ovulation (see Chapter 3.3), cows with systemic signs (e.g. inappetance,

dullness, dehydration, rectal temperature >39.5 ^{o}C) of toxic puerperal metritis (n=14), mastitis (n=25) or both (n=10) were excluded from the evaluation (5 animals concurrently had severe lameness).

Body condition scores for Day 1, Day 30 and Day 60 PP were estimated by second-degree polynomial regression curve fitting on individual data. Major losses in BCS occurred between Day 1 and Day 30 PP and mild or no further changes took place between Day 30 and 60 after calving, therefore BCS loss in the first 30 d PP ($BCSL_{30}$) was used in the statistical models. Values of $BCSL_{30}$ did not follow normal distribution pattern and were logarithmically transformed ($100LogBCSL_{30} = 100 \times Log(BCSL_{30})$) in order to achieve better approximation to normality. Results (LSM ± SEM) obtained from logarithmical transformation were back-transformed ($BackBCSL_{30}$: mean = $e^{100Logmean/100}$, SD = $e^{100Logsd/100}$-1) for better understanding. Monthly milk recordings provided data for milk yield and for fat- and protein content during the first 150 d of lactation. A second-degree polynomial regression curve was fitted individually on monthly performance data of each cow to estimate total (cumulative), fat and protein corrected milk yield *(Korver, 1982)* in the first 30 d PP (TMY_{30}). Cows were divided by parity into primiparous (n=116) and multiparous (n=191) animals. Cows were categorized by the presence (n=45) or absence (n=262) of metritis (puerperal metritis without systemic signs). Survival analysis with proportional hazards (Cox) regression was used to evaluate the cumulative proportion of cows with regard to the time of first ovulation PP (FSTOV; in days) over time. Distribution of FSTOV failed the normality test and was, therefore, logarithmically transformed in the model (100LogFSTOV= 100 x Log(FSTOV)) and was back-transformed (BackFSTOV: mean = $e^{100Logmean/100}$, SD = $e^{100Logsd/100}$-1) after results were obtained. Independent variables in the survival analysis were *Alu*I genotype, $100LogBCSL_{30}$, TMY_{30}, parity, metritis and herd. All valid cases were uncensored.

A single trait linear model with fixed effects and two-way interactions was used:

$Y_{ijklmn} = \mu + H_i + G_{j(i)} + P_{k(i)} + M_{l(i)} + C_{m(i)} + (GP)_{jk(i)} + (GM)_{jl(i)} + (GC)_{jm(i)} + (GF)_{jn(i)} + e_{ijklmn}$

where,

Y_{ijklmn} = TMY_{30} or $100LogBCSL_{30}$

μ = overall mean,

H_i = fixed effect of herd (1-4),

$G_{j(i)}$, $P_{k(i)}$, $M_{l(i)}$, $C_{m(i)}$ = fixed effects of *Alu*I genotype (1= LL, 2= LV and VV), metritis (1= presence, 2= absence), parity (1= primiparous, 2= multiparous) and covariates of 100LogFSTOV and $100LogBCSL_{30}$ or TMY_{30}, all nested within herd effect,

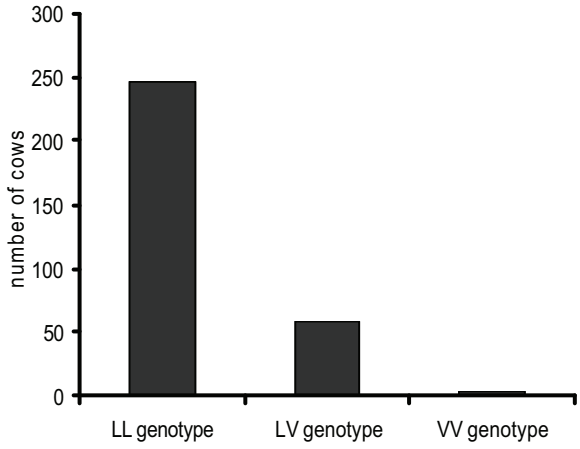
$(GP)_{jk(i)}$, $(GM)_{jl(i)}$, $(GC)_{jm(i)}$, $(GF)_{jn(i)}$ = interactions between fixed effect of genotype and fixed effects of parity, metritis and covariates (100LogFSTOV and $100LogBCSL_{30}$ or TMY_{30}), all nested within herd effect,

e_{ijklmn} = residual variation.

5.1.2. Results

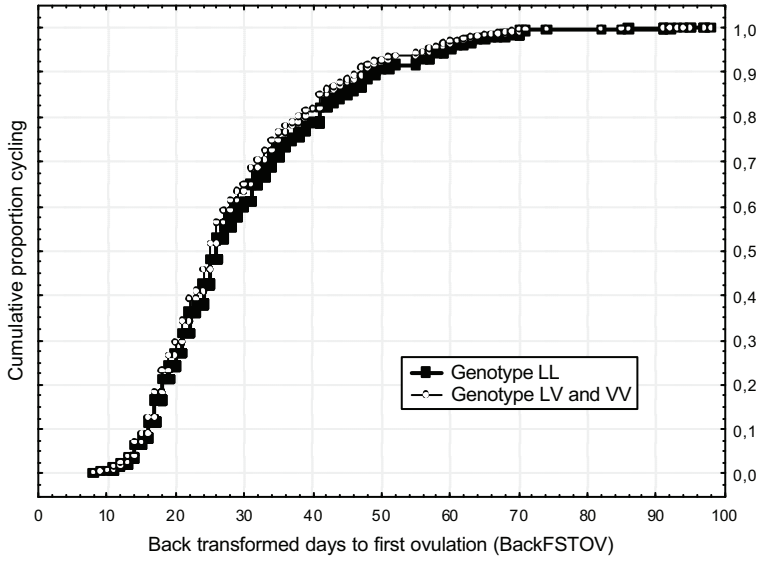
Data of 307 cows was included in the final evaluation. The majority of cows were homozygous for the leucine allele (n=246, 80.13 %), whereas 18.89 % (n=58) were heterozygous and only 0.98 % (n=3) were homozygous for the valine allele (*Fig. 5.1.1.*). Therefore, allele frequencies were $p_{leucine} = 0.896$ and $q_{valine} = 0.104$. Pearson's Chi-square test ($?^2 = 0.039$, df=1, $P > 0.05$) indicated that the genetic pool was in Hardy-Weinberg equilibrium (expected genotype frequencies were LL=80.29%, LV=18.63%, VV=1.07%).

Fig. 5.1.1. Distribution of *Alu*I genotypes in all herds (n=307; *Exp. 1)*



All animals became cyclic during the study period and the average interval to first ovulation PP was 27.6 ± 0.69 d (mean ± SD). All heterozygous cows resumed ovarian cyclicity by 64 d after parturition and the latest ovulation of the leucine homozygous group occurred on Day 98 PP. However, GH genotype did not affect the time of the first PP ovulation (P=0.503; *Fig. 5.1.2.*). On the other hand, parity (P=0.058), degree of $BCSL_{30}$ (P<0.001) and herd (P=0.008) accounted for the differences in the interval from calving to first ovulation. Briefly (details are not given), multiparous cows ovulated sooner after calving than primiparous cows. The greater extent of BCS loss cows had during the first 30 d the longer they took to restore cyclicity. The occurrence of metritis did not delay the first PP ovulation (P=0.927) and milk yield (TMY_{30}) did not influence resumption of cyclicity, either (P=0.335). Goodness-of-fit of the Cox's regression model was $?^2 = 123.8$ and $P < 0.001$.

Fig. 5.1.2. Survival curves with proportional hazards (Cox) regression of GH genotype groups (heterozygous, n=61; leucine homozygous, n=246) re-establishing ovarian cyclicity over time (P=0.503). Values of the x axis are Days to first ovulation back-transformed from their logarithmically transformed values *(Exp. 1)*



Results of the single trait linear model are shown in *Table 5.1.1.* *Alu*I genotype and herd had no influence on cumulative milk production and body condition changes. Primiparous cows produced less milk in the first 30 d after calving than multiparous cows (P<0.001). Metritis was present in 45 cows. Cows with metritis had more severe BCS losses than healthy animals (P=0.031), but showed no significant drop in milk production during the first PP month. Total 30-day milk yield did not affect $BCSL_{30}$ or vice versa.

Table 5.1.1. The association between milk yield and body condition score with *Alu*I genotype, herd, parity, metritis and first PP ovulation *(Exp. 1)*

Effects and Interactions	N	TMY_{30} (kg) P value* LSM	SEM	$100LogBCSL_{30}$ P value* LSM	SEM	$BackBCSL_{30}$ Mean	SD
Herd		0.649*		0.462*			
Herd 1	107	694	54.8	-76.1	11.2	0.47	0.12
Herd 2	92	828	42.6	-42.0	8.5	0.66	0.09
Herd 3	51	828	100.5	-55.4	21.5	0.57	0.24
Herd 4	57	691	63.7	-61.7	14.9	0.54	0.16
$100LogBCSL_{30}$		0.980*		-			
TMY_{30}		-		0.682*			
100LogFSTOV		0.336*		<0.001*			
Genotype		0.799*		0.700*			
LL	246	764	45.3	-51.1	10.2	0.60	0.11
LV and VV	61	756	115.4	-66.5	24.2	0.51	0.27
Parity		<0.001*		0.595*			
Primiparous	116	660	74.9	-61.0	17.5	0.54	0.19
Pluriparous	191	860	76.9	-56.6	16.8	0.57	0.18
Metritis		0.311*		0.031*			
Present	45	693	139.5	-44.7	30.4	0.64	0.36
Absent	262	828	53.9	-73.0	12.2	0.48	0.13
Genotype x Parity		0.172*		0.961*			
Genotype x Metritis		0.720*		0.310*			
Genotype x $100LogBCSL_{30}$		0.663*		-			
Genotype x TMY_{30}		-		0.127*			
Genotype x 100LogFSTOV		0.812*		0.434*			

N: total number of cows; TMY_{30}: total (cumulative) fat and protein corrected milk yield in the first 30 d PP; $100LogBCSL_{30}$: BCS loss in the first 30 days PP after logarithmical transformation; $BackBCSL_{30}$: mean = $e^{100Logmean/100}$, SD = $e^{100Logsd/100}$-1; 100LogFSTOV: Days from calving to first ovulation after logarithmical transformation; LSM: least square of the mean; SEM= standard error of the mean, SD= standard deviation

5.1.3. Discussion

Leucine allele was more frequent than valine (0.896 vs 0.104), so that most of the cows (80.13 %) were homozygous for the leucine allele, 18.89 % were heterozygous and only 0.98 % were homozygous for the valine allele. These findings on allele and genotype frequencies are similar to those previously reported in the HF breed *(Lucy et al., 1993; Lee et al., 1996; Kovács et al., 2006)*.

We did not find a significant association between GH genotype and the interval from calving to first ovulation. There are only a few studies investigating the relationship between *Alu*I polymorphism and reproductive traits, but results yielded no differences between genotypes in breeding values of dairy bulls or in oocyte and embryo characteristics (*Lechniak et al., 1999, 2002b;* see Chapter 3.2). Unfortunately our study did not aim to investigate other fertility traits, e.g. conception rate, days open that could further help to reveal whether *Alu*I polymorphism is indeed involved and to what extent in reproductive functions of dairy cows.

Other factors namely parity, BCS loss and herd were clearly associated with the time of first PP ovulation. Multiparous cows ovulated sooner after calving than primiparous cows which is in accordance with studies of *Opsomer et al, (1998)* and *Tanaka et al. (in press)* possibly due to the higher energy demand imposed on first parity animals by simultaneous growth and lactation. However, the advantage of multiparous cows over primiparous ones was only present in 2nd and 3rd parity and no difference was noted after the 4th lactation *(Opsomer et al., 2000)*. Unlike above reports, *Darwash et al. (1997)* showed that the interval from calving to first PP ovulation progressively increased by 2.2% with each parity in British Friesian cows, which would predict a total of 11.5% increase over a productive lifetime. *Benzaquen et al. (2007)* found longer calving to first service intervals in the first 150 d PP in multiparous compared to primiparous cows.

In Chaper 3.3 we have already discussed in details the association between NEB and commencement of ovarian activity PP. The assessment of BCS and its change after calving can be an indirect, on-farm tool for the evaluation of EB *(Moore et al., 2005)*. In our study cows that lost more BCS during the first 30 d PP took longer to restore ovarian cyclicity. Severe BCS loss was associated with impaired reproductive performance compared to minor or moderate losses as reported by *Butler and Smith (1989)*. A higher percentage of those cows re-initiated cyclic ovarian function within 2 months PP that scored higher at calving and during early lactation or had lesser degree

of BCS losses *(Yamada et al., 2003; Santos et al., in press)*. A negative relationship between BCS and resumption of ovarian activity on the genetic level was also proven by *Royal et al. (2002)*. Genetically thinner cows with a low average BCS were more likely to have a delayed first ovulation after calving and each BCS unit (1 to 9) increase translated to a ~22% (6 days) shorter onset of cyclicity. Because of the positive association between early commencement of ovulatory cycles and conception rates *(Butler and Smith, 1989; Butler, 2000; Butler, 2001)*, it is of great importance that cows resume cyclicity soon after calving. In that regard, conception rates following an ovulation synchronization/fixed-time AI protocol (0. d GnRH, 7. d prostaglandin F_{2a}, 9. d GnRH, then AI 16-19 hours after second GnRH) were significantly higher when cows ovulated within 34 d PP compared to after Day 56 PP *(Yamada et al., 2003)*. It is the degree of loss of BCS rather than the BCS per se, that affects conception rates *(Domecq et al., 1997)*.

Here we found that the presence of puerperal metritis without systemic signs of illness on Day 6-10 PP did not delay re-establishment of ovulatory ovarian cycles. It is in agreement with our previous trial where delayed first PP ovulation occurred only in cows with severe toxic puerperal metritis, but not in cows without systemic signs or in healthy animals *(Kulcsár et al., 2005a)*. *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, several studies reported irregular cycles and prolonged days to first ovulation associated with abnormal uterine content and with various forms of (endo)metritis *(Huszenicza et al., 1999; Opsomer et al., 2000; Royal et al., 2000; Sheldon at al., 2008)*. For further details we refer to Chapter 3.3. This inconsistency may partly result from discrepancies in the description and determination of uterine abnormalities occurring at various stages of the periparturient period, although recent compiling rewiews have been published *(Földi et al., 2006; Sheldon et al., 2006)* in order to set up the necessary guidelines.

Cumulative milk yield during the first 30 days was not different between leucine homozygous and heterozygous cows. Similarly, *Yao et al. (1996)* and *Lechniak et al. (2002a)* could not establish a relationship between *Alu*I polymorphism of the GH gene and genetic merit for milk production traits in dairy bulls, but reports on the advantage of both the L or the V allele exist (see Chapter 3.2 for details). In our study, cows were average producers (7401.6 ± 1995.2 kg, mean ± SEM) and genotype differences might

be only expressed at a higher production level according to *Lee et al. (1996)*. We found that parity was the only significant contributor to differences in TMY_{30} and multiparous cows produced significantly more milk than primiparous cows which is in agreement with *Taylor et al. (2004)* and *Whates et al. (2007). Zwierzchowski et al. (2002)* also demonstrated that third lactation cows yielded more milk than younger cows and that among all factors studied parity was the most significant contributor to differences in milk production traits and *Alu*I genotype had the smallest effect. Recording individual milk yields daily would provide a more accurate and reliable basis to evaluate the rise of the lactational curve and lactation yields in relation to genotype.

We could not demonstrate an association between *Alu*I genotype and the degree of BCS loss PP. On the other hand, cows with puerperal metritis experienced more severe losses compared to healthy animals. The catabolic state that characterizes the onset of lactation *(Ingvartsen, 2006)* can be further aggravated by inflammatory conditions. Cows with puerperal metritis had reduced dry matter intake and increased NEFA and ß-hydroxybutyrate plasma concentrations *(Hammon et al., 2006)* indicating 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 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 the degree of BCS loss during the first month after calving in Holstein-Friesian cows. A larger extent of BCS loss shortly after calving translates to delayed resumption of ovarian cyclicity. Multiparous cows ovulate sooner and produce more milk compared to primiparous cows. The presence of puerperal metritis without systemic signs initiates a more severe BCS loss PP than what healthy animals usually encounter.

5.2. Interrelationship of growth hormone *AluI* polymorphism and hyperketonemia with plasma hormones and metabolites in the beginning of lactation in dairy cows (Exp. 2)

In Chapter 3.1 we have seen the metabolic consequences of NEB in the immediate PP period. The homeorhetic adaptation to the increased nutrient uptake of the lactating mammary gland and the incongruency between energy demand and availability often leads dairy cows to subclinical or clinical ketosis. Therefore, it is crucial to know which factors might be important in the development of hyperketonemia and may predispose cows to increased fat mobilization and ketogenesis.

We know that *Alu*I polymorphism is associated with milk yield and hormonal differences related to genotypes (see Chapter 3.2). Interestingly, studies on how it may influence blood metabolites and metabolic hormone levels were carried out on cattle breeds where the leucine allele was less frequent and the valine allele was more frequent than in the HF breed. Furthermore, these experiments involved growing calves and AI bulls where endocrine and metabolic adaptations to growth processes and/or various feeding regimes are very different from those of the dairy cow in the beginning of lactation.

Therefore, our objective was to investigate the putative link of *Alu*I polymorphism with hyperketonemia status and plasma concentrations of metabolic hormones in the first two weeks after calving in lactating HF cows.

5.2.1. Description of study conditions

This study was conducted on 379 group-fed HF cows (=2nd lactation, BCS at calving 3.00-3.75, average 305-d yield in previous lactation 7764 kg) calving from February to mid-June, 2004 in seven large-scale dairy herds. Cows were milked twice daily and were dried off approximately 60 d before calving.

Daily ration was provided in two portions per day after the morning and evening milkings on all seven farms. Supplementation with vitamins, minerals and trace elements was continuous. On each farm during the first 6 weeks of the dry period cows were fed a mixture of corn silage (10 to 12 kg / cow), alfalfa silage (0 to 2 kg) and concentrate (0.8 to 1.5 kg), whereas grass hay (6.0 to *ad libitum*) was given separately (calculated dry matter intake: 11.8-12.3 kg, energy content: 5.4-5.9 MJ/kg NE_L, crude protein content: 11.3-14.0 %). Two to three weeks before calving cows were moved into preparatory groups (composition of TMR: 11-15 kg corn silage, 0-1.5 kg alfalfa silage,

1-3 kg alfalfa hay, 2-5 kg grass hay and 2.0 to 3.5 kg concentrate; calculated dry matter intake: 11.3-12.0 kg, energy content: 6.10-6.50 MJ/kg NE_L, crude protein: 13.5-15.8 %). One to two days before the expected due date they were transferred into maternity units where they calved and stayed until 3-4 d PP (composition of TMR: as in the preparatory period). Thereafter, cows left the maternity barn and were kept and fed in groups throughout lactation (composition of TMR for fresh cows: 14-24 kg corn silage, 0-7.0 kg alfalfa silage, 0-3.5 kg alfalfa hay, 0-2.0 kg grass hay, 0-8.0 kg sugar beet slices, 0-1.5 kg molasses and 6.0-10.5 kg concentrate enriched with by-pass protein and inert fat sources without propylene-glycol or glycerol supplementation; calculated dry matter intake: 18.0-18.5 kg, energy content: 7.20-7.60 MJ/kg NE_L, crude protein: 18.1-18.9 %; TMR in subsequent 8-10 weeks i.e. during peak lactation consisted of the same components with similar energy and protein content, but calculated dry matter intake increased to 22.0-23.0 kg). Quantity of concentrate was gradually decreased afterwards according to actual milk production.

We did not involve primiparous cows because they are less likely to develop hyperketonemia compared to multiparous cows (Baird, 1982; Andersson and Emanuelson, 1985). They also had higher prepartum IGF-I and leptin concentrations that decreased more sharply reaching to lower IGF-I and leptin (Meikle et al., 2004) or higher IGF-I (Taylor et al., 2004; Whates et al., 2007) levels PP than in multiparous cows. On Day 4-13 after calving all multiparous cows were subjected to an initial general physical examination, vaginoscopy and palpation per rectum by the herd veterinarian on each farm. Cows with clinical signs of ketosis, hypocalcemia, toxic puerperal metritis, clinical mastitis, laminitis, chronic gastro-intestinal illnesses and those that received glucocorticoids, non-steroidal anti-inflammatory drugs or any glucoplastic agents in the previous two weeks were excluded from the study (n=122).

Blood samples were taken on 4-13 d PP for GH genotype and plasma BHB, insulin, IGF-I and leptin determination. BHB in plasma was used for the detection of hyperketonemia due to its stability in the peripheral circulation *(Tyopponen and Kauppinen, 1980)* and the threshold was set at 1.2 mmol/L *(Duffield et al., 1997)*. Plasma insulin and BHB values did not follow a normal distribution pattern and were, therefore, logarithmically transformed in order to achieve better approximation to normality.

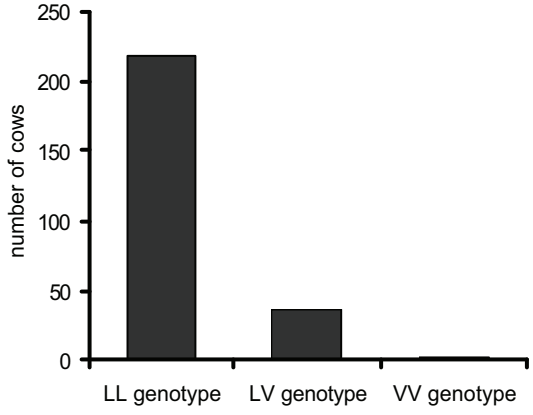
Yates corrected Chi-square test was used to compare the proportion of *Alu*I genotype groups (LL and LV+VV) by hyperketonemia status in all 7 herds. A single

trait linear model was used: $Y_{ijkl} = \mu + H_i + G_{j(i)} + K_{k(i)} + C_{l(i)} + e_{ijkl}$, where Y_{ijk} was plasma 100LogBHB, 100LogInsulin, IGF-I or leptin, μ was the overall mean, H_i was the major fixed effect of herd (1-7), $G_{j(i)}$ was the minor fixed effect of GH genotype (1: LL and 2: LV+VV) nested within herd, $K_{k(i)}$ was the minor fixed effect of hyperketonemia status (1: plasma BHB > 1.2 mmol/L, 2: = 1.2 mmol/L) nested within herd, $C_{l(i)}$ were covariates (parity [2-9], previous [standardized for 305-day] lactation yield, day of blood sampling [4-13] PP) all nested within herd, and e_{ijkl} was the residual variation. Final results were adjusted for 3rd parity, for 7715 kg milk yield and for Day 8 PP. Pearson's correlation coefficient was applied to evaluate the strength of association between plasma BHB and insulin, IGF-I and leptin levels.

5.2.2. Results

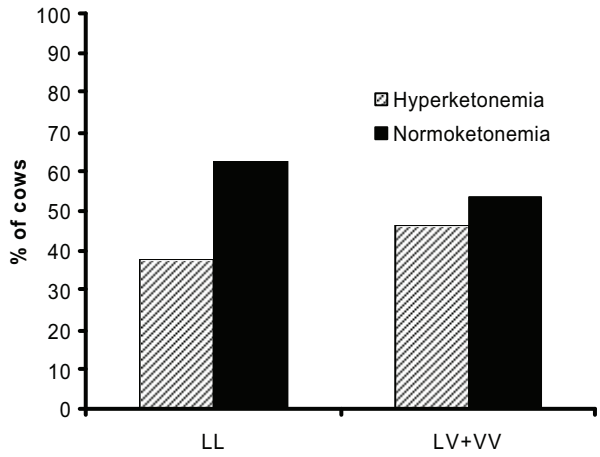
A total of 257 cows were evaluated among which 218 animals were homozygous for the leucine allele, 37 were heterozygous and 2 cows were homozygous for the valine allele (*Fig. 5.2.1.*). Allele frequencies were p_L=0.920 and q_V=0.080. Based on the observed vs. expected genotype frequencies (expected: LL n=217.5, LV n=37.8, VV n=1.7), the whole pool was in Hardy-Weinberg genetic equilibrium (?^{2}=0.098, df=1, P >0.05). Distribution of the heterozygous cows was unequal among farms, because the majority (n=28; 71.79 %) belonged to Herd 4 and 6. The proportion of LL and LV+VV was not different between Herd 4 and Herd 6 (72.31%, 27.69% and 71.43%, 28.57%, respectively, P=0.889). Allele frequencies were p_L=0.850 and q_V=0.150 and observed genotype frequencies were n=72 (LL), n=26 (LV), n=2 (VV). The genetic pool was in Hardy-Weinberg equilibrium (?^{2}=0.036, df=1, P >0.05; expected genotype frequencies were n=72.25 (LL), n=25.5 (LV), n=2.25 (VV).

Fig. 5.2.1. Distribution of *Alu*I genotypes in all herds (n=257; *Exp. 2)*



Plasma BHB concentrations ranged between 0.11 and 5.18 mmol/L (1.25 ± 0.95 mmol/L for mean ± SD). A total of 100 cows had plasma BHB levels above the predetermined threshold of 1.2 mmol/L, and 157 cows were normoketonemic. There was no difference (P=0.316) in the proportion of heterozygous vs. leucine homozygous cows within the hyperketonemic and normoketonemic groups (*Fig. 5.2.2.*).

Fig. 5.2.2. Distribution of heterozygous and leucine homozygous cows by hyperketonemia status (n=257; *Exp. 2*)



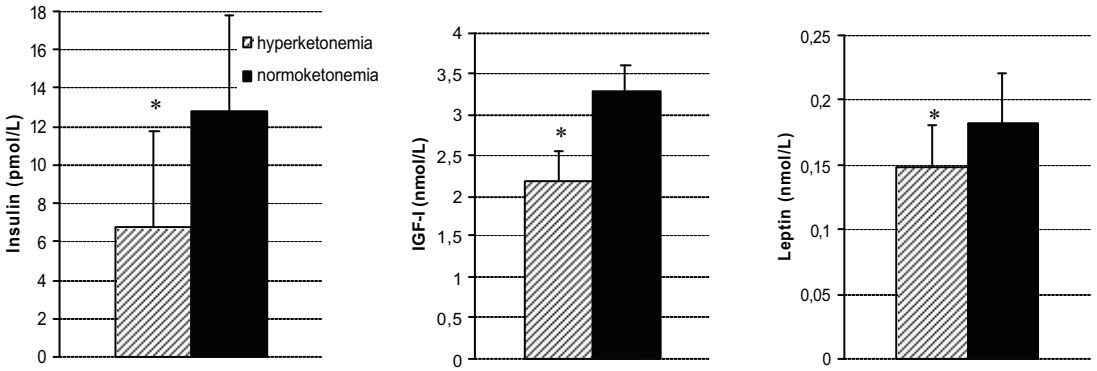
Neither in all 7 herds (n=257) nor in Herd 4 and 6 alone (n=100) could we demonstrate a significant relationship between GH genotype and any of the plasma parameters studied (P>0.27). Results from all animals on the association of *Alu*I polymorphism and plasma measurements as well as general characteristics of the LL and the LV+VV group are presented in Table *5.2.1.*

Table 5.2.1. General characteristics of GH genotypes and their relationship with plasma BHB, insulin, IGF-I and leptin concentrations in all herds (n=257; *Exp. 2*)

	P	LL (mean ± SD)	LV+VV (mean ± SD)
Plasma measurements			
BHB (mmol/L)	0.27	1.01 ± 0.98	1.20 ± 0.74
Insulin (pmol/L)	0.72	9.05 ± 6.39	9.41 ± 4.81
IGF-I (nmol/L)	0.57	2.74 ± 0.64	2.73 ± 0.65
Leptin (nmol/L)	0.61	0.17 ± 0.04	0.17 ± 0.05
N	-	218	39
Day of blood sampling PP	-	8.3 ± 1.3	8.5 ± 1.3
Previous 305-day lactation yield (kg)	-	7726 ± 1645	7885 ± 1621
Parity	-	3.1 ± 1.4	3.0 ± 1.1

The presence or absence of hyperketonemia, on the other hand, strongly contributed to differences in plasma metabolic hormone concentrations (P<0.001). Cows with BHB above 1.2 mmol/L had significantly lower insulin, IGF-I and leptin levels than normoketonemic animals (*Fig. 5.2.3.*).

Fig. 5.2.3. The association of hyperketonemia status with plasma insulin, IGF-I and leptin concentrations in all cows *(*n=257; *Exp. 2)*



*P<0.001

Plasma insulin was influenced by the number of days elapsed since calving (P=0.001) and was different by herds (P=0.013). IGF-I levels tended to vary among herds (P=0.055) and plasma BHB was related to previous 305-day lactation yield (P=0.020). Although first parity cows were not involved in this study, leptin was associated with parity number (P=0.045).

We found moderate to close negative correlations between BHB and insulin, IGF-I and leptin levels (*Fig. 5.2.4.*). IGF-I, insulin and leptin were significantly and positively related to each other (*Fig. 5.2.5.*).

Fig. 5.2.4. Correlations of plasma BHB with IGF-I, insulin and leptin (n=257; *Exp. 2*)

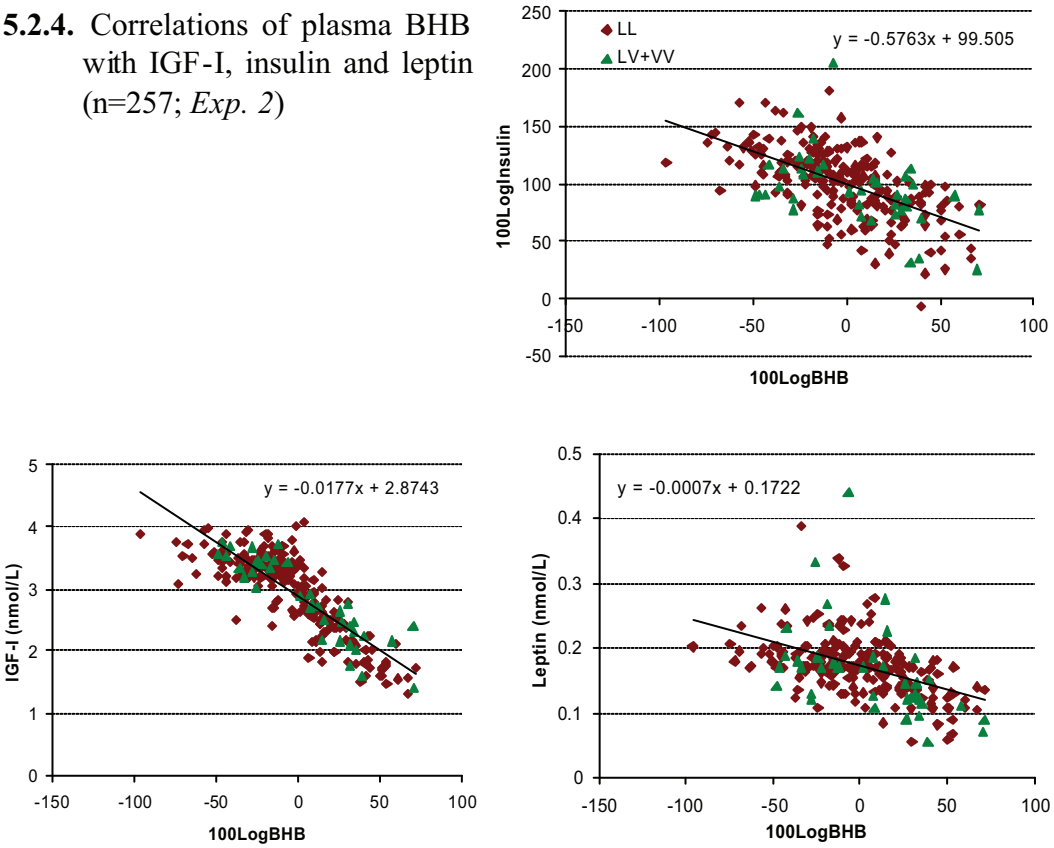
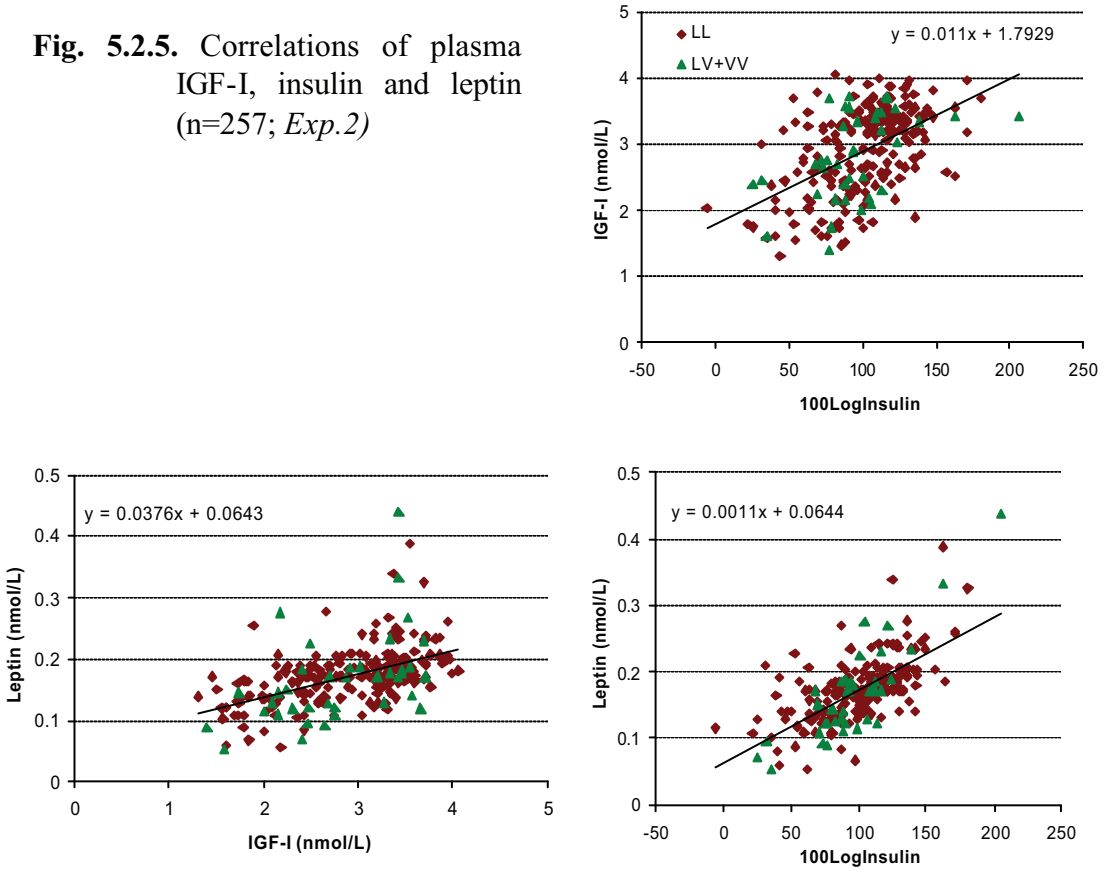


Fig. 5.2.5. Correlations of plasma IGF-I, insulin and leptin (n=257; *Exp.2)*



Correlation coefficients (R) are presented in *Table 5.2.2*. All correlations were significant (P<0.001).

Table 5.2.2. Correlation coefficients (R) between plasma parameters on Day 4-13 PP in all cows (n=257; *Exp 2*)

	100LogBHB	100LogInsulin	IGF-I	Leptin
100LogBHB	1	-0.606*	-0.843*	-0.493*
100LogInsulin	-	1	0.512*	0.626*
IGF-I	-	-	1	0.513*

*P<0.001

5.2.3. Discussion

Allele frequencies were p=0.850-0.920 for leucine and q=0.080-0.150 for valine, which are in agreement with those previously reported in the Holstein-Friesian breed (Lucy et al., 1993; Shariflou et al., 2000; Kovács et al., 2006).

In this study *Alu*I genotype was not associated with the incidence of hyperketonemia or any metabolic hormone measurements. Similarly, *Ge et al. (2003)* could not relate postweaning IGF-I levels and growth traits to *Alu*I polymorphism in growing Angus calves. *Schlee et al. (1994b)* found a tendency to higher IGF-I concentrations in heterozygous Simmental bulls, while LL calves showed the highest insulin and IGF-I levels and VV animals had significantly higher leptin and triglyceride concentrations *(Grochowska et al., 2001; Katoh et al., 2008)*. Nevertheless, 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 (e.g. growth vs. lactation).

Our results showed a significant negative association between peripheral ketone body status and plasma metabolic hormone concentrations within the first two weeks of lactation. Accordingly, cows with increased BHB levels were reported to have decreased glucose, insulin, IGF-I and thyroid hormone and higher NEFA concentrations *(Dann et al., 2005; Huszenicza et al., 2006). Meikle et al. (2004)* also showed a negative correlation between NEFA/BHB and T_4, T_3, insulin, IGF-I and leptin levels, while all hormones were positively related to each other. However, insulin did not correlate with leptin and NEFA, and was weakly, positively related to BHB two weeks PP in multiparous cows (W*hates et al., 2007).* Several studies reported that NEB was associated with decreased plasma insulin, IGF-I, T_4, T_3 and leptin concentrations *(Block et al., 2001; Huszenicza et al., 2001 and 2002; Butler et al., 2003)* and increased BHB and NEFA levels *(Beam and Butler, 1998; Fenwick et al., 2008)*. Therefore, hormonal

profiles of our hyperketonemic cows may suggest that they were at a deeper point of NEB compared to normoketonemic cows.

Despite the short study period (9 days) we noticed that insulin concentrations were associated with the number of days elapsed since calving. *Meikle et al. (2004)* and showed that insulin nadir occurred in the first week after calving, increased thereafter and returned to prepartum levels by day 30 PP. It seems that insulin does not follow recovery of EB so closely, since positive levels of EB were only reached by 8-12 weeks PP *(Block et al., 2001; Jorritsma et al., 2005; Andersen et al., 2005). Beam and Butler (1998)* also found that serum IGF-I was more closely and positively related to daily EB, than plasma insulin.

Previous 305-day lactation yield of cows in the present experiment was significantly related to plasma BHB concentrations on 4-13 d after calving. *Andersson and Emanuelson (1985)* demonstrated a low, but significant correlation between highest individual milk yield and highest milk acetone level. A significant negative correlation was shown between actual milk yield and BHB levels 7 weeks after calving in multiparous cows, while in primiparous cows this relationship was a weak positive 4 weeks PP *(Whates et al., 2007).* In a study of *Kornalijnslijper et al. (2003)* PP metabolic characteristics were similar in high and low producing dairy cows so that yield could not be related to increased plasma BHB levels. *Ingvartsen and Friggens (2005)* found that differences in milk production between individual cows were partly explained by differences in days after calving, levels of hormones and metabolites and by the extent of digestible energy intake. The order of importance in which certain metabolites contributed to yield differences was glucose first, followed by BHB and then NEFA.

In the first two weeks after calving we could not demonstrate any effect of *Alu*I polymorphism on plasma concentrations of ß-hydroxybutyrate and metabolic hormones. Hyperketonemia, on the other hand, was associated with a significant decrease in insulin, IGF-I and leptin blood levels. Insulin, IGF-I and leptin were positively correlated with each other. 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.

5.3. Interrelationships of growth hormone *Alu*I polymorphism, insulin resistance, milk production and reproductive performance in Holstein-Friesian cows (Exp. 3)

In dairy cows in the beginning of lactation IR develops to help directing nutrients from insulin sensitive tissues such as skeletal muscle and adipose tissue to the mammary gland *(Holtenius and Traven, 1990; Bell, 1995)*. A complex mechanism of endocrine and metabolic factors is implicated in this process. Milk yield and basal secretion and stimulated release of GH as well as other metabolite and hormone levels were different between *Alu*I genotypes, however, results often disagree (for further details see Chapter 3.2). Still, cows with leucine or valine allele may vary in the degree of insulin sensitivity early PP due to differences in their endocrine adaptation mechanism and/or in daily milk yield and in the rise of the lactational curve.

We are not aware of any reports on the association of *Alu*I polymorphism and IR in lactating high-yielding dairy cows. Knowledge on the relationship of reproductive performance with genotype is also limited. Our objectives were to estimate the degree of insulin resistance through an intravenous glucose tolerance test (ivGTT) in the second week of lactation and relate it to GH genotype, and to investigate whether milk yield and reproductive performance (primarily the resumption of ovarian cyclicity PP) may vary between cows with the leucine or the valine allele.

5.3.1. Description of study conditions

Our study was carried out on winter-calving (January-February, 2002) multiparous cows (n=32, parity: 2-4) in a large-scale dairy herd. Cows were dried off at 7-month pregnant. During the close-up period (from 21 days before expected due date onwards) they were regrouped and stayed in preparatory pens. Within one week of expected calving date cows were transferred into maternity units (max. 4 individuals in each), remained there until 4-5 days PP and then joined the lactating herd where they were kept in groups of 50 to 80 animals according to stage of lactation and yield performance (e.g. to the group of fresh cows first, 4 weeks later to groups producing ≥43 kg or 40 kg milk/day). Cows were milked three times per day (6am, 2pm, 10pm). Feed was distributed after each milking (3x/day). Natural ingredients and nutritive value of daily ration are shown in *Table 5.3.1* and *Table 5.3.2*. During the periparturient period niacin and propylene-glycol were also added to TMR in order to support liver function.

Table 5.3.1. Natural ingredients of cows' daily ration in kg (average live weight: 700 kg, 3.5% fat-corrected milk; *Exp. 3)*

	Dry cow I	Dry cow II	Fresh cow	Lactation (40L/day)	Lactation (43L/day)
Corn silage	11.5	12.5	15.0	18.5	17.5
Grass hay	6.0	5.0	-	-	-
Alfalfa haylage	2.0	1.0	2.0	2.5	2.5
Alfalfa hay	-	-	3.5	3.0	3.0
Chopped alfalfa	-	-	0.5	2.0	1.5
Concentrate I	1.5	-	-	-	-
Concentrate II	-	2.5	-	-	-
Concentrate III	-	-	5.0	8.2	9.0
Wet sugar beet pulp	-	-	2.0	3.0	3.0
Dry sugar beet pulp	-	-	0.5	-	-
Brewer's grain, wet	-	-	0.5	1.0	1.0
Corn gluten	-	0.6	2.6	1.8	2.4
Water	-	-	1.2	1.2	1.2
Molasses	-	-	0.6	0.6	0.6
Additives	-	0.15*	0.4*	-	0.2*

Dry cow I: first 5 weeks of the dry period; Dry cow II: last 3 weeks of the dry period; fresh cow: from calving to 30 d PP;
Concentrate I: 30% corn, 20% barley, 30% sunflower meal, extr. solvent, 10% corn distiller dried grain, 10% mineral-vitamin premix
Concentrate II: 31.3% corn, 20% full-fat soybean, 20% corn gluten, 10% corn distiller dried grain, 14.7 % mineral-vitamin premix, 4% *Saccharomyces cereovisiae* living culture
Concentrate III: 55.5% corn, 10% full-fat soybean, 10% barley, 5% corn distiller dried grain, 4% sunflower meal, extr. solvent, 13.5% mineral-vitamin premix, 2% *Saccharomyces cereovisiae* living culture
*Additives: propylene-glycol, monensin, niacin, cobalt chloride and *Saccharomyces cereovisiae*

Table 5.3.2. Nutritive value of cows' daily ration *(Exp. 3)*

	Dry cow I	Dry cow II	Fresh cow	Lactation (40L/day)	Lactation (43L/day)
As fed weight (kg)	21.0	21.8	33.8	42.8	41.9
Dry matter (kg)	12.1	11.8	18.1	22.9	22.7
NE_M (MJ)	71.7	64.2	-	-	-
NE_G (MJ)	42.3	38.5	-	-	-
NE_L (MJ)	68.6	73.8	133.9	167.9	170.9
Crude protein (g)	1379	1655	3303	4022	4064
Ether extract (g)	341	398	990	1362	1300
Crude fiber (g)	2961	2659	2811	3461	3233
Calcium (g)	72	71	182	235	220
Phosphorus (g)	54.5	72.6	82.9	119.1	119.8
Sodium (g)	23	27	38	53	56
Magnesium (g)	34	29	69	96	101
Vitamin A (IU)	112,500	225,000	147,000	195,000	191,000
Vitamin D (IU)	15,000	45,000	36,750	48,750	47,750
Vitamin E (mg)	600	1500	708	988	998
RUP (% of crude protein)	-	-	40	38	40
NDF (kg)	6.55	5.40	5.86	7.19	6.99
ADF (kg)	3.78	3.06	3.57	4.39	4.22
NSC (kg)	-	-	7.03	9.22	9.24
MPE (g)	1013	1160	1943	2432	2505
MPN (g)	902	1104	2101	2574	2633
MPN-MPE (g)	-11.04	-5.56	15.79	14.28	12.87

Dry cow I: first 5 weeks of the dry period; Dry cow II: last 3 weeks of the dry period; fresh cow: from calving to 30 d PP;
NE_M= net energy maintenance; NE_G= net energy gain; NE_L= net energy lactation; IU: international unit; RUP= rumen undegraded (intake) protein; NDF= neutral detergent fiber; ADF= acid detergent fiber; NSC= non-structural carbohydrate; MPE= metabolizable protein - energy; MPN= metabolizable protein - nitrogen; MPN-MPE= rumen N-balance

On Day 9-14 PP cows were subjected to a general physical examination, vaginoscopy and palpation per rectum, and those with clinical signs of metabolic disorders and/or inflammatory diseases (e. g. puerperal metritis, mastitis) were excluded (n=10) based on the same criteria as described in *Exp. 1*. Thereafter, healthy cows were weighed and body condition scores were assessed the same day and again on Day 24-28 after calving.

On Day 10-15 PP healthy animals were subjected to an intravenous glucose tolerance test and sampled for several plasma metabolites, metabolic hormones, enzymes and *Alu*I genotype determination. The night before the ivGTT animals were moved to a tie-stall barn with straw bedding. Fasting of cows started 2 hours before and continued throughout the experiment.

Daily milk yields of the current lactation between 4-45 d PP for each cow were obtained from recordings of the automated milking system. Resumption of cyclic ovarian activity PP was monitored by individual milk P_4 profiles. Day of first observed estrus, days from calving to conception and number of services per conception within the first 200 d PP were also recorded.

Intravenous glucose tolerance test: one hour after the morning milking a basal (0. min.) blood was drawn from the jugular vein and immediately thereafter 0.15 g/kg body weight (BW) glucose (as an iv. infusion of 40% glucose solution given in < 3 min.) was administered into the superficial cranial epigastric (mammary) vein. Placement of a jugular catheter was not permitted by the farm management, so blood samples were further collected by jugular venipuncture at 5, 15, 30, 45, 60, 75, 90, 120, 150 and 180 min. following glucose infusion.

Blood and milk samples were collected into appropriate plastic tubes and handled as described in Chapter 4.2 in order to assay plasma glucose, insulin, leptin, BHB, NEFA, TCh, AST, T_4, T_3, cortisol, to determine GH genotype and milk P_4. Plasma glucose, insulin and leptin were assayed at all time points, while BHB, NEFA, TCh, AST, T_4, T_3 and cortisol were measured only at 0. min. All laboratory procedures were carried out as indicated in Chapter 4.3.

From serial glucose and insulin measurements of the ivGTT the following parameters were calculated and used in the statistical analysis: basal concentration (t_0), peak concentration, increment (peak- t_0), area under the curve in the first 60 min. (AUC_{60}), mean concentration between 75-180 min. ($mean_{t75-180}$), clearance rate (CR, %/min), half-life ($t_{1/2,}$ min.), time to reach basal concentration ($t_{basal,}$ min.). Area under

the curve was calculated using the trapezoidal method and corrected for the baseline concentration *(Holtenius et al., 2003)*. All parameters were actual concentrations except for glucose and insulin CR, $t_{1/2}$ and t_{basal}, which were estimated by exponencial curve fitting between t_5 and t_{60} based on the equation of $F(t)=Ae^{-kt}$, where F(t) is the concentration at time t, A is the estimated maximum value, t is the time (min.) and k is the regression coefficient *(Pires et al., 2007a)*. Thereafter, calculations of CR=100 x k, $t_{1/2}=1/k \times \log(t_5/2A)$ and $t_{basal}=1/k \times \log(t_0/A)$ were carried out. Glucose half-life was disregarded due to the smaller than two-fold glucose increment from baseline. From the serial measurements of plasma leptin $mean_{t60}$ and $mean_{t180}$ (average concentration in the first 60 min. and 180 min., respectively) were used. Single trait linear models were built with dependant variables of glucose (basal and peak concentrations, increment, AUC_{60}, $mean_{75\text{-}180}$, CR, t_{basal}), insulin (basal and peak levels, increment, AUC_{60}, $mean_{75\text{-}180}$, CR, $t_{1/2}$, t_{basal}) and leptin ($mean_{t60}$ and $mean_{t180}$) parameters. Independent variables were genotype (1: LL and 2: LV), parity (2-4), previous 305-day lactation yield (kg), average milk yield (kg) of the current lactation (4-45 d PP), BHB and NEFA concentrations at t_0, BW and BCS one day before glucose challenge, BW change and BCS change (between two periods of 9-14 d and 24-28 d PP), and in case of glucose and leptin parameters plasma cortisol at t_0 was also included. These models were then subjected to a stepwise variable selection procedure *(Venables and Ripley, 2002)*.

The Revised Quantitative Insulin Sensitivity Check Index (RQUICKI) adapted from human medicine to cattle practice by *Holtenius and Holtenius (2007)* was calculated following the equation of RQUICKI = $1/[\log(t_0 \text{ glucose})+\log(t_0 \text{ insulin})+\log(t_0 \text{ NEFA})]$. In general, a higher value of the index means increased insulin sensitivity. Hyperketonemia has previously been reported to interfere with insulin resistance. Depressed pancreatic insulin secretion following ivGTT and lower glucose and insulin clearance rates have been shown by several authors *(Hove, 1978; Sakai et al., 1993 and 1996; Samanc et al, 1996, Steen et al., 1997)*. Therefore, we further modified the RQUICKI adding basal plasma concentration of BHB into the equation, which then became $RQUICKI_{BHB} = 1/[\log(t_0 \text{ glucose})+\log(t_0 \text{ insulin})+\log(t_0 \text{ NEFA})+\log(t_0 \text{ BHB})]$. We expect that the inclusion of BHB will help to quickly, easily and more accurately assess insulin sensitivity.

Student's two-sample t-test allowing for unequal variances was used to compare indexes of insulin sensitivity (RQUICKI and $RQUICKI_{BHB}$) and measurements of body condition (BW, BW change, BCS, BCS change), current (4-45 d PP) and previous 305-

day lactation yields and reproductive parameters (time to first ovulation and to first observed estrus PP) between the two genotype groups (LL and LV).

Pearson's correlation coefficient was used to evaluate the strength of association between plasma traits (BHB, NEFA, AST, T_4, T_3, TCh, cortisol, basal glucose, basal insulin and basal leptin) and between calculated glucose, insulin and leptin measurements of the ivGTT with RQUICKI, $RQUICKI_{BHB}$, BHB, NEFA, BCS, BCS change, BW, BW change.

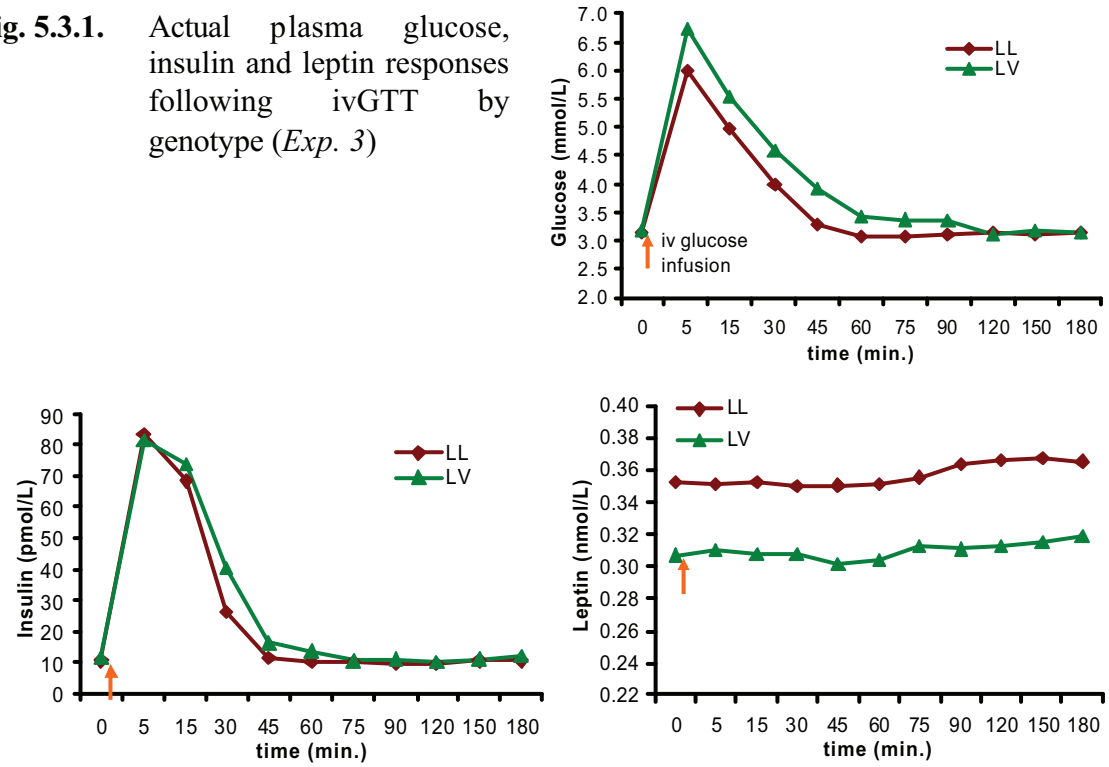
5.3.2. Results

Out of all cows examined on Day 9-14 (n=32) only 22 animals were included in the study and 10 were excluded for having clinical symptoms of (toxic) puerperal metritis. Among cows that participated in the experiment 18 were LL, 4 were LV and there were no VV animals in the population. The frequency of the leucine allele was $p_{leucine} = 0.909$ and the valine allele was $q_{valine} = 0.091$. Expected genotype frequencies were LL: n=18.2, LV: n=3.6, VV: n=0.2, so based on Pearson's Chi-square test the pool was in Hardy-Weinberg genetic equilibrium ($?^2$=0.246, df=1, P>0.05).

In the beginning of lactation (4-45 d PP) LL and LV cows produced approximately the same amount of milk (36.9 ± 5.8 kg and 38.7 ± 2.3 kg, respectively, P=0.31) and there was a mild tendency (P=0.13) for LV cows to have higher previous 305-day lactation yields (LV: 11060.5 ± 1386.8 kg and LL: 9647.4 ± 1145.3 kg).

In general, following the intravenous glucose infusion, all cows reached peak glucose concentration 5 min. later, which returned to baseline level in the 45-75. min. Accordingly, all cows mounted an insulin response to the glucose challenge that reached peak concentration 5 to 15 min. later and returned to baseline between 45-75 min. Plasma leptin did not change significantly after glucose infusion. *Alu*I genotype was not associated with any of the calculated glucose parameters of the ivGTT (P>0.64). Heterozygous cows, on the other hand, had higher $mean_{t75\text{-}180}$ plasma insulin (P=0.002), larger AUC_{60} (P=0.032), longer time to reach half of the maximal insulin concentration (P=0.035) and a tendency for increased t_0 and t_{basal} levels (P=0.064 and P=0.054, respectively) compared to leucine homozygous cows. Leptin response to the glucose challenge was not associated with genotype (P>0.58). The actual plasma glucose, insulin and leptin responses to ivGTT by genotype are shown in *Fig. 5.3.1*.

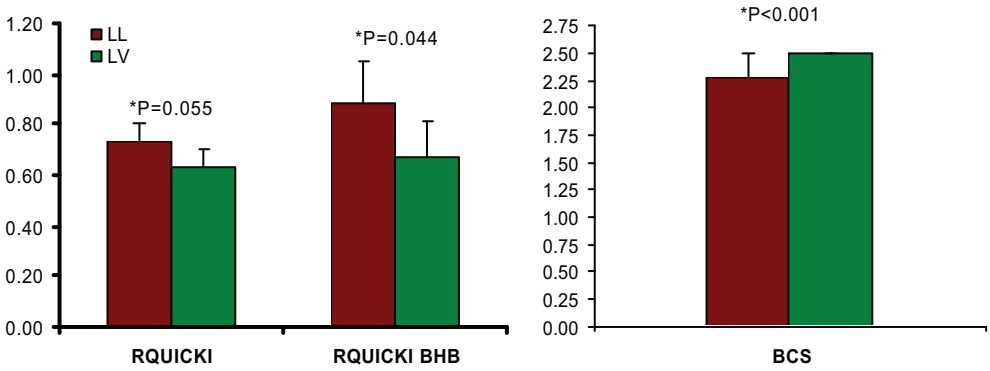
Fig. 5.3.1. Actual plasma glucose, insulin and leptin responses following ivGTT by genotype (*Exp. 3*)



Higher plasma NEFA levels accounted for higher glucose peak and increment, longer time to reach baseline glucose concentration and larger glucose AUC_{60} ($P<0.05$). Plasma BHB was negatively associated with insulin traits e.g. peak, increment, clearance rate, AUC_{60} ($P<0.001$) and t_{basal} ($P=0.045$). Higher average milk yield was related to decreased plasma glucose peak and AUC_{60} ($P=0.058$ and $P=0.040$, respectively), a shorter time to reach baseline glucose concentration ($P=0.005$) and higher leptin levels ($P<0.018$). Previous lactation yield was inversely related to t_0 and $mean_{t75\text{-}180}$ insulin levels ($P=0.007$ and $P<0.001$, respectively). Plasma cortisol lowered glucose clearance rate ($P=0.040$) and prolonged time to reach basal concentration ($P=0.006$). As parity number increased so did baseline glucose level ($P=0.025$). Older cows also had higher peak insulin concentrations and insulin increment ($P=0.042$ and $P=0.058$, respectively) and tended to have lower leptin levels during ivGTT ($P>0.068$). Cows that lost more weight PP had higher glucose peak concentration and longer time to reach t_0 ($P=0.055$ and $P=0.024$, respectively). Plasma leptin levels were higher in cows with more BW ($P<0.001$) and/or less weight loss ($P<0.009$).

Based on values of RQUICKI and $RQUICKI_{BHB}$, LV cows showed decreased insulin sensitivity on 10-15 d PP and they were also in a slightly better body condition compared to LL animals (*Fig. 5.3.2.*). There were no differences in BW, BW change and BCS change between genotypes.

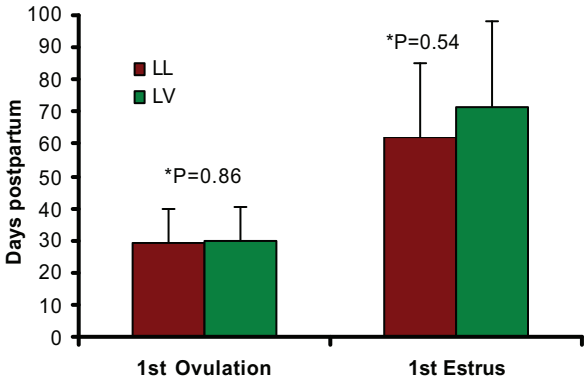
Fig. 5.3.2. Indexes of insulin sensitivity and BCS by genotype (*Exp. 3*)



*Student's t-test

All cows became cyclic and showed signs of estrus during the study period. There were no differences between LL and LV animals in the onset of ovarian activity and in the time of first observed estrus PP (*Fig. 5.3.3.*). From the LL group 17 cows were inseminated out of which 12 conceived (mean days open 119.8 ± 66.2) and on average needed 1.9 services per conception. Three of the LV cows were served and conceived (mean days open 110.7 ± 30.0) and needed 2 services per conception.

Fig. 5.3.3. Time of first ovulation and first observed estrus after calving by genotype (*Exp. 3*)



*Student's t-test

There was a moderate positive correlation between plasma BHB and NEFA and both were positively related to AST, and negatively to T_4 and T_3. AST was also negatively correlated with TCh, meanwhile T_4 and T3 depended closely and positively on each other (*Table 5.3.3*)**.**

Table 5.3.3. Correlation coefficients between basal plasma parameters (only significant relationships are shown; *Exp. 3*)

	BHB	**NEFA**	**AST**	**T_4**	**T_3**	**TCh**
BHB	1	0.529**	0.631**	-0.412*	-0.647***	NS
NEFA	-	1	0.510*	-0.485*	-0.510*	NS
AST			1	NS	NS	-0.422*
T_4				1	0.779***	NS
T_3					1	NS
TCh						1

NS: not significant; *P=0.05, **P=0.01, ***P=0.001

Glucose peak, glucose increment, t_{basal} and glucose AUC_{60} were all negatively correlated with both insulin sensitivity indexes and at the same time, positively with BHB and/or NEFA. On the other hand, insulin peak, insulin increment, insulin CR and AUC_{60} were negatively related to BHB and/or NEFA. Insulin increment and CR showed a positive correlation with RQUICKI and $RQUICKI_{BHB}$, while resting and $mean_{t75\text{-}180}$ insulin had an inverse relationship with estimates of insulin sensitivity (*Table 5.3.4*).

Table 5.3.4. Correlation coefficients of glucose and insulin measurements of ivGTT with insulin sensitivity indexes, plasma- and body condition parameters (only significant relationships are shown; *Exp. 3*)

	RQUICKI	**RQUICKI$_{BHB}$**	**BHB**	**NEFA**	**BW**	**BW change**
glucose peak	-0.634**	-0.422*	NS	0.628**	NS	-0.418*
glucose increment	-0.645***	-0.487*	NS	0.720***	NS	-0.479*
t_{basal} glucose	-0.605**	-0.558**	0.580**	0.602**	NS	NS
AUC_{60} glucose	-0.663***	-0.565**	0.416*	0.735***	NS	NS
t_0 insulin	-0.493*	NS	NS	NS	NS	NS
insulin peak	NS	NS	-0.601**	NS	NS	NS
insulin increment	NS	0.388§	-0.600**	-0.360§	NS	NS
CR insulin	0.417*	0.473*	-0.635**	-0.383§	NS	NS
$t_{1/2}$ insulin	NS	NS	NS	0.467*	NS	-0.406§
t_{basal} insulin	NS	NS	NS	NS	0.427*	NS
AUC_{60} insulin	NS	NS	-0.509*	NS	NS	NS
$mean_{t75-180}$ insulin	-0.448*	NS	NS	NS	NS	NS

NS: not significant; CR: clearance rate; AUC_{60}: area under the curve t_0-t_{60}; $t_{1/2}$: time to reach half of peak concentration; t_{basal}: time to reach basal concentration; §P=0.1, *P=0.05, **P=0.01, ***P=0.001

Leptin ($mean_{60}$ and $mean_{180}$) was not an indicator of insulin sensitivity, but showed a moderate correlation (R=0.492; P=0.02) with body weight. Both BCS and BW were negatively related to RQUICKI$_{BHB}$ (R=-0.414, P=0.05 and R=-0.459, P=0.03, respectively) and BW also showed a positive correlation with BHB and NEFA (R=0.490, P=0.02 and R=0.427, P=0.04, respectively). BCS change and BW change were also moderately related to each other (R=0.633, P=0.002).

5.3.3. Discussion

Average milk production in the beginning of lactation (4-45 d PP) was not different between *Alu*I genotypes similarly to results on cumulative 30-d yields in *Exp. 1.* Nevertheless, heterozygous cows showed a tendency for higher 305-day lactation yields. Several reports have already investigated the association of GH genotype with milk production traits (see Chapter 3.2). A recent study carried out in six large-scale Holstein herds in Hungary showed the advantage of LV dams over LL cows in 305-day lactation and test-day milk yields *(Kovács et al., 2006)*.

Patterns of plasma glucose and insulin responses to ivGTT in this study were comparable to those described elsewhere *(Holtenius et al., 2003; Pires et al., 2007a).* Leptin concentrations were not affected by the glucose challenge test. *Gabai et al. (2002)* and *Chagas et al. (2006)* could not induce a leptin response by infusing glucose or amino acid into late pregnant or lactating Simmenthal cows and to primiparous Holstein-Friesian cows, either.

Resting glucose concentrations, glucose peak and glucose disappearance during ivGTT were similar in LV and LL cows despite higher baseline and mean$_{175\text{-}180}$ insulin levels, longer half-life and larger insulin AUC$_{60}$ in LV animals, reflecting a state of IR. Basal and challenged leptin levels were not associated with genotype. *Katoh et al. (2008)* found higher insulin levels in LL Japanese Black calves than in LV animals despite similar glucose concentrations, and the highest plasma leptin levels in VV calves. Valid comparison of our results to this study is not possible due to breed, age and physiological differences between the two populations. In lactating dairy cows IR develops concomittantly with depressed pancreatic insulin secretory capacity *(Holtenius and Traven, 1990; Holtenius et al., 2003).* In our study, LV cows had larger insulin AUC which might be due to increased insulin secretion and/or decreased insulin metabolism. Glucose disappearance after ivGTT is the sum of glucose utilization by peripheral tissues, absorption from the intestine, hepatic glucose output and excretion through the kidney. Glucose utilization by the mammary gland is significantly increased PP *(Holtenius et al., 2003)* and the degree of glucose drain from the circulation depends on milk yield. In our study milk production was not different between LL and LV cows. They received the same diet and were both fasted shortly before and during ivGTT, so glucose absorption from the intestine should have been minimal. We have no knowledge about the effect of ivGTT on renal glucose excretion. Although lactating

cows have increased hepatic gluconeogenesis *(Ingvartsen, 2006)*, high insulin levels reached during ivGTT should efficiently suppress gluconeogenesis and hepatic glucose output *(Brockman and Laarveld, 1986)*. Therefore, glucose uptake by insulin-dependant peripheral tissues should have been similar in both genotypes as glucose clearance rate was not different. However, LV cows needed more insulin to trigger the same glucose response without provoking hypoglycemia compared to LL cows which often accompanies IR conditions *(Kushibiki et al., 2001)*. Accordingly, both RQUICKI and $RQUICKI_{BHB}$ were reduced in LV cows further pointing to lower insulin sensitivity in this genotype. Due to the small number of LV cows (n=4) these results should be interpreted with caution.

Both RQUICKI and $RQUICKI_{BHB}$ showed significant negative correlations with many of the glucose parameters of the ivGTT and with basal and $mean_{t75\text{-}180}$ insulin concentrations and were positively related to insulin clearance rate. Therefore, we infer that both indexes may be useful for the rapid estimation of insulin sensitivity in dairy cows.

In our study NEFA and BHB were negatively associated with peripheral glucose utilization and pancreatic insulin secretion and insulin disappearance during ivGTT. NEFA predominantly compromised glucose response, while high BHB levels mostly accounted for decreased insulin response and CR. NEFA not only impairs insulin actions in several pathways, but could also impair pancreatic insulin output. *Bossaert et al. (2008)* found that plasma NEFA was negatively related to insulin AUC and peak, but did not influence glucose parameters in PP cows. *Pires et al. (2007a and 2007b)* showed that elevated triglycerid, and more closely, NEFA levels promoted IR that could be reversed by the administration of nicotinic acid. Several studies reported marked reduction of pancreatic insulin secretory capacity and decreased insulin responsiveness in hyperketonemic cows *(Hove, 1978; Sakai et al., 1993 and 1996)* that is in agreement with our findings. We showed that plasma cortisol was associated with decreased glucose CR and prolonged time to reach baseline levels. Cortisol is usually increased in early lactation *(Ingvartsen, 2006)* and its actions on blood glucose concentration are opposite to that of insulin promoting hyperglycemia, therefore it may impair glucose clearance.

Cows that lost more weight PP had higher glucose peak and glucose increment, needed longer time to reach t_0 and had longer insulin half-life, all these changes representing a state of insulin resistance. In periparturient dairy cows blood NEFA, and

consequently BHB increases as lipolysis advances *(Pullen et al., 1989; Rukkwamsuk et al., 1999)*, so decreased peripheral insulin sensitivity in cows with more weight loss may be due to higher NEFA and BHB levels.

Both BCS and BW were negatively related to $RQUICKI_{BHB}$ indicating that cows with higher weight or in better condition probably have diminished insulin sensitivity. *Holtenius et al. (2003)* previously found that overconditioned periparturient cows were more likely to develop IR and glucose intolerance than their herdmates. Indeed, there was a significant negative linear relationship between BCS and RQUICKI in all animals using the same dataset (*Holtenius and Holtenius, 2007).* BW and BW change were also positively associated with plasma leptin concentration during the glucose challenge test. In general, plasma leptin reflects the lipid content of fat depots as well as current EB also in cattle *(Delavaud et al., 2002 and 2004; Liefers et al., 2003)*. Cows with lower plasma leptin during lactation usually had lower BW and with improving EB and re-accumulation of fat depots leptin concentration increased *(Liefers et al., 2003; Accorsi et al., 2005)*. However, others reported no difference in PP leptin levels between cows with lower or higher BW and BCS *(Holtenius et al., 2003; Chagas et al., 2006).*

There were no differences in the commencement of luteal activity and in the time of the first observed estrus by *Alu*I genotype. We have only limited information about the involvement of *Alu*I polymorphism in reproductive traits in cattle *(Lechniak et al., 1999 and 2002b),* and neither of those reports investigated fertility characteristics of the high-yielding dairy cow. Results from *Exp. 1.* showed no advantage of neither the leucine nor the valine allele in resuming ovarian activity sooner after calving, which supports findings of our current experiment even though numbers of participating cows were much smaller here and that should 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 thus 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.

6. Overview of results

The following results obtained from the studies above represent novelty that has not been reported elsewhere:

1) In Holstein-Friesian cows *Alu*I polymorphism of the GH gene is not related to the interval from calving to first ovulation and to short-term milk production. *Alu*I genotype is not associated with the extent of body condition loss shortly after calving. Cows with puerperal metritis undergo more severe body condition losses than healthy animals (*Exp. 1*).

2) Changes in plasma ß-hydroxybutyrate, insulin, IGF-I and leptin concentrations early post partum occur irrespectively of *Alu*I genotype. Hyperketonemia triggers a significant decrease in insulin, IGF-I and leptin blood levels. Peripheral insulin, IGF-I and leptin are positively related to each other in the beginning of lactation (*Exp. 2*).

3) Holstein-Friesian cows heterozygous for *Alu*I polymorphism of the GH gene are more likely to develop insulin resistance during early lactation and reach higher lactation yields than leucine homozygous cows. It appears that GH genotype is not associated with the onset of postpartum ovarian activity and first observed estrus. 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 (*Exp. 3*).

7. References

Accorsi P.A., Govoni N., Gaiani R., Pezzi C., Seren E. and Tamanini C. (2005): Leptin, GH, PRL, Insulin and metabolic parameters throughout the dry period and lactation in dairy cows. Reprod. Dom. Anim. 40. 217–223.

Ahima R.S., Prabakaran D., Mantzoros C., Qu D., Lowell B., Maratos E.F., Flier J.S. (1996): Role of leptin in the neuroendocrine response to fasting. Nature 382. 250-252.

Amstalden M., Garcia M.R., Stanko R.L., Nizielski S.E., Morrison C.D., Keisler D.H., Williams G.L. (2002): Central infusion of recombinant ovine leptin normalizes plasma insulin and stimulates a novel hypersecretion of luteinizing hormone after short-term fasting in mature beef cows. Biol. Reprod. 66. 1555-1561.

Amstalden M., Zieba D.A., Edwards J.F., Harms P.G., Welsh T.H., Stanko R.L., Williams G.L. (2003): Leptin acts at the bovine adenohypophysis to enhance basal and GnRH mediated release of LH: differential effects are dependent upon nutritional history. Biol. Reprod. 69. 1539-1544.

Andersen J.B., Mashek D.G., Larsen T., Nielsen M.O. and Ingvartsen K.L. (2002): Effects of hyperinsulinaemia under euglycaemic condition on liver fat metabolism in dairy cows in early and mid-lactation. J. Vet. Med. A 49. 65–71.

Andersen J.B., Madsen T.G., Larsen T., Ingvartsen K.L. and Nielsen M.O. (2005): The effects of dry period versus continuous lactation on metabolic status and performance in periparturient cows. J. Dairy Sci. 88. 3530–3541.

Anderson L.L., Jeftinija S., Scanes C.G., Stromer M.H., Lee J.-S., Jeftinija K., Glavaski-Joksimovic A. (2005): Physiology of ghrelin and related peptides. Dom. Anim. Endocrinol. 29. 111–144.

Andersson L. (1988): Subclinical ketosis in dairy cows. Metabolic diseases of ruminant livestock. Vet. Clin. North Am. Food Anim. Pract. 4. 233–251.

Andersson L. and Emanuelson U. (1985): An epidemiological study of hyperketonaemia in Swedish dairy cows; determinants and the relation to fertility. Prev. Vet. Med. 3. 449-462.

Baird G.D. (1982): Primary ketosis in the high-producing dairy cow: clinical and subclinical disorders, treatment, prevention, and outlook. J. Dairy Sci. 65. 1-10.

Barb C.R., Kraeling R.R. (2004): Role of leptin in the regulation of gonadotropin secretion in farm animals. Anim. Reprod. Sci. 82-83. 155-167.

Battaglia D.F., Bowen J.M., Krasa H.B., Thrun L.A., Viguie C., Karsch F.J. (1997): Endotoxin inhibits the reproductive neuroendocrine axis while stimulating adrenal steroids: a simultaneous view from hypophyseal portal and peripheral blood. Endocrinology 138. 4273–4281.

Battaglia D.F., Krasa H.B., Padmanabhan V., Viguie C., Karsch F.J. (2000): Endocrine alterations that underlie endotoxin-induced disruption of the follicular phase in ewes. Biol. Reprod. 62. 45–53.

Bauman D.E. and Currie W.B. (1980): Partitioning of nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63. 1514-1529.

Bauman D.E., Vernon R.G. (1993): Effects of exogenous bovine somatotropin on lactation. Annual Rev. Nutr. 13. 437-461.

Baumgard L.H., Weber W.J., Kazmer G.W., Zinn S.A., Hansen L.B., Chester-Jones H., and Crooker B.A. (2002): Effects of selection for milk yield on growth hormone response to growth hormone releasing factor in growing Holstein calves. J. Dairy Sci. 85. 2529–2540.

Bazan J.F. (1990): Structural design and molecular evolution of a cytokine receptor superfamily. Proc. Natl. Acad. Sci. USA 87. 6934–6938.

Beam S.W., Butler W.R. (1997): Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol. Reprod. 56. 133-142.

Beam S.W., Butler W.R. (1998): Energy balance, metabolic hormones, and early postpartum follicular development in dairy cows fed prilled lipid. J. Dairy Sci. 81. 121-131.

Beam S.W., Butler W.R. (1999): Effects of energy balance on follicular development and first ovulation in postpartum dairy cow. J. Reprod. Fert. Suppl. 54. 411-424.

Beerepoot G.H.M., Freeman A.E. and Detilleux J.C. (1991): Effect of season, genetic line, and sire on growth concentrations of somatotropin in serum of Holstein cows in early lactation. J. Dairy Sci. 74. 3202-3208.

Bell A.W. (1995): Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73. 2804-2819.

Benzaquen M.E., Risco C.A., Archbald L.F., Melendez P., Thatcher M.-J., and Thatcher W.W. (2007): Rectal temperature, calving-related factors, and the incidence of puerperal metritis in postpartum dairy cows. J. Dairy Sci. 90. 2804–2814.

Bernal Santos G. (1982): Changes in glucose and energy homeostasis with onset of lactation. M. S. Thesis. Cornell Univ., Ithaca, NY

Berry D.P., Buckley F., Dillon P., Evans R.D., Rath M., and Veerkamp R.F. (2003): Genetic relationships among body condition score, body weight, milk yield, and fertility in dairy cows. J. Dairy Sci. 86. 2193–2204.

Block S.S., Butler W.R., Ehrhardt R.A., Bell A.W., Van Amburgh M.E. and Boisclair Y.R. (2001): Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance. J. Endocrinol. 171. 339–348.

Block S.S., Rhoads R.P., Bauman D.E., Ehrhardt R.A., McGuire M.A., Crooker B.A., Griinari J.M., Mackle T.R., Weber W.J., Van Amburgh M.E., Boisclair YR.. (2003): Demonstration of a role for insulin in the regulation of leptin in lactating dairy cows. J. Dairy. Sci. 86. 3508-3515.

Blum J.W., Reding T., Jans F., Wanner M., Zemp M., Bachmann K. (1985): Variations of 3-methylhistidine in blood of dairy cows. J. Dairy Sci. 68. 2580–2587.

Blum J.W., Bruckmaier R.M. and Vacher P.-Y. (1999): Insulin-dependent whole-body glucose utilization and insulin-responses to glucose in week 9 and week 19 of lactation in dairy cows fed rumen-protected crystalline fat or free fatty acids. Dom. Anim. Endocrinol. 16.123–134.

Bobe G., Young J.W., and Beitz D.C. (2004): *Invited Review:* Pathology, etiology, prevention, and treatment of fatty liver in dairy cows. J. Dairy Sci. 87. 3105–3124.

Bossaert P., Leroy J.L.M.R., De Vliegher S., Opsomer G. (2008): Interrelations between glucose-induced insulin response, metabolic indicators, and time of first ovulation in high-yielding dairy cows. J.Dairy Sci. 91. 3363-3371.

Breier B.H. (1999): Regulation of protein and energy metabolism by the somatotropic axis. Dom. Anim. Endocrinol. 17. 209–218.

Briard N., Dadoun F., Pommier G., Sauze N., Lebouc Y., Oliver C., Dutour A. (2000): IGF-I / IGFBPs system response to endotoxin challenge in sheep. J. Endocrinol. 164. 361-369.

Brockman R.P. and Laarveld B. (1986): Hormonal-regulation of metabolism in ruminants—A review. Livest. Prod. Sci. 14. 313–334.

Butler W.R. (2000): Nutritional interactions with reproductive performance in dairy cattle. Anim. Reprod. Sci. 60-61. 449-457.

Butler W.R. (2001): Nutritional effects on resumption of ovarian cyclicity and conception rate in postpartum dairy cows. In: Fertility in the high-producing dairy cow, Diskin MG (Ed.), British Society of Animal Science, Edinburgh, Occasional publications, 26(I). 133-145.

Butler W.R. (2003): Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livest. Prod. Sci. 83. 211-218.

Butler W.R., Everett R.W., Coppock C.E. (1981): The relationships between energy balance, milk production and ovulation in postpartum Holstein cows. J. Anim. Sci. 53. 742-748.

Butler W.R., Smith R.D. (1989): Interrelationship between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci. 72. 767-783.

Butler S.T., Marr A.L., Pelton S.H., Radcliff R.P., Lucy M.C. and Butler W.R. (2003): Insulin restores GH responsiveness during lactation-induced negative energy balance in dairy cattle: effects on expression of IGF-I and GH receptor 1A. J. Endocrinol. 176, 205–217.

Butler S.T., Pelton S.H. and Butler W.R. (2004): Insulin increases 17b-estradiol production by the dominant follicle of the first postpartum follicle wave in dairy cows. Reproduction 127. 537–545.

Cadorniga C.P., Grummer R.R., Armentano L.E., and Donkin S.S. (1992): Effects of extracellular fatty acid concentration, estriol, insulin, and glucagon on hepatic triglyceride accumulation, acid soluble product formation, and gluconeogenesis. J. Dairy Sci. 75(Suppl. 1). 183.(Abstract)

Canfield R.W. and Butler W.R. (1990): Energy balance and pulsatile LH secretion in early postpartum dairy cattle. Dom. Anim. Endocrinol. 7. 323-330.

Canfield R.W. and Butler W.R. (1991): Energy balance, first ovulation and the effect of naloxone on LH secretion in early postpartum dairy cows. J. Anim. Sci. 69. 740-746.

Chagas L.M., Rhodes F.M., Blache D., Gore P.J.S., Macdonald K.A., Verkerk G.A. (2006): Precalving effects on metabolic responses and postpartum anestrus in grazing primiparous dairy cows. J. Dairy Sci. 89. 1981-1989.

Chikuni K., Nagatsuma T., Tabata T., Monma M., Saito M., Ozawa S., Ozutsumi K. (1994): Genetic variants of the growth hormone gene in Japanese cattle. Anim. Sci. Technol. 65. 340-346.

Childs G.V. (2000): Growth hormone cells as co-gonadotropes: partners in the regulation of the reproductive system. Trends in Endocrinology and Metabolism 1l. 168-175.

Chilliard Y. (1999): Metabolic adaptations and nutrient partitioning in the lactating animal. In: J. Martinet, L.M. Houdebine and HH. Head (Editors), Biology of Lactation. Collection Mieux Comprendre, INRA Editions Paris (FRA). 503-552.

Chilliard Y., Bonnet M., Delavaud C., Faulconnier Y., Leroux C., Djiane J., Bocquier F. (2001): Leptin in ruminants. Gene expression in adipose tissue and mammary gland, and regulation of plasma concentration. Dom. Anim. Endocrinol. 21. 271–295.

Clark R. (1997): The somatogenic hormones and insulin-like growth factor-1: stimulators of lymphopoiesis and immune function. Endo. Rev. 18. 157-179.

Csernus V. (1982): Antibodies of high affinity and specificity for radioimmunological determination of progesterone, testosterone, estradiol-17β and cortisol. In: Görög, S. (ed.): Advances in steroid analysis I. Akadémia Kiadó, Budapest, pp. 171-177.

Dann H. M., Morin D. E., Bollero G. A., Murphy M. R. and Drackley J. K. (2005): Prepartum intake, postpartum induction of ketosis, and periparturient disorders affect the metabolic status of dairy cows. J. Dairy Sci. 88. 3249-3264.

Darwash A.O., Lamming G.E., Woolliams J.A. (1997): Estimation of genetic variation in the interval from calving to postpartum ovulation of dairy cows. J. Dairy Sci. 80. 1227-1234.

de Boer G., Trenkle A., and J.W.Young (1985): Glucagon, insulin, growth hormone, and some blood metabolites during energy restriction ketonemia of lactating cows. J. Dairy Sci. 68. 326-337.

DeFronzo R.A., Bonadonna R.C. and Ferrannini E. (1992): Pathogenesis of NIDDM. Diabetes Care 3. 318–368.

de Kretser D.M., Robertson D.M. (1989): The isolation and physiology of inhibin and related proteins. Biol. Reprod. 44. 33-47.

Delavaud C., Bocquier F., Chilliard Y., Keisler D.H., Gertler A., Kann G. (2000): Plasma leptin determination in ruminants: effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. J. Endocrinol. 165. 519-526.

Delavaud C., Ferlay A., Faulconnier Y., Bocquier F., Chilliard Y. (2002): Plasma leptin concentration in adult cattle: effect of breed, adiposity, feeding level and meal intake. J. Anim. Sci. 80. 1317-1328.

Delavaud C., Agenas S., Holtenius K., Chilliard Y. (2004): Interactions between energy intake level, physiological state and methodology for determination of plasma leptin in bovine species. Reprod. Nutr. Dev. 44. 151.

Denbow C.J., Perera K.S., Gwazdauskas F.C., Akers R.M., Pearson R.E., and McGilliard M.L. (1986): Effect of season and stage of lactation on plasma insulin and glucose following glucose injection in holstein cattle. J. Dairy Sci. 69. 211-216.

de Vries M.J. and Veerkamp R.F. (2000): Energy balance of dairy cattle in relation to milk production variables and fertility. J. Dairy Sci. 83. 62–69.

Doepel L., Lapierre H., Kennelly J.J. (2002): Peripartum performance and metabolism of dairy cows in response to prepartum energy and protein intake. J. Dairy Sci. 85. 2315–2334.

Dohoo I.R. and Martin S.W. (1984): Subclinical ketosis: prevalence and associations with production and disease. Can. J. Comp. Med. 48. 1-5.

Doll K., Sickinger M., Seeger T. (2008): New aspects in the pathogenesis of abomasal displacement. Vet. J. in press (doi:10.1016/j.tvjl.2008.01.013).

Domecq J.J., Skidmore A.L., Lloyd J.W., Kaneene J.B. (1997): Relationship between body condition scores and conception at first artificial insemination in a large dairy herd of high yielding Holstein cows. J. Dairy Sci. 80. 113-120.

Driancourt M.A., Thatcher W.W., Terqui M., Andrieu D. (1991): Dynamics of ovarian follicular development in cattle during the estrus cycle, early pregnancy and in response to PMSG. Dom. Anim. Endocrinol. *8*. 209-221.

Duffield T.F., Kelton D.F., Leslie K.E., Lissemore K.D., Lumsden J.H. (1997): Use of test day milk fat and milk protein to detect subclinical ketosis in dairy cattle in Ontario. Can. Vet. J. 38. 713-718.

Dunaif A., Segal K.R., Futterweit W., Dobrjansky A (1989): Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes 38. 1165–1174.

Dybus A. (2002): Associations between *Leu/Val* polymorphism of growth hormone gene and milk production traits in Black-and-White cattle. Arch. Tierz. 45(5). 421-428.

Echternkamp S.E., Spicer L.J., Gregory K.E., Canning S.F., Hammond J.M. (1990): Concentrations of insulin-like growth factor-I in blood and ovarian follicular fluid of cattle selected for twins. Biol. Reprod. 43. 8-14.

Edens A. and Talamantes F. (1998): Alternative processing of growth hormone receptor transcripts. Endocrine Rev. 19. 559-582.

Elsasser T.H., Fayer R., Rumsey T.S., Hartnell G.F. (1994): Recombinant bovine somatotropin blunts plasma tumor necrosis factor-a, cortisol, and thromboxane-B2 responses to endotoxin in vivo. Endocrinology 134. 1082–1088.

Enjalbert F., Nicot M.C., Bayourthe C., and Moncoulon R. (2001): Ketone bodies in milk and blood of dairy cows: relationship between concentrations and utilization for detection of subclinical ketosis J. Dairy Sci. 84. 583–589.

Eppard P.J., Bentle L.A., Violand B.N., Ganguli S., Hintz R.L., Kung I. Jr, Krivi G.G., Lanza G.M. (1992): Comparison of the galactopoietic response to pituitary-derived and recombinant-derived variants of bovine growth hormone. J. Endocrinol. 132. 47-56.

Etherton T.D. and Bauman D.E. (1998): Biology of somatotropin in growth and lactation of domestic animals. Physiol. Rev. 78. 745-761.

Fenwick M.A., Fitzpatrick R., Kenny D.A., Diskin M.G., Patton J., Murphy J.J., Wathes D.C. (2008): Interrelationships between negative energy balance (NEB) and IGF regulation in liver of lactating dairy cows. Dom. Anim. Endocrinol. 34. 31–44.

Flint D.J., Knight C.H. (1997): Interactions of prolactin and growth hormone (GH) in the regulation of mammary gland function and epithelial cell survival. J. Mammary Gland Biol. Neoplasia 2. 41–48.

Földi J., Kulcsár M., Pécsi A., Huyghe B., de Sa C., Lohuis J.A.C.M., Cox P., Huszenicza Gy. (2006): Bacterial complications of postpartum uterine involution in cattle. Anim. Reprod. Sci. 96. 265–281.

Gabai G., Cozzi G., Rosi F., Andrighetto I. and Bono G. (2002): Glucose or essential amino acid infusions in late pregnant and early lactating Simmenthal cows failed to induce a leptin response. J. Vet. Med. A 49. 73–80.

Gatford K.L., Egan A.R., Clarke I.J., Owens P.C. (1998): Sexual dimorphism of the somatotropic axis. J. Endocrinol. 157. 373-389.

Gazal O.S., Leshin L.S., Stanko R.L., Thomas M.G., Keisler D.H., Anderson L.L., Williams G.L. (1998): Gonadotropin-releasing hormone secretion into third-ventricle cerebrospinal fluid of cattle: correspondence with tonic and surge release of luteinizing hormone and its tonic inhibition by suckling and neuropeptid Y. Biol. Reprod. 59. 676-683.

Ge W., Davis M.E., Hines H.C., Irvin K.M., and Simmen R.C.M. (2003): Association of single nucleotide polymorphisms in the growth hormone and growth hormone receptor genes with blood serum insulin-like growth factor I concentration and growth traits in Angus cattle. J. Anim. Sci. 81. 641–648.

Geishauser T., Leslie K., Duffield T., and Edge V. (1997): An evaluation of milk ketone tests for the prediction of left displaced abomasum in dairy cows. J. Dairy Sci. 80. 3188–3192.

Giustina A., Veldhuis J.D. (1998): Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr. Rev. 19. 717-797.

Goff J.P. and Horst R.L. (1997): Physiological changes at parturition and their relationship to metabolic disorders. J. Dairy Sci. 80. 1260-1268.

Gong J.G., Lee W.J., Garnsworthy P.C. and Webb R. (2002): Effect of dietary-induced increases in circulating insulin concentrations during the early postpartum period on reproductive function in dairy cows. Reproduction 123. 419–427.

Grochowska R., Sørensen P., Zwierzchowski L., Snochowski M., and Løvendahl P. (2001): Genetic variation in stimulated GH release and in IGF-I of young dairy cattle and their associations with the leucine/valine polymorphism in the GH gene. J. Anim. Sci. 79. 450–476.

Grummer R.R. (1993): Etiology of lipid-related metabolic disorders in periparturient dairy cows. J. Dairy Sci. 76. 3882-3896.

Grummer R.R., Mashek D.G., Hayirli A. (2004): Dry matter intake and energy balance in the transition period. Vet. Clin. Food Anim. 20. 447–470.

Hammon D.S., Evjen I.M., Dhiman T.R., Goff J.P., Walters J.L. (2006): Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet. Immunol. Immunopathol. 113. 21–29.

Hauser S.D., McGrath M.F., Collier R.J. and Krivi G.G. (1990): Cloning and in vivo expression of bovine growth hormone receptor mRNA. Mol. Cell. Endocrinol. 72. 187–200.

Hayirli A., Grummer R.R., Nordheim E.V. and Crump P.M. (2002): Animal and dietary factors affecting feed intake during the prefresh transition period in Holsteins. J. Dairy Sci. 85. 3430-3443.

Hayirli A. (2006): The role of exogenous insulin in the complex of hepatic lipidosis and ketosis associated with insulin resistance phenomenon in postpartum dairy cattle. Vet. Res. Comm. 30. 749–774.

Havel P.J. (2004): Update on adipocyte hormones: regulation of energy balance and carbohydrate/lipid metabolism. Diabetes 53. Suppl. 1. 143-151.

Hoeben D., Burvenich C., Trevisi E., Bertoni G., Hamann J., Bruckmaier R.M., Blum JM. (2000): Role of endotoxin and TNFa in the pathogenesis of experimentally-induced coliform mastitis in periparturient cows. J. Dairy Res. 67. 503–514.

Hollenbeck C.B., Chen N., Chen Y.-D.I., Reaven G.M. (1984): Relationship between the plasma insulin response to oral glucose and insulin stimulated glucose utilization in normal subjects. Diabetes 33. 460–463.

Holtenius P., Traven M. (1990): Impaired glucose tolerance and heterogeneity of insulin responses in cows with abomasal displacement. J. Vet. Med. A. 37. 445-451.

Holtenius P., Olsson G., Björkman C. (1993): Periparturient concentrations of insulin, glucagon and ketone bodies in dairy cows fed two different levels of nutrition and varying concentrate / roughage ratios. J. Vet. Med. A. 40 118-127.

Holtenius K., Agenäs S., Delavaud C., and Chilliard Y.(2003): Effects of feeding intensity during the dry period. 2. Metabolic and hormonal responses. J. Dairy Sci. 86. 883–891.

Holtenius P. and Holtenius K. (2007): A model to estimate insulin sensitivity in dairy cows. Acta Vet. Scand. 49. 29-31.

Houseknecht K.L., Portocarrero C.P., Ji S., Lemenager R., Spurlock M.E. (2000): Growth hormone regulates leptin gene expression in bovine adipose tissue: correlation with adipose IGF-1 expression. J. Endocrinol. 164. 51–57.

Hove K. (1978): Insulin secretion in lactating cows: responses to glucose infused intravenously in normal, ketonemic and starved animals. J. Dairy Sci. 61. 1407-1413.

Huszenicza Gy., Jánosi Sz., Kulcsár M., Kóródi P., Dieleman S.J., Bartyik J., Rudas P. and Ribiczei-Szabó P. (1998): Gram-negative mastitis in early lactation may interfere with ovarian and certain endocrine functions and metabolism in dairy cows. Reprod. Dom. Anim. 33. 147-153.

Huszenicza Gy., Fodor M., Gács M., Kulcsár M., Dohmen M.J.W., Vámos M., Porkoláb L., Kégl T., Bartyik J., Lohuis J.A.C.M., Jánosi Sz. and Szita G. (1999): Uterine bacteriology, resumption of cyclic ovarian activity and fertility in postpartum cows kept in large-scale dairy herds. Reprod. Dom. Anim. 34. 237-245.

Huszenicza Gy., Kulcsár M., Nikolic J.A., Schmidt J., Kóródi P., Kátai L., Dieleman S., Ribiczei-Szabó P., Rudas P. (2001): Plasma leptin concentration and its interrelation with some blood metabolites, metabolic hormones and the resumption of cyclic ovarian function in postpartum dairy cows supplemented with Monensin or inert fat in feed. In: Diskin MG (ed.): Fertility in the high-producing dairy cow. Br. Soc. Anim. Sci. (Edinburgh, UK), Occasional publications, No. 26. Vol. 2, pp. 405-409.

Huszenicza Gy., Kulcsár M., Rudas P. (2002): Clinical endocrinology of thyroid gland function in ruminants: A review of literature. Vet. Med. Cz. 47. 191-202.

Huszenicza Gy., Kulcsár M., Dankó G., Balogh O., Gaál T (2003): The interrelation of nutrition, milk production and reproduction in high-yielding dairy cows. Part 4: Form and consequences of increased ketone production. A review of literature (In Hungarian). Magy. Állato. Lapja 125(58). 203-208.

Huszenicza Gy., Jánosi Sz., Gáspárdy A., Kulcsár M. (2004): Endocrine aspects in pathogenesis of mastitis in postpartum dairy cows. Anim. Reprod. Sci. 82-83. 389-400.

Huszenicza Gy., Jánosi Sz., Kulcsár M., Kóródi P., Reiczigel J., Kátai L., Peters A.R. and De Rensis F. (2005): Effects of clinical mastitis on ovarian function in post-partum dairy cows. Reprod. Dom. Anim. 40. 199–204.

Huszenicza Gy., Kulcsár M., Kóródi P., Bartyik J., Rudas P., Ribiczei-Szabó P., Nikolic J.A., Samanc H., Ivanov I. and Gvozdic D. (2006): Adrenocortical and thyroid function, hormone and metabolite profiles and the onset of ovarian cyclicity in dairy cows suffering from various forms of ketosis. Acta Vet. (Beograd). 56. 25-36.

Ingvartsen K.L. (2006): Feeding- and management-related diseases in the transition cow. Physiological adaptations around calving and strategies to reduce feeding-related diseases. Anim. Feed Sci. Technol. 126. 175–213.

Ingvartsen K.L., Dewhurst R.J., Friggens N.C. (2003): On the relationship between lactational performance and health: is it yield or metabolic imbalance that cause production diseases in dairy cattle? A position paper. Livest. Prod. Sci. 73. 277–308.

Ingvartsen K.L., Friggens N.C. (2005): To what extent do variabilities in hormones, metabolites and energy intake explain variability in milk yield? Domest. Anim. Endocrinol. 29. 294–304.

Jánosi Sz., Kulcsár M., Kóródi P., Kátai L., Reiczigel J., Dieleman S.J., Nikolic J.A., Sályi G., Ribiczei-Szabó P., Huszenicza Gy. (2003): The energy imbalance related predisposition for mastitis in group-fed high-producing postpartum dairy cows. Acta Vet. Hung. 51. 409–424.

Jaster E.H., and Wegner T.N. (1981): Beta-adrenergic receptor involvement in lipolysis of dairy cattle subcutaneous adipose tissue during dry and lactating state. J. Dairy Sci. 64. 1655.

Jiang H., Okamura C.S. and Lucy M.C. (1999): Isolation and characterization of a novel promoter for the bovine growth hormone receptor gene. J. Biol. Chem. 274. 7893–7900.

Jiang H., Okamura C.S., Boyd C.K., and Lucy M.C. (2000): Identification of Sp1 as the transcription factor for the alternative promoter P2 of the bovine growth hormone receptor gene. J. Mol. Endocrinol. 24. 203–214.

Jiang H., and Lucy M.C. (2001): Variants of the 5-untranslated region of the bovine growth hormone receptor mRNA: Isolation, expression and effects on translational efficiency. Gene 265. 45–53.

Jiang H., Lucy M.C., Crooker B.A., and Beal W.E. (2005): Expression of growth hormone receptor 1A mRNA is decreased in dairy cows but not in beef cows at parturition. J. Dairy Sci. 88. 1370–1377.

Jones J.I., Clemmons D.R. (1995): Insulin-like growth factors and their binding proteins: biological actions. Endo. Rev. 16. 3-34.

Jorritsma R., Langendijk P., Kruip T.A.M., Wensing T.H. and Noordhuizen J.P.T.M. (2005): Associations between energy metabolism, LH pulsatility and first ovulation in early lactating cows. Reprod. Dom. Anim. 40. 68–72.

Kadokawa H., Blache D., Yamada Y., Martin G.B. (2000): Relationships between changes in plasma concentrations of leptin before and after parturition and the timing of first post-partum ovulation in high-producing Holstein dairy cows. Reprod. Fertil. Dev. 12. 405-411.

Kadokawa H., Blache D., Martin G.B. (2006): Plasma leptin concentrations correlate with luteinizing hormone secretion in early postpartum Holstein cows. J. Dairy Sci. 89. 3020-3027.

Kahn C.R. (1978): Insulin resistance, insulin sensitivity, and insulin unresponsiveness: a necessary distinction. Metabolism 27. 1893–1902.

Katoh K., Kouno S., Okazaki A., Suzuki K., Obara Y. (2008): Interaction of GH polymorphism with body weight and endocrine functions in Japanese black calves. Dom. Anim. Endocrinol. 34. 25–30.

Kawashima C., Fukihara S., Maeda M., Kaneko E., Amaya Montoya C., Matsui M., Shimizu T., Matsunaga N., Kida K., Miyake Y.-I., Schams D., Miyamoto A. (2007): Relationship between metabolic hormones and ovulation of dominant follicle during the first follicular wave postpartum in high-producing dairy cows. Reproduction 133. 155–163.

Kleppe B.B., Aiello R.J., Grummer R.R., and Armentano L.E. (1988) Triglyceride accumulation and very low density lipoprotein secretion by rat and goat hepatocytes in vitro. J. Dairy Sci. 71. 1813.

Kobayashi Y., Boyd C.K., Bracken C.J., Lamberson W.R., Keisler D.H., and Lucy M.C. (1999): Reduced growth hormone receptor (GHR) messenger ribonucleic acid in liver of periparturient cattle is caused by a specific down-regulation of GHR 1A that is associated with decreased insulin-like growth factor I. Endocrinology 140. 3947–3954.

Kölle S., Sinowatz F., Boie G., Lincoln D. (1998): Developmental changes in the expression of the growth hormone receptor messenger ribonucleic acid and protein in the bovine ovary. Biol. Reprod. 59. 836-842.

Koller A., Reist M., Blum J.W., Kupfer U. (2003): Time empty and ketone body status in the early postpartum period of dairy cows. Reprod. Dom. Anim. 38. 41-49.

Komatsu T., Itoh F., Kushibiki S. and Hodate K. (2005): Changes in gene expression of glucose transporters in lactating and nonlactating cows. J. Anim. Sci. 83. 557-564.

Kornalijnslijper E., Beerda B., Daemen I., van der Werf J., van Werven T., Niewold T., Rutten V., Noordhuizen-Stassen E. (2003): The effect of milk production level on host resistance of dairy cows, as assessed by the severity of experimental *Escherichia coli* mastitis. Vet. Res. 34. 721–736.

Korver S. (1982): Feed intake and production in dairy breeds dependent on the ration. PhD thesis Wageningen Agricultural University, Wageningen, The Netherlands.

Kovács K., Völgyi-Csík J., Zsolnai A., Györkös I. and Fésüs L. (2006): Associations between the AluI polymorphism of growth hormone gene and production and reproduction traits in a Hungarian Holstein-Friesian bull dam population. Arch. Tierz. 49(3). 236-249.

Kremer W.D.J., Noordhuizen-Stassen E.N., Grommers F.J., Schukken Y.H., Heeringa R., Brand A., Burvenich C. (1993): Severity of experimental *Escherichia coli* mastitis in ketonemic and nonketonemic dairy cows. J. Dairy Sci. 76. 3428-3436.

Kulcsár M. (2007): Clinical endocrinology of leptin in ruminants. PhD thesis. Szent István University, Postgraduate School of Veterinary Science, Budapest, Hungary; http://phd.univet.hu

Kulcsár M., Kátai L., Balogh O., Pécsi A., Dalevaud C., Földi J., Hirvonen J., Faigl V., Chilliard Y., Huszenicza Gy. (2005a): Metabolic and endocrine changes, inflammatory proteins and ovarian activity in dairy cows with acute puerperal metritis. Reprod. Dom. Anim. 40. 407 (Abstract).

Kulcsár M., Jánosi Sz., Lehtolainen T., Kátai L., Delavaud C., Balogh O., Chilliard Y., Pyörälä S., Rudas P., Huszenicza Gy. (2005b): Feeding-unrelated factors influencing the plasma leptin level in ruminants. Domest. Anim. Endocrinol. 29. 214-226.

Kulcsár M., Dankó G., Magdy, H.G.I., Reiczigel J., Forgach T, Proháczik A., Delavaud, A., Magyar K., Chilliard, Y., Solti L., Huszenicza Gy. (2006a): Pregnancy stage and number of fetuses may influence maternal plasma leptin in ewes. Acta Vet. Hung. 54. 221-234.

Kulcsár M., Dankó G., Delavaud, C., Mircu, C., Nikolic, A.J., Gáspárdy A., Cernescu, H., Chilliard, Y., Cseh S., Rudas P., Huszenicza Gy. (2006b): Endocrine characteristics of late pregnant hyperketonaemic ewes and their reproductive performance following the induction of ovarian cyclicity out of the breeding season. Acta Vet. Hung. 54. 235-249.

Kushibiki S., Hodate K., Shingu H., Ueda Y., Mori Y., Itoh T., Yokomizo Y. (2001a): Effects of long-term administration of recombinant bovine tumor necrosis factor-a on glucose metabolism and growth hormone secretion in steers. Am. J. Vet. Res. 62. 794–798.

Kushibiki S., Hodate K., Shingu H., Ueda Y., Shinoda M., Mori Y., Itoh T., Yokomizo Y. (2001b): Insulin resistance induced in dairy steers by tumor necrosis factor alpha is partially reversed by 2,4 –thiazolidinedione. Dom. Anim. Endocrinol. 21. 25–37.

Kushibiki S., Hodate K., Shingu H., Obara Y., Touno E., Shinoda M. and Yokomizo Y. (2003): Metabolic and lactational responses during recombinant bovine tumor necrosis factor-*a* treatment in lactating cows. J. Dairy Sci. 86. 819–827.

Lamming G.E., Whates D.C., Peters A.R. (1981): Endocrine patterns of the post partum cow. J. Reprod. Fert. 30. 155-170.

Lavau M., Fornari V., Hashim S.A. (1978): Keystone metabolism in brain slices from rats with diet induced hyperketonemia. J. Nutr. 108. 621-629.

Lean I.J., Bruss M.L., Baldwin R.L., Troutt H.F. (1992): Bovine ketosis: A review II. Biochemistry and prevention Vet. Bull. 61. 1-14.

LeBlanc S.J., Duffield T.F., Leslie K.E., Bateman K.G., Keefe G.P., Walton J.S., Johnson W.H. (2002a): Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. J. Dairy Sci. 85. 2223–2236.

LeBlanc S.J., Leslie K.E., and Duffield T.F. (2005): Metabolic predictors of displaced abomasum in dairy cattle. J. Dairy Sci. 88. 159-170.

Lechniak D., Machnik G., Szydlowski M., Switonski M. (1999): Growth hormone gene polymorphism and reproductive performance of AI bulls. Theriogenology 52. 1145-1152.

Lechniak D., Strabel T., Przybyla D., Machnik G., Switonski M. (2002a): GH and CSN3 gene polymorphisms and their impact on milk traits in cattle. J. Anim. Feed Sci. 11. 39-45.

Lechniak D., Adamowicz T., Stanislawski D., Kaczmarek D. (2002b): In vitro maturation and fertilisation of bovine oocytes in relation to GH gene polymorphism (Leu/Val). Reprod. Nutr. Dev. 42. 275-280.

Lee B.K., Lin G.F., Crooker B.A., Murtaugh M.P., Hansen L.B., Chester-Jones H. (1996): Association of somatotropin (BST) gene polymorphism at the 5th exon with selection for milk yield in Holstein cows. Dom. Anim. Endocrinol. 13(4). 373-381.

Le Roith D., Bondy C., Yakar S., Liu J., and Butler A. (2001): The somatomedin hypothesis. Endocr. Rev. 22. 53–74.

Leroy J.L.M.R., Vanholder T., Opsomer G., Van Soom A. and de Kruif A. (2006): The in vitro development of bovine oocytes after maturation in glucose and ß-hydroxybutyrate concentrations associated with negative energy balance in dairy cows. Reprod. Dom. Anim. 41. 119–123.

Leslie K.E., Duffield T.F., Schukken Y.H., LeBlanc S.J. (2000): The influence of negative energy balance on udder health. Proc. Natl. Mast. Counc. Regional Meeting pp. 25-33.

Leury B.J., Baumgard L.H., Block S.S., Segoale N., Ehrhardt R.A., Rhoads R.P., Bauman D.E., Bell A.W., Boisclair Y.R. (2003): Effect of insulin and growth hormone on plasma leptin in periparturient dairy cows. Am. J. Physiol. (Regul. Integr. Comp. Physiol.) 285. R1107-1115.

Liefers S.C., Veerkamp R.F., te Pas M.F.W., Delavaud C., Chilliard Y., van der Lende T. (2003) Leptin concentrations in relation to energy balance, milk yield, intake, live weight, and estrus in dairy cows. J. Dairy Sci. 86. 799-807.

Lomax M.A., Baird G.D., Mallinson C.B. and. Symonds H.W. (1979): Differences between lactating and non-lactating dairy cows in concentration and secretion rate of insulin. Biochem. J. 180. 281-289.

Løvendahl P., Angus K.D., Woolliams J.A. (1991): The effect of genetic selection for milk yield on the response to growth hormone secretagogues in immature cattle. J. Endocrinol. 128. 419-424.

Lucy M.C., Hauser S.D., Eppard P.J., Krivi G.G., Collier R.J. (1991a): Genetic polymorphism within the bovine somatotropin (bST)gene detected by polymerase chain reaction and endonuclease digestion. J. Dairy Sci. 74(Suppl. 1). 284.

Lucy M.C., Staples C.R., Michel F.M., Thatcher W.W. (1991b): Energy balance and size and number of ovarian follicles detected by ultrasonography in early postpartum dairy cows. J. Dairy Sci. 74. 473-482.

Lucy M.C., Hauser S.D., Eppard P.J., Krivi G.G., Clark J.H., Bauman D..E, Collier R.J. (1993): Variants of somatotropin in cattle: gene frequencies in major dairy breeds and associated milk production. Dom. Anim. Endocrinol. 10(4). 325-333.

Lucy M.C., Boyd C.K., Koenigsfeld A.T., Okamura C.S. (1998): Expression of somatotropin receptor messenger ribonucleic acid in bovine tissues. J. Dairy Sci. 81. 1889–1895.

Lucy M.C., Jiang H., and Kobayashi Y. (2001): Changes in the somatotrophic axis associated with the initiation of lactation. J. Dairy Sci. 84(E. Suppl.). E113-E119.

Lukes A.J., Barnes M.A. and Pearson R.E. (1989): Response to selection for milk yield and metabolic challenges in primiparous dairy cows. Dom. Anim. Endocrinol. 6. 287-298.

Mashek D.G., Ingvartsen K.L., Andersen J.B., Vestergaard M., Larsen T. (2001): Effects of a four-day hyperinsulinemic-euglycemic clamp in early and mid-lactation dairy cows on plasma concentrations of metabolites, hormones, and binding proteins. Dom. Anim. Endocrinol. 21. 169–185.

Mason T.M., Goh T., Tchipashvili V., Sandhu H., Gupta N., Lewis G.F., and Giacca A. (1999): Prolonged elevation of plasma free fatty acids desensitizes the insulin secretory response to glucose in vivo in rats. Diabetes 48. 524–530.

McCann J.P. and Reimers T.J. (1985): Glucose response to exogenous insulin and kinetics of insulin metabolism in obese and lean heifers. J. Anim. Sci. 61. 612-618.

McCann J.P. and Reimers T.J. (1986): Effects of obesity on insulin and glucose metabolism in cyclic heifers. J. Anim. Sci. 62. 772-782.

McMahon C.D., Elsasser T.H., Gunter D.R., Sanders L.G., Steele B.P., Sartin L (1998): Estradiol/progesterone implants increase food intake, reduce hyperglycemia and increase insulin resistance in endotoxic steers. J. Endocrinol. 159. 469-478.

McNamara J.P. (1988): Regulation of bovine adipose tissue metabolism during lactation. 4. Dose-responsiveness to epinephrine as altered by stage of lactation. J. Dairy Sci. 71. 643.

Meikle A., Kulcsár M., Chilliard Y., Fébel H., Delavaud C., Cavestany D., Chilibroste P. (2004): Effects of parity and body condition score at calving on endocrine and reproductive parameters of the dairy cow under grazing conditions. Reproduction 127. 727-737.

Moore C.E., Kay J.K., VanBaale M.J. and Baumgard L.H. (2005): Calculating and improving energy balance during times of nutrient limitations. Proc. Southwest Nutr. Conf. 173-185.

Morisson C.D., Daniel J.A., Holmberg B.J., Djiane J., Raver N., Gertler A., Keisler D. H. (2001): Central infusion of leptin into well-fed and undernourished ewe lambs: effects on feed intake and serum concentrations of growth hormone and luteinizing hormone. J. Endocrinology 168. 317-324.

Moss G.E., Parfet J.R., Marvin C.A., Allrich R.D., Diekman M.A. (1985): Pituitary concentrations of gonadotropins and receptors for GnRH in suckled beef cows at various intervals after calving. J. Anim. Sci. 60. 285-291.

Nagy P., Solti L., Kulcsár M., Reiczigel J., Huszenicza Gy., Abaváry-Mihály K., Wöfling A. (1998): Progesterone determination in equine plasma using different immunoassays. Acta Vet. Hung. 46. 501-513.

Nakao T., Moriyoshi M. and Kawata K. (1992): The effect of postpartum ovarian dysfunction and endometritis on subsequent reproductive performance in high and medium producing dairy cows. Theriogenology 37. 341-349.

National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Sci., Washington, D. C.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, D. C.

Nikolic J.A., Samanc H., Begovic J., Krsmanovic J., Aleksic S., Miscevic B., Huszenicza Gy., Damjanovic Z. (1998): Basal serum cortisol concentrations in cattle. Acta Vet. (Beograd) 48. 265-276.

Nikolic J.A., Kulcsár M., Kátai L., Nedic O., Jánosi Sz., Huszenicza Gy. (2003): Periparturient endocrine and metabolic changes in healthy cows and in those affected by various forms of mastitis. J. Vet. Med. A 50. 22-29.

Oetzel G.R. (2004): Monitoring and testing dairy herds for metabolic disease. Ve.t Clin. Food Anim. 20. 651–674.

Ohtsuka H., Koiwa M., Hatsugaya A., Kudo K., Hoshi F., Itoh N., Yokota H., Okada H., and Kawamura S. (2001): Relationship between serum TNF activity and insulin resistance in dairy cows affected with naturally occurring fatty liver. J. Vet. Med. Sci. 63. 1021–1025.

Oikawa S. and Oetzel G.R. (2006): Decreased insulin response in dairy cows following a four-day fast to induce hepatic lipidosis. J. Dairy Sci. 89. 2999–3005.

Opsomer G., Coryn M., Deluyker H. and de Kruif A. (1998): An analysis of ovarian dysfunction in high yielding dairy cows after calving based on progesterone profiles. Reprod. Dom. Anim. 33. 193-204.

Opsomer G., Wensing Th., Laevens H., Coryn M., de Kruif A. (1999): Insulin resistance: the link between metabolic disorders and cystic ovarian disease in high yielding dairy cows? Anim. Reprod. Sci. 56. 211–222.

Opsomer G., Grohn Y.T., Hertl J., Coryn M., Deluyker H., de Kruif A. (2000): Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. Theriogenology 53. 841–857.

Perry R.C., Corah L.R., Cochran R.C., Beal W.E., Stevenson J.S., Minton J.E., Simms D.D. and Brethour J.R. (1991): Influence of dietary energy on follicular development, serum gonadotropins, and first postpartum ovulation in suckled beef cows. J. Anim. Sci. 69. 3762-3773.

Perseghin G., Caumo A., Caloni M., Testolin G., Luzi L. (2001): Incorporation of the fasting plasma FFA concentration into quicki improves its association with insulin sensitivity in nonobese individuals. J. Clin. Endocrinol. Metab. 86. 4776-4781.

Pires J.A.A., Souza A.H. and Grummer R.R. (2007a): Induction of hyperlipidemia by intravenous infusion of tallow emulsion causes insulin resistance in Holstein cows. J. Dairy Sci. 90. 2735–2744.

Pires J.A.A., Pescara J.B., and Grummer R.R. (2007b): Reduction of plasma NEFA concentration by nicotinic acid enhances the response to insulin in feed-restricted Holstein cows. J. Dairy Sci. 90. 4635–4642.

Pravettoni D., Doll K., Hummel M., Cavallone E., Re M., Belloli A.G. (2004): Insulin resistance and abomasal motility disorders in cows detected by use of abomasoduodenal electromyography after surgical correction of left displaced abomasums. Am. J. Vet. Res. 65. 1319–1324.

Pullen D.L., Emery R.S., and Ames N.K. (1988): Turnover of hepatic and plasma triacylglycerol in sheep. J. Anim. Sci. 66. 1538.

Pullen D.L., Palmquist D.L., and Emery R.S. (1989): Effect on days of lactation and methionine hydroxy analog on incorporation of plasma fatty acids into plasma triglycerides. J. Dairy Sci. 72. 49-58.

Pullen D.L., Liesman J.S., and Emery R.S. (1990): A species comparison of liver slice synthesis and secretion of triacylglycerol from nonesterified fatty acid in the media. J. Anim. Sci. 68. 1395.

Putnam D.E., Varga G.A., Dann H.M. (1999): Metabolic and production responses to dietary protein and exogenous somatotropin in late gestation dairy cows. J. Dairy Sci. 82. 982-995.

R Development Core Team (2006): R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Radcliff R.P., McCormack B.L., Crooker B.A., and Lucy M.C. (2003): Growth hormone (GH) binding and expression of GH receptor 1A mRNA in hepatic tissue of periparturient dairy cows. J. Dairy Sci. 86. 3933–3940.

Radcliff R.P., McCormack B.L., Keisler D.H., Crooker B.A., and Lucy M.C. (2006): Partial feed restriction decreases growth hormone receptor 1A mRNA expression in postpartum dairy cows. J. Dairy Sci. 89. 611–619.

Rajamahendran R., Taylor C. (1990): Characterization of ovarian activity in postpartum dairy cows using ultrasound imaging and progesterone profiles. Anim. Reprod. Sci. 22. 171-180.

Rajaram S., Baylink D.J., Mohan S. (1997): Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions. Endo. Rev. 18. 801-831.

Reist M., Erdin D.K., von Euw D., Tschumperlin K.M., Leuenberger H., Hammon H.M., Morel C., Philipona C., Zbinden Y., Kunzi N., Blum J.W. (2003): Postpartum reproductive function: association with energy, metabolic and endocrine status in high yielding dairy cows. Theriogenology 59. 1707-1723.

Renaville R., Hammadi M., Portetelle D. (2002): Role of the somatotropic axis in the mammalian metabolism. Dom. Anim. Endocrinol. 23. 351–360.

Rigamonti A.E., Marazzi N., Cella S.G., Cattaneo L., Muller E.E. (1998): Growth hormone responses to growth hormone-releasing hormone and hexarelin in fed and fasted dogs: effect of somatostatin infusion or pretreatment with pirenzepine. J. Endocrinol. 156. 341-348.

Rose M.T., Obara Y., Fuse H., Itoh F., Ozawa A., Takahashi Y., Hodate K. and Ohashi S. (1996): Effect of growth hormone-releasing factor on the response to insulin of cows during early and late lactation. J. Dairy Sci. 79. 1734-1745.

Royal M.D., Darwash A.O., Flint A.P.F., Webb R., Wooliams J.A., Lamming G.E. (2000): Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. Anim. Sci. 70. 487–501.

Royal M.D., Pryce J.E., Woolliams J.A., and Flint A.P.F. (2002): The genetic relationship between commencement of luteal activity and calving interval, body condition score, production and linear type traits in Holstein-Friesian dairy cattle. J. Dairy Sci. 85. 3071–3080.

Ruan H., Lodish H.F. (2003): Insulin resistance in adipose tissue: direct and indirect effects of tumor necrosis factor-a. Cytokine & Growth Factor Rev. 14. 447–455.

Rukkwamsuk T., Wensing T. and Kruip T.A.M. (1999): Relationship between triacylglycerol concentration in the liver and first ovulation in postpartum dairy cows. Theriogenology 51. 1133-1142.

Rutter L.M., Randel R.D. (1984): Postpartum nutrient intake and body condition: effect on pituitary function and onset oestrus in beef cattle. J. Anim. Sci. 58. 265-274.

Sabour M.P., Lin C.Y., Smith C. (1997): Association of genetic variants of bovine growth hormone with milk production traits in Holstein cattle. J. Anim. Breed. Genet. 114. 435-442.

Sakai T., Hayakawa T., Hamakawa M., Ogura K. and Kubo S. (1993): Therapeutic effects of simultaneous use of glucose and insulin in ketotic dairy cows. J. Dairy Sci. 76. 109-114.

Sakai T., Hamakawa M., Kubo S. (1996): Glucose and xylitol tolerance tests for ketotic and healthy dairy cows. J. Dairy Sci. 79. 372-377.

Samanc H., Nikolic A.J., Stojic V., Dokovic R., Damjanovic Z, Ivanov I. (1996): Glucose tolerance and propionate loading tests in the assessment of endocrine pancreatic function in healthy and ketotic cows. Acta Vet. Beograd 46. 245-254.

Sano H., Nakai M., Kondo T. and Terashima Y. (1991): Insulin responsiveness to glucose and tissue responsiveness to insulin in lactating, pregnant, and nonpregnant, nonlactating beef cows. J. Anim. Sci. 69. 1122–1127.

Sano H., Narahara S., Kondo T., Takahashi A. and Terashima Y. (1993): Insulin responsiveness to glucose and tissue responsiveness to insulin during lactation in dairy cows. Dom. Anim. Endocrinol. 10. 191–197.

Santos J.E.P., Rutigliano H.M., Sá Filho M.F. Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows. Anim. Reprod. Sci. in press

Savio J.D., Boland M.P., Hynes N., Roche J.F. (1990): Resumption of follicular activity in the early postpartum period of dairy cows. J Anim. Sci. 70. 1271-1282.

Schillo K.K. (1992): Effects of dietary energy on control of luteinizing hormone secretion in cattle and sheep. J. Anim. Sci. 70. 1271-1282.

Schlee P., Graml R., Rottmann O., Pirchner F. (1994a): Influence of growth-hormone genotypes on breeding values of Simmental bulls J. Anim. Breed. Genet. 111. 253-256.

Schlee P., Graml R., Schallenberger E., Schams D., Rottmann Q., Olbrich-Bludau A., and Pirchner F. (1994b): Growth hormone and insulin-like growth factor-I concentrations in bulls of various growth hormone genotypes. Theor. Appl. Genet. 88. 497–500.

Shariflou M.R., Moran C, Nicholas F.W. (2000): Association of the Leu(127) variant of the bovine growth hormone (bGH) gene with increased yield of milk, fat, and protein in Australian Holstein-Friesians. Australian J. Agricultural Res. 51(4). 515-522.

Sheldon I.M., Noakes D.E., Rycroft A.N., Pfeiffer D.U., Dobson H. (2002b): Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. Reproduction 123. 837–845.

Sheldon M.I., Lewis G.S., LeBlanc S., Gilbert R.O. (2006): Defining postpartum uterine disease in cattle. Theriogenology 65. 1516–1530.

Sheldon I.M., Williams E.J., Miller A.N.A., Nash D.M., Herath S. (2008): Uterine diseases in cattle after parturition. Vet. J. 176. 115–121.

Shrestha H.K., Nakao T., Suzuki T., Higaki T., Akita M. (2004): Effects of abnormal ovarian cycles during pre-service period postpartum on subsequent reproductive performance of high-producing Holstein cows. Theriogenology 61. 1559–1571.

Sjogren K., Liu J.L., Blad K., Skrtic S., Vidal O., Wallenius V., LeRoith D., Tornell J., Isaksson O.G., Jansson J.O., and Ohlsson C. (1999): Liver-derived insulin-like growth factor I (IGF-I) is the principal source of IGF-I in blood but is not required for postnatal body growth in mice. Proc. Natl. Acad. Sci. 96. 7088–7092.

Skidmore A.L., Peeters K.A.M., Sniffen C.J., Bzand A. (1997): Monitoring dry period management. Appendix: Body condition scoring and interpretation. In: Herd health and production management in dairy practice, Brand A, Noordhuizen JPTM, Schukken YH (Eds.), Wageningen Pers, Wageningen, The Netherlands, pp. 191-197.

Sorensen A., Knight C.H. (2002): Endocrine profiles of cows undergoing extended lactation in relation to the control of lactation persistency. Dom. Anim. Endocrinol. 23. 111–123.

Sørensen P., Grochowska R., Holm L., Henryon M., and Løvendahl P. (2002): Polymorphism in the bovine growth hormone gene affects endocrine release in dairy calves. J. Dairy Sci. 85. 1887–1893.

Spicer L.J. (2001): Receptors for insulin-like growth factor-I and tumor necrosis factor-a are hormonally regulated in bovine granulosa and thecal cells. Anim. Reprod. Sci. 67. 45–58.

Spicer L.J. (2003): The effect of leptin on ovarian steroidogenesis. In: Henson MC, Castracane VD (eds): Leptin and reproduction. Kluwer Academic / Plenum Publishers, New York, pp 97-110.

Spicer L.J., Alpizar E., Echternkamp S.E. (1993): Effects of insulin, insulin-like growth factor I, and gonadotropins on bovine granulosa cell proliferation, progesterone production, estradiol production, and (or) insulin-like growth factor I production in vitro. J. Anim. Sci. 71. 1232-1241.

Spicer L.J., Stewart R.E. (1996): Interactions among basic fibroblast growth factor, epidermal growth factor, insulin, and insulin-like growth factor-I (IGF-I) on cell numbers and steroidogenesis of bovine thecal cells: role of IGF-I receptors. Biol. Reprod. 54. 255-263.

Spicer L.J., Chamberlain C.S., Francisco C.C. (2000): Ovarian action of leptin: effects on insulin-like growth factor-I-stimulated function of granulosa and thecal cells. Endocrine 12. 53-59.

Spicer L.J., Alonso J., Chamberlain C.S. (2001) Effects of thyroid hormones on bovine granulosa and thecal cell function in vitro: dependence on insulin and gonadotropins. J. Dairy Sci. 84. 1069-1076.

Steen A., Gronstol H., Torjesen P.A. (1997): Glucose and insulin responses to glucagon injection in dairy cows with ketosis and fatty liver. J. Vet. Med.. A 44. 521-530.

Stein D.T., Esser V., Stevenson B.E., Lane K.E., Whiteside J.H., Daniels M.B., Chen S., McGarry J.D. (1996): Essentiality of circulating fatty acids for glucose-stimulated insulin secretion in the fasted rat. J. Clin. Invest. 97. 2728–2735.

Strang B.D., Bertics S.J., Grummer R.R., Armentano L.E (1998): Relationship of tryglyceride accumulation to insulin clearance and hormonal responsiveness in bovine hepatocytes. J. Dairy Sci. 81. 740-747.

Subiyatno A., Mowat D.N. and Yang W.Z. (1996): Metabolite and hormonal responses to glucose or propionate infusions in periparturient dairy cows supplemented with chromium. J. Dairy Sci. 79. 1436-1445.

Suriyasathaporn W., Daemen A.J.J.M., Noordhuizen-Stassen E.N., Dieleman S.J., Nielen M., Schukken Y.H. (1999): ßhydroxybutyrate levels in peripheral blood and ketone bodies supplemented in culture media affect the in vitro chemotaxis of bovine leukocytes. Vet. Immun. Immunopath. 68. 177-186.

Suriyasathaporn W., Heuer C., Noordhuizen-Stassen E.N., Schukken Y.H. (2000): Hyperketonemia and the impairment of udder defense: a review. Vet. Res. 31. 397–412.

Svennersten-Sjaunja K., Olsson K. (2005): Endocrinology of milk production. Dom. Anim. Endo. 29. 241–258.

Tanaka T., Arai M., Ohtani S., Uemura S., Kuroiwa T., Kim S., Kamomae H. Influence of parity on follicular dynamics and resumption of ovarian cycle in postpartum dairy cows. Anim. Reprod. Sci. in press

Taponen J., Kulcsár M., Kátai L., Huszenicza Gy., Rodríguez-Martínez H., Katila T. (2002): Short estrous cycles and estrous signs after premature ovulations induced with cloprostenol and gonadotropin-releasing hormone in cyclic dairy cows. Theriogenology 58. 1291-1302.

Taylor V.J., Cheng Z., Pushpakumara P.G., Beever D.E., Wathes D.C. (2004): Relationships between the plasma concentrations of insulin-like growth factor-I in dairy cows and their fertility and milk yield. Vet. Rec. 155. 583-588.

Taylor V.J., Beever D.E., Bryant M.J., Wathes D.C. (2006): Pre-pubertal measurements of the somatotrophic axis as predictors of milk production in Holstein–Friesian dairy cows. Dom. Anim. Endocrinol. 31. 1–18.

Thissen J.-P., Ketelslegers J.-M., Underwood L.E. (1994): Nutritional regulation of the insulin-like growth factors. Endo. Rev. 15. 80-101.

Tse E.O., Gregoire F.M., Reusens B., Remacle C., Hoet J.J., Johnson P.R. and Stern J.S. (1998): Changes of islet size and islet distribution resulting from protein-malnutrition in lean (Fa/Fa) and obese (fa/fa) Zucker rats. Obesity Res. 5. 563–571.

Tyopponen J., Kauppinen K. (1980): The stability and automatic determination of ketone bodies in blood samples taken in field conditions. Acta Vet. Scand. 21. 55–61.

Vanderkooi W.K., Vandehaar M.J., Sharma B.K., Binelli M., Tucker H.A., Akers R.M., and Moseley W.M. (1995): Comparison of growth hormone-releasing factor and somatotropin: The somatotropic axis in lactating primiparous cows. J. Dairy Sci. 78. 2140-2149.

Van der Werf, J.H.J., Verburg F.J., and Garssen G.J. (1996): Evidence for a strong effect of the AluI polymorphism in the growth hormone gene on yield characteristics in dairy cattle. In: Proc. 47th Meeting EAAP. (Abstract). Lillehammer, Norway 1. 310

van Epps-Fung M., Williford J., Wells A. and Hardy R.W. (1997): Fatty acid-induced insulin resistance in adipocytes. Endocrinology 138. 4338–4345.

Vanholder T., Leroy J.L.M.R., Van Soom A., Coryn M., de Kruif A. and Opsomer G. (2006): Effects of ß-OH butyrate on bovine granulosa and theca cell function in vitro. Reprod. Dom. Anim. 41. 39–40.

Vazquez-Anon M., Bertics S., Luck M., Grummer R.R., Pinheiro J. (1994): Peripartum liver triglyceride and plasma metabolites in dairy cows. J. Dairy. Sci. 77. 1521-1528.

Veldhuis J.D., Anderson S.M., Shah N., Bray M., Vick T., Gentili A., Mulligan T., Johnson M.L., Weltman A., Evans W.S., and Iranmanesh A. (2001): Neurophysiological regulation and target-tissue impact of the pulsatile mode of growth hormone secretion in the human. Growth Horm. IGF Res. 11 Suppl. A. S25–S37.

Venables W.N. and Ripley B.D. (2002): Modern Applied Statistics with S. Springer (4th ed), New York.

Vicini J.L., Buonomo F.C., Veenhuizen J.J., Miller M.A., Clemmons D.R., and Collier R.J. (1991): Nutrient balance and stage of lactation affect responses of insulin, insulin-like growth factors I and II, and insulin-like growth factor-binding protein 2 to somatotropin administration in dairy cows. J. Nutr. 121. 1656–1664.

Wallis M. (1992): The expanding growth hormone/prolactin family. J. Mol. Endocrinol. 9. 185-188.

Walsh R.B., Kelton D.F., Duffield T.F., Leslie K.E., Walton J.S., and LeBlanc S.J. (2007a): Prevalence and risk factors for postpartum anovulatory condition in dairy cows. J. Dairy Sci. 90. 315–324.

Walsh R.B., Walton J.S., Kelton D.F., LeBlanc S.J., Leslie K.E., and Duffield T.F. (2007b): The effect of subclinical ketosis in early lactation on reproductive performance of postpartum dairy cows. J. Dairy Sci. 90. 2788–2796.

Wathes D.C., Cheng Z., Bourne N., Taylor V.J., Coffey M.P., Brotherstone S. (2007): Differences between primiparous and multiparous dairy cows in the inter-relationships between metabolic traits, milk yield and body condition score in the periparturient period. Domest. Anim. Endocrinol. 33. 203–225.

Weber W.J., Baumgard L.H., Kazmer G.W., Zinn S.A., Hansen L.B., Chester-Jones H., and Crooker B.A. (2005): Growth hormone response to growth hormone releasing hormone in calves that differ in genetic merit for milk yield. J. Dairy Sci. 88. 1723–1731.

Weisdorf D.J., Craddock P.R., Jacob H.S. (1982): Granulocytes utilize different energy sources for movement and phagocytosis. Inflammation 6. 245-256.

Whisnant C.S., Kiser T.E., Thompson F.N., Hall J.B. (1985): Effect of nutrition on the LH response to calf removal and GnRH. Theriogenology 24. 565-573.

Whitlock B.K., Daniel J.A., Wilborn R.R., Elsasser T.H., Carroll J.A., Sartin J.L. (2008): Comparative aspects of the endotoxin- and cytokine-induced endocrine cascade influencing neuroendocrine control of growth and reproduction in farm mammals. Reprod. Domest. Anim. In press

Williams C.Y., Harris T.G., Battaglia D.F., Viguie C., Karsch F.J. (2001): Endotoxin inhibits pituitary responsiveness to gonadotropinreleasing hormone. Endocrinology 142. 1915–1922.

Williams E.J., Fischer D.P., Noakes D.E., England G.C.W., Rycroft A., Dobson H., Sheldon I.M. (2007): The relationship between uterine pathogen growth density and ovarian function in the postpartum dairy cow. Theriogenology 68. 549–559.

Williams G.L., Amstalden M., Garcia M.R., Stanko R.L., Nizielski S.E., Morrison C.D., Keisler D.H. (2002): Leptin and its role in the central regulation of reproduction in cattle. Dom. Anim. Endocrinol. 23. 339-349.

Wiltbank M.C., Gumen A., and Sartori R. (2002): Physiological classification of anovulatory conditions in cattle. Theriogenology 57. 21–52.

Wiltbank M., Lopez H., Sartori R., Sangsritavong S., Gümen A. (2006): Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology 65. 17–29.

Wood D.C., Salsgiver W.J., Kasser T.R., Lange G.W., Rowold E., Violand B.N., Johnson A., Leimgruber L.M., Parr G.R., Siegel N.R., Kimack N.M., Smith C.E., Zobel J.F., Ganguli S.M., Garbow J.R., Bild G., and Krivi G.G. (1989): Purification and characterization of pituitary bovine somatotropin. J. Biol. Chem. 264. 14741-14747.

Wood G.M., Boettcher P.J., Kelton D.F., and Jansen G.B. (2004): Phenotypic and genetic influences on test-day measures of acetone concentration in milk. J. Dairy Sci. 87. 1108–1114.

Woychik R.P., Camper S.A., Lyons R.H., Horowitz S., Goodwin E.C., Rottman F.M. (1982): Cloning and nucleotid sequencing of the bGH gene. Nucleic Acids Res. 10. 7197-7220.

Wylie A.R., Woods S., Carson A.F., McCoy M. (2008): Periprandial changes in metabolite and metabolic hormone concentrations in high-genetic-merit dairy heifers and their relationship to energy balance in early lactation. J. Dairy Sci. 91. 577-586.

Yamada K., Nakao T., Isobe N. (2003): Effects of body condition score in cows peripartum on the onset of postpartum ovarian cyclicity and conception rates after ovulation synchronization / fixed-time artificial insemination. J. Reprod. Dev. 49. 381-388.

Yao J., Aggrey S.E., Zadworny D., Hayes J.F., Kuhnlein U. (1996): Sequence variations in the bovine growth hormone gene characterized by single-strand conformation polymorphism (SSCP) analysis and their association with milk protein traits in Holsteins. Genetics 144. 1809-1816.

Yokota I., Hayashi H., Matsuda J., Saijo T., Naito E., Ito M., Ebina Y., Kuroda Y. (1998): Effect of growth hormone on the translocation of GLUT4 and its relation to insulin-like and anti-insulin action. Biochem. Biophys. Acta 1404. 451-456.

Zhang H.M., Brown D.R., DeNise S.K. and Ax R.L. (1992): Nucleotide sequence determination of a bovine somatotropin allele. Anim. Genet. 23. 578.

Zhou L., Chen H., Up P., Cong L.N., Sciacchitano S., Li Y., Graham D., Jacobs A.R., Taylor S.I. and Quon M.J. (1999): Action of insulin receptor subtrate-2 (IRS-3) and IRS-4 to stimulate translocation of GLUT4 in rat adipose cells. Mol. Endocrinol. 13. 505–514.

Zieba D.A., Amstalden M., Williams G.L. (2005): Regulatory role of leptin in reproduction and metabolism: a comparative review. Domest. Anim. Endocrinol. 28. 166-185.

Zinn S.A., Kazmer G.W., Rycroft H., Campbell R.M. (1994): Growth hormone response after administration of growth hormone-releasing factor to proven dairy sires. Livest. Prod. Sci. 40. 157-164.

Zwierzchowski L., Krzyzewski J., Strzalkowska N., Siadkowska E., Ryniewicz Z. (2002): Effects of polymorphism of growth hormone (GH), Pit-1, and leptin (LEP) genes, cow's age, lactation stage and somatic cell count on milk yield and composition of Polish Black-and-White cows. Anim. Sci. Pap. Rep. 20(4). 213-227.

8. The candidate's publications related to the present 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, L. Kátai, **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)

3. Abstracts published in peer-reviewed journals in English

3.1. **O. Balogh**, K. Kovács, M. Kulcsár, M. Keresztes, H. Fébel, V. Faigl, A. Gáspárdy, A. Zsolnai, L. Fésüs and Gy. Huszenicza: Effect of growth hormone genotype (*Alu*I polymorphism) on plasma levels of metabolic hormones and ketones in postpartum holstein cows. *Reprod. Dom. Anim*., 2006. 41. 347.

3.2. **O. Balogh**, K. Kovács, M. Kulcsár, A. Gáspárdy, A. Zsolnai, J. Reiczigel, L. Kátai, L. Fésüs, Gy. Huszenicza: Possible role of the STH genotype (*Alu*I

polymorphism) in the length of postpartum acyclic period in dairy cows. *Livest. Prod. Sci*., 2005. 98. 176-177.

3.3. M. Kulcsár, L. Kátai, **O. Balogh**, A. Pécsi, C. Dalevaud, J. Földi, J. Hirvonen, V. Faigl, Y. Chilliard, Gy. Huszenicza: Metabolic and endocrine changes, inflammatory proteins and ovarian activity in dairy cows with acute puerperal metritis. *Reprod. Dom. Anim*., 2005. 40. 407.

3.4. M. Kulcsár, Sz. Jánosi, L. Kátai, P. Kóródi, **O. Balogh**, C. Delavaud, Y. Chilliard: Mastitis-related endocrine alterations in postpartum dairy cows. *Reprod. Dom. Anim*., 2004. 39. 289.

3.5. M. Kulcsár, T. Lehtolainen, **O. Balogh**, C. Delavaud, Y. Chilliard, S. Pyörälä: Experimental endotoxin mastitis fails to alter the plasma leptin level in dairy cows. *Reprod. Dom. Anim*., 2004. 39. 290.

3.6. **O. Balogh**, K. Kovács, M. Kulcsár, L. Kátai, A. Zsolnai, A. Gáspárdy, J. Reiczigel, L. Fésüs, Gy. Huszenicza: Interrelations of the STH genotype (*Alu*I polymorphism) and first ovulation in postpartum dairy cows. *Biotechnology, Agronomy, Society and Environment*, 2004. 8. 36.

3.7. **O. Balogh**, M. Kulcsár, R. Szeleczky, H. Fébel, J. Reiczigel, A. Gáspárdy, E. Szucs, C. Delavaud, Y. Chilliard, P. Rudas, Gy. Huszenicza: Whole-body insulin resistance and first ovulation in post-partum dairy cows. *Reprod. Dom. Anim*., 2003. 38. 361-362.

4. Full-text papers published in conference proceedings

4.1. **O. Balogh**, K. Kovács, M. Kulcsár, A. Gáspárdy, A. Zsolnai, J. Reiczigel, L. Kátai, L. Fésüs, Gy. Huszenicza: Possible role of the STH genotype (*Alu*I polymorphism) in the length of postpartum (pp) acyclic period in dairy cows. *Proc. of the 15th Hungarian and 5th Middle-European Buiatrics Congress* (2-5 June 2004, Hajdúszoboszló, Hungary), pp. 172-177.

4.2. Gy. Huszenicza, M. Kulcsár, L. Kátai, **O. Balogh**, A. Meikle, H. Fébel, C. Delavaud, A. Pécsi, J. Földi, D. Cavestany, Y. Chilliard, S. Fekete: Metabolic factors influencing the onset of cyclic ovarian function and fertility in postpartum dairy cows. *Proc. of the 8th International Conference of the European Society of Veterinary and Comparative Nutrition* (Sept. 23-25, 2004, Budapest, Hungary), pp. 17-37.

Further publications not related to the current thesis

M. Kulcsár, Sz. Jánosi, T. Lehtolainen, L. Kátai, C. Delavaud, **O. Balogh**, Y. Chilliard, S. Pyörälä, P. Rudas, Gy. Huszenicza: Feeding-unrelated factors influencing the plasma leptin level in ruminants. *Dom. Anim. Encocrinol.*, 2005. 29. 214-226. **(IF:** 1.559)

D. Cavestany, M. Kulcsár, D. Crespi, Y. Chilliard, A. La Manna, **O. Balogh,** M. Keresztes, C. Delavaud, G. Huszenicza and A. Meikle: Effect of prepartum energetic supplementation on productive and reproductive characteristics, and metabolic and hormonal profiles in dairy cows under grazing conditions. *Reprod. Dom. Anim.*, in press (**IF:** 1.50)

Acknowledgement

First of all, I would like to express my great appreciation to *Prof. Dr. Gyula Huszenicza* for his continuous help and encouragement throughout the years and without whom I would have never succeeded to reach my professional goals.

A special thank has to be granted to *Dr. Margit Kulcsár* for her contribution in the experiments and above all, for her kind and loving personality.

It is my honor to acknowledge *Profs. Walter R. Butler* and *Robert O. Gilbert* at Cornell University, Ithaca (USA) for their generous help and advices, and the great comments to *Prof. Yves Chilliard* at INRA (Saint-Genes-Champanelle, France) to improve my work.

This thesis would have never been accomplished without the academic staff, co-workers, fellow students and technicians in my alma mater whose support needs to be recognized: *Prof. László Solti* as the head of the department, *Drs. Jeno Reiczigel* and *András Gáspárdy* for their help in the statistical evaluations, *Prof. Sándor György Fekete* for his help in the nutritional analyses, *Drs. Vera Faigl, Mónika Keresztes* and *Alíz Márton* for their cooperation in the laboratory and for sharing the winding road of PhD students with me, *Alice Vonáné Nagy* for her great analytical work and friendship, *Erzsébet Kiss* and *Ibolya Czigányné Simon* for their outstanding laboratory assistance.

My fellow colleague and friend, *Dr. Katalin Kovács* and her supervisor, *Prof. László Fésüs* together with *Dr. Hedvig Fébel* from the Research Institute for Animal Breeding and Nutrition (Herceghalom) have undeniable merits in all the work that is presented here.

Drs. Levente Kátai and *József Földi* helped me with the practical implementation of my experiments. I would like to specially thank *Drs. Orsolya Szepes*, *Regina Szeleczky* and *Leif Reidar Ronning* their hard work, persistence and cheerful partnership in the coldest winter or hottest summer of the Hungarian dairies. I am grateful to *Dr. János Bartyik* and all other resident veterinarians, managers and personnel on the farms who made my studies work with their generous help.

My reviewers, *Profs. Zsuzsanna Neogrády, László Zöldág* and *Miklós Mézes* deserve all my appreciation for their time and effort to revise my work and for their precious advices so that the final format of this thesis could hopefully be also the best one.

The financial support was granted by the *National Foundation for Basic Research* (Hungary, project number: OTKA-T/046826).

Last, but not least I would like to acknowledge my family for their endless sacrifices, love and support so that I could stand where I am now: my mother, *Ilona*, my sister, *Andrea*, my husband and love, *Javier* and my second mother, *Kati.*

Budapest, 12th November, 2008.

Orsolya Balogh