23. Hülsenberger Gespräche
H. Wilhelm Schaumann Stiftung
Lübeck, 2.-4. Juni 2010

Störungen im Energiestoffwechsel im Zyklus der Reproduktion bei Milchkühen

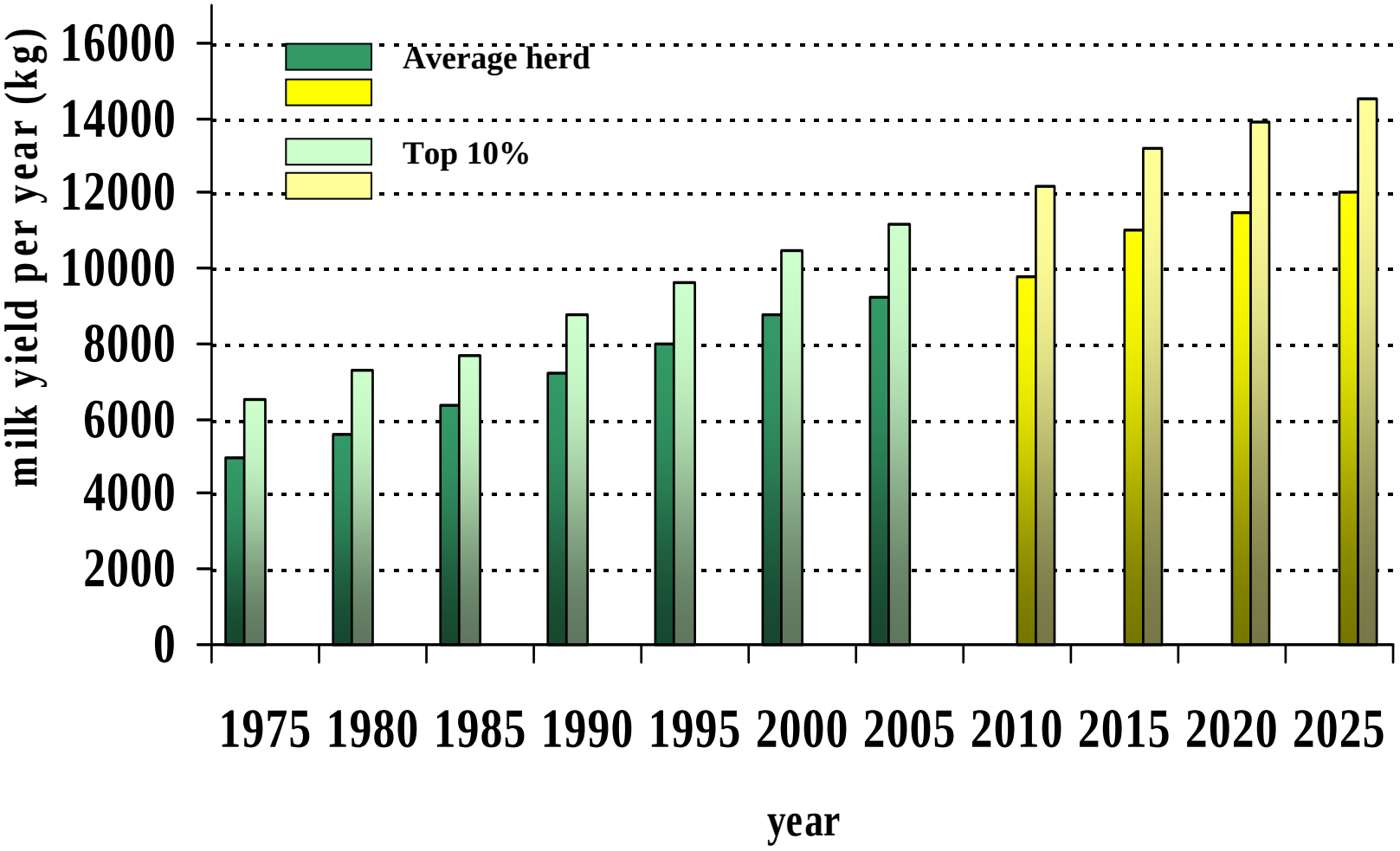
Juergen Rehage

Klinik für Rinder
Stiftung Tierärztliche Hochschule Hannover

- Einleitung
- Stoffwechsel
- Interaktion Stoffwechsel – Reproduktion
- Schlussfolgerungen

Durchschnittliche Jahresmilchleistung

(Osnabrück Holstein Genetics)



Zwischenkalbezeit

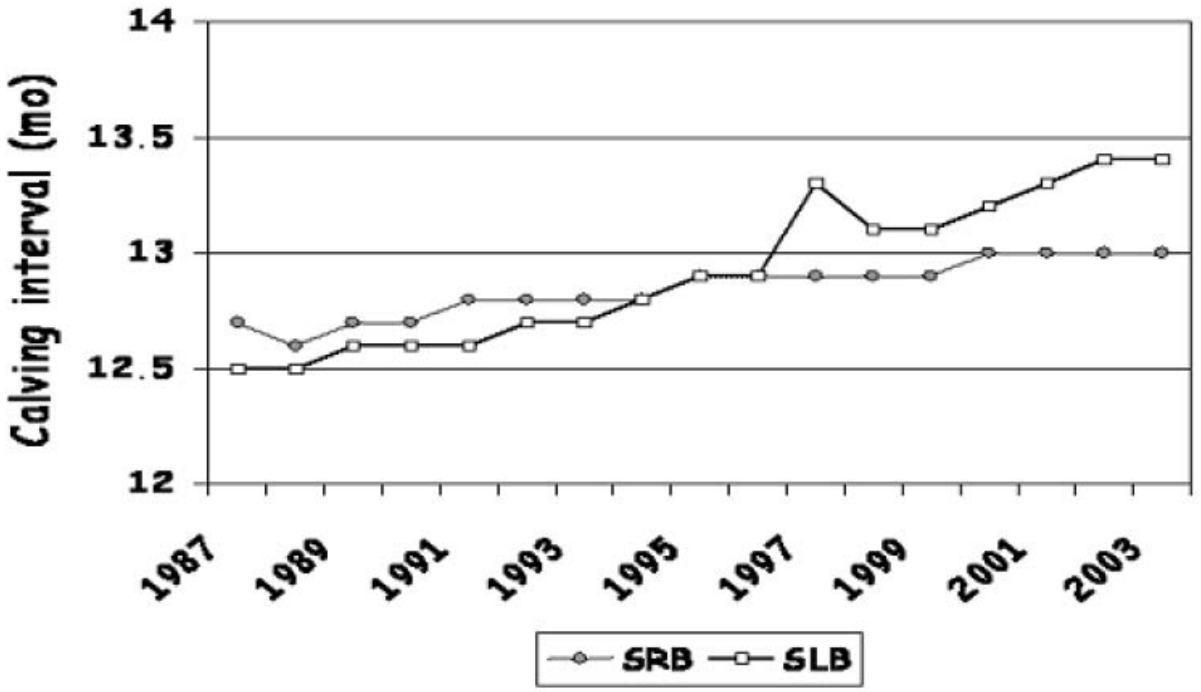


Figure 5: The average calving interval from 1987 to 2003 for the two major Swedish dairy breeds.

Annex to the EFSA Journal (2009) 1143, 41-284

➢ Inzidenz/Praevalenz von Produktionskrankheiten

- Milchfieber: 1 bis 20 %
- Subklinische Ketosen: 2 bis 20 %
- SARA: 0 bis 50 %
- Labmagenverlagerung: 0 bis 30 %
- Lahmheiten: 5 bis 50 %
- Mastitis: 15 bis 70 %

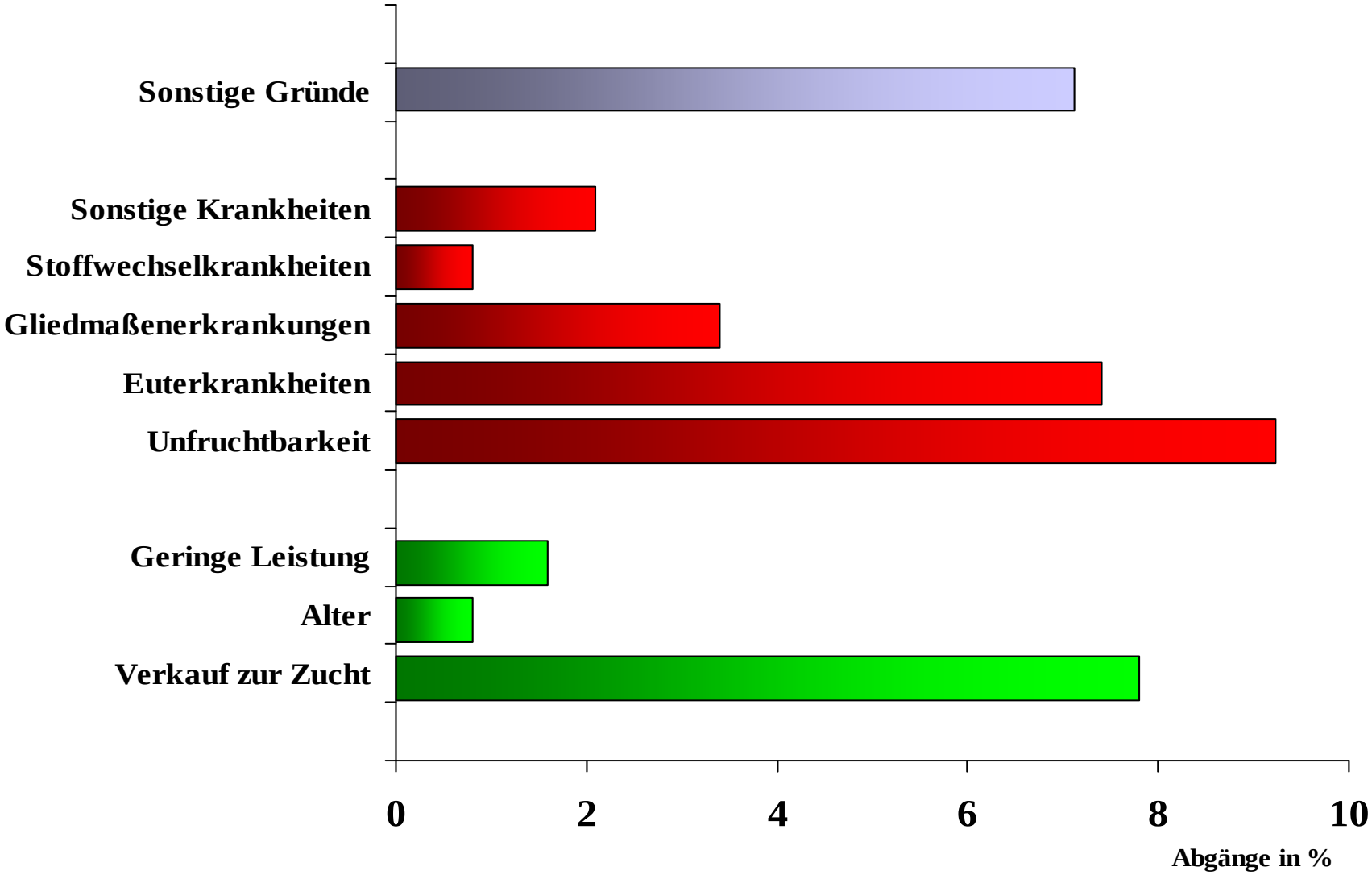
Korrelationen zwischen Milchleistung und Produktionskrankheiten

- Genetische Korrelationen
  - Im Durchschnitt etwa 10 – 35%
    (Oltenacu et al. 1991, Uribe et al 1996, Dematawewa and Berger 1998, Royal et al. 2000, Pryce and Veerkamp 2001, Roxstrom 2001, Veerkamp et al. 2003)

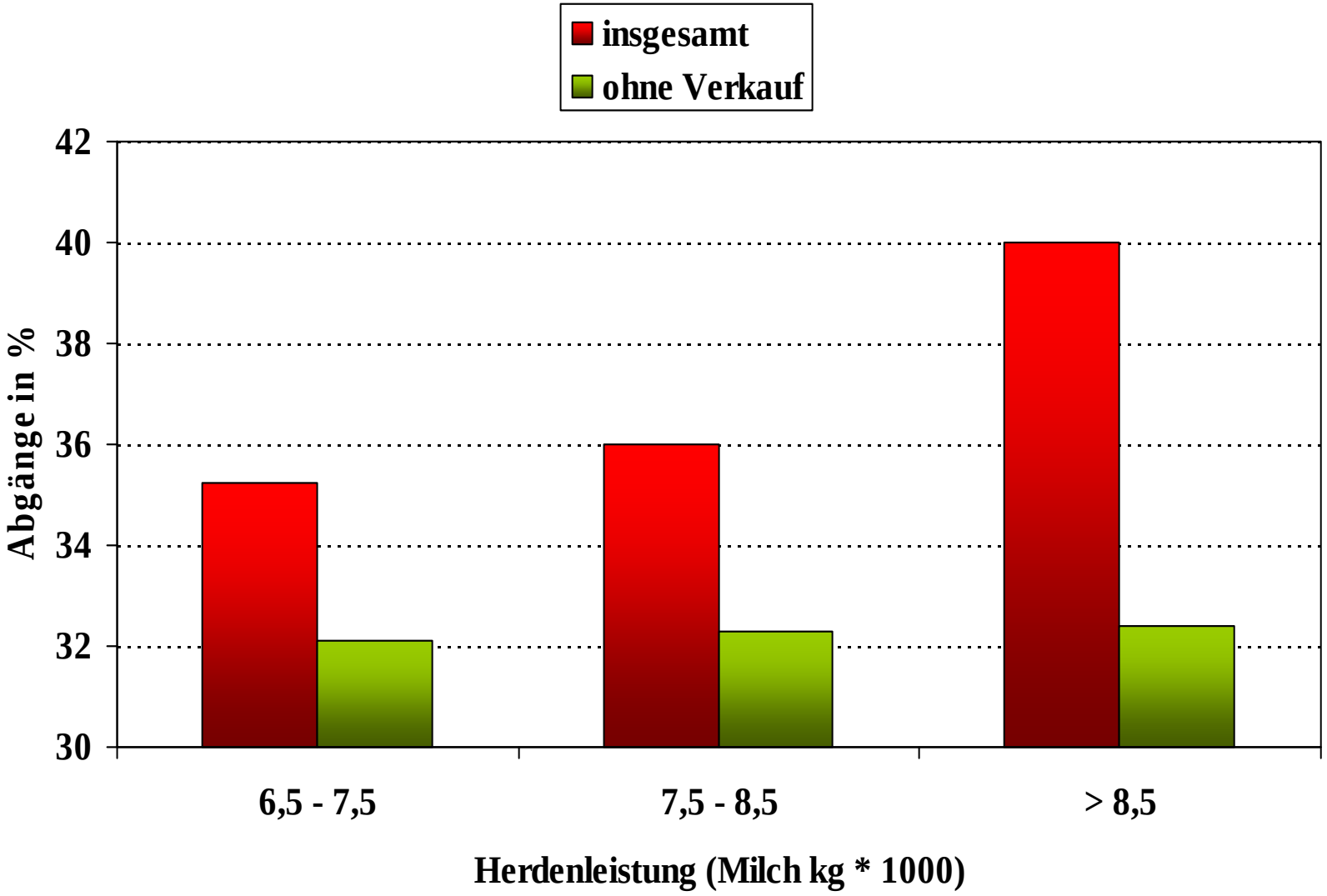
- Phänotypische Korrelationen
  - Keine, mit Ausnahme von Mastitis und Ovarcysten
    (Mrode and Swanson 1996, Pryce and Brotherstone 1999, Rupp and Boichard 1999, Ingvartsen et al 2003)

Ursachen für Abgänge von Milchkühen aus Herden > 8500 kg Milch

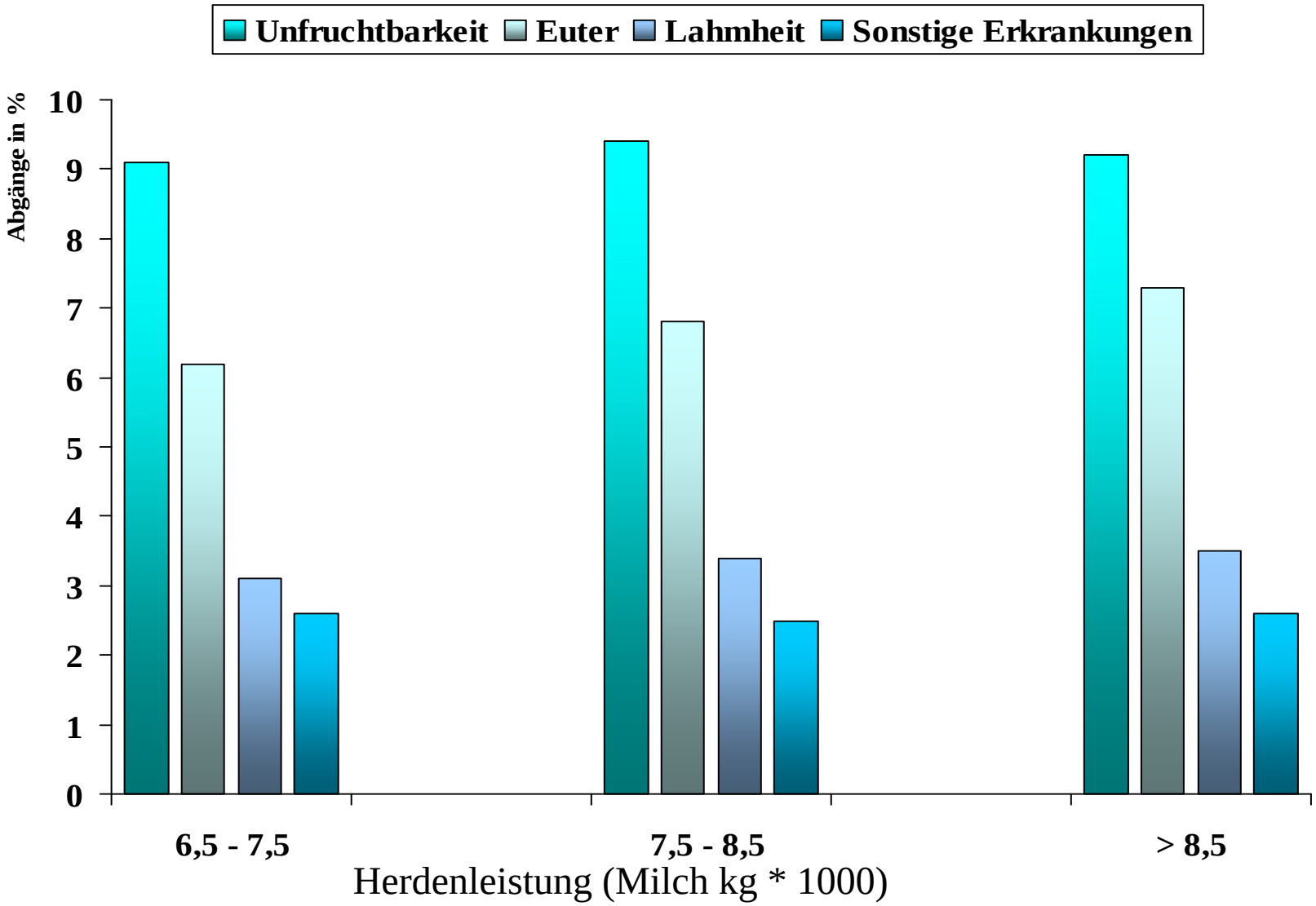
(Niedersachsen, Quelle VIT)



Abgänge von Milchkühen (Niedersachsen, Quelle VIT)



Ursachen für Abgänge von Milchkühen (Niedersachsen, Quelle VIT)



Relatives Risiko der Verwertung

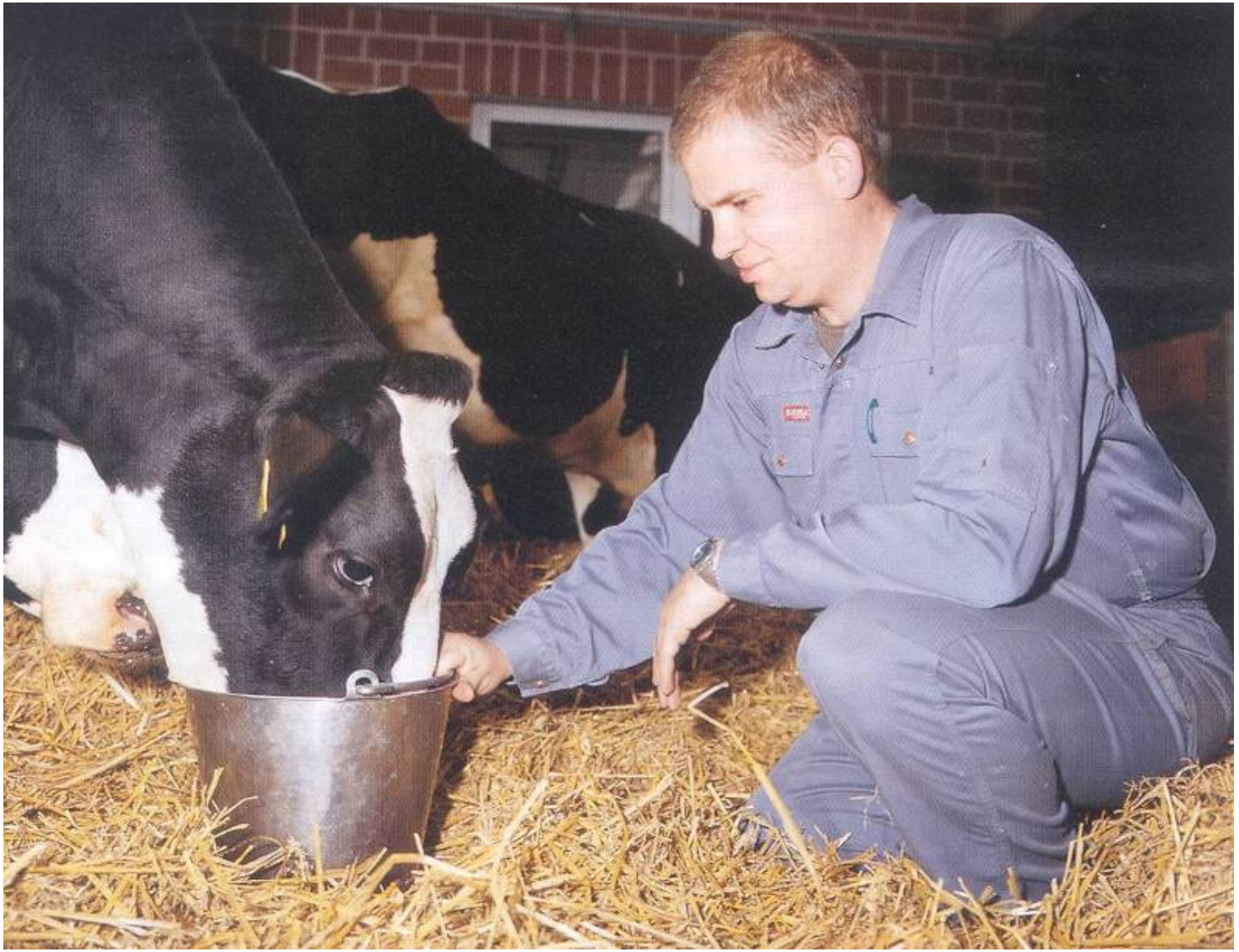
Table 1. Effect of management practices and labor situations on relative risk of culling for low- and high-producing cows, as compared with average-producing cows, during the period from 1996 to 2000.

Variable	Category	Number		Risk vs. average cows	
		Herds	Cows	Low cows	High cows
Rolling herd average (lb.)	< 20,000	49	14,854	2.48	0.65[a]
	20,000 to 25,000	108	42,110	2.64	0.67[a]
	> 25,000	29	15,492	2.37	0.75[b]

Weigel et al. 2003: J. Dairy Sci. 1482 - 1486

Strukturwandel in der Milchviehwirtschaft

- **Abnahme der Zahl der Betriebe**
- **Zunahme der Größe der Betriebe**
- **Ganzjährige Stallhaltung (mit Auslauf)**
- **Abnahme der Zahl qualifizierten Personals pro Kuh**
- **Automatisierung und Computerisierung**



Bedeutung der Trockensubstanzaufnahme (TM)

Genetische Korrelation zwischen Milchleistung und TM Aufnahme: 0,46 - 0,65
(Jensen et al. 1991, Persaud et al. 1991, Svendsen et al. 1994, Van Arendonk et al. 1991, Veerkamp et al. 1995, Veerkamp & Brotherstone 1997)

↓

Selektion der Kühe nach Milchleistung führt automatisch auch zur Steigerung der täglichen TM Aufnahme,
aber nur etwa 40 - 50 % des gleichzeitig gesteigerten Energiebedarfs werden gedeckt

(Veerkamp, J. Dairy Sci. 1998, 81, 1109-1119)

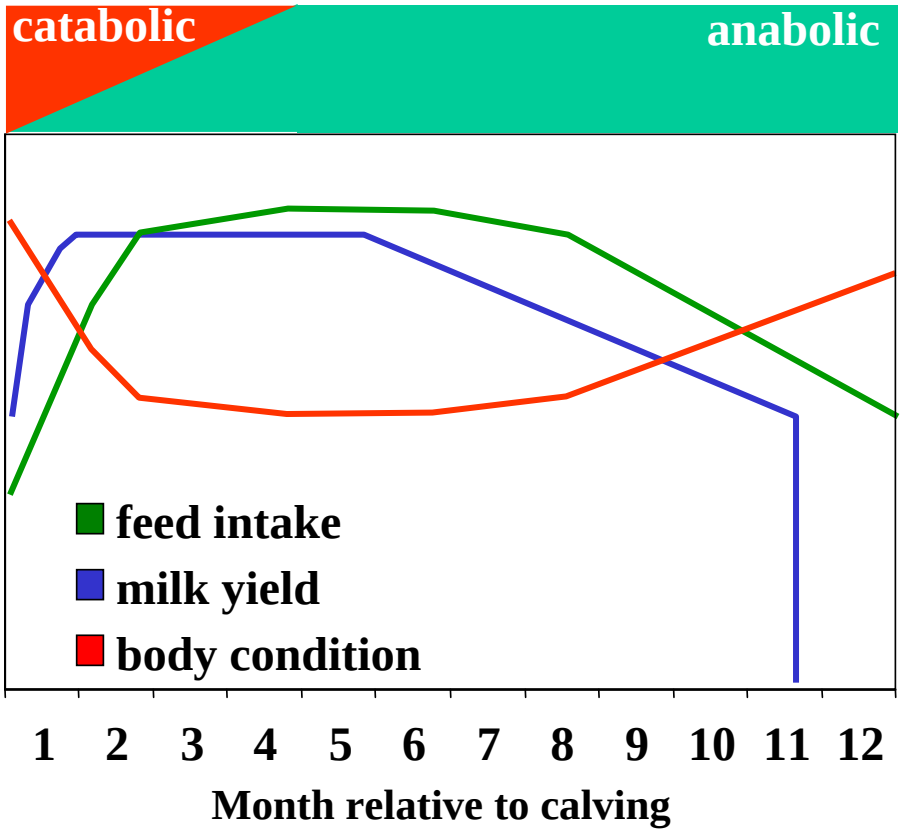
↓

Genetische Korrelation zwischen Milchleistung und Energiebilanz: -0,70
Daher führt höhere Milchleistung automatisch zu verstärkter NEB
(Svendsen et al., J. Anim. Sci. 1994, 72, 1441-1449)

↓

NEB erhöht das Risiko für Ketose und Leberverfettung

Energieaufnahme, Milchleistung, Körperkondition in Relations zum Laktationsmonat



Hormonelle Adaptation:

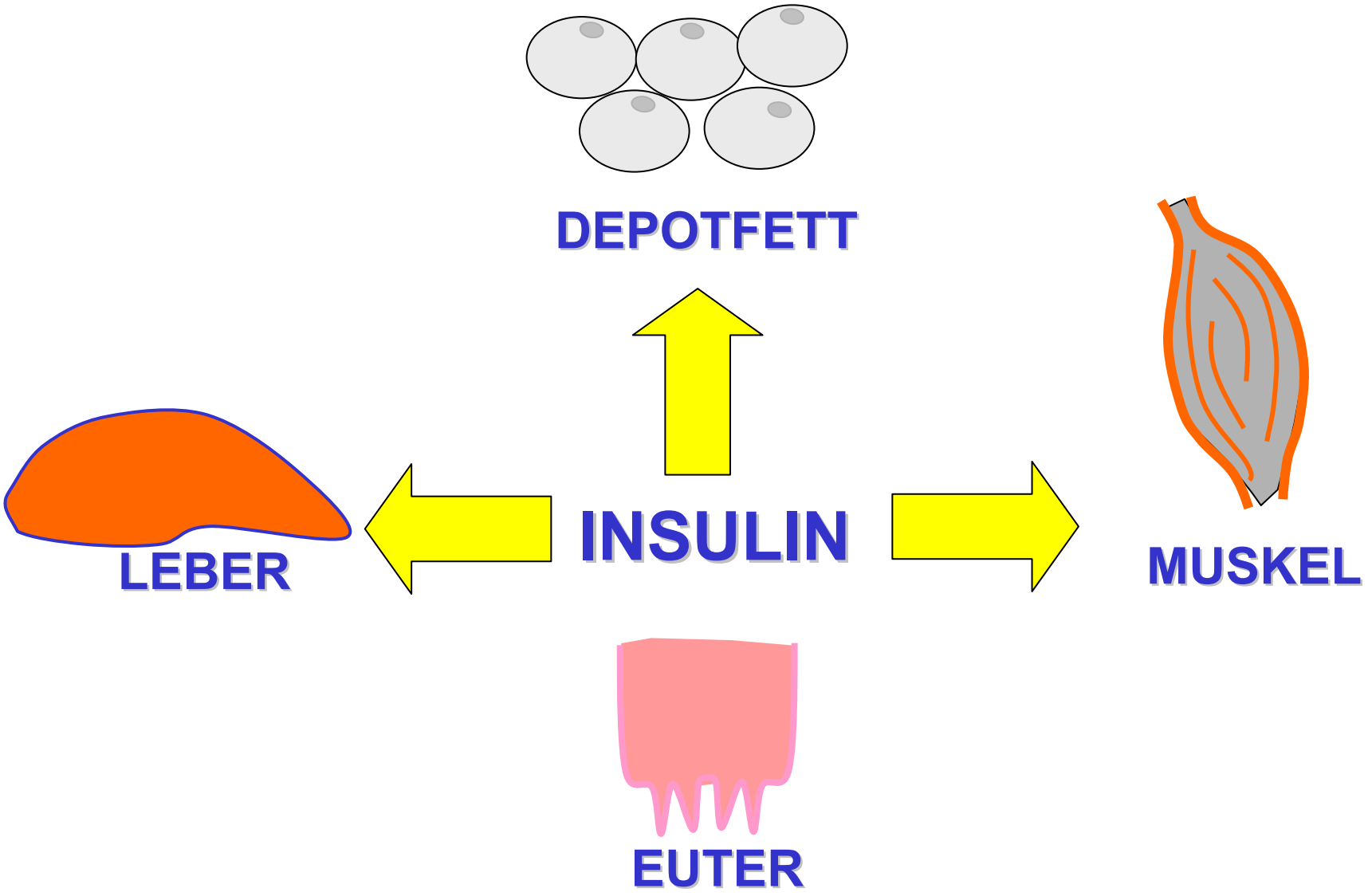
- Insulin ↓
- bGH ↑
- Glucagon ↑
- Cortisol ↑

Kompensation des Energiedefizits:

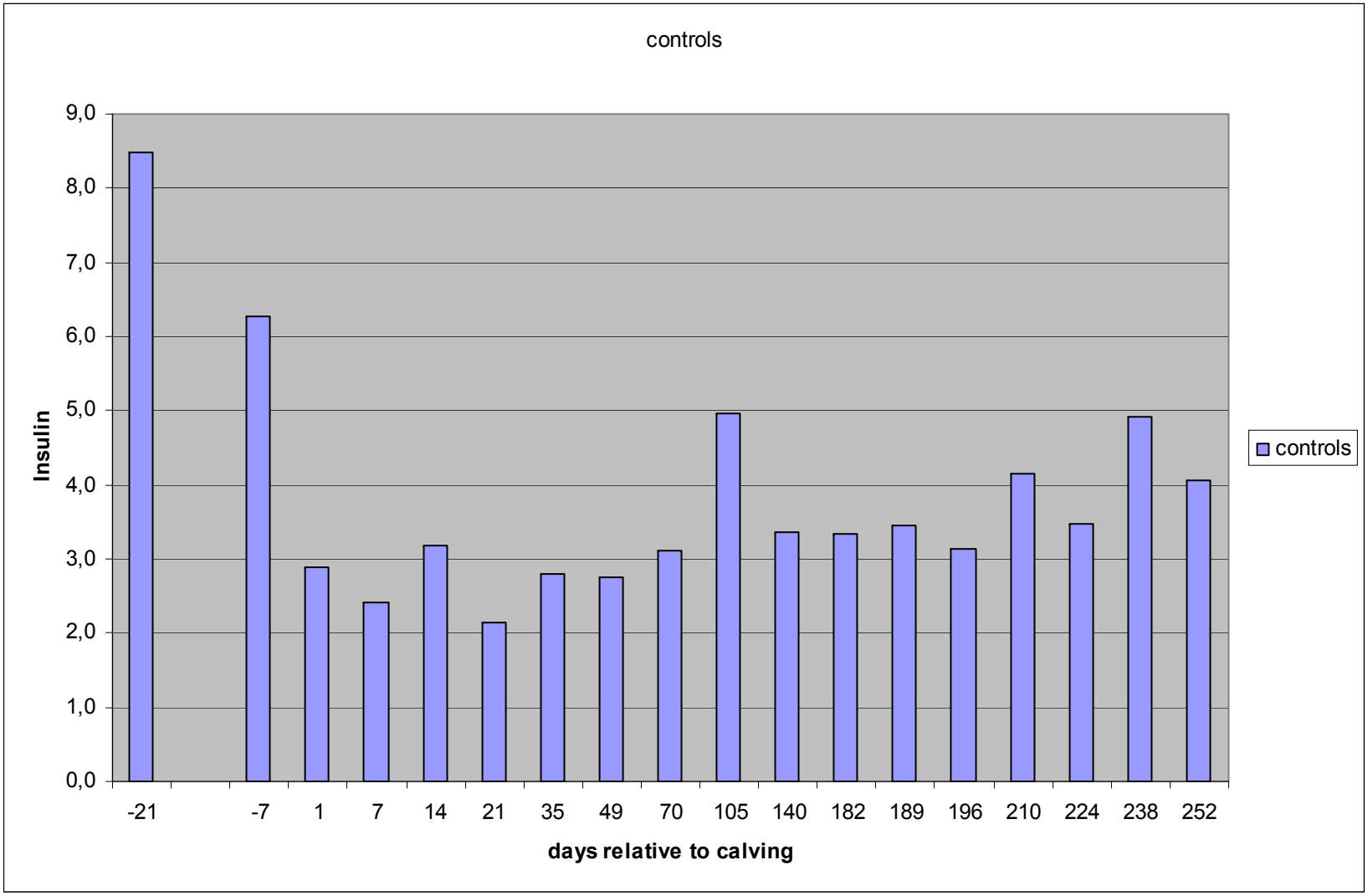
* Lipomobilisation
* Proteinolyse
* Verminderte extramammäre Substrat-Utilisation

Adapted from Bell (1995): J. Anim. Sci. 73, 2804-2819

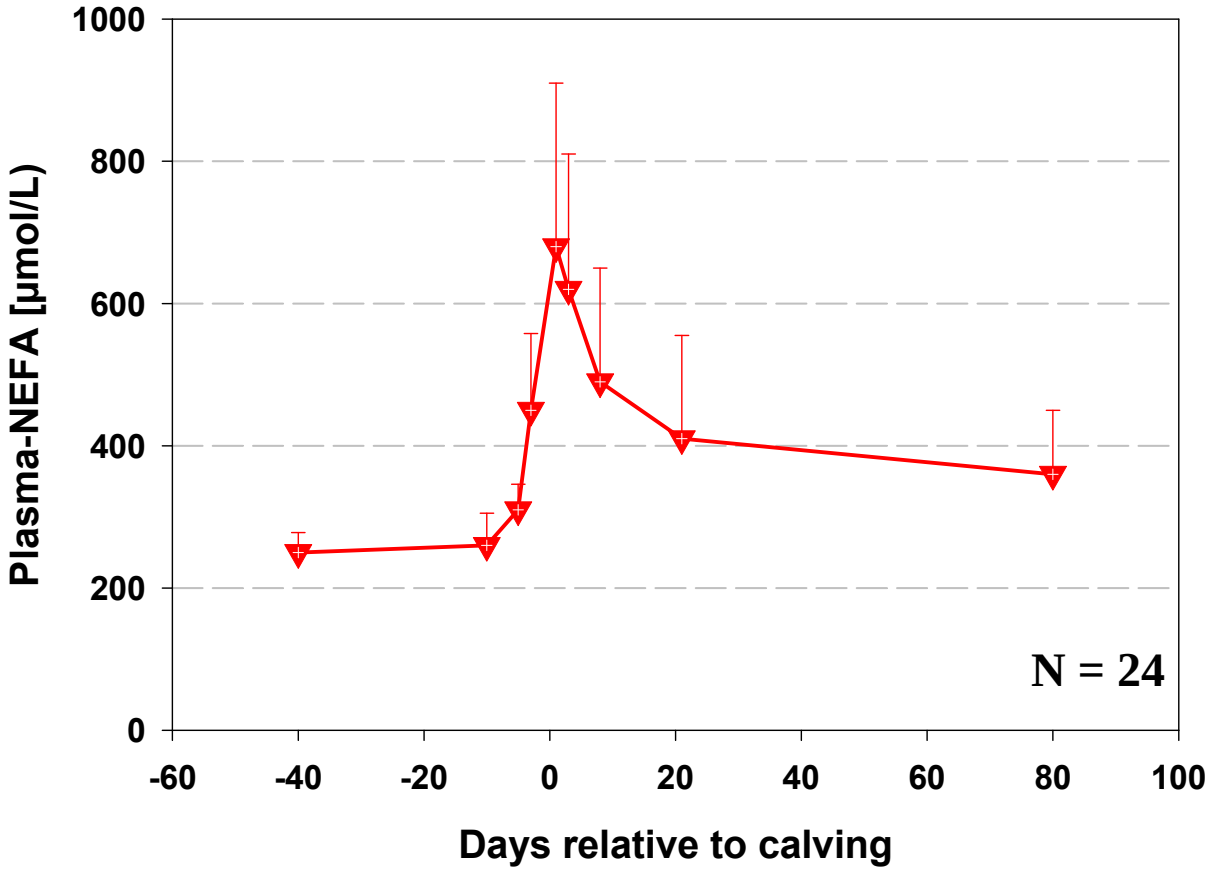
Zelluläre Glucoseaufnahme insulinabhängig, Ausnahme: Milchdrüse



Plasma Insulin

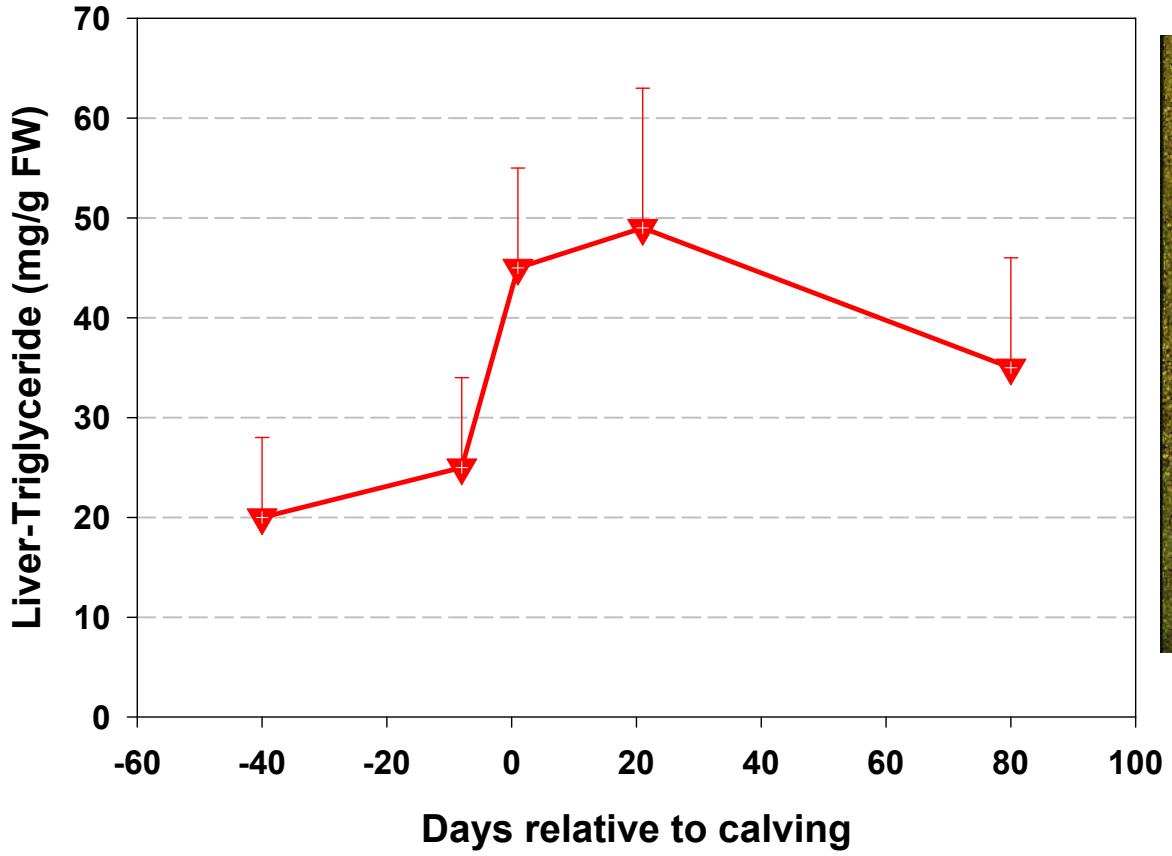


Plasma NEFA während der Transition Period



Hepatischer Triglycerid Gehalt während der Transition Periode

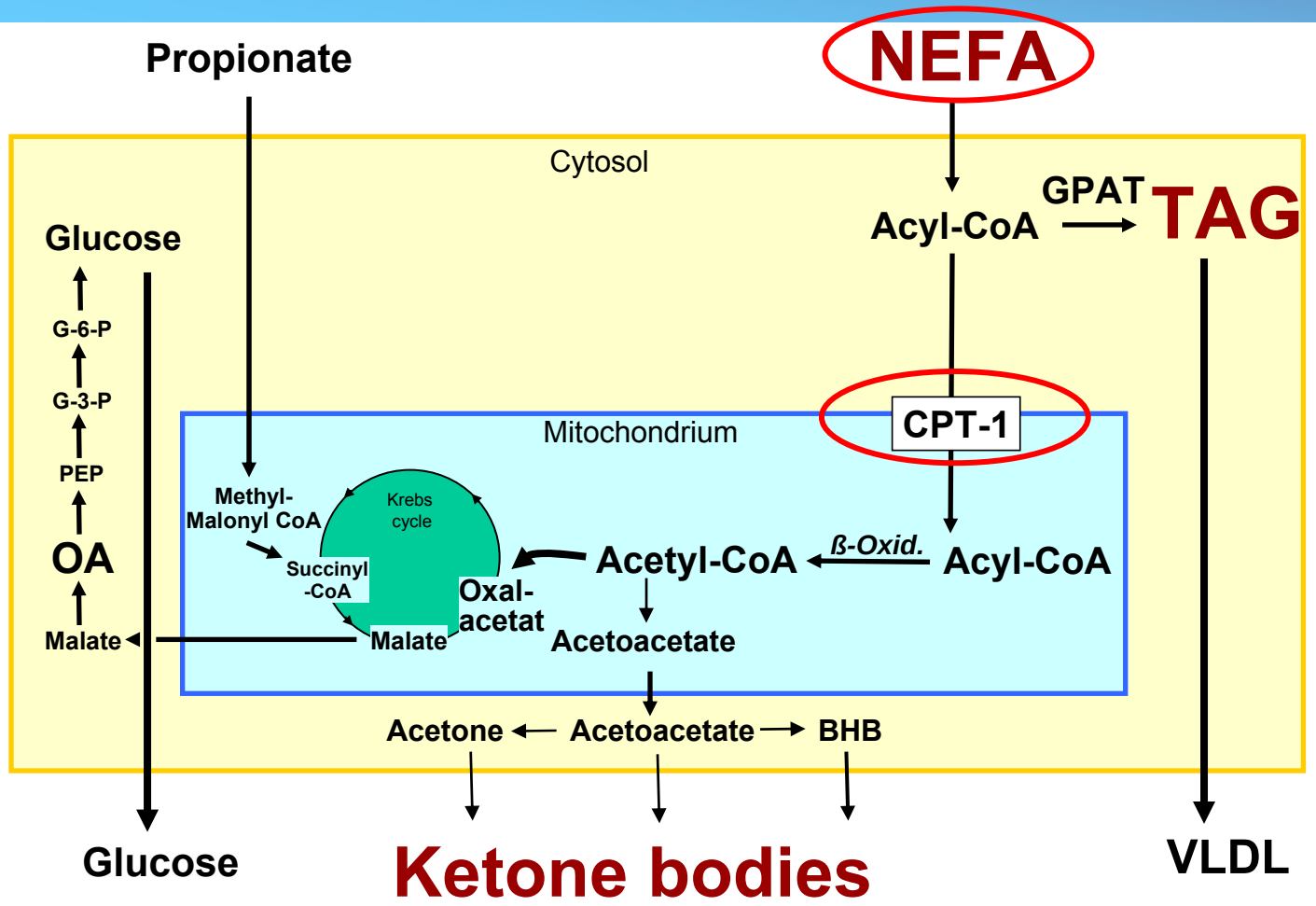
Hepatischer TAG Gehalt:
Mild: < 50 Moderate: 50 – 100 Hoch : > 100 mg/g FW



Etwa 50% der Kühe entwickeln mittel- bis hochgradige Leberverfettung

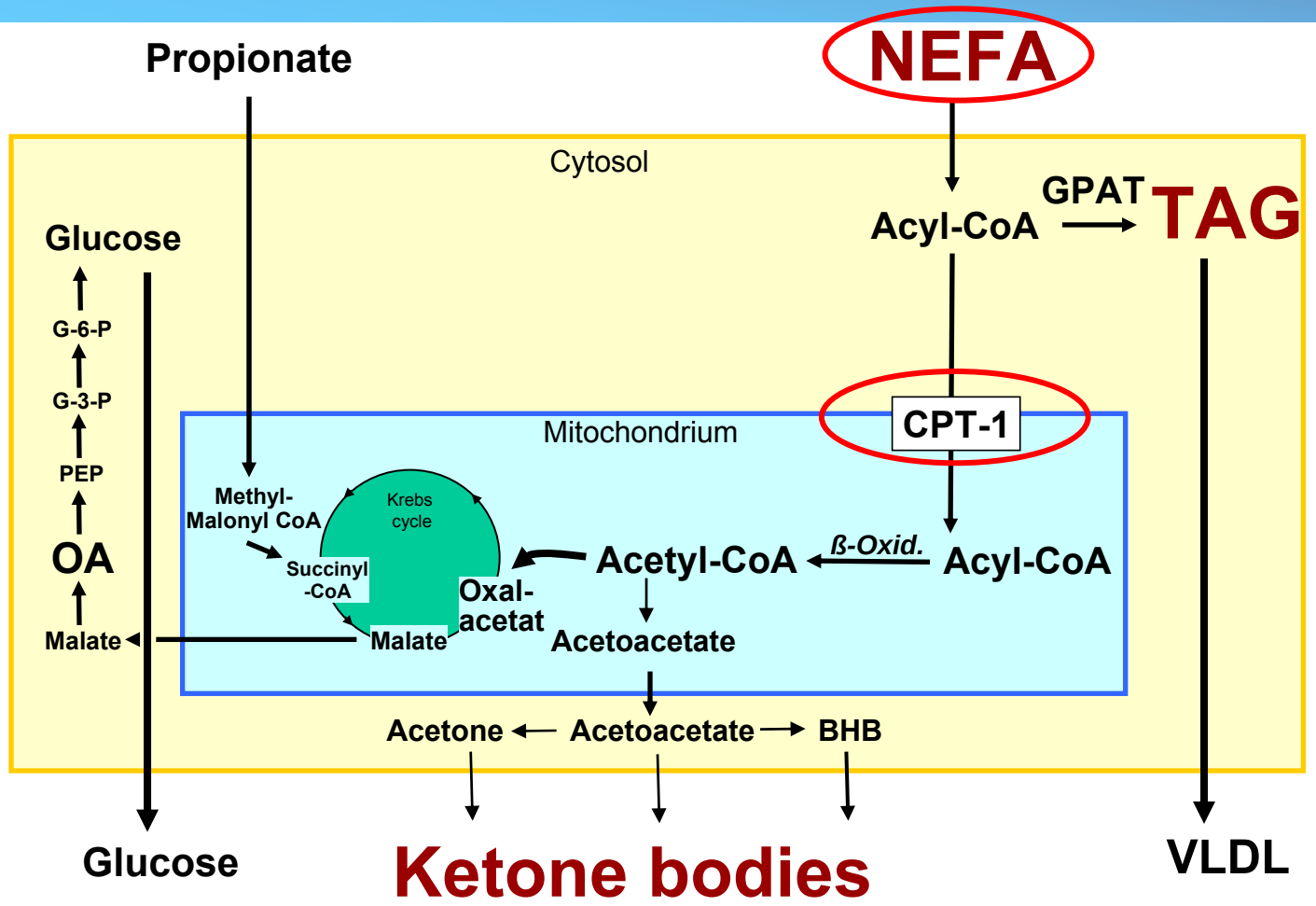
(Jorritsma et al. 2000, 2001)

Hepatischer Energiestoffwechsel



Insulin
wirkt antilipolytisch (Vernon 1992, Andersen et al 2002, Kaske et al 2002)
Vermindert die hepatische ß-oxidation (Jesse et al 1986, Drackley et al 1991, Andersen et al 2002)
Erhöht TAG Synthese (Cadorniga-Valino et al 1997)

Hepatischer Energiestoffwechsel



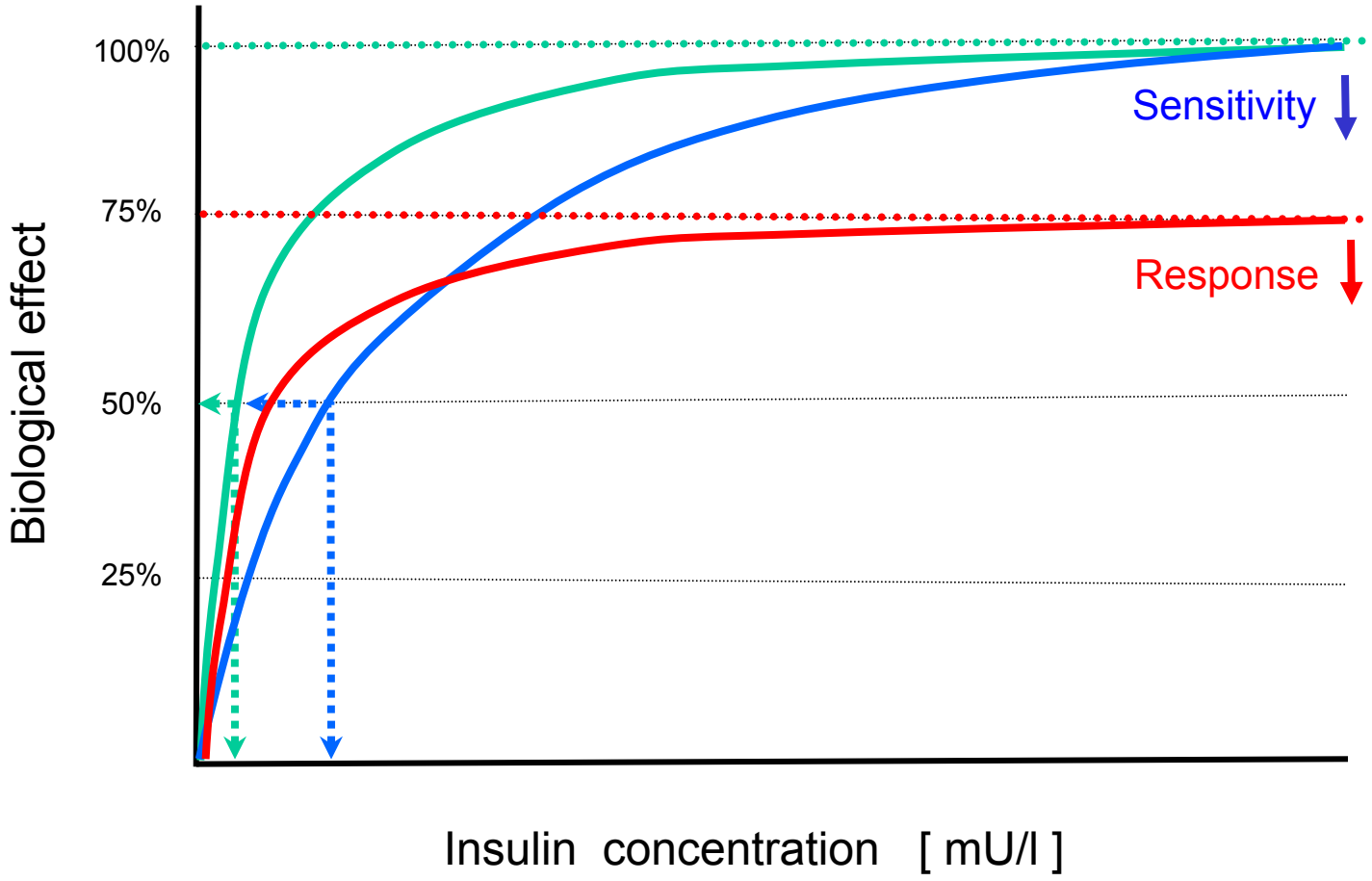
Bei excessiver Lipomobilization entwickeln Kühe entweder

Ketose Typ 1: **Insulin niedrig, Plasma Ketone hoch, wenig Leberfett oder**

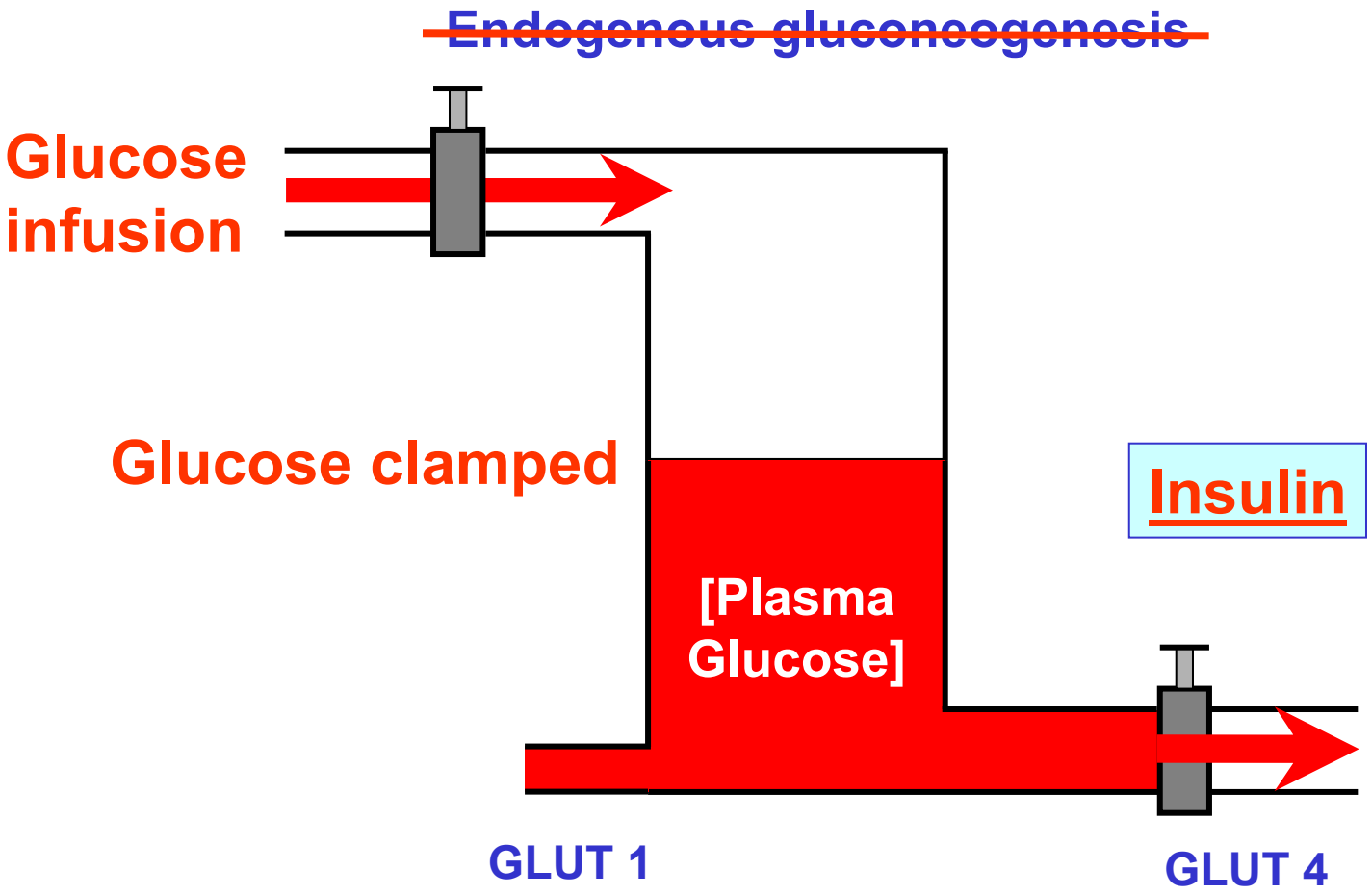
Ketose Typ 2: **Insulin hoch, Plasma Ketone niedrig, viel Leberfett**

(Holtenius& Holtenius 1996, Holtenius et al 2000)

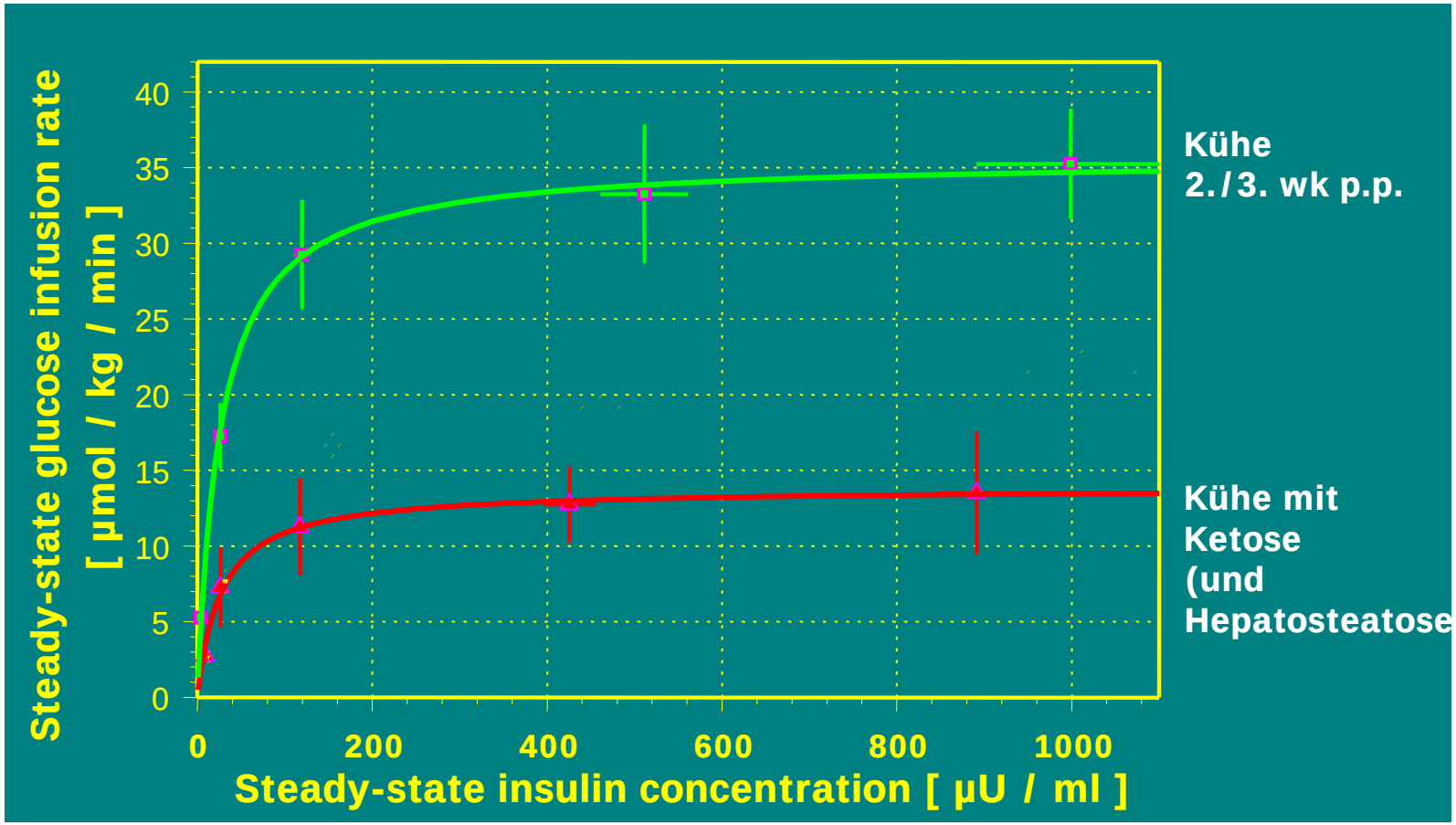
Insulin action



Principle of hyperinsulinemic, euglycemic Clamps

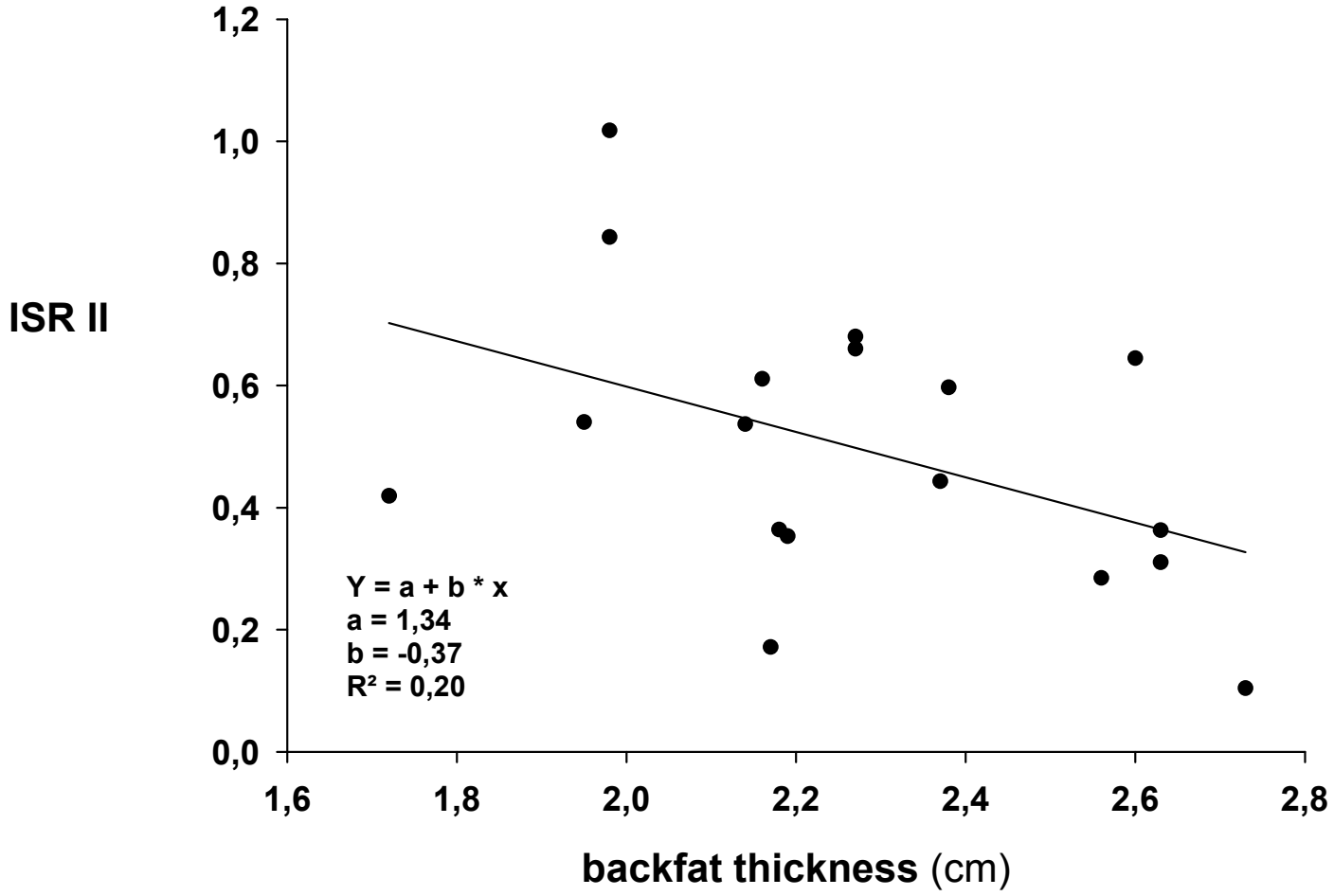


Insulin Resistenz bei Milchkühen mit Ketose/Leberverfettung

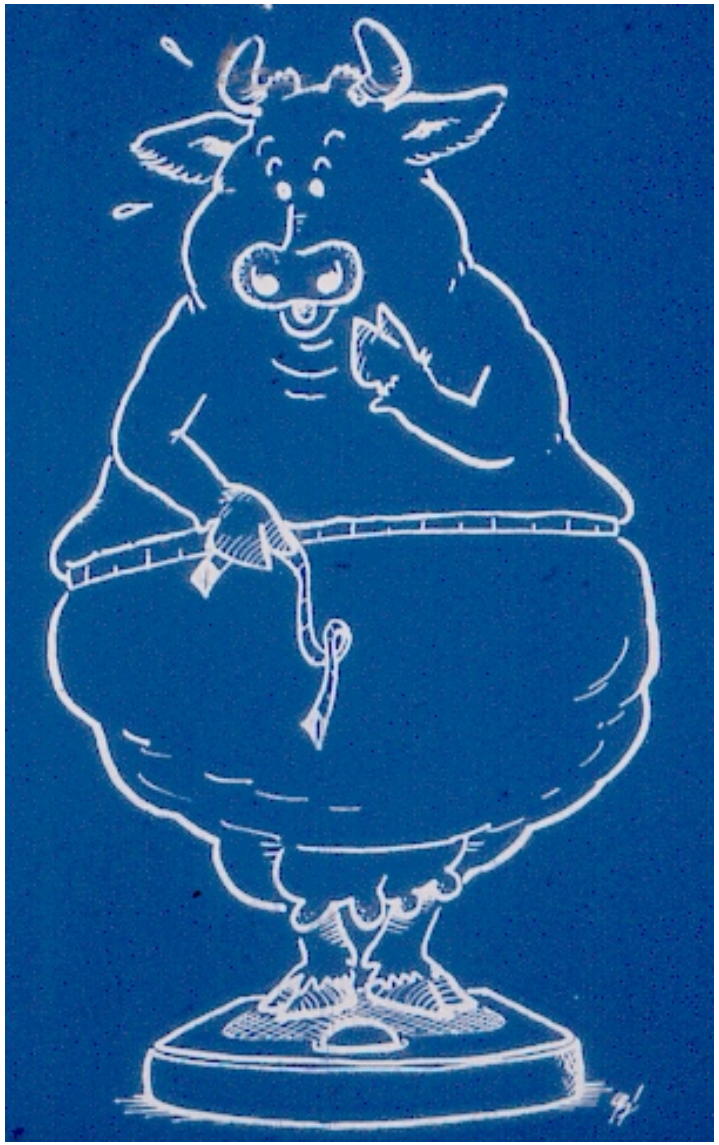


Kräft 2004

Insulin-Sentivitätsindex vs. Rückenfettdicke Kühe 180 Tage pp

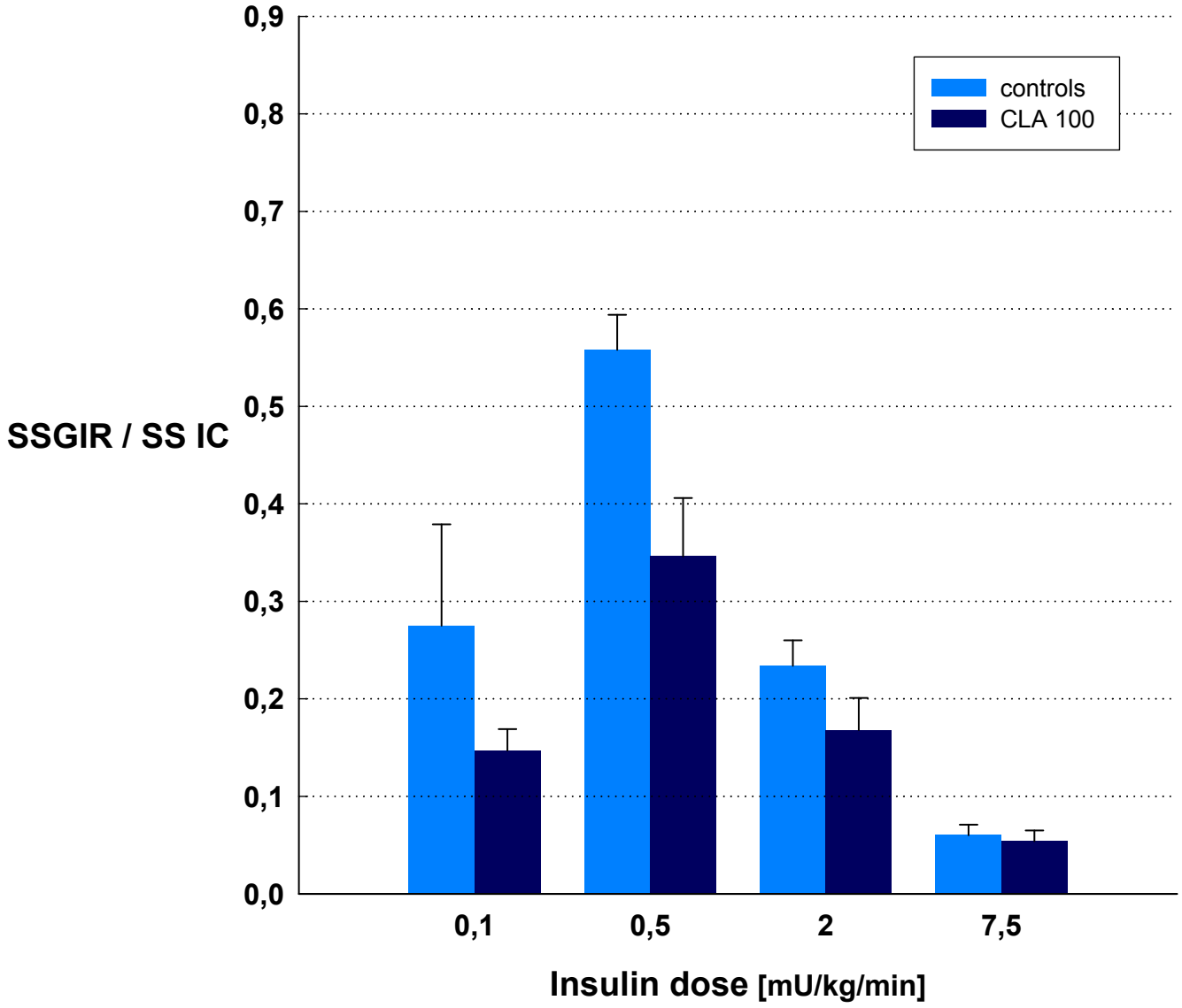


Verfettung der Kühe vermeiden

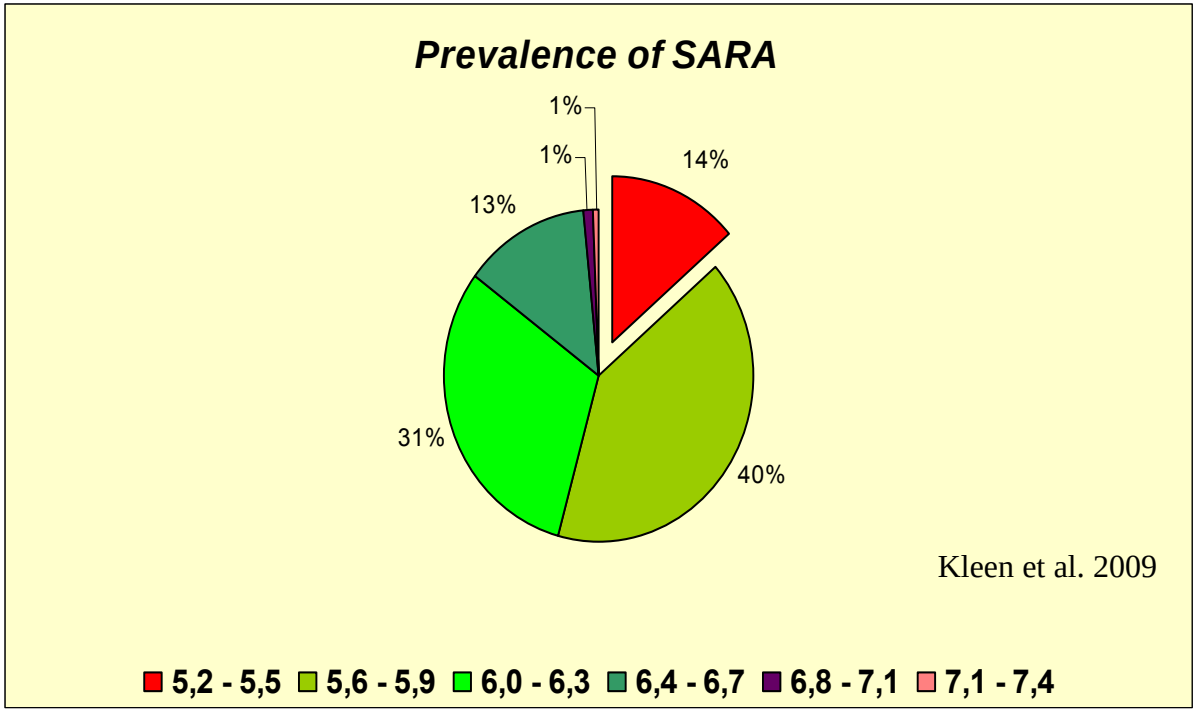


- Insulinresistenz
- Geringe TM Aufnahme post partum
- Neigung zu Schwergeburten
- Neigung zur Ret. sec
- Neigung zu Milchfieber

Insulin sensitivity ratio - G/I



Subakute Pansenazidose



Subakute Pansenazidose:

- Verminderte pankreatische Insulinausschüttung (Oetzel et al 1998) und vermutlich auch
- Periphere Insulinresistenz

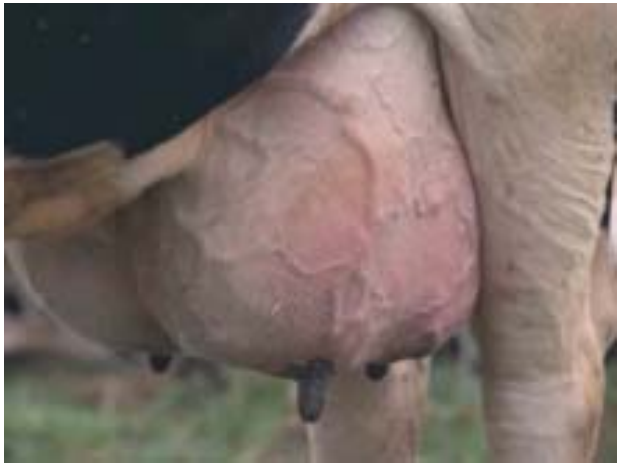
Entzündliche Krankheiten können Insulin Resistenz verursachen

Hepatosteatose vermindert die hepatische Endotoxin Entgiftung
(Andersen et al. 1996)

Endotoxine führen zu erhöhten TNF-α Blutspiegeln:

- **NEFA ↑**
- **Cortisol ↑**
- **Insulin Resistenz**

(Ohtsuka et al. 2001, Kushibiki et al. 2002)



Indices zur Bestimmung der Insulinsensitivität aus Plasmaparametern

HOMA = Glucose(mmol/l) x Insulin(µU/ml)

HOMA-IR = Glucose(mmol/l) x Insulin(µU/ml) / 22,5

QUICKI = 1/(log(Glucose(mg/dl)) + log(Insulin(µU/ml)))

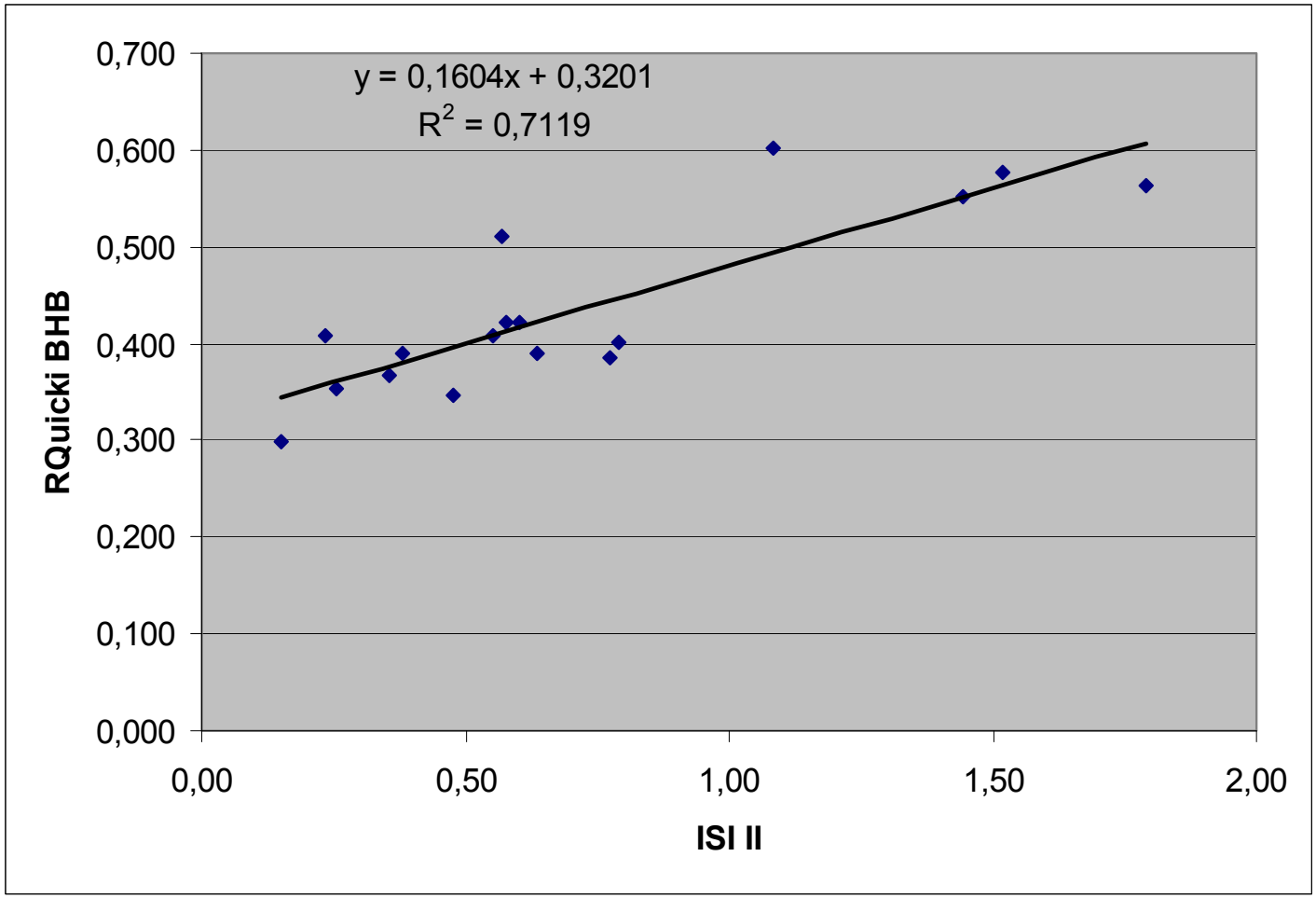
RQUICKI = 1/(log(Glucose(mg/dl)) + log(Insulin(µU/ml) + log(NEFA(mmol/l)))

QUICKI-BHB (Q-BHB) = 1/(log(Glucose(mg/dl)) + log(Insulin(µU/ml) + log(BHB(mmol/l)))

RQUICKI-BHB (RQ-BHB) = 1/(log(Glucose(mg/dl)) + log(Insulin(µU/ml) + log(NEFA(mmol/l) + log(BHB(mmol/l)))

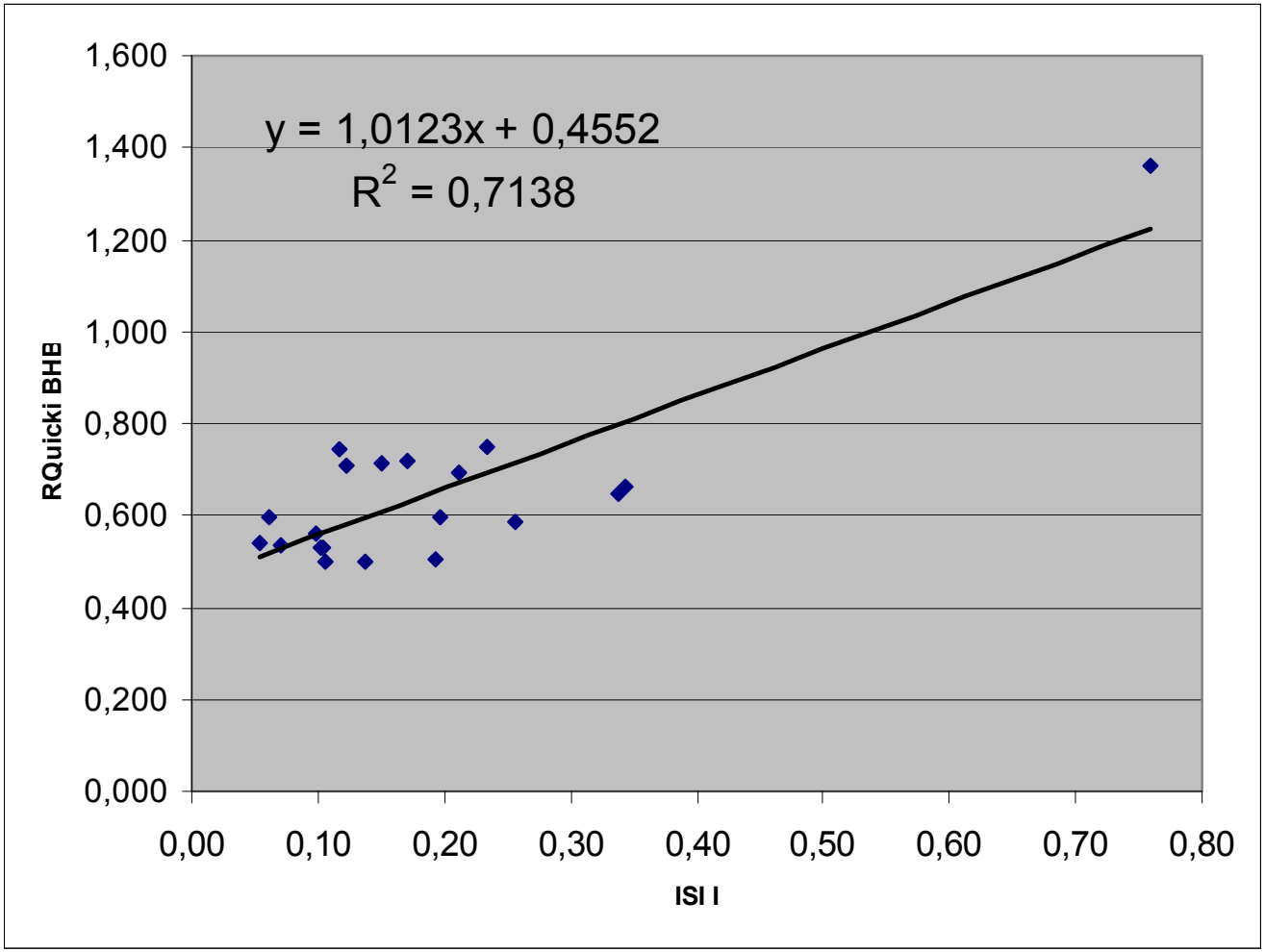
Holtenius et al 2004

RQUICKI-BHB vs ISI aus EHGC: Klinikpatienten



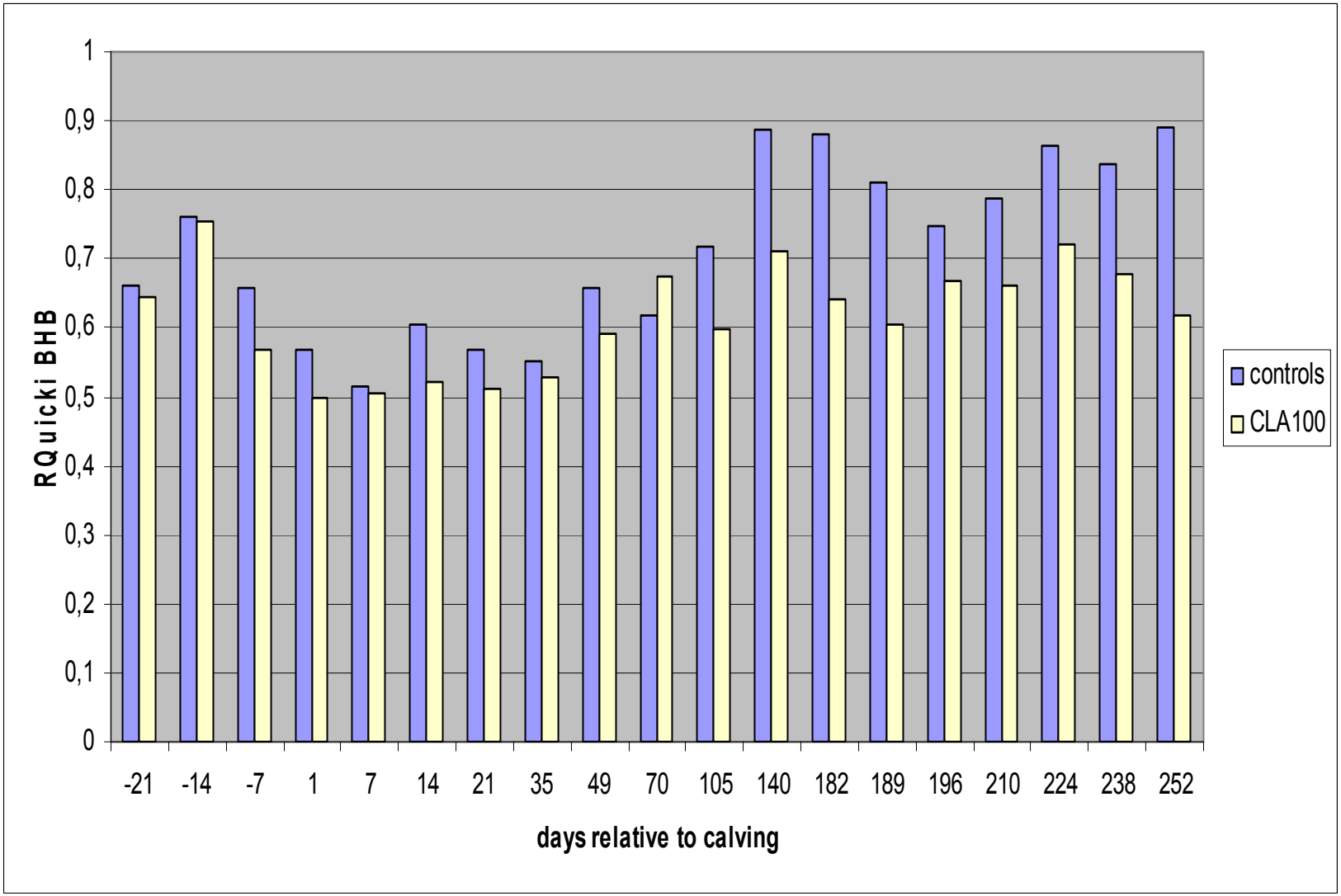
Haarstrich et al, unveröffentlicht

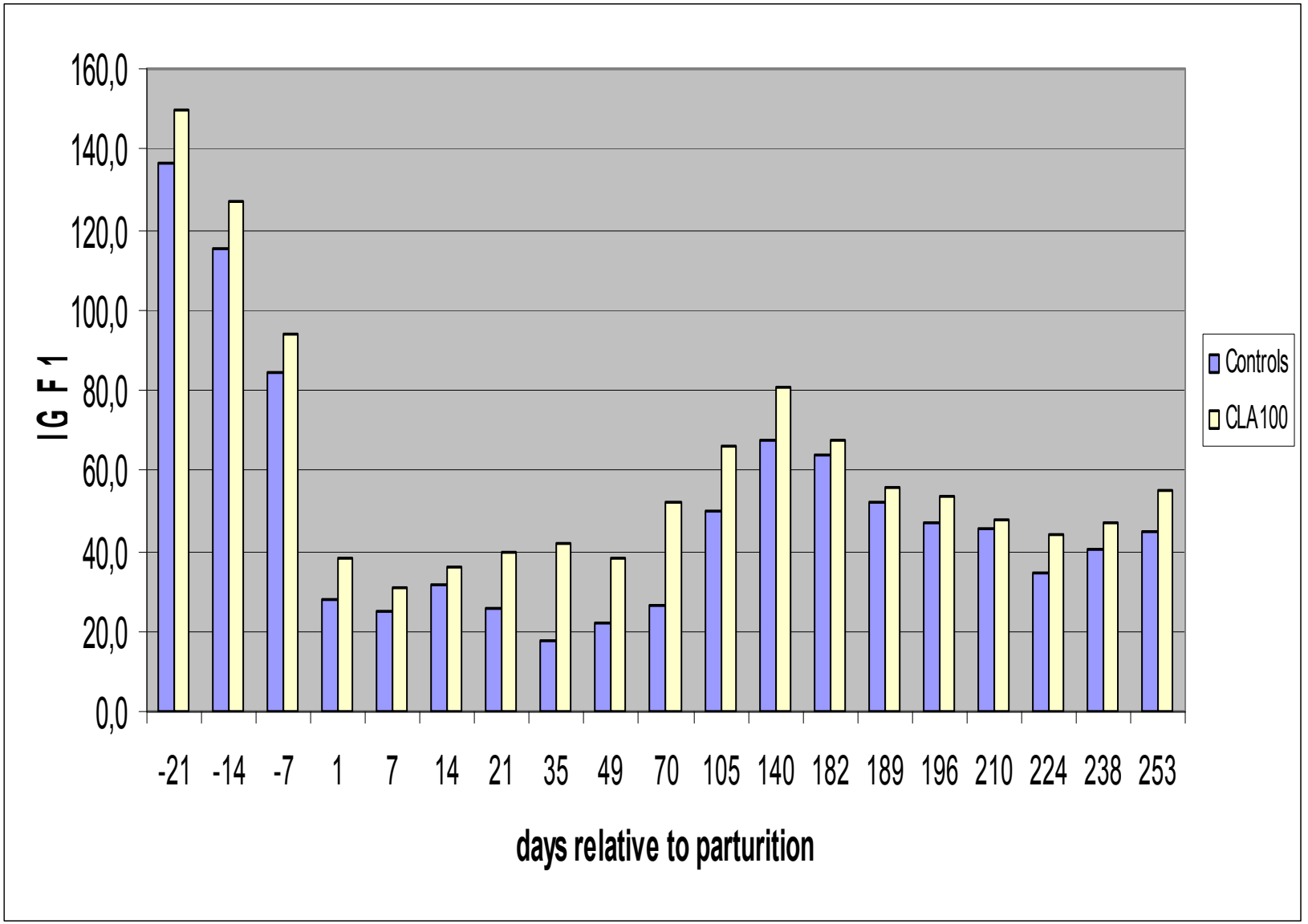
RQUICKI-BHB vs ISI aus EHGC: Gesunde laktierende Kühe 180 Tage pp

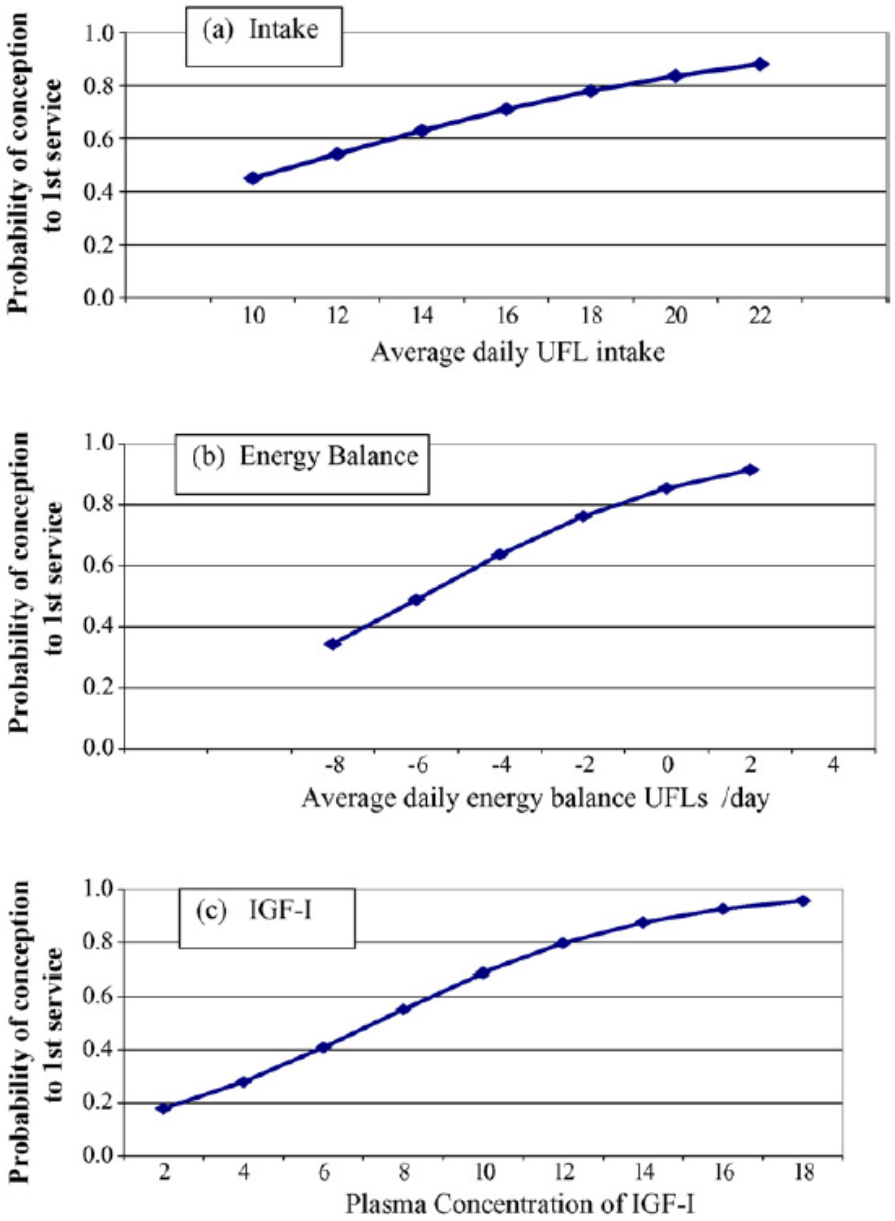


Haarstrich et al, unveröffentlicht

RQuicki BHB: CLA induzierte Insulinresistenz




160,0
140,0
120,0
100,0
80,0
60,0
40,0
20,0
0,0
IGF1
-21
-14
-7
1
7
14
21
35
49
70
105
140
182
189
196
210
224
238
253
days relative to parturition
Controls
CLA100



M.G. Diskin et al. / Animal Reproduction Science 96 (2006) 297–311

Fig. 5. Relationships between (a) average daily intake (UFL/day), (b) average daily EB (UFL/day and (c) plasma concentration of IGF-I during first 28 days of lactation and probability of conception rate to first service in dairy cows (source Patton et al., in press).

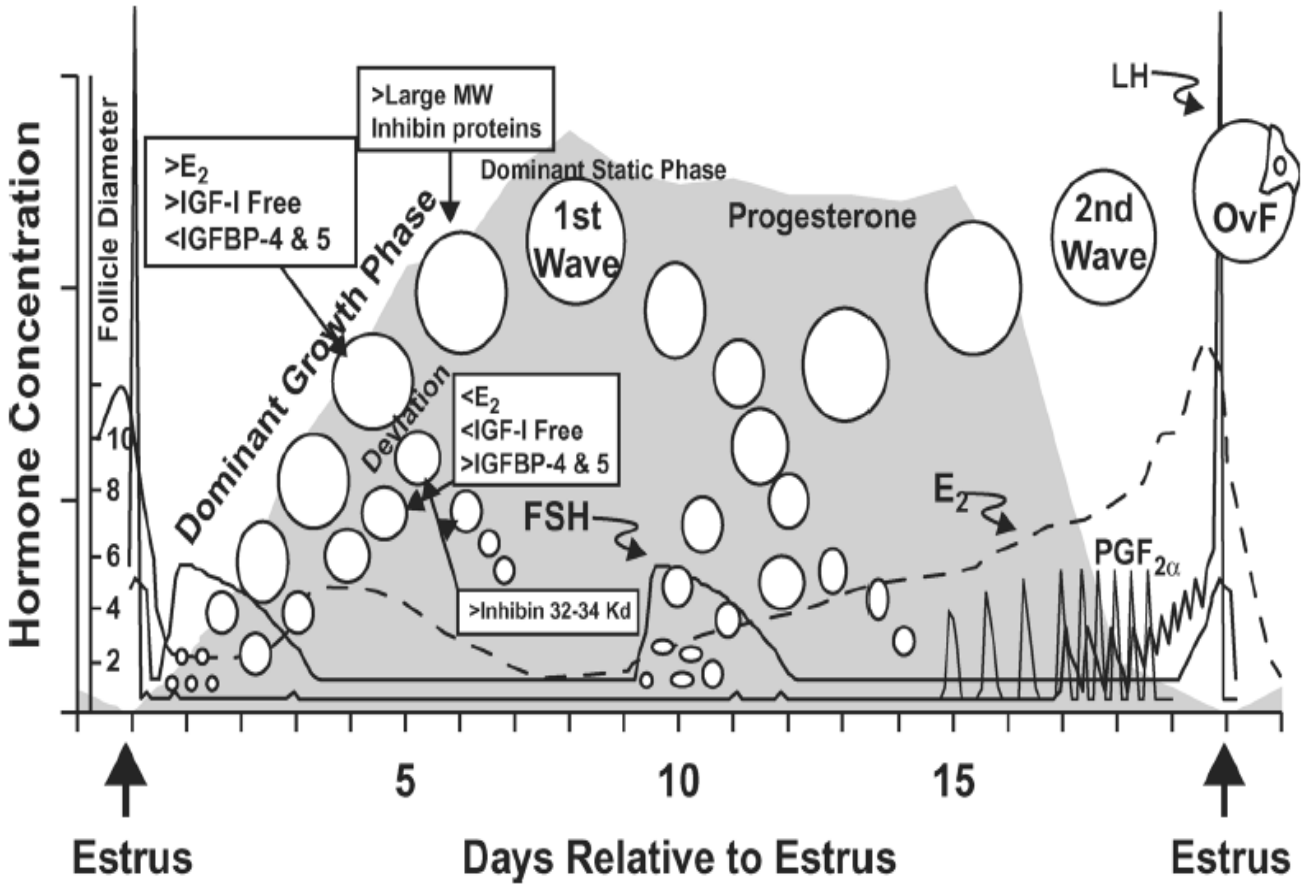
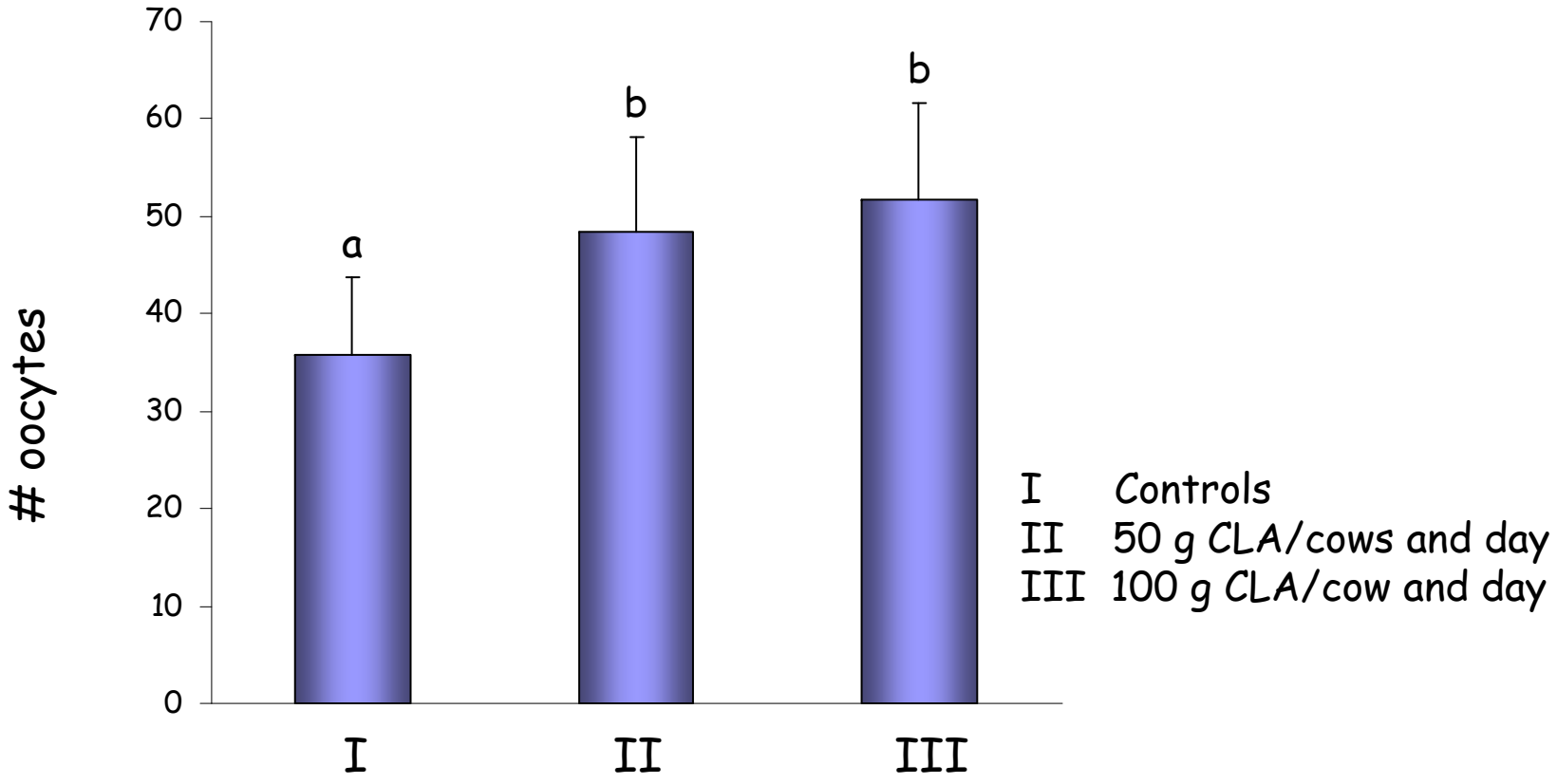


Figure 1. Ovarian follicular and corpus luteum development correlated with endocrine changes during the bovine estrous cycle. E_2 = Estradiol; IGFBP-4 and -5 = insulin-like growth factor binding proteins 4 and 5; OvF = ovulatory follicle, OvF.

K. Moore and W. W. Thatcher: J. Dairy Sci. 89 (2006):1254–1266

Number of collected oocytes / session and cow

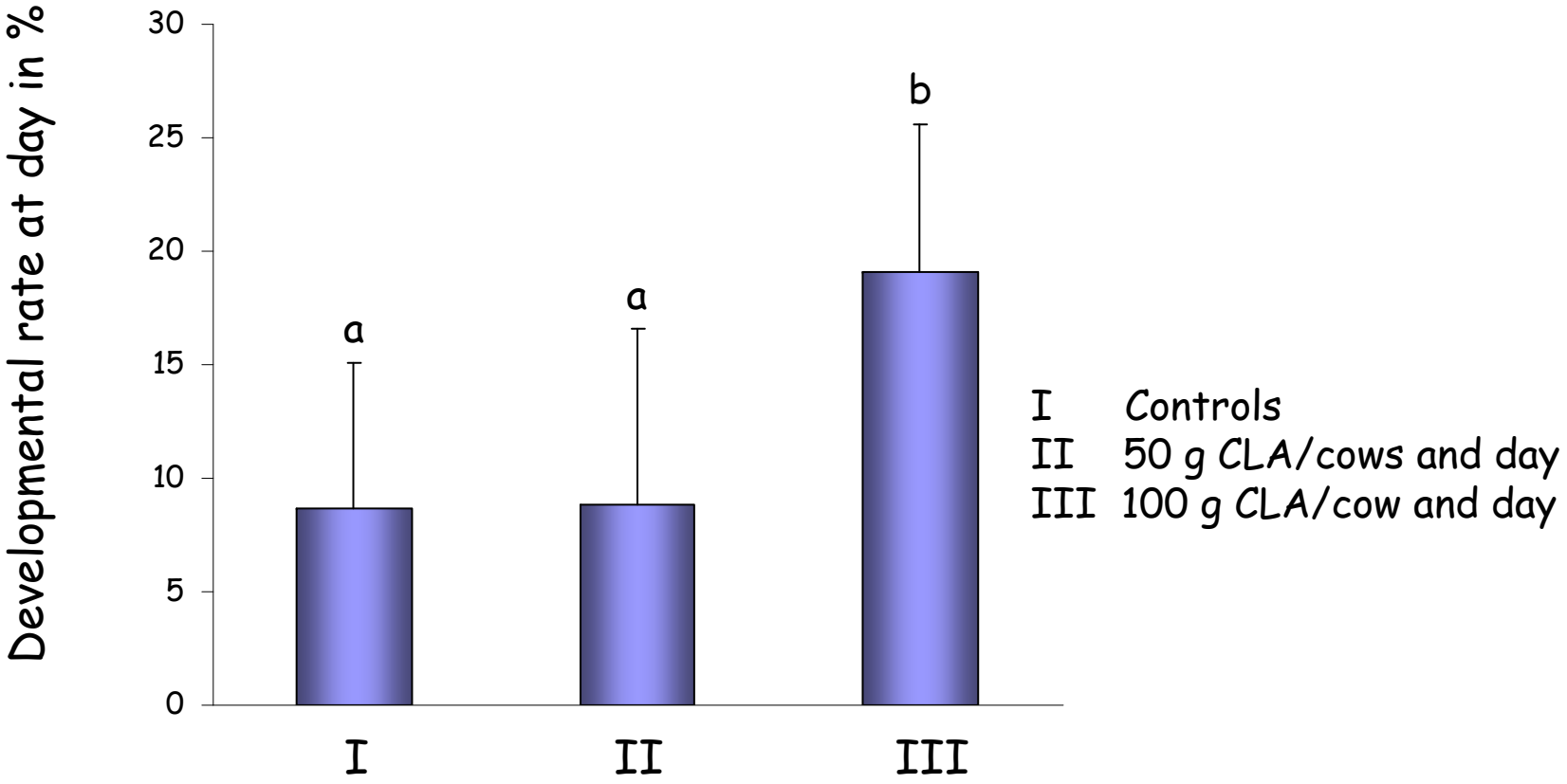
Höffmann K, A Hanstedt, E Onnen-Lübben, H Stinshoff, S Wilkening-Krass, H Bollwein and C Wrenzycki (2008)



a:b p<0,05

Developmental rate (blastocytes)

Höffmann K, A Hanstedt, E Onnen-Lübben, H Stinshoff, S Wilkening-Krass, H Bollwein and C Wrenzycki (2008)



a:b $p<0,05$

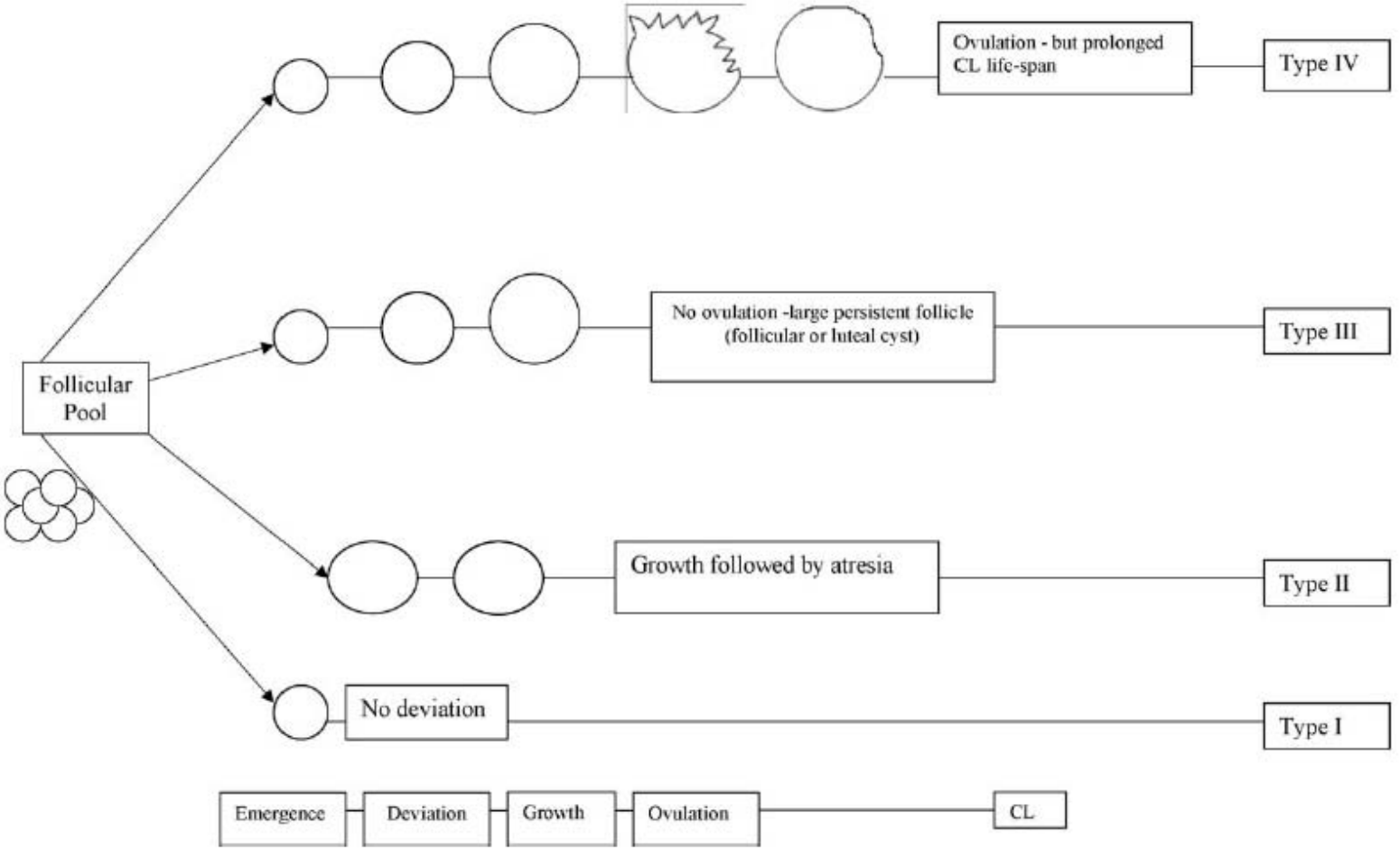


Fig. 1. Schematic representation of types of anestrous conditions based on the physiology of ovarian follicular luteal dynamics. See the text for details.

A.T. Peter et al. / Theriogenology 71 (2009) 1333–1342

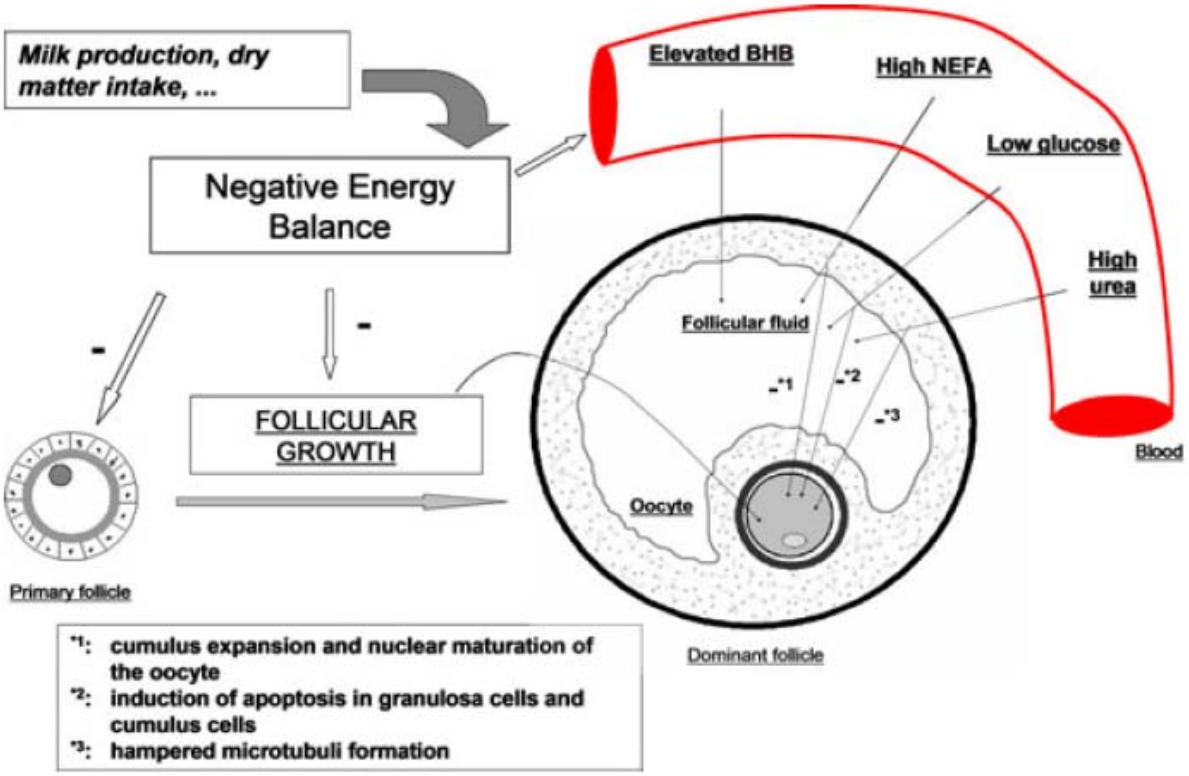


Fig. 1. Metabolic mechanisms linking negative energy balance and oocyte quality in high producing dairy cows. A status of negative energy balance is hypothesized to affect the health of the primary follicles which may have a carry-over effect on oocyte quality. An altered follicular growth pattern might impair oocyte developmental competence. Biochemical parameters, associated with a negative energy status, are well reflected in the follicular fluid and can directly affect oocyte competence. NEFA: non-esterified fatty acids, β-OHB: β-hydroxybutyrate

JLMR Leroy, G Opsomer, A Van Soom, IGF Goovaerts and PEJ Bols: Reprod Dom Anim 43, 612–622 (2008)

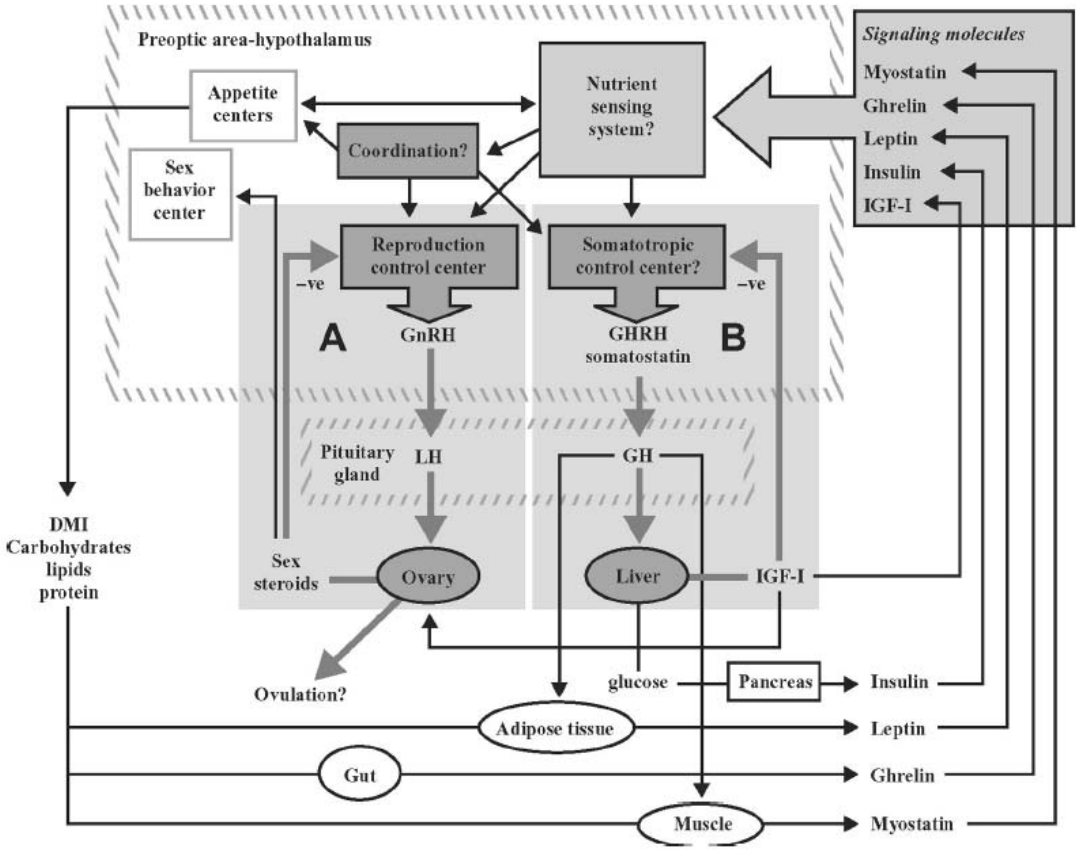
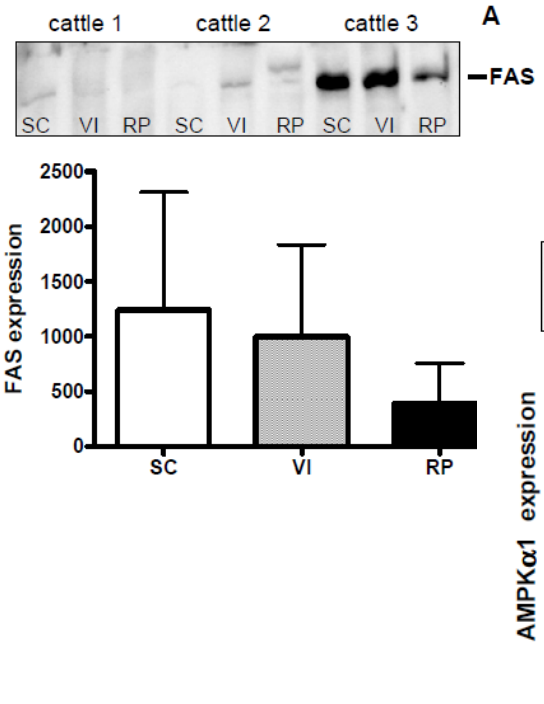
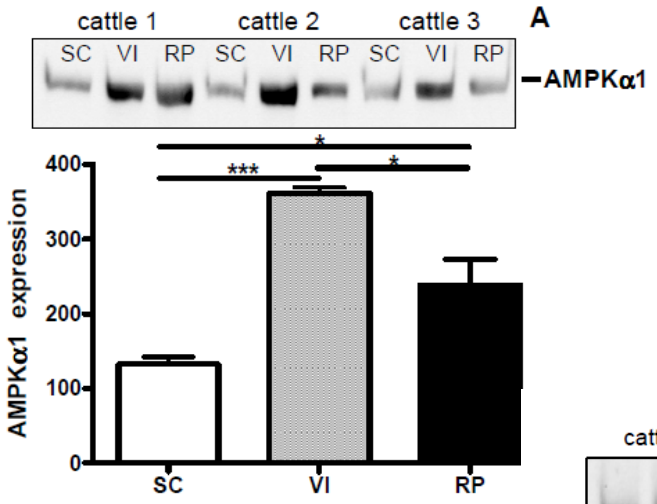
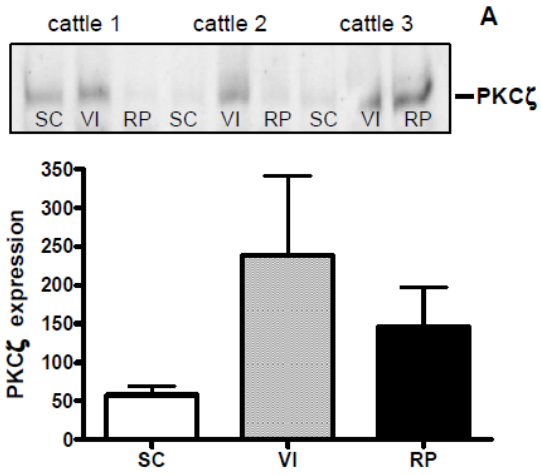


Figure 2. The feedback-regulated systems that control the reproductive axis (shaded area A) and the somatotropic axis (shaded area B) interact at several levels and thus link nutritional and metabolic inputs into the reproductive process. Note that for the reproductive axis, FSH is omitted because it does not appear to limit dairy cow fertility. In addition, for the sake of clarity, the thyroid and adrenocortical axes have been omitted: they are both regulated by their own feedback loops in the brain-pituitary system, both are intimately linked with lactation, both respond to metabolic inputs, and both affect the reproductive and somatotropic systems, so they introduce inputs from stressors such as high ambient temperature, disease, and the antagonistic interactions associated with establishment of social dominance. With respect to sex-steroid feedback, positive feedback for induction of the preovulatory surge has been omitted.

LM Chagas et al: J. Dairy Sci. 90 (2007):4022–4032







Huber et al, unveröffentlicht

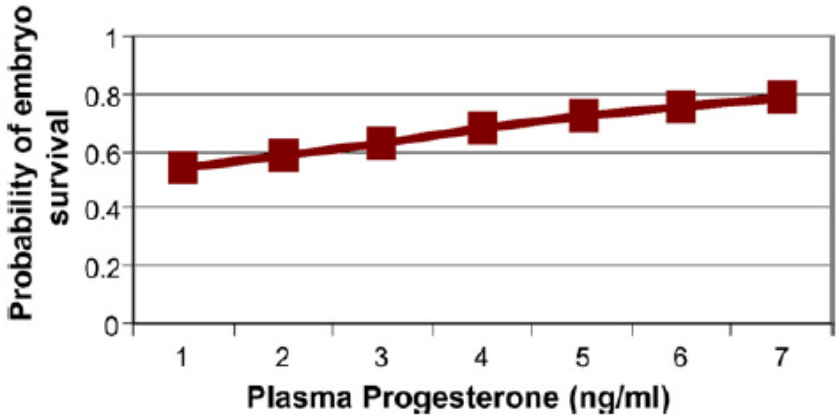
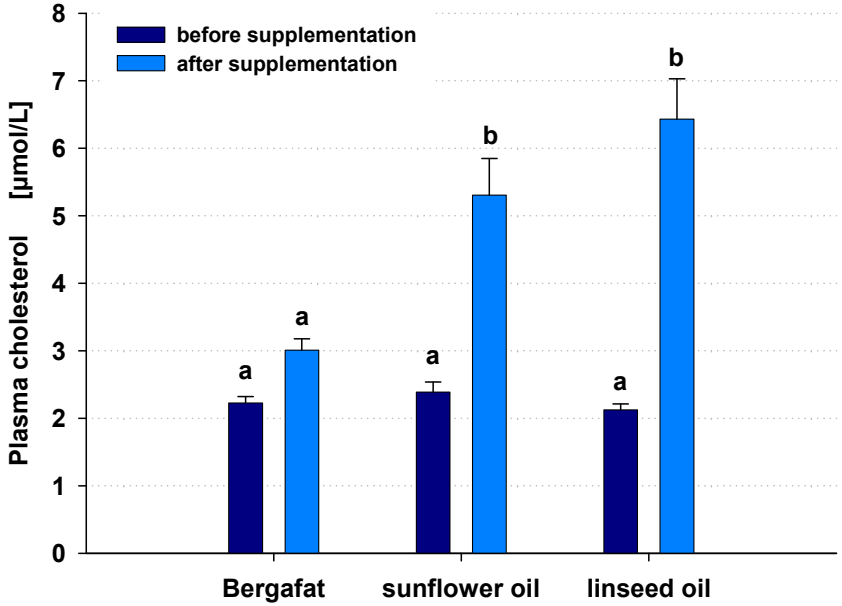


Fig. 2. Relationship between plasma concentrations of progesterone on the day of induced luteolysis and subsequent embryo survival rate.

M.G. Diskin et al. / Animal Reproduction Science 96 (2006) 297–311

Strategies to improve reproduction performance by fat supplementation

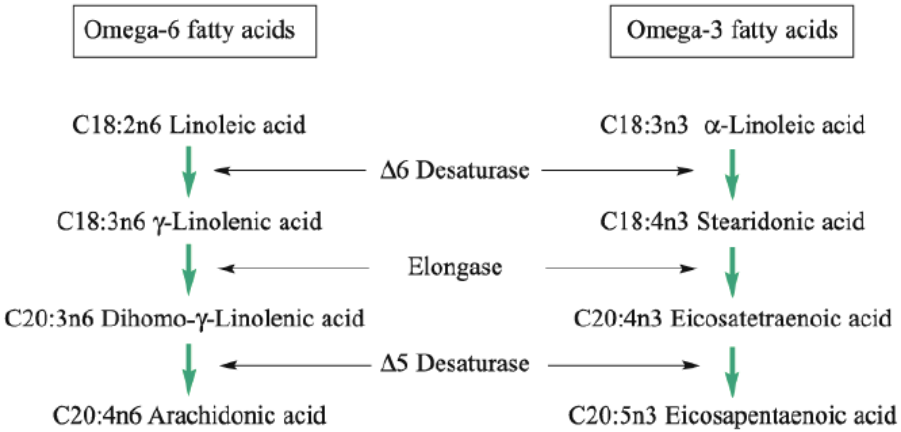
- Supplementary fats are likely to affect fertility because fatty acids (FA) are the precursors both of prostaglandins (PG) and, via cholesterol, the steroid hormones (Lucy et al., 1992)
- Omega 3 FA increase plasma progesterone (Staples et al, 1997, Petit et al, 2002)



Strategies to improve reproduction performance by fat supplementation

- Treatments that reduce ovarian and endometrial synthesis of PGF2*α and* favour PGF3*α* production may contribute to a reduction in embryonic mortality (Mattos et al., 2000).
- Feeding omega3 FA (linseed, maritine FA) increase plasma PGF3*α (Petit et al, 2002)*

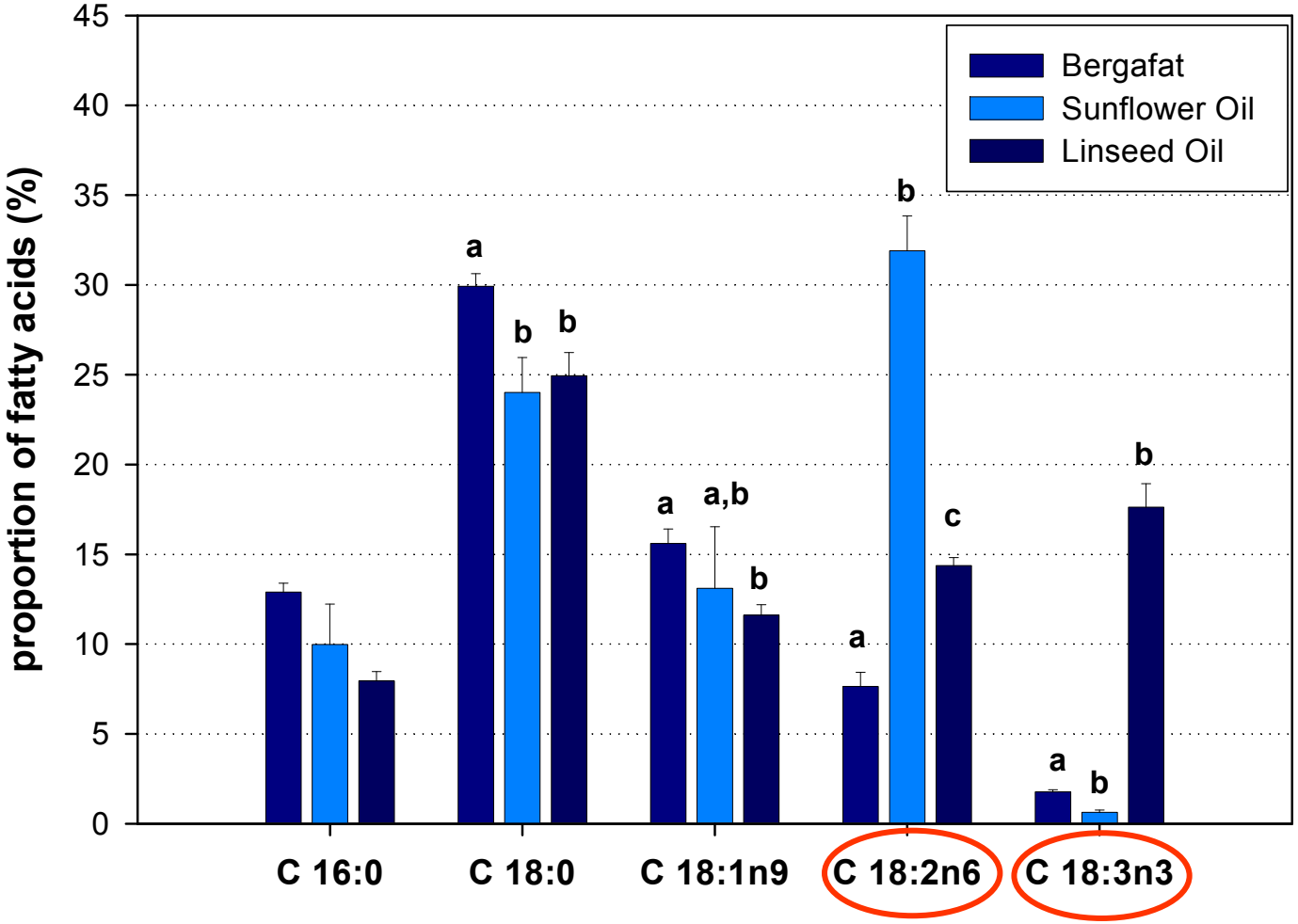
Figure 2. Schematic pathway of omega-6 and omega-3 fatty acid synthesis.



PGF2α

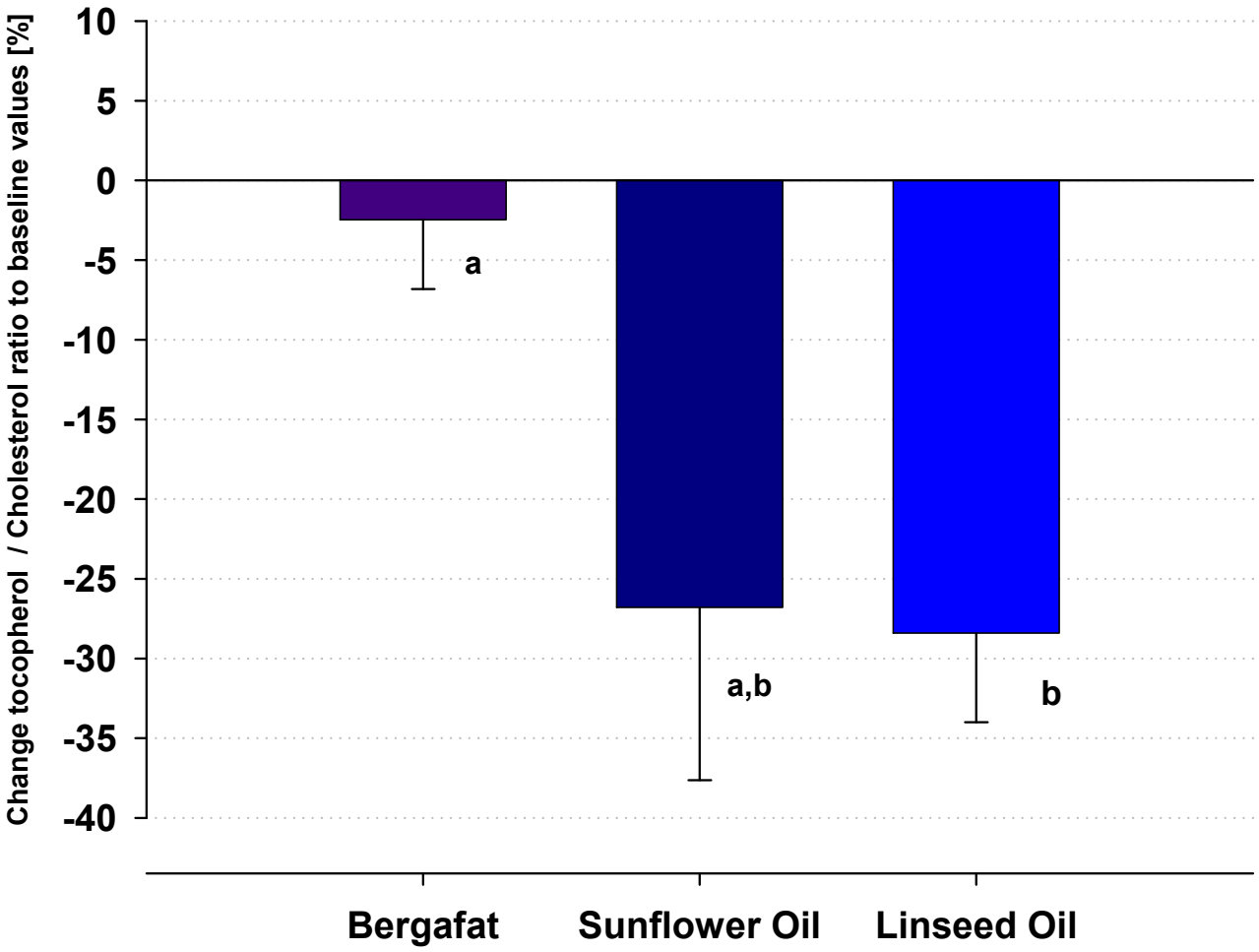
PGF3α (little biological activity)

Hepatic fatty acid composition of phospholipids after fat supplementation



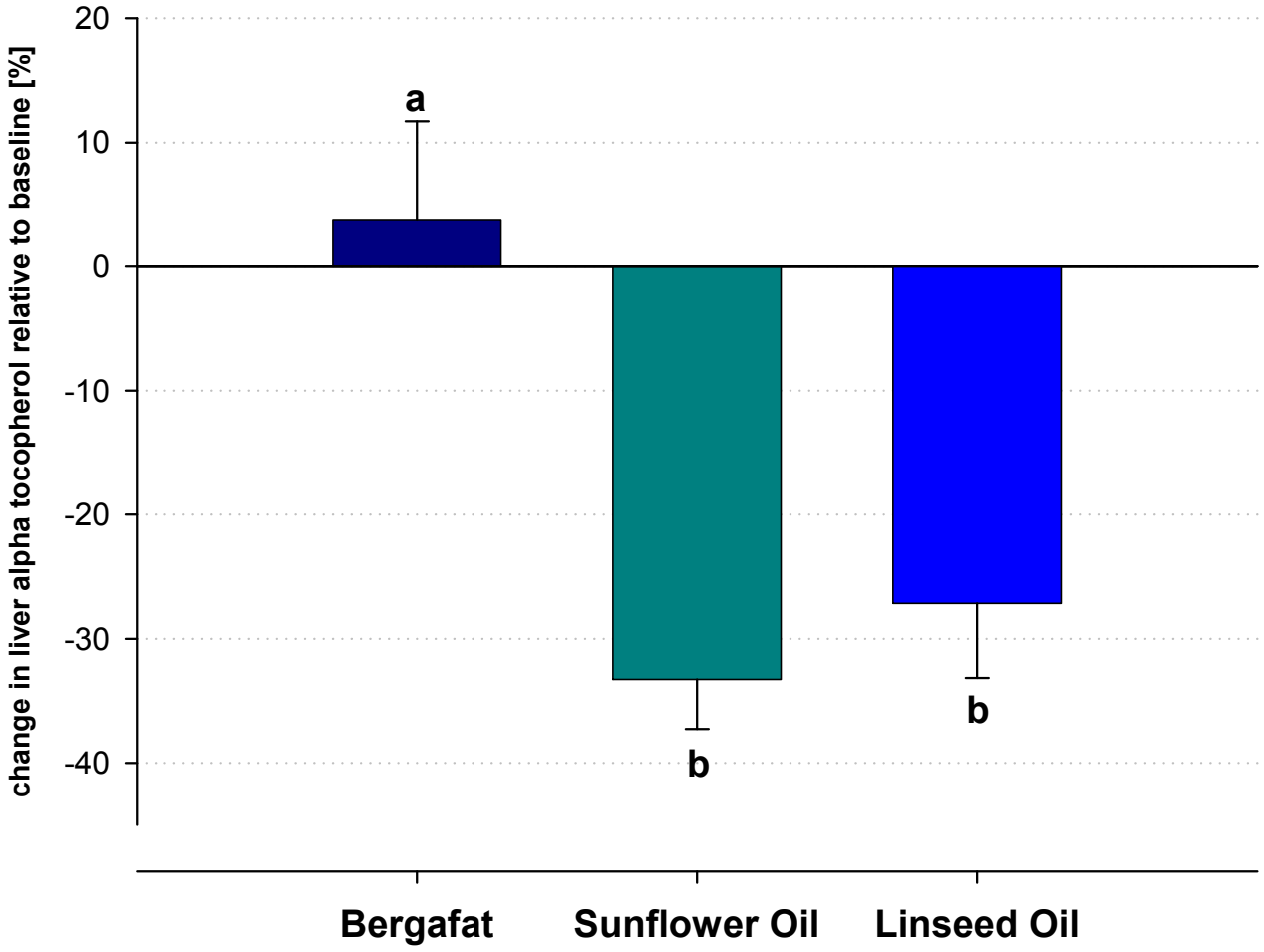
Portmann et al 2002

Plasma alpha tocopherol / cholesterol ratio



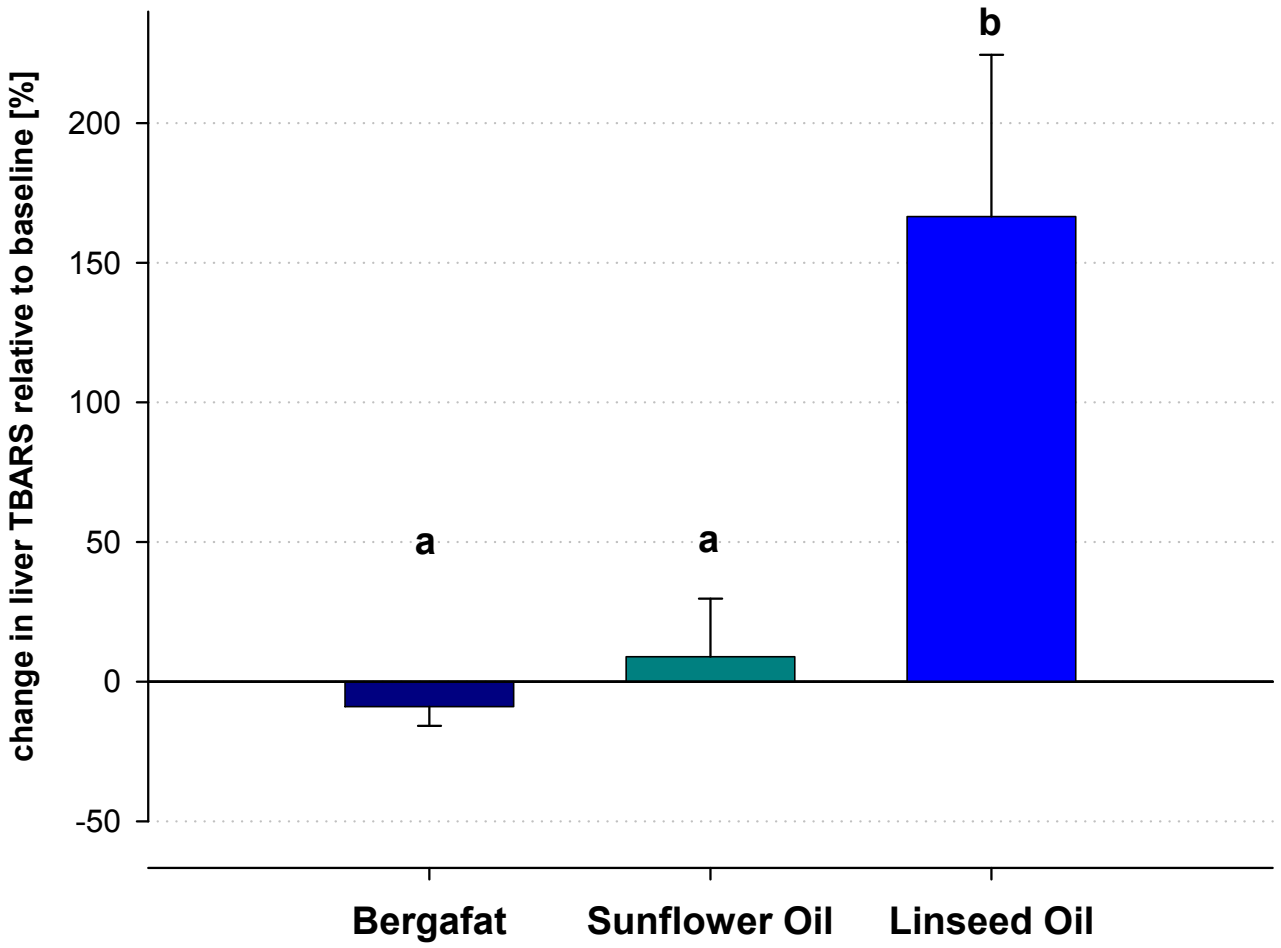
Portmann et al 2002

Hepatic alpha tocopherol



Portmann et al 2002

Liver malondialdehyde (TBARS)



Portmann et al 2002

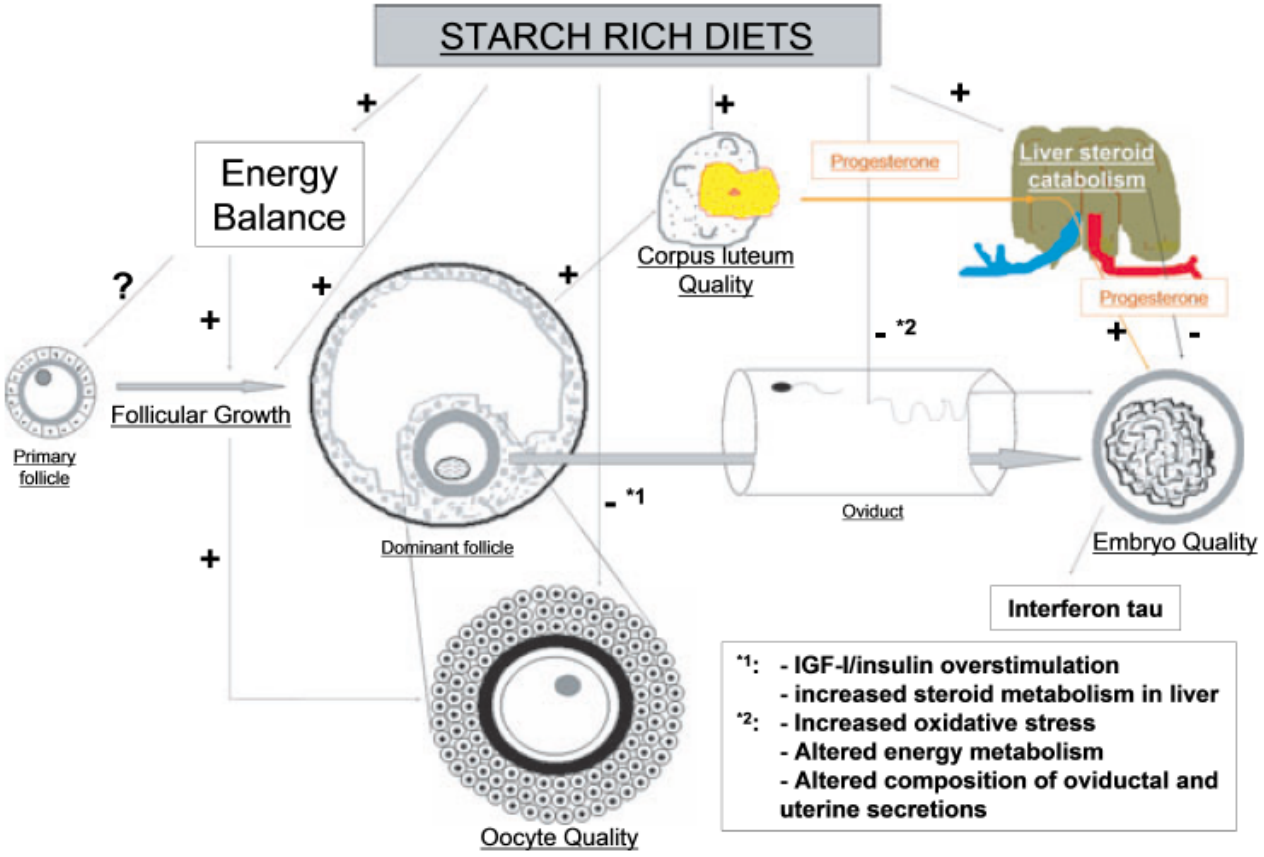


Fig. 1. Diagrammatic presentation of the major mechanisms through which starch-rich diets can affect oocyte and embryo quality in high-producing dairy cows. Starch-rich diets have a glucogenic effect on the energy balance which promotes follicular growth and steroid production which has a positive effect on oocyte quality. A sound follicle gives rise to a healthy corpus luteum, producing adequate amounts of progesterone, capable of supporting early pregnancy

JLMR Leroy, A Van Soom, G Opsomer, IGF Goovaerts and PEJ Bols: Reprod Dom Anim 43, 623–632 (2008)

I. Martin Sheldon, James Cronin, Leopold Goetze, Gaetano Donofrio, and Hans-Joachim Schuberth: BIOLOGY OF REPRODUCTION 81, 1025–1032 (2009)

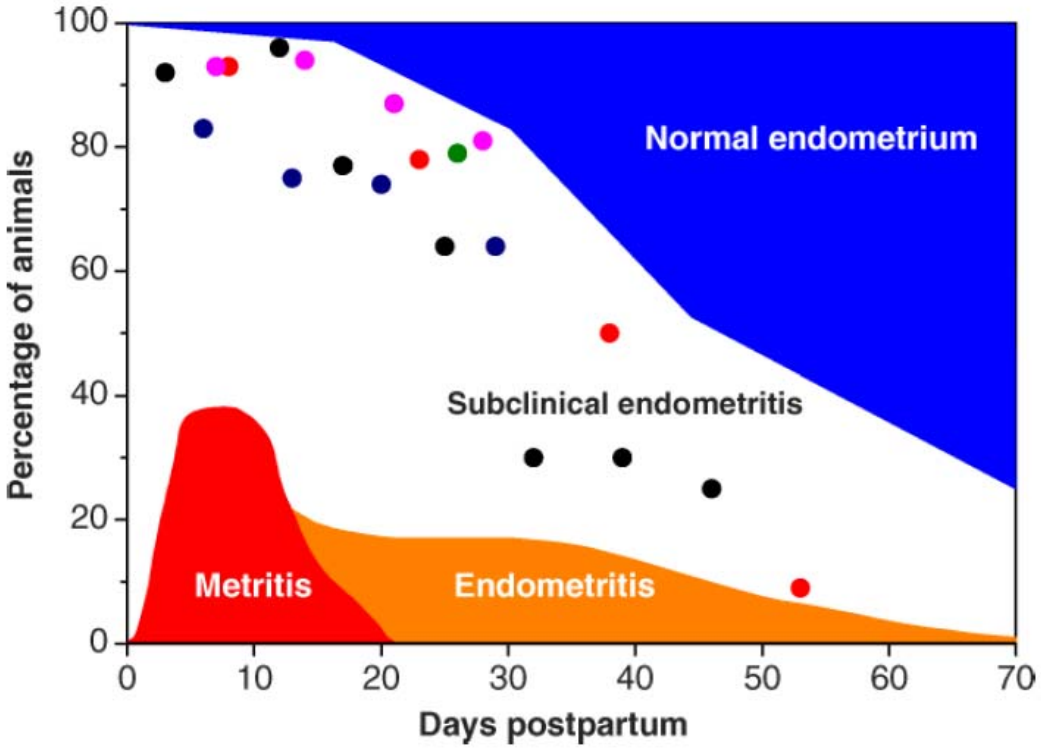


FIG. 1. The incidence of uterine bacterial infection and disease in postpartum dairy cattle. Bacteria can be isolated from the uterus of most cows during the postpartum period; each marker (circle) indicates the percentage of animals with bacteria isolated from the uterine lumen [10–14]. The shaded areas represent estimates of the proportion of animals with metritis (red), clinical endometritis (orange), or a normal uterus (blue); the remainder of animals have subclinical endometritis [15].

I. Martin Sheldon, James Cronin, Leopold Goetze, Gaetano Donofrio, and Hans-Joachim Schuberth: BIOLOGY OF REPRODUCTION 81, 1025–1032 (2009)

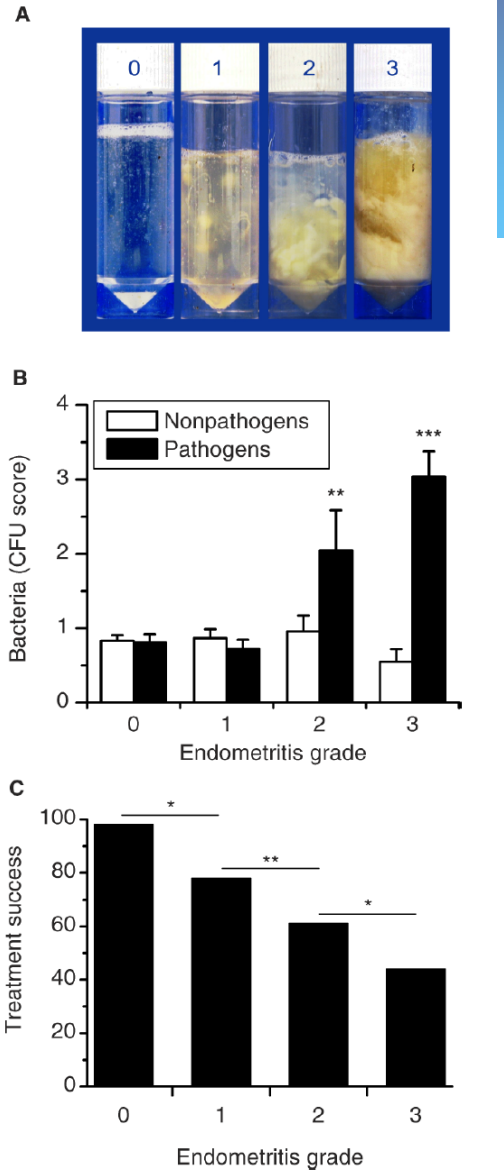


FIG. 2. Grading scheme for clinical endometritis. **A**) Vaginal mucus character is graded as 0 (clear or translucent mucus), 1 (mucus containing flecks of white or off-white pus), 2 (exudate containing <50% white or off-white mucopurulent material), or 3 (exudate containing ≥50% purulent material, usually white or yellow but occasionally sanguineous) [2]. **B**) Endometritis grades reflect the number of pathogenic (black bars) but not opportunist nonpathogenic (white bars) bacteria isolated from the uterus of cattle [11]; data are presented as semiquantitative scores of the number of colony-forming units (CFU) from uterine swabs, where CFU is scored as 0 (no growth), 1 (<10 CFUs), 2 (10–100 CFUs), 3 (101–500 CFUs), or 4 (>500 CFUs). Values differ from endometritis grade 0, $^{**}P < 0.01$ and $^{***}P < 0.001$. **C**) Endometritis grade is prognostic for treatment success [22]; treatment success rates were determined as the percentage of

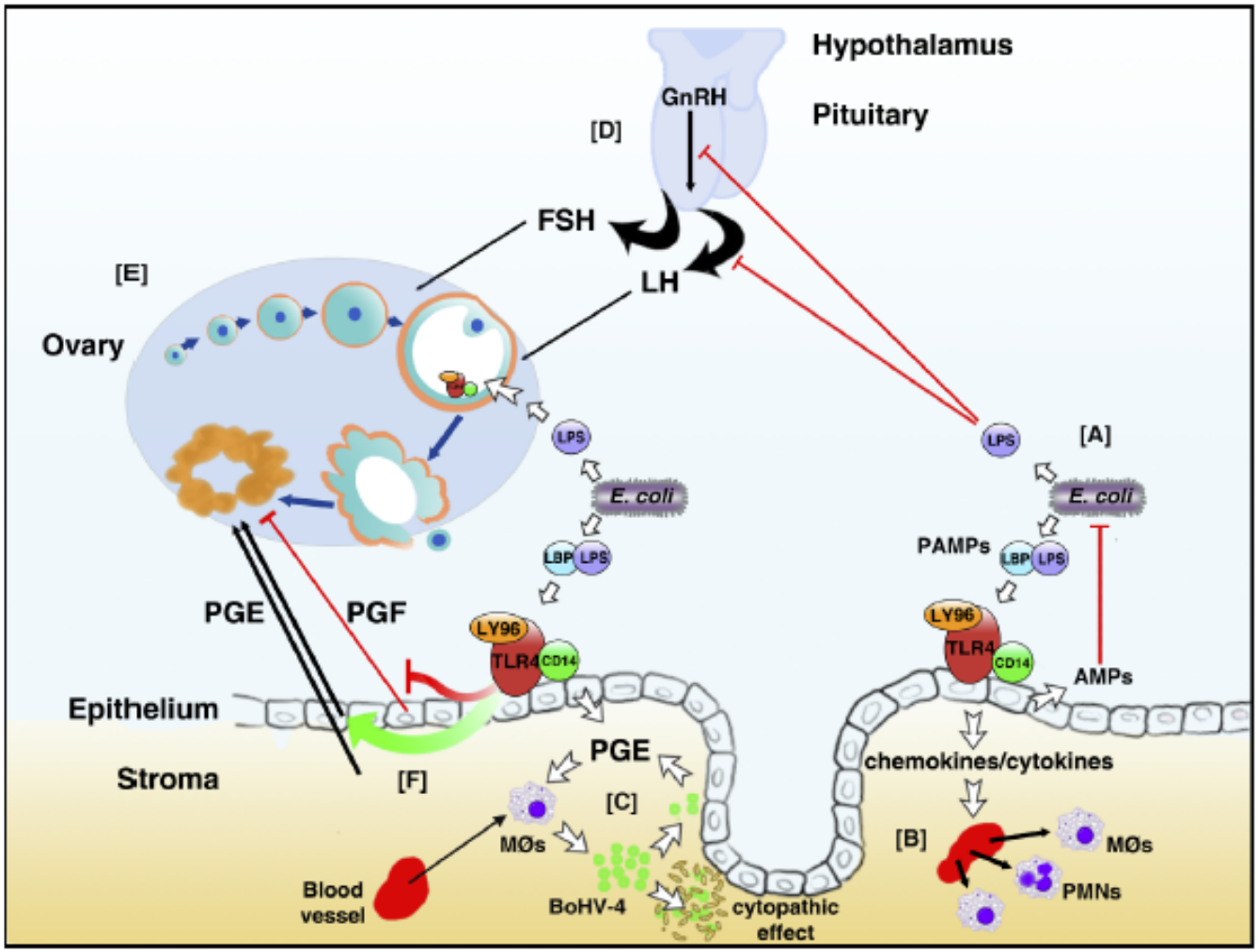


FIG. 3. The mechanisms underlying infertility associated with uterine disease. **A**) Bacterial infection with *E. coli* and *A. pyogenes* is common after parturition [10]. The innate immune system is alerted by endometrial cell TLRs detecting pathogen-associated molecules (such as bacterial DNA and lipids) and *E. coli* LPS, which is bound to LPS-binding protein (LBP) [4, 5]. The bovine endometrial cells secrete cytokines and chemokines to direct the immune response, increase the expression of AMPs, and secrete principally PGE rather than PGF [9, 46]. Bacterial infection causes endometrial damage and inflammation, reducing the chance of conception. **B**) Cytokines and chemokines direct the immune response. Chemokines attract neutrophils (PMNs) and macrophages (MØs) to eliminate the bacteria. However, neutrophil function is often compromised in cattle around the time of parturition [47]. Persistence of PMNs in the endometrium in the absence of bacteria is thought to be the primary characteristic of subclinical endometritis [15, 24]. **C**) It is thought that viral replication may be stimulated in macrophages that are persistently infected with BoHV-4 by PGE and LPS [34]. The BoHV-4 can then infect the endometrial stromal and epithelial cells, causing further tissue damage [45]. **D**) Follicle-stimulating hormone (FSH) concentrations from the pituitary are unaffected by uterine disease, and so waves of ovarian follicles emerge in the first weeks after parturition [10]. However, the release of GnRH from the hypothalamus and LH from the pituitary can be suppressed by LPS, reducing the ability to ovulate a dominant follicle [48, 49]. **E**) Cows with endometritis have slower growth of dominant follicles in the ovary and lower peripheral plasma estradiol concentrations and so are less likely to ovulate [10]. Follicular fluid contains LPS in animals with endometritis, granulosa cells express the TLR4/CD14/LY96 (MD2) complex required to detect LPS, and LPS perturbs estradiol secretion from granulosa cells by reducing aromatase expression [7]. **F**) If cows with endometritis ovulate, they form a corpus luteum secreting progesterone and reinitiate ovarian cycles. However, the peripheral plasma concentrations of progesterone are lower than those in normal fertile animals [32]. Cytokines may perturb luteal cell steroidogenesis [50]. Luteolysis is probably disrupted, and luteal phases are often extended because bacteria switch the endometrial epithelial secretion of prostaglandins from the F series to the E series [9].

I. Martin Sheldon, James Cronin, Leopold Goetze, Gaetano Donofrio, and Hans-Joachim Schuberth: BIOLOGY OF REPRODUCTION 81, 1025–1032 (2009)

D. Claire Wathes, Zhangrui Cheng, Waliul Chowdhury, Mark A. Fenwick, Richard Fitzpatrick, Dermot G. Morris, Joe Patton, and John J. Murphy: *Physiol Genomics* 39: 1–13, 2009

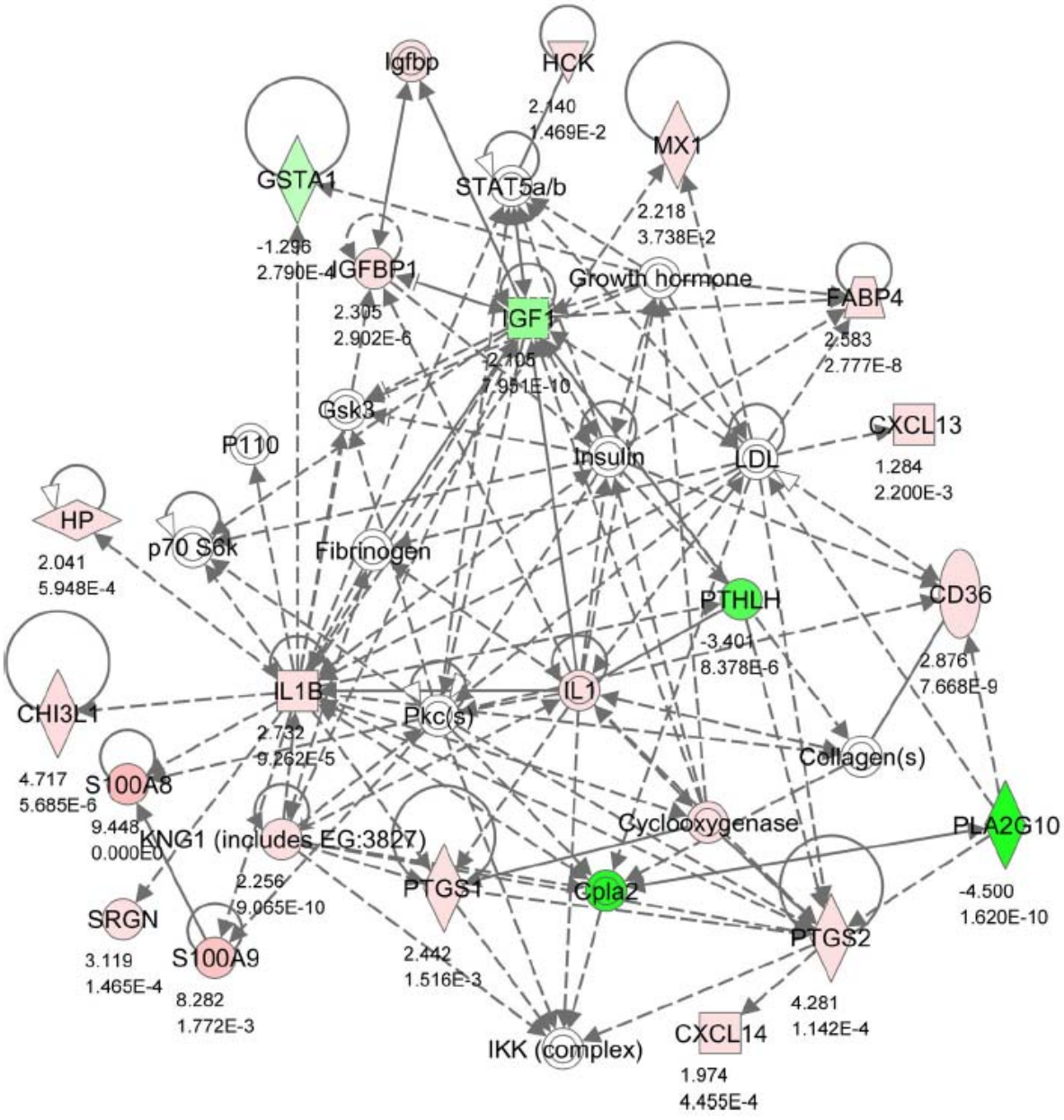


Fig. 3. IPA Network 2. Differentially regulated genes in endometrium involved in cellular movement, hematological system development and function and immune cell trafficking with 20 focus molecules and a score of 40. The network is displayed graphically as nodes (gene/gene products) and edges (the biological relationship between nodes). The node color intensity indicates the expression of genes: red upregulated, green downregulated in SNEB vs. MNEB endometrium. The fold value and *P* values are indicated under each node. The shapes of nodes indicate the functional class of the gene product as shown in the key given in Fig. 2. Solid lines indicate a direct interaction, and dotted lines an indirect interaction.

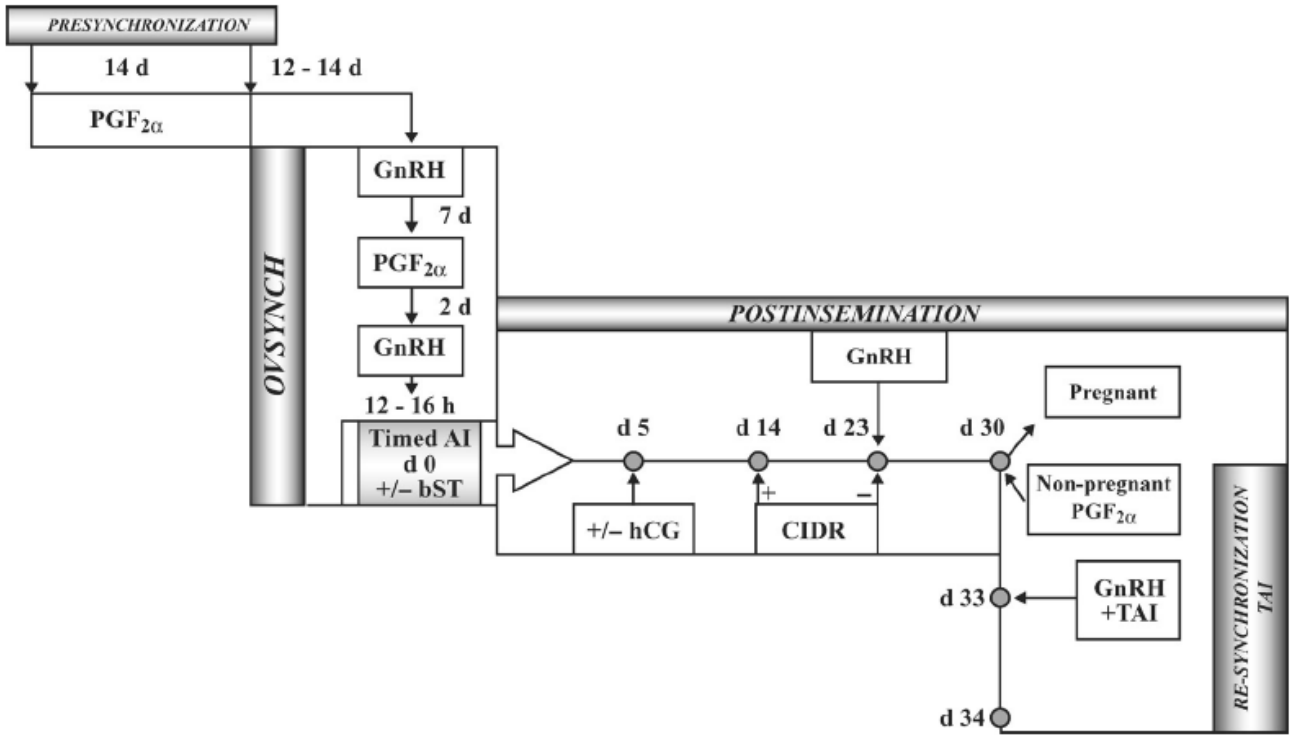


Figure 2. Reproductive management alternatives to improve reproductive performance of lactating dairy cows with the use of presynchronization, Ovsynch for timed artificial insemination (TAI), postinsemination endocrine treatments, and resynchronization for TAI. Endocrine treatments involve injection of bovine somatotropin (bST) at TAI and injection of human chorionic gonadotropin (hCG) at d 5 after TAI. Resynchronization of nonpregnant cows involves the insertion of an intravaginal progesterone device (CIDR) between d 14 and 23 after artificial insemination and injection of GnRH at d 23 at the time of CIDR withdrawal. Cows diagnosed nonpregnant at d 30 receive an injection of $PGF_{2\alpha}$, and on d 33 are injected with GnRH and concurrently inseminated.

K. Moore and W. W. Thatcher: J. Dairy Sci. 89 (2006):1254–1266

Table 1
Data from a reproductive management program derived from cows first inseminated or examined 45–70 days postpartum: pregnancies at AI and overall number of ovarian and uterine disorders

Year	*n*	Overall AI (*n*)[1]	Pregnant (%)[2,3]	AI cool period (*n*)[4]	Pregnant (%)[2]	AI warm period (*n*)[4]	Pregnant (%)[2,3]	Inactive ovaries (%)[3,5]	Ovarian cysts (%)[5]	Uterine disorders (%)[3,5]	Milk yield cow/year (kg)
1991	1,118	968	42.3	594	46	374	36.4	1.6	7.6	4.2	7,800
1992	1,250	1,141	39.2	676	43.9	465	32.3	2.2	7.8	3.9	8,000
1993	1,311	1,128	38.5	620	46	508	29.3	2.9	7.6	3.4	8,300
1994	1,295	1,103	38.9	612	46.1	491	29.7	5.9	6	2.9	8,500
1995	1,332	1,123	36.8	617	42	506	30.4	6.4	6.8	2.5	8,900
1996	1,412	1,168	36.3	700	45	468	23.3	7.9	7.2	2.2	9,100
1997	1,617	1,326	35.2	692	42.5	634	27.3	9.6	6.6	1.8	9,400
1998	978	785	34.8	471	46.9	314	16.6	10.8	7	1.9	9,700
1999	1,139	909	34.7	509	43.4	400	23.5	11.1	7.6	1.6	9,900
2000	1,259	985	33.1	518	43.1	467	22.1	11.8	8.1	1.8	10,200
Total	12,711	10,636	37	6,009	44.4 a	4,627	27.4 b	7	7.3	2.6	

Proportions were compared in 10×2 contingency tables using the Chi-squared test. The different letters denote significant differences when compared in a 2×2 contingency table using the Chi-squared test (a,b: $P < 0.00001$).

[1] Total number of inseminated cows.

[2] Number of pregnant cows as a percentage of the total number of inseminated cows in each group.

[3] Proportions were different ($P < 0.00001$).

[4] Number of inseminated cows during the cool and the warm period, respectively.

[5] Number of cows with inactive ovaries, ovarian cysts, and uterine disorders, respectively, as a percentage of the total number of cows.

Table 2
Data from a reproductive management program derived from cows first inseminated or examined 45–70 days postpartum: cyclicity and ovarian disorders during the cool and the warm period

Year	*n*	Cyclic cows (%)[1,2]	Number of cows. Cool period (*n*)[3]	Cyclic cows (%)[4]	Number of cows. Warm period (*n*)[3]	Cyclic cows (%)[2,4]	Inactive ovaries. Cool period (%)[5,6]	Inactive ovaries. Warm period (%)[2,5,6]	Ovarian cysts. Cool period (%)[6]	Ovarian cysts. Warm period (%)[6]	Milk yield cow/year (kg)
1991	1,118	86.6	640	92.8	478	78.2	0.8	2.7	2	15.2	7,800
1992	1,250	91.3	723	93.5	527	88.2	0.6	4.4	2.5	16.9	8,000
1993	1,311	86	665	93.2	646	78.6	0.9	4.9	4.7	12.8	8,300
1994	1,295	85.2	655	93.4	640	76.7	1.2	10.6	2	10.2	8,500
1995	1,332	84.3	663	93.1	669	75.6	1.2	11.5	2.7	10.9	8,900
1996	1,412	82.7	743	94.2	669	70	1.2	15.4	2.4	12.4	9,100
1997	1,617	82	734	94.3	883	71.8	1.6	16.3	2	10.3	9,400
1998	978	80.3	504	93.5	474	66.2	1.8	20.5	2	12.3	9,700
1999	1,139	79.8	540	92.6	599	66.8	1.3	19.9	2.6	12.9	9,900
2000	1,259	78.2	558	92.8	701	66.6	1.3	19.3	2.9	12.3	10,200
Total	12,711	83.7	6,425	93.5 a	6,286	73.6 b	1.2 c	12.9 d	2.4 e	12.3 f	

Proportions within the same column were compared in 10×2 contingency tables using the Chi-squared test. The different letters denote significant differences when compared in 2×2 contingency tables using the Chi-squared test (a–b, c–d, e–f: $P < 0.00001$).

[1] Total number of inseminated cows as a percentage of the total number of cows.
[2] Proportions were different ($P < 0.00001$).
[3] Number of possible cyclic cows during the cool and the warm period, respectively.
[4] Percentage of inseminated cows (cyclic) during the cool and warm period, respectively.
[5] Number of cows with inactive ovaries during the cool and the warm period, respectively, as a percentage of the number of possibly cyclic cows in each group.
[6] Number of cows with ovarian cysts during the cool and the warm period, respectively, as a percentage of the number of possibly cyclic cows in each group.

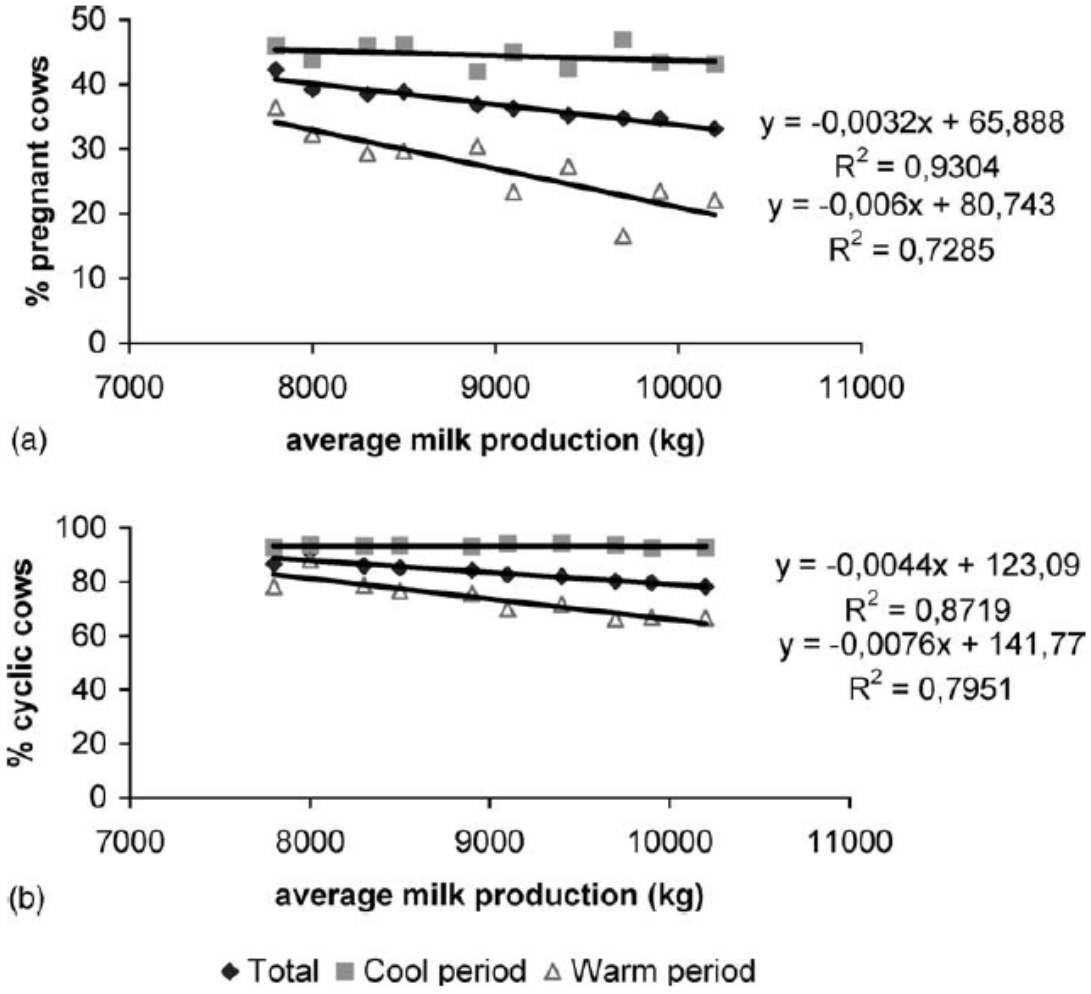


Fig. 1. Pregnancy rate (a) and cyclicity (b) in terms of average milk production.

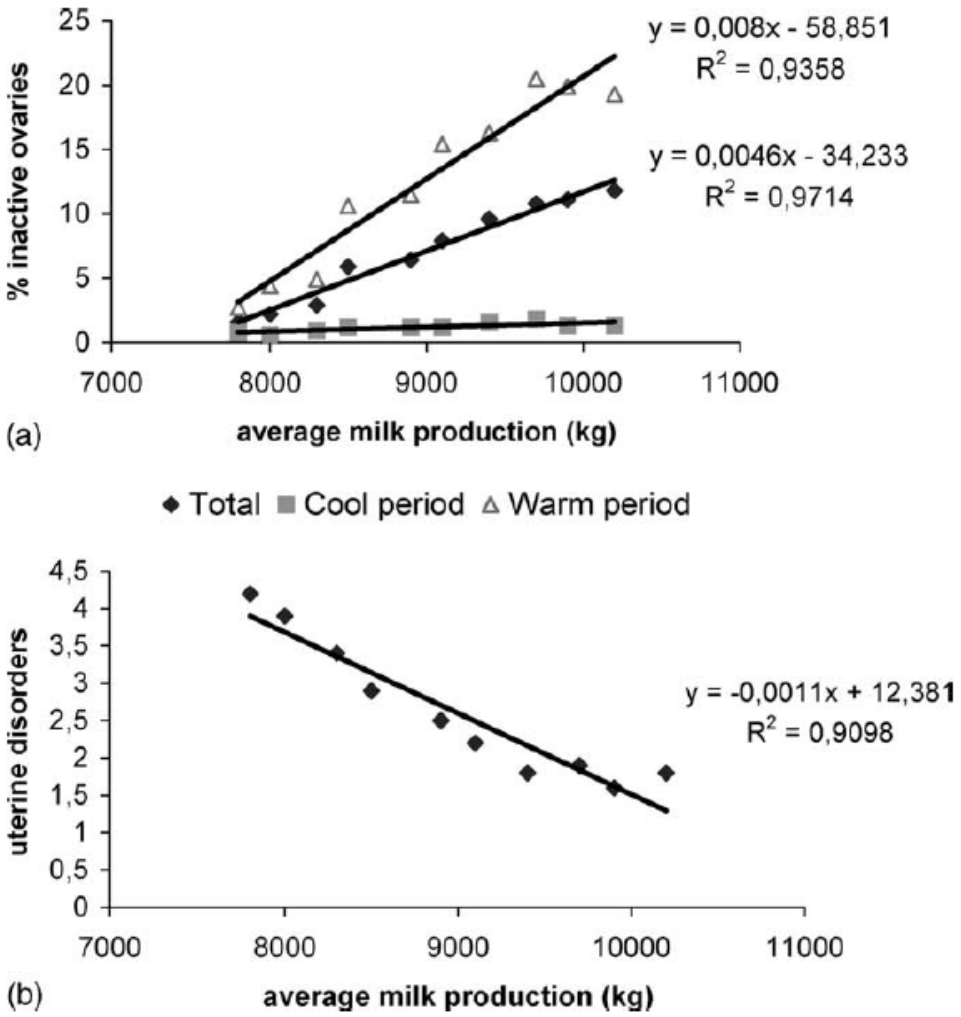


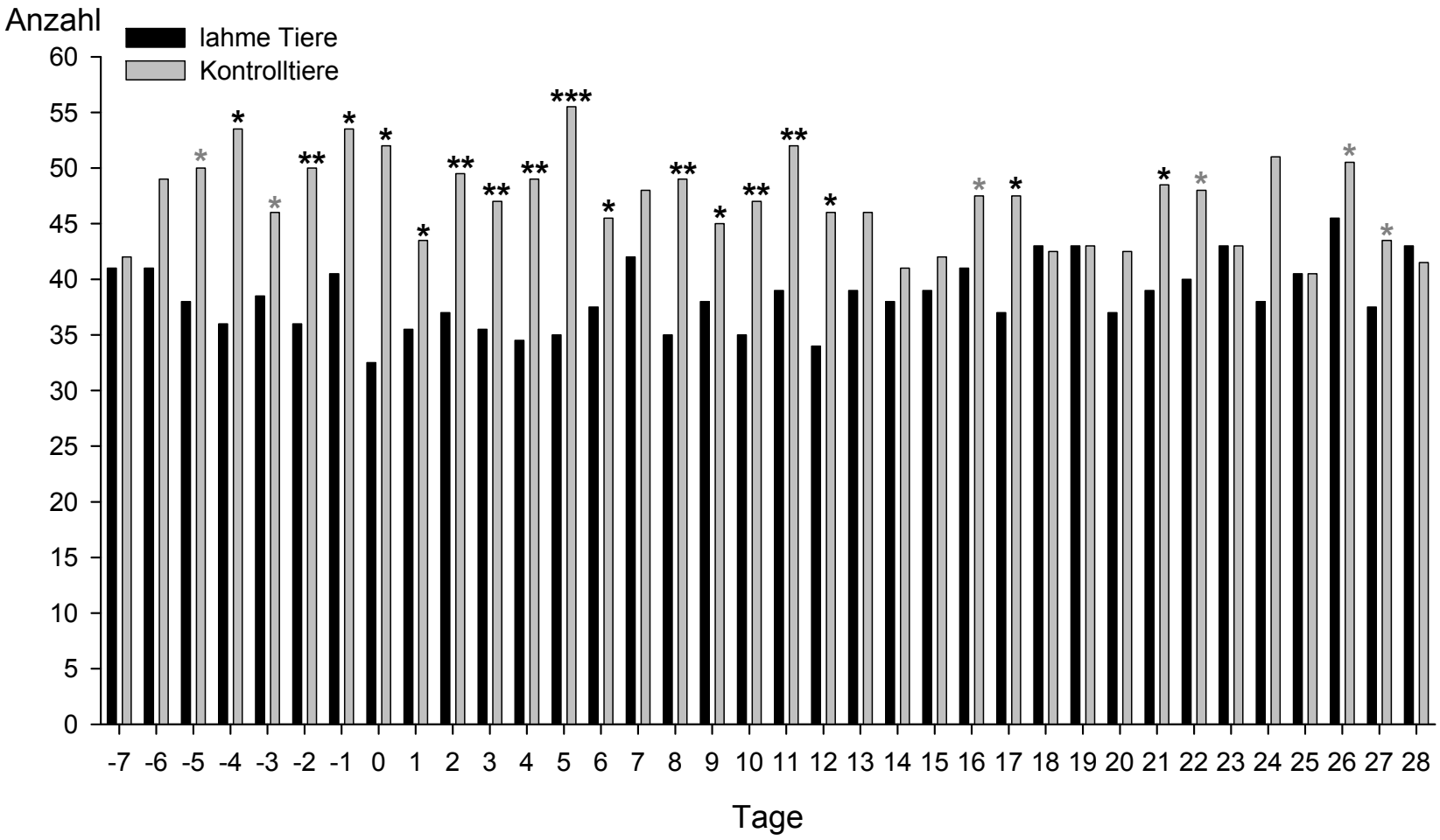
Fig. 2. Rates of inactive ovaries (a) and uterine disorders (b) in terms of average milk production.

Lahmheiten

Inzidenz: 15 bis 30%
Praevalenz: etwa 25%

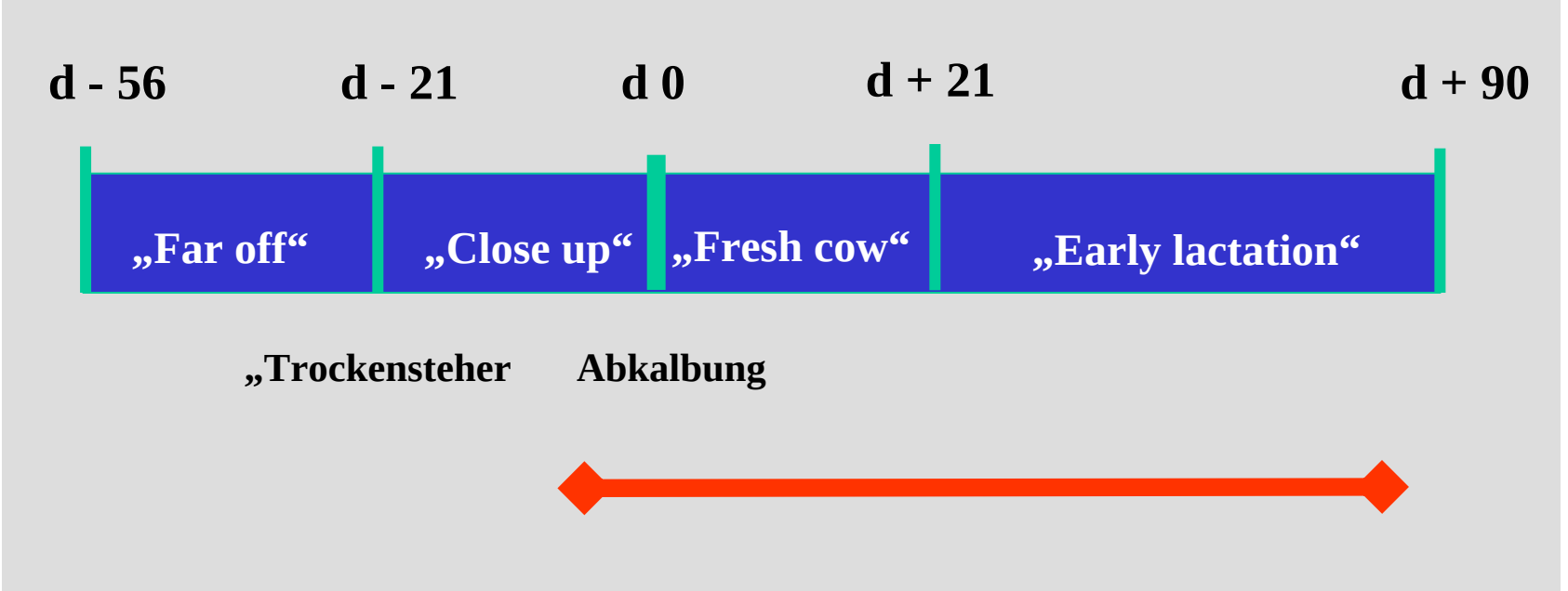
Durchschnittliche Dauer einer Lahmheit bei Behandlung: 4 Wochen

Grundfutter: Anzahl der Mahlzeiten / Tag



Haschka 2009

Produktionskrankheiten in der “Transition Period” bei Milchkühen



Ketose
Leberverfettung
Milchfieber
Nachgeburtsverhaltung
SARA

Labmagenverlagerung
Mastitis
Lahmheit
Metritis

Hohe Milchleistung erhöht die Wahrscheinlichkeit stärkerer und länger anhaltender NEB und die Praevalenz von Ketose und Leberverfettung

Insulinsensitivität spielt in der Kompensation der NEB eine zentrale Rolle

Bei Milchkühen ist NEB negativ mit der Wahrscheinlichkeit der erneuten Trächtigkeit zu einem vor 40 Jahren definierten Zeitpunkt korreliert, bisherige Interventionen haben dies nicht nachhaltig geändert.

Niedrige Trächtigkeitsraten 70 bis 80 Tage pp sind nicht zwingend ein Indikator für eine Verletzung von „Animal Wellfare“

Fruchtbarkeitsstörungen sind weit überwiegend bedingt durch Management, Fütterung und Haltung

Züchtung muss Fitnessparameter höher bewerten

Insulinsensitivität vs. Milchleistung
180 Tage pp