Energy Status Related to Production and Reproduction in Dairy Cows

Prevention of Decreased Fertility and Detection of Cows at Risk

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Energy Status Related to Production and Reproduction in Dairy Cows. Prevention of Decreased Fertility and Detection of Cows at Risk

Abstract

Decreased fertility in dairy cows is widespread and economically undesirable. Current management strategies to prevent decreased fertility are exploiting the close relationship between negative energy balance in transition cows and subsequent decreased fertility. However, there is a continuous need for more information regarding the effects of different strategies on fertility. This thesis evaluated the effect of supplemental feeding with glycerol or propylene glycol, the usefulness of measuring metabolic indicators in blood samples to predict decreased fertility and investigated potential risk factors. In two field studies, the metabolic status, milk yield and fertility of cows in 17 herds, fed either a glycerol or propylene glycol supplement or no supplement (control) 0-21 days after calving, were evaluated. A separate study evaluated the accuracy of metabolic indicators when used to predict decreased fertility. The results were based on a single blood sample taken in early lactation and different test cut-off values were applied. Finally, potential risk factors for decreased fertility related to housing, feeding and the cow herself, were evaluated in approximately 750 Swedish herds.

Cows fed glycerol produced significantly 1 kg more milk during the first 90 days in milk and cows fed propylene glycol tended to produce more milk without a subsequent decrease in metabolic status or fertility. The test performance of the metabolic indicators was in general low and was influenced by cow parity, cow breed and the prevalence of decreased fertility in the population studied. Cows experiencing a change in system (e.g. in housing or milking system or from conventional to organic production) had lower fertility than cows not experiencing such a change. In addition, cows with severe claw lesions and cows displaying a rise in somatic cell counts had a lower probability of pregnancy at first insemination.

In conclusion, supplemental feeding with glycerol or propylene glycol, as a general strategy in a herd, does not seem to influence fertility or energy status and could increase milk yield. Measures to prevent a decrease in fertility could be more effective if applied to cows in physiological imbalance, rather than all cows in a herd. However, the use of metabolic indicators in a single blood sample may not be optimal for detecting cows at risk. The identified risk factors for decreased fertility could be used when devising preventive strategies.

Keywords: dairy cow, glycerol, propylene glycol, fertility, negative energy balance, metabolic indicators, risk factor, field study, test accuracy

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Dedication

To my Family

A cow saying mooh, drawing by Fritiof.

Det finns ingen genväg till det perfekta ljudet
Farbror Barbro

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Lomander H, Frössling J, Ingvartsen K L, Gustafsson H, & Svensson C (2012). Supplemental feeding with glycerol or propylene glycol of dairy cows in early lactation- Effects on metabolic status, body condition and milk yield. *Journal of Dairy Science* (95), 2397 - 2408.

II Lomander H, Gustafsson H, Frössling J, Ingvartsen K L, Larsen T & Svensson C (2012). Effect of supplemental feeding with glycerol or propylene glycol in early lactation on the fertility of Swedish dairy cows. *Reproduction in Domestic Animals*. DOI:10.1111/j.1439-0531.2012.02004.x

III Lomander H, Gustafsson H, Svensson C, Ingvartsen K L & Frössling J (2012). Test accuracy of metabolic indicators in predicting decreased fertility. Accepted for publication in *Journal of Diary Science*. DOI:10.3168/jds.2012-5534.

IV Lomander H, Svensson C, Hallén-Sandgren C, H. Gustafsson & Frössling J (2012). Associations between decreased fertility and management factors, claw health and somatic cell count in Swedish dairy cows (Manuscript).

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Abbreviations

AI	Artificial insemination
ANEST	Anoestrus
BCS	Body condition score
BHBA	β-hydroxy butyrate
C	Control
CI	Confidence interval
CON	Conception
DCON	Delayed conception
DFAI	Delayed first artificial insemination
DIM	Days in milk
DMI	Dry matter intake
ECM	Energy corrected milk
FAI	First artificial insemination
FLA	First luteal activity
FSH	Follicle stimulating hormone
GLY	Glycerol
HG	Heart girth
HR	Hazard ratio
IGF-1	Insulin-like growth factor 1
LH	Luteinising hormone
NADRS	National disease data base
NEB	Negative energy balance
NEFA	Non-esterified fatty acids
NINS	Number of inseminations per animal submitted to AI
PAI	Pregnant (or not) at first AI
PG	Propylene glycol
PMR	Partial mixed ratio
PV-	Negative predictive value

PV+	Positive predicted value
SCC	Somatic cell count
SD	Standard deviation
Se	Sensitivity
SEP	Separate feeding of concentrates and roughages and individual feeding of concentrates
SH	Swedish Holstein breed
SOMRS	Swedish official milk recording scheme
Sp	Specificity
SR	Swedish Red breed
TG-ROC	Two graph receiver operator characteristic
TMR	Total mixed ratio

1 Background

1.1 The dairy cow

1.1.1 History

Cattle were domesticated between 8000 and 10,000 years ago (Tucker, 2009) and humans collected cow's milk already in 2000 BC, as shown by Egyptian paintings (Jensen, 1995). In Sweden, detailed information about dairy cows is available from the 16^{th} century, thanks to a recording system introduced by the King Gustav Vasa, although it is believed that cows were introduced into the country much earlier (Björnhag, 1997). However, the highly specialised dairy cow of today has little in common with the ancient cow, which was much smaller and produced considerably less milk.

1.1.2 Swedish dairy herds

Swedish dairy herds are structurally changing, and the last 20 years have seen a typical change from a small tie-stall barn to a larger loose-housing system. Furthermore, the numbers of automatic milking systems (AMS) are gradually increasing and by 2011, 755 AMS had been installed in Swedish herds (Lakic, 2011). At the same time, many small herds, predominantly those with less than 100 cows have disappeared. Today, the mean number of cows across the remaining 5600 herds in Sweden is 62 (Swedish Board of Agriculture, 2011). Tie-stalls are still the predominant housing system, with 53% of Swedish cows held in tie-stalls and 47 % in loose-housing systems (Marie Mörk, Swedish Dairy Association, pers. comm. 2012). As of 2012, approximately 600 herds are organic (Johan Eriksson, KRAV, pers. comm. 2012).

The main breeds are the Swedish Red (SR; 45%) and Swedish Holsteins (SH; 55%). SR cows generally produce less milk (9200 kg ECM on average on a yearly basis) than SH cows (nearly 9800 kg ECM/year) (Swedish Dairy Association, 2012). Swedish cows are inseminated following oestrus detection, 52 % using farmer-operated AI.

Eighty-five percent of Swedish dairy cows (*i.e.* 270 000 cows in 4000 herds) are affiliated with the Swedish official milk recording scheme (SOMRS) which is administered by the Swedish Dairy Association (Swedish Dairy Association, 2012). In SOMRS, data on monthly milk yield and composition and on fertility and health in the associated herds are gathered to provide a basis for *e.g.* herd health surveillance and feed advice.

Swedish dairy herds have in general a low incidence of several infectious diseases that are known to affect fertility. According to national surveillance programmes, the dairy cow population is free from brucellosis and bovine herpes virus I and the occurrence of bovine viral diarrhoea virus is limited to small regions of the country (National Veterinary Institute, 2012). The herd-level prevalence of *Neospora caninum* was 8.3% in 2008, which is low in comparison to other European countries (Frössling *et al.* 2008). The annual prevalence of salmonellosis is less than 1% in the collected population of food-producing animals (National Veterinary Institute, 2012).

1.1.3 Milk and glucose

The main constituents in milk are lactose, fat, protein, calcium and water (Jenness, 1985). Because lactose is the most important osmotically active component in milk, it is a key driver of milk production (Sandholm *et al.*, 1995). Lactose is synthesised from glucose and there is a large uptake of glucose by the mammary gland to meet the glucose requirements in the high-yielding cow (Baumann, 2000). Ingested carbohydrates are effectively fermented to short-chain fatty acids in the rumen and only a minor amount of glucose in the feed is absorbed in non-fermented form. Therefore, adult ruminants are dependent on hepatic (>90%) and, to a smaller extent, renal gluconeogenesis to fulfil their glucose requirements (Aschenbach *et al.*, 2010; Figure 1). The main precursor for gluconeogenesis is the short-chain fatty acid propionate, and to a lesser extent valerate and butyrate, all from ruminal fermentation of carbohydrates (McDonald *et al.*, 2002). Besides short-chain fatty acids, lactate, amino acids from muscle tissue break down and glycerol (GLY) from lipid mobilisation can be used for glucose synthesis (Aschenbach *et al.*, 2010). The gluconeogenesis is highly prioritised by the cow and is under hormonal regulation mainly by insulin, glucagon and growth hormone (Aschenbach *et al.*, 2010).

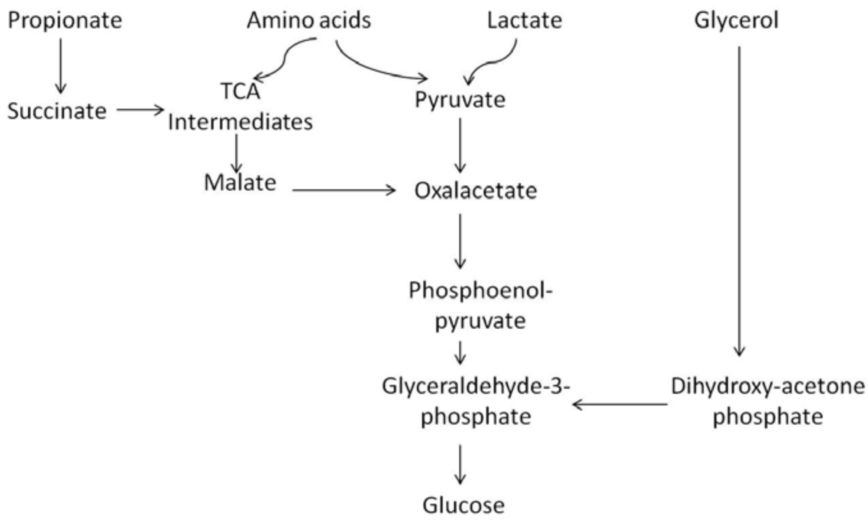

Figure 1. Major pathways in gluconeogenesis in cows (McDonald *et al.* 2002).

1.2 Reproduction in dairy cows

1.2.1 Ovarian cyclicity and hormonal regulation

Cows are polyoestrus and oestrus occurs approximately every 21 days after the onset of puberty, which occurs approximately between 7 and 18 months of age (Noakes, 2001). The average duration of oestrus (*i.e.* standing heat) is 8 h (Forde *et al.*, 2011). Milk yield influences the length of the cycle and the oestrus of high yielders is generally shorter than that of low-yielders (Lopez *et al.*, 2004).

Follicular growth and atresia occurs throughout the oestrus cycle and there are normally one or two follicle waves before the dominant follicle ovulates (Webb *et al.*, 1992; Figure 2). Ovulation is spontaneous and occurs on average 12 h after the end of standing heat. The corpus luteum (CL), which produces progesterone, is developed at the site of the ovulatory follicle within 2-3 days after ovulation. The subsequent period of 14-18 days is therefore called the luteal phase. If no fertilisation occurs, prostaglandin F2α from the uterus is released and regresses the CL on day 17-18 after ovulation (Miyamoto *et al.*, 2009). When the CL regresses, progesterone concentrations decline back to basal levels.

The ovarian cyclicity is under hormonal control via a positive and negative feedback system by gonadotrophin-releasing hormone from the hypothalamus, and follicle stimulating hormone (FSH) and luteinising hormone (LH) from the anterior pituitary gland (Webb *et al.*, 1992). In addition, progesterone, oestradiol and inhibins from the ovaries and prostaglandin F2α from the uterus are also involved (Forde *et al.*, 2011; Webb *et al.*, 1992; Figure 3). At the end of the luteal phase of the cycle, progesterone concentrations decrease and oestradiol from the dominant follicle increases, this induces a pre-ovulatory surge of gonadotrophin-releasing hormone, leading to a surge of both FSH and LH. Pulses of LH occur regularly throughout the cycle, in the beginning with large frequency but low amplitude and in mid cycle with low frequency and low amplitude. Ovulation occurs when LH pulses occur with high frequency leading to the LH surge (Forde *et al.*, 2011; Figure 3).

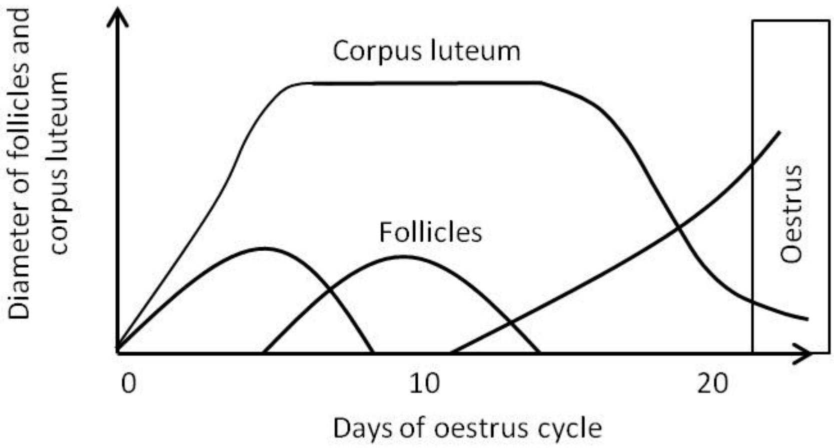

Figure 2. Schematic diagram showing the development of corpus luteum and follicular waves throughout the oestrus cycle of the cow (modified after Noakes, 2001).

1.2.2 Measuring reproductive performance

Reproductive performance can be described using a variety of indicators. The interval between two subsequent calvings (calving interval), the interval from calving to first AI (CFI) and the interval from calving to conception (CCI) are commonly used in Sweden. The two other indicators, namely 56 days non-return rate to first AI (percentage of animals not showing oestrus within 56 days after first AI) and number of inseminations per animals submitted for AI (NINS) are also used. These indicators are all influenced by external factors such as nutrition, bull fertility, occurrence of infectious diseases and the farmers skills' in heat detection, timing and conducting AI. The reproductive performance is also influenced by the ability of the embryo and foetus to develop and by the survival of the calf (Rodriguez-Martinez *et al.*, 2008; Parkinson, 2001).

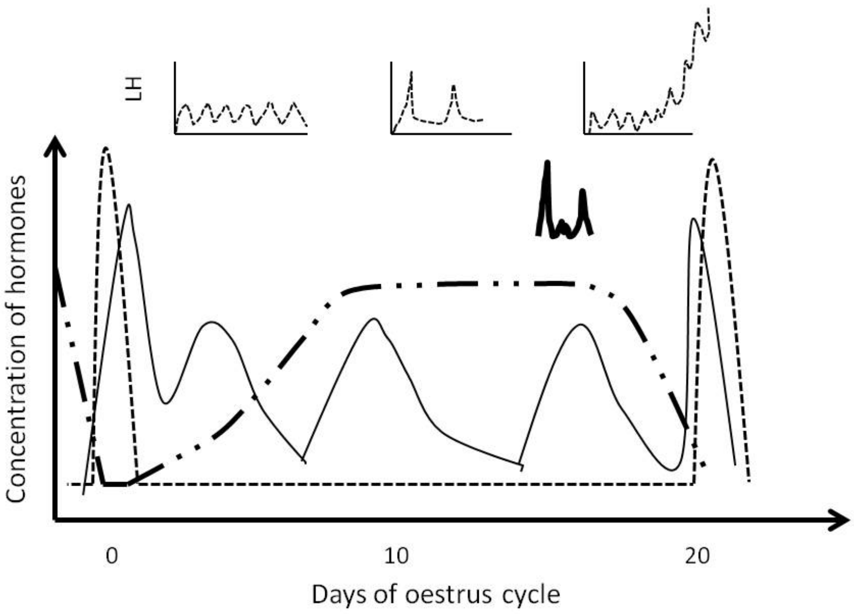

Figure 3. Schematic diagram showing differences in concentrations of luteinising hormone (LH; ----), follicle stimulating hormone (thin solid line), progesterone (–¨–¨–) and prostaglandin f2α (thick solid line) during the oestrus cycle of a cow (modified after Forde *et al*., 2011 and Gustafsson, 1987).

In addition, management decisions such as the individual voluntary waiting period for each individual cow or herd have a large impact on the outcomes of inseminations. It is well known that the probability of pregnancy increases with increasing days from calving because cows are given time for resumption of ovarian activities, for clearing the uterus of minor bacterial contamination *etc* (Wathes *et al.*, 2007b; Parkinson, 2001). Recently, a reproductive performance indicator adjusted for the herd's individual voluntary waiting period was evaluated and found to be useful (Löf *et al.*, 2012).

Even though the available measures of reproductive efficiency and fertility may be 'noisy' and may underestimate or overestimate the reproductive efficiency, they are used in both practice and in research. In this thesis the word 'fertility' is used as a synonym for 'reproductive performance'.

1.2.3 Declining fertility and its consequences for dairy production

The reproductive performance of the cow is important in dairy production. If the cow does not conceive, no subsequent lactation will follow and a potential replacement heifer will be lost. For many years, concerns about declining dairy cow fertility were raised both world-wide (Lucy, 2001) and in Sweden (Rodriguez-Martinez *et al.*, 2008). In Sweden, the calving interval had increased from 12.6 months in 1988 to 13.4 months in 2008. Today, the decrease seems to be slightly halted (Berglund, 2008) with a calving interval of 13.3 across all breeds (Swedish Dairy Association, 2012). However, reproductive problems are still the major reason for premature and involuntary culling in Swedish herds (Swedish Dairy Association, 2012) and should consequently be given much attention.

The general recommendation to Swedish dairy herds is to obtain one calf per cow and year. In order to reach this goal, cows need to conceive no later than 90 days after calving. In contrast, the average interval from calving to last AI in Swedish herds is currently 128 days (Swedish Dairy Association, 2012). Having an extended calving interval can be beneficial for reproductive efficiency, as shown in a study of two herds aiming for an interval of 15 and 12 months, respectively (Larsson & Berglund, 2000). Cows with a planned calving interval of 15 months required fewer hormonal treatments for anoestrus and smaller number of AI to conceive. A key factor for an extended calving interval to become economically favourable is to maintain lactation persistency. It has been shown that the lactation persistency in cows with an 18 month calving interval could be improved by management factors such as increased milking frequency (Sørensen *et al.*, 2008). Despite this, an extended calving interval is still considered economically non-beneficial (Inchaisri *et al.*, 2010). However, postponed first AI (*i.e.* long voluntary waiting period) and thus a longer calving interval, could be favourable in herds with low reproduction efficiency and when beef prices are taken into account (Sørensen & Østergaard, 2003).

The calving interval is influenced by *e.g.* the voluntary waiting period, the optimum of which varies between herds and cows. A voluntary waiting period of approximately 42 days was found to be economically optimal for most Dutch cows (Inchaisri *et al.*, 2011). No information on the average length of the voluntary waiting period in Swedish herds is available, but the average interval from calving to first AI is 90 days (Swedish Dairy Association, 2012).

Although sub-fertility is not generally a disease *per se,* it could be considered an animal welfare indicator, as it indicates that the cow has not been able to simultaneously produce milk and maintain her reproductive functions. Concerns have also been raised that consumer awareness about the declining fertility in dairy cows could lead to a public opinion turning against the modern dairy industry (Oltenacu & Algers, 2005).

1.3 Negative energy balance

1.3.1 Transition physiology

The period comprising the last 3 weeks of gestation and first 3 weeks of lactation is often referred to as the transition period, because the cow 'transits' from the dry period to lactation. During the transition period the final growth of the foetus and calving occur and milk production starts. The cow is also challenged by other stressors such as separation from her calf, immune challenges when the uterus is cleared (Salasel *et al.*, 2010), increased demands for energy, nutrients and minerals (LeBlanc, 2010), and most often also exposure to a new feeding regime and regrouping (Schirmann *et al.*, 2011). Thus, it is not surprising that there is an over-all increased risk of health disturbances during the transition period (Ingvartsen, 2006).

Concurrently with the dramatically increased need for nutrients, for the growing foetus but particularly for lactation, a temporary physiological decrease in dry matter intake (DMI) leads to a shortage of glucogenic precursors from exogenous sources. Although DMI increases after calving, the increase is slow compared with the increased need for nutrients for lactation, with maximum intake reached between 5 and 7 weeks post-partum (Grummer *et al.*, 2004; Ingvartsen & Andersen, 2000). As a result, a period of negative energy balance (NEB) follows calving in almost all cows.

To support the increased glucose requirements of the mammary gland, homeorhetic regulations change nutrient partitioning (Ingvartsen & Andersen, 2000). This involves increased hepatic gluconeogenesis, decreased peripheral glucose utilisation mediated via insulin resistance, increased lipolysis and increased amino acid mobilisation from muscle tissue (Bell, 1995). As a result, there is an increased contribution of endogenous precursors to gluconeogenesis, such as lactate, alanine from muscle tissue breakdown and GLY from lipolysis (Aschenbach *et al.*, 2010).

When GLY is released from body fat tissue, there is an increase in non-esterified fatty acids (NEFA), which are the main source of energy for the dairy cow under NEB. NEFA may be completely oxidised in the liver, but when the oxidative capacity of the liver is exceeded, NEFA are partially oxidised to ketone bodies such as acetoacetate and β-hydroxy butyrate (BHBA). NEFA can also be re-esterified to tri-acyl glycerides and stored or excreted from the liver in very low density lipoproteins (Drackley & Andersen, 2006; Figure 4).

Negative energy balance in dairy cows after calving is characterised by low blood glucose, low blood insulin, high blood NEFA, high blood BHBA and low blood insulin-like growth factor I (IGF-1) (Ingvartsen & Andersen, 2000).

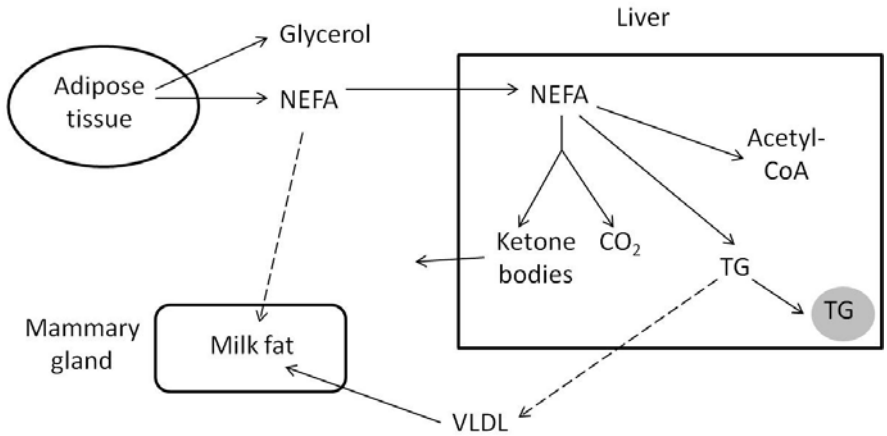

Figure 4. Schematic picture showing lipid metabolism in adipose tissue, liver and in the mammary gland. NEFA= non-esterified fatty acids, VLDL= very low density lipoproteins, TG=Triglyceride. Dashed lines indicate a process that occur in a low rate (Modified after Drackley, 1999).

1.3.2 Impacts of negative energy balance on fertility

Numerous studies have shown associations between NEB and decreased reproductive performance. Cows in severe NEB have been shown to have a longer interval from calving to the onset of ovarian cyclicity compared with cows without NEB (Wathes *et al.*, 2007a; Opsomer *et al.* 2000; Butler *et al.*, 1981), indicating that cows simply postpone their reproductive functions until energy balance does not compromise survival of the embryo or foetus. It has been found that an early resumption of ovarian activity leads to a subsequent improvement in fertility (Wathes *et al.*, 2007a). Furthermore, cows in severe NEB may have suppressed LH pulse frequencies and reduced ovarian sensitivity to LH (Canfield & Butler, 1989). A follicle developed under such conditions is more likely to become non-ovulatory and hence delay cyclicity than a follicle developed during normal conditions.

In addition to the hormonal effects, direct effects of metabolites from lipid metabolism on the ovaries have been suggested. Plasma hormones and metabolites related to NEB have been found to be correlated with the concentrations of metabolites and hormones in follicular fluid of the dominant follicle (Leroy *et al.*, 2004; Jorritsma *et al.*, 2003); *e.g.* oocytes cultivated in media with NEFA concentrations resembling those of cows in NEB or in low glucose concentrations together with BHBA concentrations resembling clinical or subclinical ketosis, show delayed maturation, fertilization and cleavage (Leroy *et al.*, 2006; Leroy *et al.*, 2005). Furhtermore, *in vitro* studies have shown that NEFA can modulate proliferation and steroidogenesis in oocyte granulosa cells (Vanholder *et al.*, 2005).

1.4 Nutritional strategies to improve energy balance and fertility

1.4.1 Impact of energy supply during dry- and transition period

Britt (1992) hypothesized that oocytes developed during a period of NEB cause reduced fertility. Furthermore, he suggested that it takes 60-80 days for an early preantral follicle to reach ovulatory size (Figure 5). Thus, to achieve one calving per cow and year, measures to prevent NEB should focus on the transition period, rather than at time of AI. However, already the dry period can have a large effect on the subsequent lactation. There are a tremendous numbers of nutritional management approaches, aimed at limiting weight gain during the dry period and minimising NEB during the transition period. Feeding a high-energy diet throughout the entire dry period can be detrimental to cow health, *e.g.* Swedish cows fed a high energy diet for at least 8 weeks pre partum had a larger decrease in DMI and a larger increase in post partum NEFA concentrations than cows fed a low energy diet (Agenäs *et al.*, 2003a; Holtenius *et al.*, 2003). A common approach in large herds in *e.g.* the US is instead to group the dry cows to allow for a low-energy diet during the early dry period and increasing energy content during the last 2-3 weeks before parturition (Overton & Waldron, 2004). Another strategy proposes that high amount of dietary fibre leads to a relatively low decrease in DMI before calving (Grummer *et al.*, 2004).

There is also a considerable amount of literature describing the effects of adding specific substances to the diets with the aim of reducing NEB and improving fertility. However, the results from studies investigating addition of protein, fats (*e.g.* dietary fats, specific lipids or rumen inert fats) or glucogenic substances (*e.g.* GLY or propylene glycol (PG)) and minerals and trace elements are inconsistent (Chagas *et al.*, 2007a). Starch-rich diets given post-partum, inducing a high concentration of insulin, have been shown to increase the proportion of cows ovulating within 50 days after calving (Gong *et al.*, 2002). However, if this type of insulinogenic diet is fed for longer periods, the high plasma insulin concentration may lead to negative effects on oocyte and embryo quality (as reviewed by Leroy *et al.*, 2008). Garnsworthy *et al.* (2009) therefore combined an insulinogenic diet to allow for early cycling, followed by a "mating diet" rich in fat (which generated lower insulin concentrations) fed from the first rise in progesterone until 120 DIM. This combination doubled the proportion of cows pregnant at 120 days compared with cows fed only the insulinogenic diet for the entire period.

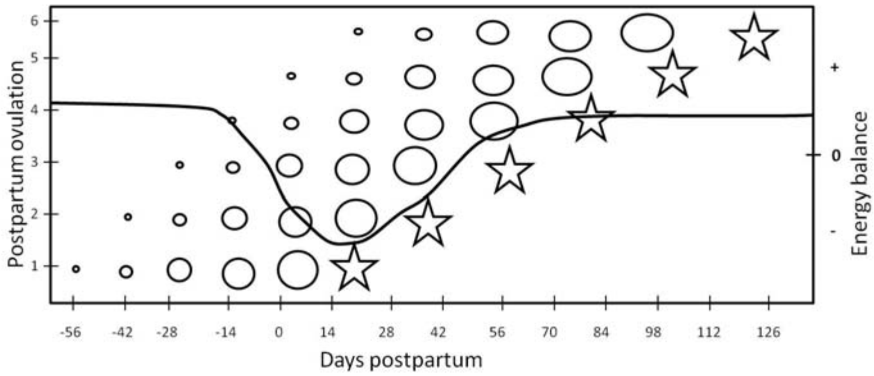

Figure 4. Schematic diagram of follicle growth from preantral (small circles to the left) to ovulatory size (indicated by stars) in relation to energy balance and days post partum (modified after Britt, 1992).

1.4.2 Glycerol

As a treatment for ketosis, GLY was used as early as 1954 (Johnson, 1954), and was further evaluated in the 1970s (Fisher *et al.*, 1973; Sauer *et al.*, 1973). When GLY is ingested, microbial fermentation to VFA is considered the main route of digestion (Rémond *et al.*, 1993), although VFA pattern results are inconsistent between studies (Donkin & Doane, 2007). However, in a recent report on four lactating cows, two-thirds of the ingested GLY was absorbed non-fermented over the epithelium (Kullberg, 2008). Thus, GLY could contribute to gluconeogenesis with both GLY and VFA as precursors.

During recent years the production of bio-diesel from oilseed crops has increased, resulting in large amounts of GLY as a by-product. Due to its low price, GLY has become interesting as a cheap feed ingredient replacing relatively expensive concentrates in cow diets (Donkin & Doane, 2007). When lactating Holsteins were fed diets with a maximum of 15% of corn grain replaced with GLY, no adverse effects on milk yield, milk composition or BCS were observed (Donkin *et al.*, 2009). Results similar to these, and also unchanged blood metabolites, were obtained by Carvalho et al (2011) in a study where GLY was fed at 11.5% and 10.8% of the dry matter ration from 28 days pre-partum to 56 DIM.

Besides the use of GLY as a treatment for ketosis or as a cheap feed ingredient, there is extensive interest in using GLY as a supplement to prevent severe NEB. In a study where 400 mL of GLY were administered as an oral drench daily from calving to 14 DIM, serum concentrations of NEFA and BHBA decreased and those of glucose increased (Osman *et al.*, 2008). In addition, cows fed 100, 200 or 300 g of GLY as a top-dress during 4-63 DIM had a higher serum concentration of glucose and lower concentrations of

NEFA and BHBA (Wang *et al.*, 2009). However, DeFrain *et al.* (2004) fed 0.43 or 0.86 kg/day dry matter for 14 days pre-partum to 21 DIM and reported decreased DMI pre-partum, decreased glucose concentrations post-partum and a tendency for decreased milk yield. However, these studies were conducted on a relatively low number of animals within the same farm, and therefore it is difficult to draw any general conclusions.

Theoretically, a less severe NEB would influence fertility positively. However, to the best of my knowledge, no previous study investigated the effect of supplemental feeding with GLY during early lactation on the fertility of dairy cows prior to Paper I. There is also a lack of large field studies using commercial farms under practical conditions to investigate the effects of supplementing GLY to cows in early lactation on their metabolic status.

1.4.3 Propylene glycol

As for GLY, PG has long been recognised as a treatment for ketosis in newly calved cows (Johnson, 1954). After ruminal fermentation, propanol and propionate are produced, of which propionate is entered into gluconeogenesis and propanol is further metabolised in the liver to propionate. In addition, a part of the ingested PG can be absorbed directly and diffuses back to the rumen for later fermentation (Kristensen & Raun, 2007). Around 10% can also be metabolised in the liver to L-lactate, which is further entered into gluconeogenesis (Kristensen *et al.*, 2002). In this way, PG contributes to gluconeogenesis with either propionate or L-lactate. Besides this, PG and propanol have insulin resistance-inducing properties, which saves glucose for the udder (Kristensen & Raun, 2007).

Used as oral drenches to transition dairy cows, PG can be effective in increasing glucose and decreasing NEFA and BHBA (as reviewed by Nielsen & Ingvartsen, 2004). Feeding PG has also been found to increase energy status and reduce body weight loss, *e.g.* when 450 mL PG was top-dressed from 1 to 63 DIM (Liu *et al.*, 2009). In contrast to GLY, there have been a substantial number of studies in which PG was either drenched or fed during transition and where the metabolic status of the supplemented cows was successfully increased without improved fertility (Miyoshi *et al.*, 2001; Hoedemaker *et al.*, 2004; Butler *et al.*, 2006; Rizos *et al.*, 2008). In particular, these studies found no improvement in early post-partum LH pulse frequency or follicle dynamics (Rizos *et al.*, 2008; Butler *et al.*, 2006) or conception rate (Hoedemaker *et al.*, 2004; Miyoshi *et al.*, 2001) in supplemented cows compared with un-supplemented cows. However, the study of Miyoshi *et al.* (2001) showed that cows drenched with PG had their first ovulation post-partum earlier than control cows.

As for GLY, there is a need for further evaluation of PG under practical farm conditions.

1.5 Predicting decreased fertility

1.5.1 Risk factors for decreased fertility

Preventive strategies may be focused on animals with certain characteristics that have been identified as a risk factor for decreased fertility. Because new techniques on farms are evolving and risk factors are not constant, there is a continuous need for new risk factor studies. Due to the strong association between NEB and decreased fertility (Friggens, 2003), it could be difficult to separate risk factors for these two problems.

There are associations between milk yield, NEB and decreased fertility (Butler *et al.,* 1981). Mild yield *per se* (*i.e.* phenotype) is not necessarily a limiting factor for fertility (Gröhn & Rajala-Schultz, 2000). However, cows that are selected for high milk yields have genetically driven energy partitioning and a larger reduction in DMI around calving (Veerkamp *et al.*, 2003) which may influence reproductive performance negatively.

Differences in reproductive performance have been reported between the two main Swedish dairy breeds, SR and SH. Generally, the SR breed has a better reproductive performance than the SH breed, exemplified by a calving interval of 13.1 months for SR cows and 13.6 months for SH cows (Swedish Dairy Association, 2012). SR cows are also more likely to resume ovarian cyclicity early than SH cows (Petersson *et al.*, 2006b). One potential reason for the observed difference is that fertility traits have been incorporated in the breeding goals for a longer period for the SR breed than the SH breed (Philipsson & Lindhé, 2003).

Parity can also affect the reproductive performance of cows. Petersson *et al.* (2006b) found that cows in first parity had a higher incidence of atypical progesterone profiles and subsequently a longer interval from calving to first AI, and from calving to conception, than cows of higher parity. A possible explanation for this is that metabolic profiles might differ between young cows and older because young cows are still growing but generally produce less milk (Wathes *et al.*, 2007a).

Several diseases have been found to be risk factors of decreased fertility in dairy cows, among others mastitis (Hockett *et al.*, 2005), retained placenta, metritis (Gröhn & Rajala-Schultz, 2000) and claw diseases (Hultgren *et al.*, 2004).

For cows in herds where calvings are evenly distributed throughout the year, the time of the year in which the calving and breeding take place affects the reproductive outcome. In Swedish herds, cows calving during the winter have been found to have a lower probability of pregnancy (Löf, 2012) and a higher incidence of delayed cyclicity (Petersson *et al.*, 2006a) than cows calving during the summer season.

In recent years, the use of total mixed ratios (TMR) or partially mixed ratios (PMR) has become common practice in dairy herds, primarily in those with loose-housing systems. According to Swedish farm advisors, there is a potential risk of over-conditioned cows, with a subsequent decrease in fertility, in these systems because the same ratio is often fed to cows in both early and late lactation (Spörndly, 2005). This concern was supported by the work of Löf *et al.* (2007b) who found that herds using TMR had a longer calving interval and CLI than herds using individual feeding.

Today, more cows are held in loose-housing systems and milked automatically. Several Swedish studies have found that cows in loose-housing systems have better reproductive performance than cows held in tie-stalls (Löf *et al.*, 2007b; Petersson *et al.*, 2006a). In general, AMS allow the cows to move more freely, but little is known on the effects on fertility (Jacobs & Siegford, 2012). In addition, there are no data on reproductive performance during the period of change from one system to another, *e.g*. from tie-stalls to loose-housing or from conventional milking to AMS.

1.5.2 Metabolic indicators

Individual cows kept under identical conditions could yet show different adaptations to metabolic stress (Kessel *et al.*, 2008) and arguments for the use of metabolic indicators to predict the risk of disturbed health in animals are raised (Ingvartsen, 2006). In the US, group-level measurements of metabolic indicators have been used to evaluate how well cows adapt during the transition period (Oetzel, 2004). In Sweden, measurements of keton bodies in milk, urine or blood have traditionally been made for diagnostic purpose on individual diseased cows. Only recently, the use of cow-side tests for BHBA with the aim of evaluating energy balance in early lactation have been more common (J. Waldner, Swedish Board of Agriculture, pers. comm., 2012).

In general, metabolites and hormones that show a large between-animal variation in plasma or serum concentrations are more ideal as indicators, in comparison to more stable ones (Ingvartsen, 2006). In the following section plasma or serum concentrations for respective indicator are referred to as simply ‘concentration’.

The concentration of ***glucose*** is under homeostatic control by insulin and shows only a limited variability, which makes it less ideal for use as an indicator (Ingvartsen *et al.*, 2003). The glucose concentration is increased by external factors such as feeding (Andersson & Lundström, 1984) and stress, (Leroy *et al.*, 2011), and can show a diurnal rhythm (Andersson & Lundström, 1984) which should be accounted for during sampling.

The ***insulin*** concentration declines sharply at calving and remains low in early lactation during the period of NEB (Ingvartsen, 2006). In cows in metabolic balance, insulin is increased in response to increased glucose concentrations to maintain glucose homeostasis (Hove, 1978).

NEFA is under homeorhetic control with large variations at cow-level and is therefore potentially useful as an indicator (Ingvartsen, 2006). In general, the increase in NEFA concentration starts before calving, peaks at calving and remains elevated for approximately 2 weeks. Generally, concentrations of NEFA are back to basal levels after 6 weeks or more (Adewuyi *et al.*, 2005). As for glucose, NEFA is affected by stressful handling of animals before sampling (Leroy *et al.*, 2011). Concentrations of NEFA are significantly higher at one hour before the first morning feeding, and within a herd or an experiment, samples must be taken at a constant time of the day (Quiroz-Rocha *et al.*, 2010).

BHBA is also under homeorhetic regulation with large variations at cow-level (Ingvartsen, 2006). Generally, BHBA increases later than NEFA and peaks at approximately 2 weeks post-partum (Hachenberg *et al.*, 2007). BHBA shows some variation related to feeding. Low concentrations have been reported after feeding in animals not fed *ad libitum* (Andersson & Lundström, 1984) and high concentrations after feeding a total mixed ratio (TMR) *ad lbitum* (Quiroz-Rocha *et al.*, 2010). Samples of BHBA should therefore be taken at the same time point during the day in a herd or within an experiment.

Concentrations of the peptide hormone ***IGF-1*** parallel the insulin concentrations with a sharp decrease at calving and a gradual increase after calving (Hachenberg *et al.*, 2007; Schams *et al.*, 1991). The concentration of IGF-1 in blood is also affected by parity, with primiparous cows having higher concentrations than multiparous cows (Taylor *et al.*, 2004). IGF-1 is considered indicative of metabolic competency for reestablishment of fertility during the post-partum period (Velazquez *et al.*, 2008).

1.6 Test evaluation

1.6.1 Sensitivity and specificity

Because of the strong association between NEB and fertility, metabolic indicators can potentially be used as a diagnostic test to predict decreased fertility. By convention, a diagnostic test refers to laboratory analysis of a sample in order to detect or exclude disease (*e.g.* presence of antibodies in a non-vaccinated animal). However, in a broader context, a diagnostic test could involve anything (*e.g.* serological sample, rectal examination or pathological-anatomical diagnosis of a biopsy) that is used to detect the status of the animal (*e.g.* pregnancy, oestrus cyclicity or presence/absence of cancer) (Dohoo *et al.*, 2009a). Two key components in test evaluation are the diagnostic sensitivity (the proportion of unhealthy individuals that test positive; Se) and the specificity (the proportion of healthy individuals that test negative; Sp) of the test (Table 1). Even though Se and Sp are characteristics of the test itself, external factors influencing Se and Sp, such as breed, sex, disease incidence and the presence or not of an eradication programme, have been identified (Greiner & Gardner, 2000). During test evaluation, influencing factors can be dealt with using a stratification of the analysis or by a logistic regression approach (Coughlin *et al.*, 1992).

Table 1. *Expressed as a two-by-two table, sensitivity = a/m_1, specificity = d/m_0, a positive predicted value = a/n_1 and a negative predicted value = d/n_0.*

.	Test positive (T+)	Test negative (T-)	Total
Diseased (D+)	a (true positive)	b (false negative)	m_1
Healthy (D-)	c (false positive)	d (true negative)	m_0
Total	n_1	n_0	n

1.6.2 Cut-off values

For tests that give outcomes on a continuous scale, the Se and Sp vary with different cut-off values. The change in Se and Sp for different cut-off values can be graphically described in a two-graph receiver operating characteristics (TG-ROC) graph (Figure 6) where Se and Sp are plotted on the y-axis against all possible cut-off values on the x-axis. (Greiner *et al.*, 1995). The most optimal cut-off value varies depending on the application of the test and is not necessarily related to the point where the lines of Se and Sp cross. In general, a cut-off value with high Se, and therefore few false negatives, is desirable when the test is used to detect disease. On the other hand, when the aim is to rule out disease and few false positives are wanted, a cut-off value with high Sp is more useful.

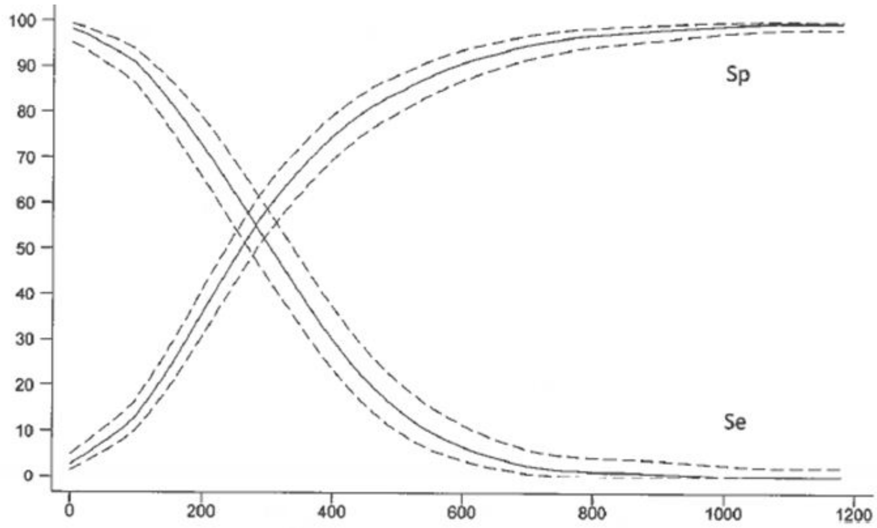

Figure 6. Example of a two-graph receiver operating characteristics graph, where Se and Sp are plotted against all possible cut-off-values on the x-axis.

1.6.3 Predictive values

While Se and Sp are characteristics of the test itself, the prevalence of the investigated health disorder may influence the test and the usefulness of the test therefore depends on the population to which the test is applied. The usefulness is measured by the predicted values of the test. The positive predicted value (PV+) is the proportion of animals testing positive that are actually diseased. Correspondingly, the negative predicted value (PV-) refers to the proportion of animals testing negative that are actually free of disease (Table 1).

When a test is evaluated, Se and Sp, PV+ and PV- and the prevalence of the event of interest in the population (*i.e.* apparent prevalence) and sample size in the investigated population are considered a minimum of factors that should be investigated (Greiner & Gardner, 2000).

2 Aims

The overall aims of this thesis were to evaluate the effect of supplemental feeding with glycerol and propylene glycol to dairy cows in early lactation, to investigate how and with what accuracy cows with a risk of decreased fertility could be identified in early lactation, and to investigate risk factors for decreased fertility at cow and herd level. Specific aims were to:

- Evaluate the effect of supplemental feeding with glycerol or propylene glycol to dairy cows in early lactation on metabolic status, body condition and milk yield.
- Evaluate whether supplemental feeding with glycerol or propylene glycol affects the onset of cyclicity in dairy cows.
- Evaluate if supplemental feeding with glycerol or propylene glycol affects the time of first AI or day of conception.
- Investigate the extent to which the different hierarchical levels (cow level or herd level) contribute to the variability in metabolites and hormones related to energy metabolism.
- Assess the usefulness of a single measurement of NEFA or BHBA in early lactation in predicting the risk of decreased fertility; and investigate how cow-level factors and different prevalences of decreased fertility affect the results of the test.
- Investigate some risk factors for decreased fertility, related to the cow and to management and housing, which could potentially be used when directing preventive measures.

3 Material and methods

An overview of the materials and methods used in this thesis is provided below. More detailed descriptions can be found in Papers I and II which describes field experimental studies, and Papers III and IV, which report observational studies. Papers I – III are all based on observations from the same herds (in total 17 herds), while Paper IV is based on questionnaire data combined with data from SOMRS (in total 759 herds).

3.1 Animals and herds

The use of animal as research objects in Papers I-III was approved by the local animal ethics committee (Gothenburg, Sweden). No ethical approval was necessary for Paper IV, as the data were obtained from a questionnaire and from SOMRS.

In Papers I-III, cows that calved from 1 October 2007 to 28 February 2009 in the same 17 commercial herds were included. Of these, 12 herds were included in Papers I and III, and 17 herds in Paper II. The herds were located in south-west Sweden, in the vicinity of the Swedish University of Agricultural Sciences, Skara. The herds had tie-stalls, were non-organic and had on average 60 calving cows per year (range 45–100). The cows were milked twice daily and the herds were affiliated to SOMRS. In all herds except one herd in Paper II, the cows were fed an individual diet of grass silage and concentrates based on monthly milk yield and BCS (Spörndly, 2003). In the remaining herd in Paper II, a TMR based on grass silage, fresh hard-pressed sugar beet pulp and grains was fed *ad libitum* twice daily. During summer, cows in all herds were on pasture for a minimum of three months.

In Paper IV, cows calving in 759 Swedish herds during the period 1 Mars 2010 to 28 February 2011 were included. The herds were geographically spread across the country and had on average 156 calving cows on a yearly basis (range 60-624). Of these, 54% had tie-stalls, 35% had loose-housing systems and the remaining herds were changing from tie-stalls to loose-housing. Eighty percent of the herds had conventional milking, 14% had an AMS and the remaining were changing from the former to the latter. Four percent were certified organic according to KRAV, 93% were of conventional production type and approximately 3% were changing to organic. Separate feeding of roughages and concentrates with individually fed concentrates (SEP) dominated among the herds (50%), 34% of the herds had PMR, 11% had TMR and for approximately 6% of the herds the feeding system was unknown. Information on number of cows across breed and parity in the Papers I-IV is presented in table 2.

Table 2. *Distribution of cows across breed and parity in Papers I-IV.*

		Paper1 (12 herds)	Paper 2 (17 herds)	Paper 3 (12 herds)	Paper 4 (759 herds)
Breed	Swedish Red	396	344	297	23,368
	Swedish Holstein	201	371	129	30,917
	Other[3]	76	81	54	9276
Parity	1	243	296	176	19,617
	2	190	224	136	18,218
	≥3	240	276	168	25,726
Total number of cows		673	798	480	63,561

3.2 Supplemental feeding

In Papers I and II, cows were randomly distributed to one of three groups at calving: group GLY, group PG or a control group (C). During 0-21 DIM, cows received supplemental feeding with (on a daily basis) 450 g of liquid GLY (refined and of 99.9% purity), 300 g of liquid PG, or no supplement (C). The supplements were given twice daily as a top-dress on concentrates or TMR (one herd in Paper II).

Originally, the doses were intended to correspond to equal amounts of energy on a molar basis. Unfortunately, the GLY dose was calculated with crude (80%) GLY in mind, but pure (99.9%) GLY was delivered to the farms. Due to this mistake, the PG dose corresponded to 80% of the energy provided by the GLY dose. Therefore comparisons were made between group C and group PG and group C and group GLY, not between GLY and PG.

The supplements were delivered to the farms in colour-coded containers. The supplements were colourless and odourless, but GLY was slightly more viscous. Therefore, water was added to the supplements so that the viscosity was more similar and to make the given amounts correspond to equal volumes (250 mL each).

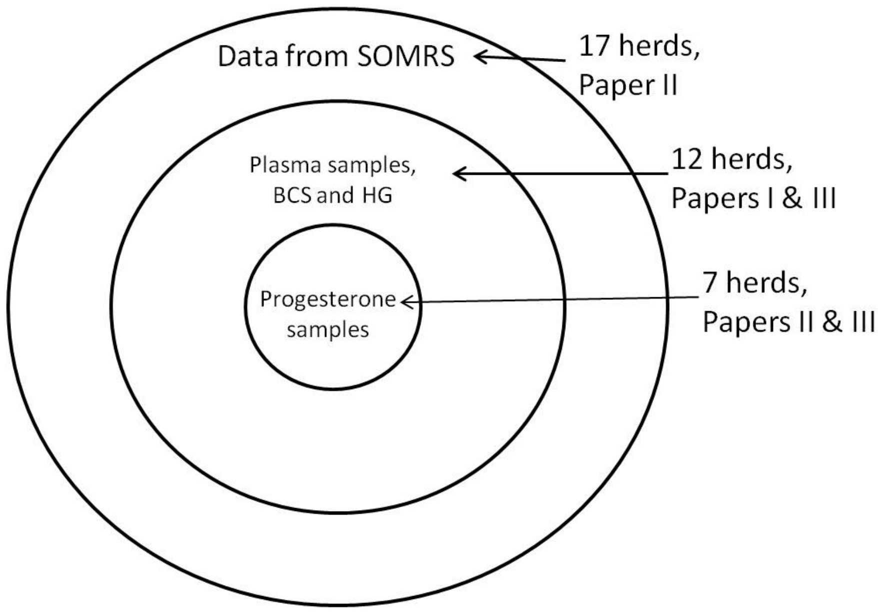

Figure 7. Description of herds subjected to different samplings in Papers I–III.

3.3 Data collection

3.3.1 Sampling

An overview of the cows and herds subjected to different samplings in Papers I-III is shown in Figure 7. Blood samples were collected in Na-heparin-coated vacuum tubes (BD Vacutainer, Plymouth, UK) on three occasions during the post-partum period at three-week intervals, *i.e.* at 1-3, 4-6 and 7-9 weeks after calving (Figure 8). The samples were taken in the coccygeal vein or artery and kept on ice until they were centrifuged at 2000 g for 20 minutes within an hour after sampling. The plasma was stored in a deep-freeze until analysis for plasma concentrations of glucose, BHBA, NEFA, insulin and IGF-1. The blood sample results were further used in Papers I and III.

On the same occasions as blood sampling, measurements of BCS and heart girth (HG) were recorded. Body condition score was obtained on a 5-point scale (Edmonson *et al.*, 1989) and the measurements were further used in Paper I.

In Papers II and III, milk samples were collected twice weekly starting at 14 DIM and ending when the first heat was detected. The milk samples were taken by the farmers after milking and stored in a deep-freeze until analysis of progesterone.

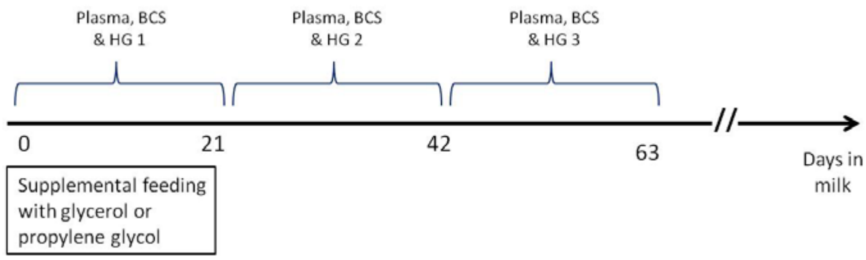

Figure 8. The sampling protocol from 0-63 days in milk used in Papers I and II in relation to period of supplemental feeding (0-21 days in milk).

3.3.2 Laboratory analyses

The plasma and milk samples were analysed at the Department of Animal Science, Aarhus University, Denmark. All plasma samples were analysed with regard to concentrations of glucose, insulin, NEFA and BHBA. Plasma samples taken 0-23 DIM were also analysed in terms of concentration of IGF-1. Samples showing evident haemolysis were excluded from the study. The analyses of plasma metabolites (*i.e.* glucose, NEFA and BHBA) were performed with an auto-analyser ADVIA 1650® Chemistry System (Siemens Medical Solutions, Tarrytown, NY 10591, USA). Insulin and IGF-1 were determined using non-competitive, time-resolved immunofluorometric assays as described by Løvendahl & Purup (2001). Milk samples were analysed for concentrations of progesterone according to the method described by Friggens *et al* (2008). The analytical methods used are further described in Papers I and II.

3.3.3 Questionnaire

In Paper IV, a questionnaire was sent to the chief feed advisors at the seven regional dairy associations in Sweden. For all herds >60 cows with which they were familiar, the feed advisors were asked to indicate whether the herd used a TMR, PMR or SEP feeding system, using the definitions of these feeding systems as shown in Table 3. The advisors were also asked to state whether the herds received advanced monthly feed advice where rations were formulated according to BCS of all cows.

Table 3. *Definitions of the three feeding systems compared in Paper IV.*

Feeding system	Description
Total mixed ratio (TMR)	All roughages and concentrates were mixed and fed together; concentrates < 1kg given during milking were not counted.
Partial mixed ratio (PMR)	Roughages and concentrates were mixed and fed together but >1kg of the concentrates were also given individually in automatic feeders.
Individual feeding (SEP)	All roughages were fed separately from concentrate, which were fed individually.

3.3.4 Data from SOMRS

For all cows in Papers I-IV, information on breed, calving date, parity, monthly test day milk yield (kg), milk composition (kg of fat and kg of protein) and somatic cell count (SCC) were collected from SOMRS. Furthermore, data on fertility, including dates of inseminations, gynaecological examinations, veterinary examinations and treatments were collected for Paper II. In Papers I –II, data on cases of diseases not treated by a veterinarian (and thus not included in SOMRS) were reported by farmers using standardised protocols.

For herds in Papers IV, information regarding milking system (conventional or AMS), housing (whether the cows were held in tie-stalls or in a loose-housing system) and whether the herd was organic or non-organic was collected from SOMRS. This data also contained information about when a herd changed from one system to another.

Because farmers sometimes report reproductive events late (Löf *et al.*, 2007a), all data were collected from SOMRS at least 3 months after the end of the follow-up period.

3.4 Data editing

3.4.1 Exclusion of animals

Sixteen cows in Papers I and 22 cows in Paper II were excluded because the farmers decided to stop their supplemental feeding, mainly due to severe illness in these cows. The remaining 673 (Paper I) and 798 (Paper II) cows were included in the studies.

In Paper II and III, cows that were subjected to treatment for ovarian cysts or other hormonal treatment before the reproductive event of interest were excluded from the analyses.

In Paper IV, questionnaire data was available for 91,162 cows in 771 herds. After removal of cows served by a bull, cows subjected to embryo transfer, cows without any recorded data on insemination or other fertility and cows with missing milk yield data, the remaining 63,561 cows in 759 herds were included.

3.4.2 Definition of reproductive performance indicators

First luteal activity (FLA) was defined as the first day after calving when a progesterone concentration above 4 ng/mL was observed (Friggens *et al.*, 2008) and when the previous samples had been taken with a maximum interval of 7 days. First AI (FAI) was defined as the first day after calving with an AI registered within SORMS. The day of an AI followed by a calving recorded within 270–290 days, or when the AI was followed by a recorded pregnancy check (rectal palpation or ultrasound examination) with positive results, was defined as the day of conception (CON). Pregnant or not at first AI (PAI) and number of AI in animals submitted to AI (NINS) were also used as reproductive performance indicators in Paper IV.

Anoestrus (ANEST), delayed first AI (DFAI) and delayed conception (DCON) were used in Paper III as reference tests of decreased fertility. If the cow did not experience the event of interest (FLA, FAI or CON) before the DIM corresponding to the 75th percentile in the study population, she was considered to have decreased fertility (Table 4).

Table 4. *Days in milk corresponding to the 75th percentile in the study population for anoestrus (ANEST), delayed first AI (DFAI) and delayed conception (DCON) (Paper III).*

Reference test	75th percentile (days in milk)
ANEST	36
DFAI	96
DCON	145

3.4.3 Definition of other parameters

In Paper I, each first case of a disease in a cow (excluding recurrences) was grouped as ketosis (ketosis, feed depression or displacement of the abomasum), mastitis (acute mastitis or fever caused by other infection), paresis (puerperal paresis or hypomagnesemia) or other disease (any record of a disease with or without a diagnosis where the cow had a disturbed general condition).

Energy corrected milk (kg ECM) was calculated using the formula *0.25 x kg milk + 12.2 x kg fat + 7.7 x kg protein* (Swedish Dairy Association, 2004).

3.5 Statistical analyses

3.5.1 Model building (Papers I – IV)

All statistical analyses were performed in Stata (Stata release 11, StataCorp LP, College Station, TX., USA).

In Paper I, the effects of supplemental feeding with GLY or PG on the plasma concentrations of glucose, insulin, NEFA and BHBA measured 4–63 DIM, monthly test day milk yield during the first 90 DIM, BCS and HG were investigated using linear mixed effects regression models (Rabe-Hesketh & Skrondal, 2005). Insulin, NEFA and BHBA data were log-transformed to better reach normal distribution. The models included a random intercept for cow and herd to account for repeated measurements within cows and the cluster of cows within herds. Because sampling was not repeated within cows, the effects of supplemental feeding on the plasma concentrations of IGF-1 were evaluated using linear mixed effects models with a random intercept only for herd.

In Paper II, the effects of supplemental feeding on the fertility parameters FLA, FAI and CON were investigated using semi-parametric survival models (Cox proportional hazards models) with a frailty term accounting for the clustering effect of herd (Dohoo *et al.*, 2009b). Cows that did not get the first AI before 150 DIM or that did not conceive before 365 DIM were not included in FAI and CON models, respectively.

In Paper III, the associations between a cut-off value in plasma concentrations of NEFA, BHBA and the outcomes representing decreased fertility (ANEST and DFAI) were evaluated using a random logistic regression model (Coughlin *et al.*, 1992). In such a model, strata-specific Se and Sp are obtained. In model evaluation, robust standard errors were applied (Dohoo *et al.*, 2009b). Further details on selection of cut-off values are given in section 3.5.2.

In Paper IV, the effects of the risk factors included on the outcomes in terms of PAI and NINS were investigated using a logistic regression model and a Poisson distributed regression model, respectively. Both models included a random intercept for herd to account for its clustering effect.

Model building strategies followed that proposed by Hosmer & Lemeshow (2000). The general full models in Papers I and II included group as main effect and breed, parity, milk yield and calving season as potential biological predictors. Milk yield was included using the mean milk yield at the monthly test days during the first 60 DIM. In Paper III only breed and parity were included as potential predictors. In Paper IV, cow-level and herd-level predictors were screened using univariate models where covariates with

P<0.25 were included in the full models. The initial full models were reduced using backwards step-wise elimination with Wald P=0.05 as a decision threshold. Biologically plausible interactions were tested to the reduced model. The functional forms of the continuous predictors and the outcomes were investigated using fractional polynomials or by adding a quadratic and cubic term to the models.

3.5.2 Evaluation of thresholds in plasma concentration (Paper III)

In Paper III, multiple cut-off values were constructed, one at each 50μeq./L step of NEFA, one at each 0.2 mM step of BHBA and one at each 10 ng/mL step of IGF-1. Using the standard cross-tabulation technique (Dohoo *et al.*, 2009a) the Se and Sp at each cut-off value in relation to ANEST, DFAI and DCON were calculated. Furthermore, TG-ROC curves (Greiner *et al.*, 2000) were constructed for each test and outcome to visually describe how test performance changed with changing cut-off values.

In general, the cut-off values with the highest combined Se and Sp would classify about half the cows in the study population as test positives. From a practical perspective, this is not useful, so cut-off values of NEFA, BHBA and IFG-1 with a high Sp (80%) were chosen and further evaluated. In this step cut-off values of NEFA and BHBA were used, while the IGF-1 cut-off value was omitted from the models and from further evaluation due to the general poor accuracy of the test (based on TG-ROC curves). Furthermore, the fertility measure DCON was omitted from model building and from further evaluation due to the multi-factorial aetiology of decreased fertility. Thus the NEFA and BHBA tests for ANEST and DFAI were further evaluated.

3.5.3 Variance decomposition (Paper I)

In Paper I, the sources of variations in the models were evaluated by comparing the random effects in the final models with a model containing only the constant. In this way, the variation at the level of farm, cow and residual was obtained.

3.5.4 Diseases (Paper I)

In Paper I, the effects of supplemental feeding on the occurrence of any of the categories ketosis, mastitis, paresis and other were investigated using Fischer's exact test. In this test, the number of non-cases and cases were compared in the C group with the corresponding numbers in the GLY or PG group.

4 Main Results

A summary of results of the studies included in this thesis is presented below. More detailed information on these results can be found in Papers I-IV.

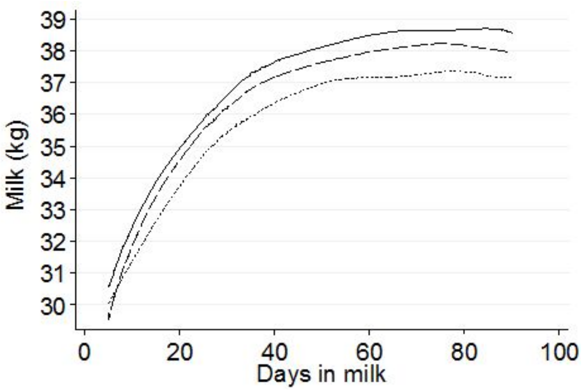

Figure 9. Milk yield at monthly test days during the first 90 days in milk among 673 cows receiving supplemental feeding with glycerol (____), propylene glycol (− − −) or control (.......) over their first 21 days in milk.

4.1 Effects of glycerol or propylene glycol (Papers I and II)

In general, there were few differences regarding the measured parameters between groups GLY and C, and between groups PG and C. Cows that were supplemented with GLY had a higher mean milk yield (P=0.03) at monthly test days during the first 90 DIM compared with control cows (Figure 9). When measured in kg, this increase in yield was 1.24 (95% CI = 0.28-2.21) while in kg ECM it corresponded to 0.95 (95% CI = 0.03-1.86) per monthly test day. For cows supplemented with PG, there was a tendency (P=0.06) for an increase in milk yield corresponding to 0.94 kg (95% confidence interval = -0.03-1.91).

There were no differences between group PG and group C when milk was measured in kg ECM.

No significant differences in plasma concentrations of glucose, NEFA, BHBA and IGF-1 were found between cows in group C and those in groups GLY or PG (Figure 10A-D). However, cows in the GLY group had lower plasma insulin concentrations compared with the C group (P=0.01). Body condition score across all groups and adjusted for other covariates was 2.9±0.04 during the first 3 weeks post-partum and 2.5±0.04 during weeks 7 to 9 post-partum. The corresponding HG was 198±0.41 cm and 194±0.45cm, respectively. No differences between treatment groups were seen with regards to BCS and HG.

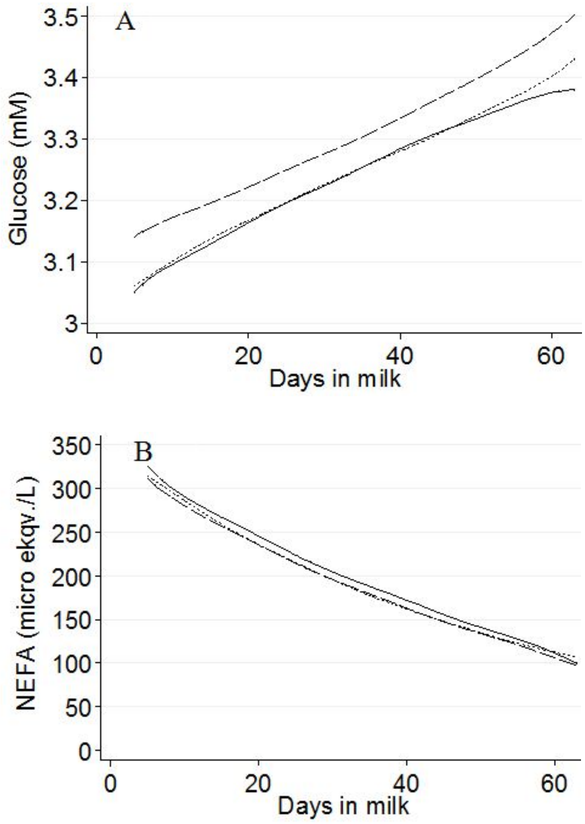

Figure 10. Predicted plasma concentrations of (A) glucose and (B) non-esterified fatty acids (NEFA) among 673 cows receiving supplemental feeding with glycerol (____), propylene glycol (− − −) or control (.......) over their first 21 days in milk.

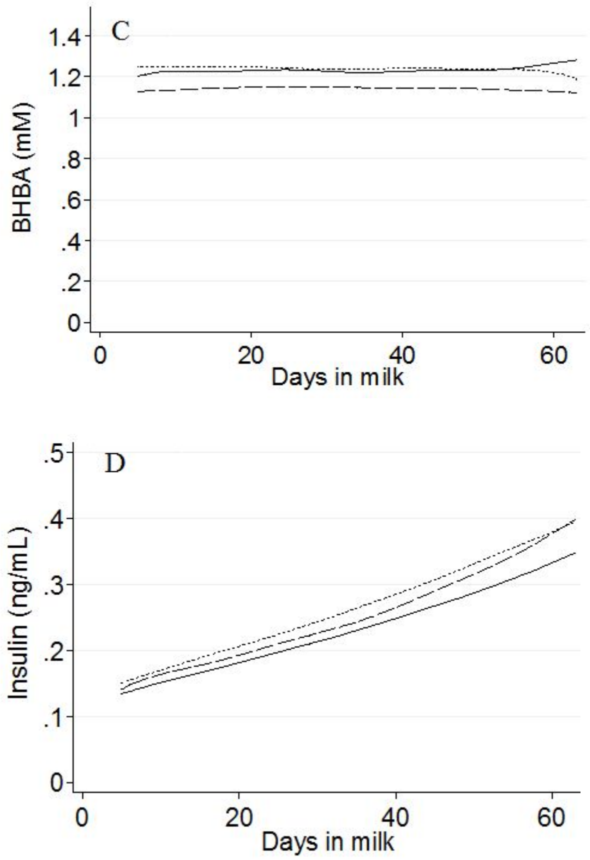

Figure 10. Predicted plasma concentrations of (C) β-hydroxy butyrate (BHBA) and (D) insulin among 673 cows receiving supplemental feeding with glycerol (——), propylene glycol (− − −) or control (⋯⋯) over their first 21 days in milk.

Of all cows in the GLY, PG and C group in Paper I, 50, 48 and 53 cows, respectively, were diagnosed with one or more of the defined diseases on one or more occasions between 0 and 90 DIM. There were no significant differences between control cows and cows treated with GLY (0.58<P<1) or PG (0.21<P<0.59).

Data on the interval from calving to FLA, FAI and CON for cows across the treatment groups in Paper II are presented in Table 5.

Table 5. *Distribution of the interval from: calving to first luteal activity (FLA), calving to first insemination (FAI) and calving to conception (CON) across cows supplemented with glycerol (GLY), propylene glycol (PG) or supplementation (C) (Paper II).*

Interval	Group	Median	25th percentile	75th percentile
Calving-FLA	GLY	23	14	61
	PG	22	15	69
	C	23	15	58
Calving-FAI	GLY	80	50	134
	PG	77	51	128
	C	75	49	128
Calving-CON	GLY	102	54	203
	PG	107	57	238
	C	102	54	239

The Kaplan-Meier graphs of FLA, FAI and CON, where the cumulative survival was plotted against time after calving, indicated that there were no major differences in the probability of experiencing these events after calving depending on supplemental feeding group (Figure 11A-C). At any point in time, there were no differences in the hazard ratio (HR) of having a FLA (P=0.55) or conceiving (P=0.49) between group C and either of the other groups. In other words, the interval from calving to FLA or FAI did not differ between the groups. Cows in the GLY group tended to have a later FAI than control cows (HR=0.84, P=0.07). No such difference was seen in the PG group (HR=0.96, P =0.14).

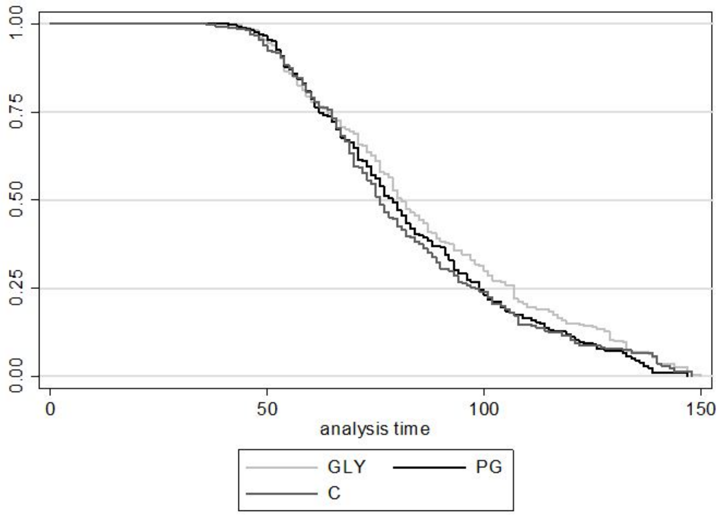

Figure 11 A.

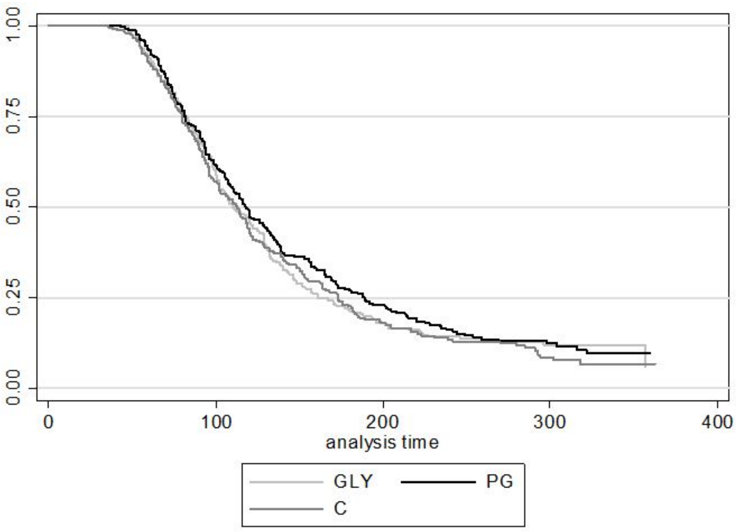

Figure 11 B.

Figure 11. Kaplan-Meier survival estimates of the probability of a first luteal activity (A) or first insemination (B) in cows given supplemental feeding with glycerol (GLY), propylene glycol (PG) or no supplementation (C).

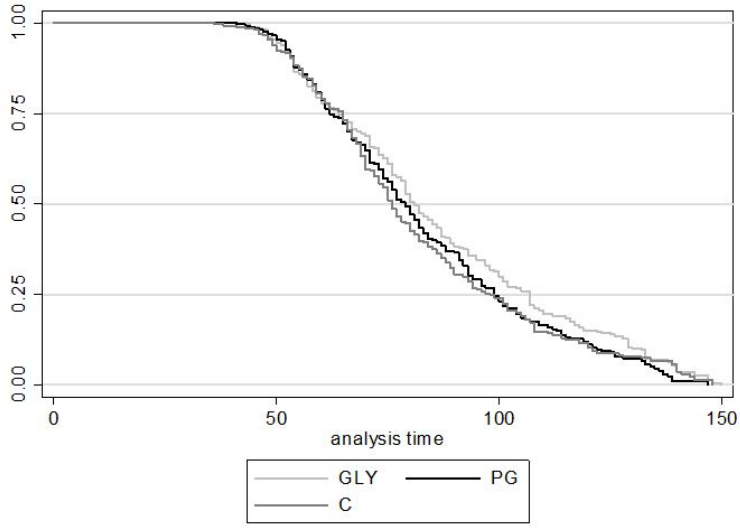

Figure 11C. Kaplan-Meier survival estimates of the probability of a conception in cows given supplemental feeding with glycerol (GLY), propylene glycol (PG) or no supplementation (C).

4.2 Variance decomposition (Paper I)

Overall, the variation in all models was lowest at the level of herd and highest at the level of cow and residual. For hormones and metabolites, between 47 and 59% of the variation was unexplained error. Of the remaining random variation, explained variation at the cow level was in general more than twice that at the herd level.

4.3 Test accuracy of metabolic indicators in predicting decreased fertility (Paper III)

The median plasma concentration (with values corresponding to the first and third quartiles in brackets) of NEFA was 278 (174-405) µeq./L. The corresponding concentration of BHBA was 1.0 (0.75-1.51) mM and that of IGF-1 97 (67-132) ng/mL.

The investigated cut-off values had either a high Se or a high Sp and the highest combined Se and Sp were in general only approximately 60%. The cut-off values for the tests that corresponded to a Sp of approximately 80% are shown in Table 6.

Table 6. *Cut-off values of plasma concentration of non-esterified fatty acids (NEFA) and β-hydroxy butyrate (BHBA) with a Sp of approximately 80% when used to predict anoestrus (ANEST) and delayed first insemination (DFAI) in dairy cows.*

Evaluated test	Reference standard	Cut-off value	Se	95% CI	Sp	95% CI
NEFA (μeq./L)	ANEST	400	0.45	0.32-0.57	0.79	0.72-0.85
	DFAI	400	0.27	0.21-0.34	0.76	0.71-0.81
BHBA (mM)	ANEST	1.8	0.30	0.18-0.42	0.80	0.73-0.86
	DFAI	1.8	0.15	0.10-0.22	0.80	0.75-0.84

Parity was significantly associated with both tests and reference standards. The estimated Sp of the different tests was higher when the tests were used in cows of parity one or two, than when used in cows of parity three or older. Breed was significant only for the BHBA test in the DFAI model, where Sp was higher when the test was used on SH cows compared with SR cows.

As expected, the prevalence of decreased fertility in the herds affected PV+ and PV-. In herds with a high prevalence of decreased fertility, PV+ was higher, but still <0.70. PV- was increased in populations with a low prevalence of decreased fertility.

4.4 Risk factors for decreased fertility (Paper IV)

Many of the covariates investigated in Paper IV were found to be significantly associated with PAI and NINS. The occurrence of disease affected fertility negatively and cows that had severe claw lesions or displayed a rise in SCC after calving had a lower probability of PAI and received more NINS than healthy cows. Other cow-level covariates that were significantly associated with fertility were breed, parity and calving season. The length of the interval calving and first AI was significantly associated with fertility. The individual milk yield at the first test day after calving was also significantly associated with PAI, but the odds ratio were equal to 1.00.

Among the herd-level variables, a significant interaction of housing and feeding system was found. There were no differences in measured reproductive performance between cows kept in a loose-housing system and fed TMR or PMR compared to cows kept in tie-stalls and fed SEP. Variables representing a change in system (*i.e.* from conventional milking to AMS; from conventional production to organic or from tie-stalls to loose-housing) were significantly associated with a lower probability of pregnancy at first AI and with receiving more NINS (not valid for change from conventional to organic) compared with cows in an unchanged system. There were no differences in fertility between cows milked in an AMS compared to cows milked conventionally. Cows in organically managed herds had a lower probability of PAI and received more NINS than cows in non-organic herds. The reproductive performance was also significantly different between some of the regions studied.

There were no significant associations between NINS or PAI and high SCC, herd size, herd level of production and whether the farm received advanced feed advice or not.

5 General Discussion

5.1 Effects of glycerol and propylene glycol

5.1.1 Milk yield

In Paper I, milk yield, measured both in kg and in kg ECM in cows supplemented with GLY 0–21 DIM increased during the first three months of lactation compared with cows in the control group. Although differences were not statistically significant, cows supplemented with PG had a higher yield of kg milk compared with control cows. When milk yield was measured in kg ECM no such difference was observed. This might indicate that PG induced a deprivation of milk fat or protein, as previously reported by Liu *et al.* (2009). Because of the uneven doses that were given, it is important to note that the milk yield results do not necessarily indicate that GLY is 'better' than PG. It is not known if a PG dose corresponding to 20% more energy, as in the GLY dose, would cause the milk yield to be significantly increased.

The results for supplemental feeding with PG are in agreement with those of earlier studies where cows supplemented with PG in early lactation showed unchanged yield or a tendency towards increased yield (Chung *et al.*, 2009a; Hoedemaker *et al.*, 2004; Juchem *et al.*, 2004). The results from previous studies of supplementation with GLY in early lactation are conflicting and have shown unaffected yield (Wang *et al.*, 2009) or a tendency towards lower milk yield (DeFrain *et al.*, 2004) compared with un supplemented controls. These conflicting results could derive from the study design used, *e.g.* the doses given, whether the substances were top-dressed onto concentrate or drenched and the physiological status of the animals (see further sections 5.1.2, 5.1.4 and 5.4).

5.1.2 Metabolic status and body condition

The plasma concentrations of glucose, NEFA, BHBA and IGF-1 were not improved in cows supplemented with GLY or PG (Paper I). However, the lack of decrease in plasma metabolites and IGF-1, together with the increased milk yield in cows given GLY, indicates better metabolic status. In earlier studies, supplementation with PG generally increased glucose and insulin, and decreased BHBA and NEFA (reviewed by Nielsen & Ingvartsen, 2004). In addition, several studies report increased glucose levels during feeding or drenching with GLY (Wang *et al.*, 2009; Osman *et al.*, 2008; DeFrain *et al.*, 2004).

In Papers I and II, the substances were fed as a top-dress on concentrates or on a TMR. Studies comparing methods of delivery (*i.e.* oral drenches, mixing with concentrates or in a TMR) have concluded that the positive metabolic effects of GLY and PG seem to be dependent upon rapid delivery, *i.e.* drenching (Chung *et al.*, 2009b; Linke *et al.*, 2004; Christensen *et al.*, 1997). It has also been found that the insulin resistance-inducing properties of PG depend on rapid delivery (Kristensen *et al.*, 2002). Therefore, it is possible that cows in Papers I and II had lower peak values of glucose and insulin after supplementation with GLY and PG than if these would have been administered in a drench, which might have influenced the result. However, the actual objective of the studies was to investigate the effects of these supplements under practical conditions using an allocation method that was easy for the farmer to use and that did not stress the animals.

Cows in Papers I and II supplemented with GLY had a decreased plasma concentration of insulin during 4-64 DIM which may indicate decreased metabolic status. However, these cows produced more milk without a subsequent decrease in reproductive performance and with unaffected concentrations of glucose, NEFA, BHBA and IGF-1, indicating that they could cope with the metabolic stress. Reduced DMI has been found to rapidly decrease insulin and glucose concentrations (Agenäs *et al.*, 2003b). In our field study, DMI was not recorded but no apparent decrease in DMI was reported by the farmers. In addition, Omazic *et al.* (2011) found that SR cows given concentrates that were top-dressed twice daily with crude or refined GLY did not have decreased DMI, compared with un-supplemented controls. The importance of the decreased insulin concentrations observed in the GLY cows remains unknown.

No differences in BCS were found between cows in the different groups during or in the weeks following the period of supplemental feeding. However, BCS as a measurement of metabolic status can have poor precision and may have poor repeatability.

5.1.3 Fertility

Overall, cows in Paper II did not have improved fertility measured as probability of luteal activity, first AI or conception, due to the supplemental feeding. Similarly, Moallem *et al.* (2007) and Hoedemaker *et al.* (2004) observed no effects in early post-partum follicle dynamics and no increase in luteal activity (defined as a serum progesterone concentration of >3.18 nmol/L) in cows fed PG compared with controls. To the best of my knowledge, no study other than Paper II has investigated the effects on early fertility measurements following supplemental feeding with GLY. An early resumption of ovarian activity has been positively associated with subsequent pregnancy (Wathes *et al.*, 2007b) and generally, cows with a less severe NEB have an earlier resumption of ovarian cyclicity after calving (Butler, 2003). The cows in Paper II supplemented with GLY increased their milk yield and cows given PG tended to do the same, which might be one reason for the lack of effect on resumption of ovarian activity. It is possible that high-yielding cows, when provided with extra energy, use this for milk lactose synthesis rather than to decrease lipid mobilisation and improve fertility.

On average, cows in the GLY group were given their first insemination later than the control cows, although the difference was not statistically significant. Time to first insemination is dependent on several factors, *e.g.* decisions made by the farmer, the cow's ability to show oestrus and the farmer's ability to detect oestrus (*i.e.* heat detection rate). In general, cows with a high milk yield have a shorter duration of oestrus than low-yielding cows (Lopez *et al.*, 2004). As shown in Paper I, cows supplemented with GLY produced 1.2 kg ECM more per day. However, it is unlikely that this difference in milk yield is sufficient to affect the heat detection rate or the farmer's decision to inseminate or not. The reason for this unexpected tendency for delayed FAI in cows given GLY remains to be identified.

No differences in the probability of conception were seen between the GLY group and controls, or the PG group and controls. This agrees with Hoedemaker *et al.* (2004), who did not find a shortened interval from calving until conception in cows supplemented with PG during late pregnancy and early lactation. On the other hand, Pehrson *et al.* (1991) found that cows fed supplements comprising of GLY, calcium propionate, soya and beet pulp between 40 and 75 DIM had a shorter interval between calving and conception. Although NEB can extend beyond the first six weeks of lactation (Nielsen & Ingvartsen, 2004) nadir often occurs at 2–3 weeks post-partum (Butler & Smith, 1989) and it was therefore decided to feed the supplements during early lactation in this thesis.

The later FAI combined with the non-affected time to CON in cows in the GLY group compared with control cows indicates that cows treated with GLY conceive more quickly. However, there were no differences in the mean number of AI per conception between the groups, which indicates that overall fertility was not improved.

5.1.4 Should Swedish herds use supplemental feeding routinely?

Altogether, feeding dairy cows with GLY and PG during the first 21 DIM could potentially increase the milk yield during the first 3 months of lactation without a subsequent decrease in metabolic status or fertility. However, it is uncertain whether this increase in milk yield is cost-effective.

In Papers I and II, the supplemental feeding with GLY and PG was given to all cows in the herds, regardless of their physiological status. However, Ingvartsen (2006) argues that cows in physiological imbalance can derive most benefit from a preventive measure and that such cows can be identified based on plasma metabolites and hormones. In Paper I, the herd variable generally explained less than 19% of the variation in metabolites and hormones, while the cow variable explained more than twice the variation explained by 'herd'. In addition, >90% of the variability in the parameter first-service conception risk and first service to conception interval in Canadian herds resided in the individual lactation (*i.e.* cow-level), and only <10% at the herd level (Dohoo *et al.*, 2001). This further supports the idea that interventions aiming to increase metabolic status and fertility should not be applied to all animals in a herd. Instead, a risk-based approach should be favoured and attention should be focused on animals in need of extra care.

In recent studies, McArt *et al.* (2012) found that PG-treated cows with subclinical ketosis (defined as a BHBA concentration of 1.2 to 2.9 mmol/L) were 1.3 times more likely to conceive at first insemination than cows with subclinical ketosis but without PG treatment. In two of the herds included in that study, the PG-treated cows also showed an increase in milk yield of 1.3–1.6 kg during the first 30 DIM. Furthermore, it was found in studies on grazing heifers in New Zealand with a poor BCS at calving, that the interval from calving to first ovulation was reduced when they were given PG (Chagas *et al.*, 2007b). As discussed in Paper I, cows that were included in that study had in general only a small loss of body condition during the follow-up period, which indicates minor mobilisation and few cases of severe NEB (Roche *et al.*, 2009). Therefore, the effect of the supplemental feeds might have differed if cows in physiological imbalance had been selected for treatment.

In conclusion, the findings presented in this thesis do not support a strategy involving supplemental feeding to all cows in Swedish dairy herds. However, a strategy to treat based on presence of clinical disease at calving, high BCS at calving (*e.g.* BCS >3.25-3.5) or indication of *e.g.* subclinical ketosis could prove useful.

5.2 Predicting decreased fertility using metabolic indicators

5.2.1 Cut-off values of metabolic indicators in plasma

In Paper III, the diagnostic abilities of plasma concentrations of NEFA, BHBA and IGF-1 in predicting decreased fertility were evaluated. Although cut-off values for this purpose have been proposed earlier (Ospina *et al.*, 2010a), Paper III used an approach that is novel in this context but is commonly used when diagnostic tests are evaluated.

In general, the highest combined Se and Sp of the tests for ANEST and DFAI were low. Here, a cut-off value with high Sp was favoured in order to make the test more practical to use (*i.e.* fewer animals to treat). The NEFA cut-off value (400 μeq./L) and BHBA cut-off value (1.8 mM) chosen for further evaluation corresponded to a Sp of approximately 80%. Ospina *et al.* (2010a) used a higher NEFA cut-off value of 720 μeq./L (serum) when evaluating the effects of increased fat mobilisation on fertility. However, that threshold was set with the purpose of predicting displaced abomasum (estimated Se = 0.75 and Sp = 0.61) and no information is available on Se and Sp at this cut-off value when the test is used to predict decreased fertility (Ospina *et al.*, 2010b). Chapinal *et al.* (2011) used a cut-off value in serum concentration of 1000 μeq./L NEFA (Se = 0.51 and Sp = 0.80) when attempting to predict displaced abomasum. It is reasonable for a cut-off value used to predict clinical disease to be higher than one used to predict decreased fertility. When the NEFA cut-off value of 1000 μeq./L was applied to the study population in Paper III, PV+ for ANEST was slightly better than that for the 400 μeq./L cut-off value (data not shown).

5.2.2 Factors influencing the usefulness of the test

When strata-specific Se and Sp were calculated for explanatory variables that were significant in the logistic models, Se was higher when the test was used in cows of higher parity compared with first parity cows. In the NEFA test for ANEST, Se was nearly twice as high when the test was used for cows of third parity or older, compared with when it was used in first parity cows. At the same time, Sp was higher in cows of first parity, than in older cows. Cows of different parity have different capacity to adapt to metabolic stress (Friggens *et al.*, 2007) which could be one explanation for the observed different accuracies of the tests. Breed was significantly associated with only the BHBA test and only when testing for DFAI. When plasma concentrations of BHBA and NEFA are measured and interpreted in the field situation, it may therefore be advisable to ignore breed but to take parity of the cow into some consideration.

In Paper III, PV+ was below 0.50 for all tests and reference standards. This indicates that given a positive test and the present prevalence of decreased fertility, the probability that the animal had decreased fertility was low. Thus, because there is a large risk of treating the 'wrong' animal, this is not a useful test. The Scandinavian countries have incorporated fertility traits into the breeding goals for decades (Philipsson & Lindhé, 2003) and although decreased fertility is considered a problem, the prevalence is relatively low. In a population with a hypothetical high prevalence of 50%, the PV+ was higher, but still <0.70. However, the possibility cannot be excluded that this test system is more useful in other countries where the prevalence of decreased fertility in general is higher.

The accuracy of tests can be increased by applying parallel testing (*i.e.* an animal is considered to be test-positive when it tests positive in one or both tests), which increases Se, or serial testing (*i.e.* only animals that test positive in both tests are considered test-positive), which increases Sp. The usefulness of the tests investigated in Paper III would probably be increased by being interpreted in combination. Serial interpretation of milk acetone and milk lactose measured during the first 4 weeks after calving predicts luteal function with Se of 84% and Sp of 86% (Reksen *et al.,* 2002). There are several cow-side tests for measuring BHBA in plasma available on the market, but NEFA and IGF-1 analyses must still be performed in the laboratory. As costs for analyses of plasma concentrations of NEFA, BHBA and IGF-1 would be high when interpreting the test in combination, this was not further evaluated and reported in Paper III, but could be considered under special circumstances.

Plasma concentrations of metabolic indicators are not constant and change with DIM. According to Jorritsma *et al.* (2003), the nadir in NEB occurs between 2 and 12 days post-partum. Here, some of the variation in the data was minimised as the period of most severe NEB was included in the sampling period. It is likely that the test results would have been more accurate if samples had been taken at the nadir of NEB for each individual cow or at a more specified time period than during the first three weeks. In addition, repeated and more frequent sampling could have improved the performance of the test systems. This has been illustrated in a study by Nielsen *et al.* (2005), where BHBA was measured frequently in milk and used in a biological model to predict clinical ketosis. However, in the present study sampling within 3 weeks was considered realistic, especially for smaller herds where veterinary visits are prone to occur at longer intervals. The mean herd size in Swedish dairy production is 63 milking cows on a yearly basis (Swedish Dairy Association, 2012) and the material in this thesis reflects the field situation.

5.3 Cows at risk of decreased fertility could be identified using risk factors

In Paper IV, associations between management, nutrition, housing, cow factors, and decreased fertility were analysed. Knowledge about such associations could be used in decisions about when preventive strategies should be used.

In Paper IV, cows held in a loose-housing system and fed a TMR or PMR in general did not have a lower fertility than cows kept in tie-stalls and given SEP feeding, despite concerns about this within the dairy sector. In addition, the results contradict earlier Swedish studies where cows in herds fed TMR had longer calving intervals and longer intervals from calving to last AI (Löf *et al.*, 2007b). However, in a recent risk factor study on cow level by Löf *et al.* (2012), the chance of being pregnant 30 days after the herds calculated voluntary waiting period did not differ between TMR and SEP cows. These conflicting results indicate that herds fed TMR and PMR as a group are heterogeneous, and that feeding system and housing alone are probably not good predictors of fertility.

In Paper IV, organically managed cows had an approximately 25% lower probability of PAI compared with conventionally managed cows. This contradicts Löf *et al.* (2012) who found that the calving interval and interval from calving to first AI at herd level were shorter in organically managed herds. Furthermore, Fall *et al.* (2009) did not find any differences in the success of pregnancy at first AI between cows in 20 Swedish organic herds and 20 conventionally managed herds. The feeding regime on conventional farms has slowly moved towards including a higher proportion of home-grown roughages in the diet without being approved as an organic farm, minimising the differences between the types of farms.

Cows that calved and were inseminated in herds that were changing from one system to another (*e.g.* from tie-stall to loose-housing; from conventional milking to AMS; or from conventional to organic management) all had a lower probability of pregnancy at first AI ($0.29<OR<0.79$) and more inseminations per animal submitted to AI ($1.10<IR<1.14$). A change on the farm is likely to interrupt the normal routines in the herd, resulting in lower heat detection rates and less cows inseminated in time. The general stress level in the herd may also be increased, which will affect the cows negatively. Paper IV also indicates that when routines (*e.g.* as regards heat detection) become adapted to the new system, the reproductive performance of the cows also stabilises. When planning for a change in herd management, extra time and labour should be made available in order to prevent a decline in reproduction. Extra attention should also be paid to cows during the transition period and the subsequent insemination period, in farms with an ongoing change.

The claw status and lameness of dairy cows is a well-known risk factor for decreased fertility (Hultgren *et al.,* 2004; Barkema *et al.*, 1994). In Paper IV, a cow diagnosed with a severe claw lesion had approximately 20% reduced probability of pregnancy at first AI and received approximately 5% more inseminations compared with cows without claw lesions. Severe claw lesions that are painful will reduce cow mobility (Walker *et al.*, 2008) and access to the feed bunk and consequently reduce intake (Palmer *et al.*, 2012). In a British study, the incidence of oestrus was not affected by lameness, but lame cows were mounted less frequently and expressed oestrus of lower intensity (Walker *et al.*, 2010). Morris *et al.* (2011) found that 29% of 37 lame cows failed to express oestrus or to ovulate and that this was associated with lower LH pulse frequency and lower oestradiol concentrations. Model results in Paper IV also showed that absence of a claw health record was associated with better reproductive performance. However, there was insufficient information available to draw conclusions about this group and a direct causal relationship is naturally not expected. Based on these results and on the literature, regular and preventive claw trimming and trimming of acute lame cows should always be included when strategies aiming for increased fertility are planned.

Cows of the Swedish Red breed had an 18% higher probability than cows of the Swedish Holstein breed of conceiving at first AI, which is in agreement with other studies (*e.g.* Petersson *et al.*, 2006a). However, parity of the cow seemed to influence reproductive performance in different ways. Based on the results in Paper IV, cows in parity two and older received more inseminations per animal submitted to AI than cows in parity one. However, the cows in parity two and older were more likely to conceive at FAI than first parity cows. This somewhat conflicting result might indicate that first parity cows conceived to a lesser extent, and that the older cows were given more chances (*i.e*. more inseminations) than younger cows. In practice, strategies with the aim of improving reproductive performance, *e.g.* intensified heat detection, could be applied especially to cows of SH breed.

In Paper IV, cows calving during the spring had a 19% lower probability of pregnancy at FAI than cows calving during the winter. Furthermore, spring-calving cows had higher NINS than winter-calving cows. This seasonal effect confirms findings by Löf *et al*. (2012). Cows calving during the spring are inseminated during the subsequent summer when, due to the national legislation, they are on pasture for 3 months (as a minimum). Thus decreased heat detection efficiency during the pasture period could have resulted in decreased reproductive performance. In addition, the temperature in Sweden can exceed 25°C during the summer, which may cause heat stress and thus an increased interval from calving to conception in high-yielding cows (De Rensis & Scaramuzzi, 2003). The reproductive performance in cows inseminated at pasture during summer could be increased by the use of heat detection devices (*e.g*. mounting detectors) and also by providing cows with shade during hot, sunny summer days.

Cows that displayed a rise in SCC after calving had half the probability of PAI of their counterparts, although having high cell counts *per se* was not associated with the fertility of cows. These findings on the effect of a rise in SCC confirm those of Lavon *et al.* (2011) and Hudson *et al*. (2012). Many of the cows that went from low to high SCC could have been suffering either clinical or sub-clinical mastitis. There are several possible links between increased SCC and reduced reproductive performance. It has been shown that herds with a high incidence of mastitis also have a high metabolic stress (Holtenius *et al*., 2004), thus increasing the risk of severe NEB and subsequent decreased fertility. Furhtermore, cows with clinical mastitis show decreased LH pulsatility, absent LH surge and reduced signs of oestrus (Hockett *et al*., 2005). Ultimately, it is likely that a management system that supports udder health will also lead to better reproductive performance.

In conclusion, based on Paper IV and on the literature, preventive measures should be focused on cows with increased SCC after calving, cows with severe claw lesions and cows calving during spring with the subsequent insemination period at pasture. Attention should also be paid to cows having their reproductive period during a change in housing, milking system or production type. Even if loose-housing *per se* was not found to be associated negatively with fertility, the risk of increased SCC and severe claw lesions may be increased in that type of housing (Hovinen *et al.*, 2009; Bergsten & Herlin, 1996). Preventive and regular claw trimming, as well as a structured approach to prevent increases in SCC, should thus be included in fertility programmes.

5.4 Methodological considerations

5.4.1 Field study versus experimental study

In three of four studies in this thesis, a field study design was used. In Papers I and II the effects of supplemental feeding with GLY and PG were evaluated in a total of 17 commercial dairy herds. Previous studies on nutritional interventions have usually been made in experimental research herds. Such herds have a well-controlled environment and management, which reduces the amount of non-controlled variation in the data and more truly shows the effect of the intervention. Research herds also have specially trained staff for use in applying measures and documentation of procedures. The field study design, on the other hand, offers an opportunity to evaluate the intervention under practical conditions and under the influence of a variety of management factors. If a difference between the supplemented cows and the control group is detected only in the research herd and not in the field situation, this could indicate that the supplement may not be applicable to all types of herds and to all type of cows. Therefore, field studies and studies in experimental herds will always complement each other.

In Papers I and II, the field design was chosen because the investigation of effects on fertility called for a large number of cows to be monitored for a long period. It was also of interest to evaluate how much the data varied between cows and herds, something that is only possible with a field design. Finally, it was of great interest to evaluate the supplemental feeds under practical on-farm conditions.

When data are collected from several herds, the clustering effect of herd must be considered. This was done by adding herd as a random effect in the models used in Papers I, III and IV and herd as a shared frailty term in the survival models in Paper II.

Due to the field design chosen, some factors were not possible to measure or use, even if they would have been advantageous for the studies. Firstly, no positive control (*i.e.* grain or other concentrate of similar energy content) was used. Therefore, based on the results presented, the possibility cannot be ruled out that a supply of concentrates with equivalent energy content to GLY or PG would induce the same effect. Secondly, there was no possibility to measure the DMI of the cows and the study had to rely on the subjective opinions of farmers. In addition, the energy balance of the individual cows could not be measured because it is not known whether cows stole feed from each other and feed refusals were not weighed.

5.4.2 Measuring metabolic status

In this thesis, metabolic status was estimated using plasma concentrations of glucose, NEFA, BHBA, insulin and IGF-1. In Paper I, cows were sampled three times at three weeks apart during 0-63 DIM, but only samples taken from 4 DIM were included in the analyses to minimise the effects of calving. The recorded NEFA and insulin profiles across all cows were in agreement with those in a previous study of Swedish dairy cows (Fall *et al.*, 2008), indicating that the long intervals between samplings did not affect the results. However, the BHBA profile recorded in study I was surprisingly flat. In general, BHBA concentrations are elevated during the first 6 weeks post-partum (Ingvartsen *et al.,* 2003) with a peak 1-2 weeks after calving. It is therefore reasonable to believe that the BHBA curve, and thus the results of Paper I, would have been different if samples had been taken more often in the beginning of the sampling period.

5.4.3 Sampling in relation to allocation

In Papers I and II, the supplemental feeding with GLY and PG was given during morning and evening feeding. The samples were mainly taken during late morning although, due to practical reasons, some farms were visited during the afternoon. Time of sampling in relation to administration of the substance may influence the result of the metabolite or hormone measured (*e.g.* a rise in insulin and glucose concentration commonly occurs 90 minutes after drenching with PG (Rizos *et al.*, 2008; Butler *et al.*, 2006; Miyoshi *et al.*, 2001). It is likely that cows sampled at midday or in the afternoon had lower plasma concentrations of glucose and insulin (*i.e.* lower metabolic status) than cows sampled at a morning visit. This might have affected the results of the study. However, cows from all treatment groups within one farm were sampled at the same time of day and therefore the bias due to differential misclassification would have been reduced

5.4.4 Measures of reproductive performance

Several indicators of reproductive performance were used in the Papers II-IV in this thesis, namely FLA, FAI, CON (all hazards), PAI (a probability) and NINS (a count), as well as the indicators of decreased fertility; ANEST, DFAI and DCON (dichotomous indicators).

Luteal activity, which is an objective measure of fertility, was measured using milk progesterone measurements, and FLA after calving was defined as the first rise in milk progesterone above 4 ng/mL (Friggens *et al.*, 2008). In Papers II and III the farmers were instructed to collect milk samples twice weekly, *i.e*. at 3-4 day intervals. However, the sampling intervals were sometimes accidently prolonged. Samples taken 2-3 times per week have been shown to have high accuracy in detecting delayed cyclicity (Petersson *et al.*, 2008), but in order not to lose too many observations, only cows with samples that were taken at longer intervals than 7 days were excluded from analyses here. The progesterone cut-off value and the sampling interval may have led to the percentage of cycling cows being underestimated in our material. In Paper II this potential underestimation would have been evenly distributed among the supplemental groups and it is therefore unlikely that it influenced the result. In

Paper III, the results might have been affected if a cow was classified as ANEST, but in reality had cycled undetected earlier.

FLA, FAI and CON are conventionally used as interval measurements indicating the time interval from calving to respective event. As mentioned before, these interval measures and other measures are highly dependent on management decisions and time after calving at which the cow is inseminated. In Paper IV, the objectivity of the indicator PAI was increased by adjusting for CFI. In Paper II, the intervals were analysed as hazards of the event using survival analysis. In that type of analysis, cows that cycled or not cycled were both included, which is a strength.

Number of inseminations per animals submitted for AI is an indicator commonly used in the Swedish dairy industry (H. Gustafsson, Swedish Dairy Association, pers. comm., 2012). It is important to emphasise that this indicator also includes animals that do not conceive or are slaughtered or just remain not pregnant throughout the lactation. Besides actual reproductive performance, NINS is also largely able to describe the farmers' personal intents, level of ambition and persistence in trying to get cows pregnant.

When validating a test, the gold standard (or reference standard) is used to classify the animal in its true status (Greiner & Gardner, 2000). In Paper III, the cut-off values were evaluated against ANEST, DFAI and DCON as the gold standards of decreased fertility. The definitions were subjectively made using the DIM corresponding to the 75th percentile in the study population. However, with the general aim of achieving one calving per cow and year, the limit of ANEST at 36 days, DFAI at 96 days and DCON at 145 days is reasonable. The 75th percentile of FAI and CON in all herds with 45-105 calving cows per year registered within SOMRS corresponded to 102 and 143 days (Swedish Dairy Association, 2009), in comparison with 96 and 145 in this thesis. Anoestrus is not routinely recorded within SOMRS, but Peterson *et al.* (2006) reported that 16% of cows had not started the cycle at 56 DIM. In this thesis cows were not followed as far as 56 DIM, but 27% were not cycling at 36 DIM.

6 Main conclusions and recommendations

Based on the results in Papers I-IV and the analysis presented in this thesis, the following conclusions were drawn:

- Supplemental feeding with glycerol or propylene glycol during early lactation can increase milk yield during the first 90 days after calving, without a subsequent decrease in metabolic status, body condition or decreased fertility.
- Supplemental feeding in early lactation with GLY or PG did not affect the metabolic status, body condition or fertility (measured as the onset of cyclicity and time to FAI or CON) of dairy cows.
- Metabolites and hormones related to metabolic status vary approximately twice as much at the cow level compared with what is explained at herd level. This implies that preventive strategies should primarily be applied on the cow level.
- Test performance is generally low for a single measurement of plasma NEFA, BHBA or IGF-1 collected 4-21 DIM in predicting decreased fertility. Test performance could be improved in herds with a high prevalence of decreased fertility. Parity number should be taken into account when interpreting test results.
- Important cow-level predictors of fertility are severe claw lesions or a rise in SCC between two monthly test days. Strategies for improved fertility should therefore also include regular claw trimmings and other measures to prevent claw lesions, and measures aimed at preventing mastitis and increased SCC.
- Cows experiencing a change in their housing, management or milking system have a considerably increased risk of decreased fertility. This is important information for farmers planning to change their production, who should provide extra facilities during such a period.

7 Future research and development

The housing systems and techniques used in the dairy industry are continuously developing. At the same time, the genetic potential for high yields is increasing in the dairy cow, leading to a larger impact of stressors such as negative energy balance at the time around calving. An increased focus on the transition period is therefore needed in the management of dairy cows.

- Little is known about how Swedish cows actually cope with the metabolic stress during the transition period and how this affects their subsequent lactation, health and reproductive performance. Further evaluation of the transition period could involve studies of the fat to protein ratio (Heuer *et al.*, 2000), the Transition Cow Index™ (Nordlund, 2006), and repeated measurements of BHBA based on data from herds using Herd Navigator™ (DeLaval International AB, Tumba, Sweden).

- This thesis focused on metabolites measured after calving. However, since the NEB period can start already during the dry period (Ingvartsen, 2000) future studies should also evaluate methods for identifying cows at risk of severe NEB and decreased fertility before calving.

- Studies have shown that cows diagnosed with subclinical ketosis or metritis after calving have a significantly decreased DMI, shorter feeding time and a different pattern of social behaviours than healthy cows (Goldhawk *et al.*, 2009; Huzzey *et al.*, 2007). There is thus a need to further evaluate if and how behavioural indicators could be used in Swedish herds to identify cows with the need of special attention.

- Feeding systems and feed ingredients differ between herds and little is known about how this actually affects reproductive performance and health at the herd level. This needs to be further investigated.

- Results from this thesis indicate that changing housing, milking system or production type on the farms have a negative effect on fertility. However, the study was not designed to investigate this specific issue. To study the effects of such changes in more detail, a longitudinal study where herds are monitored before, during and after change could be useful

- There is a need for evaluation of Swedish claw trimming data. Even though most cows are probably trimmed at least once a year, a high proportion of herds do not record claw status routinely. Thus claw trimmers and farmers need to be encouraged to routinely record claw status. When claw trimming data become more frequent and reliable, they can be used to further evaluate the relationships between fertility, claw health and negative energy balance in dairy cows.

8 Populärvetenskaplig sammanfattning

Associationen mellan energistatus, produktion och reproduktion hos mjölkkor. Att förebygga och att upptäcka kor som riskerar nedsatt fruktsamhet

8.1 Bakgrund

Nedsatt fruktsamhet hos mjölkkor är kostsamt och kor som haft problem med att bli dräktiga slaktas ofta i förtid. Under de senaste 20 åren har mjölkkornas fruktsamhet försämrats runt om i världen. Detta har setts även i Sverige, bland annat genom att det tar längre tid för en ko att bli dräktig idag än för 20 år sedan. Man vet inte riktigt varför fruktsamheten har försämrats men några orsaker kan vara att kornas mjölkavkastning har ökat kraftigt och att besättningarna blivit allt större och använder fler tekniska hjälpmedel.

Mjölken innehåller mjölksocker (laktos) och för att bilda det behöver kon mycket energi. Innan kon kalvar sjunker hennes foderintag naturligt. Efter kalvningen ökar visserligen foderintaget men inte i samma takt som energibehovet för mjölkproduktionen. Som ett resultat får de flesta kor vid kalvningen ett energiunderskott, det vill säga en negativ energibalans. Kon kan till viss del kompensera för den negativa energibalansen genom att bilda socker via nedbrytning av kroppsfett och muskler. Vid kraftig nedbrytning av kroppsfett klarar inte levern av att ta hand om alla restprodukter från blodet. Det kan leda till att dessa kor i högre grad drabbas av sjukdomar, nedsatt fruktsamhet och minskad mjölkproduktion. Kor med kraftig negativ energibalans har längre tid från kalvning till första ägglossning och de har svårare att bli dräktiga än kor som har en liten negativ energibalans.

Att förebygga negativ energibalans och den nedsatta fruktsamhet som den kan leda till, kräver metoder som är effektiva, enkla och billiga. Glycerol och propylenglykol har sedan 50-talet använts som tillskott till kor med så kallad foderleda (upphörd matlust, acetonemi) som de fått till följd av en stor negativ energibalans. Många studier har visat att en stötdos (dvs att hälla i kon preparatet genom munnen med slang eller flaska) ökar blodsocker-halten och minskar fettnedbrytningen. Teoretiskt sett borde en ökad blodsocker-halt och minskad fettnedbrytning förbättra kons fruktsamhet, men endast små och osäkra effekter har rapporterats från tidigare studier.

Det finns nu studier som tyder på att det kan vara effektivare att ge de kor som har störst behov ett tillskott av glycerol eller propylenglykol, istället för att ge det till alla kor i besättningen. Ett sätt att hitta kor med behov av tillskott skulle kunna vara att mäta kornas energibalans genom analys av halten restprodukter från fettnedbrytningen i ett blodprov. För att ett sådant blodprov skall vara användbart behöver valet av gränsvärde för att testet skall anses visa på ökad risk för det man vill undersöka (till exempel sjukdom eller nedsatt fruktsamhet) utvärderas noggrant.

8.2 Sammanfattningar av studier och resultat

I studie I och II utvärderades effekten av tillskottutfodring av kor med 450 g glycerol eller 300 g propylenglykol jämfört med inget tillskott (kontrollgrupp) under 0-21 dagar efter kalvning på mjölkproduktion, energibalans och fruktsamhet. Hos 798 kor i 17 västsvenska besättningar följdes fruktsamheten med hjälp av data från Kokontrollen. Hos 308 kor i 7 av dessa besättningar mättes halten av progesteron i mjölk för att bestämma när första ägglossning skett. Hos 673 kor i 12 av besättningarna bestämdes energibalansen genom blodprov med upprepade mätning av blodsocker, hormoner (insulin och IGF-1) samt restprodukter från fettnedbrytning (NEFA och BHBA). Dessutom mättes hull och bröstomfång och data för mjölkproduktion hämtades från Kokontrollen. Data analyserades statistiskt med så kallade linjära mixade regressionsmodeller för att kunna ta hänsyn till olika faktorer som kan påverka resultaten.

Kor som tillskottsutfodrats med glycerol producerade med statistisk säkerhet 1 kg mer mjölk per dag under de första 90 dagar efter kalvningen. Även hos kor som tillskottsutfodrades med propylenglykol sågs en förbättring av mjölkproduktionen men denna skillnad var inte statistiskt säker. Kor som tillskottutfodrades med glycerol hade något lägre insulinnivåer i blodet men i övrigt sågs inga skillnader i energibalans mellan grupperna. Tillskottsutfodringen påverkade inte hur mycket hull och bröstomfång som korna tappade efter kalvnigen. Kor som fått tillskott med glycerol eller propylenglykol hade inte bättre fruktsamhet, än kor som inte fått något tillskott alls. Sammanfattningsvis så medförde ökningen i mjölkproduktion ingen samtidig nedgång i energibalans eller minskning av fruktsamhet. Resultaten tyder på att kor som fick tillskottsutfodring lade den extra energin på en ökad mjölkproduktion snarare än på att förbättra sin energibalans eller sin fruktsamhet.

I studie III undersöktes hur väl ett enskilt blodprov taget inom 3 veckor efter kalvningen kan förutsäga om kon riskerar att få nedsatt fruktsamhet eller inte. I denna studie användes blodprov från 480 kor från 12 besättningar samt 241 progesteronprov från 7 besättningar. Studien utvärderade hur väl olika gränsvärden av NEFA, BHBA eller IGF-1 korrekt klassar en ko som ”sjuk” eller ”frisk”. Generellt var det stor sannolikhet att felaktigt klassa en ko med ökad risk för nedsatt fruktsamhet som ”frisk” och användbarheten var därför generellt låg.

I studie IV samlades data över ko-faktorer (såsom ras, laktationsnummer, mjölkavkastning, sjukdomsförekomst och fruktsamhet) samt besättningsdata (såsom besättningsstorlek och avkastning, vilken husdjursförening som besättningen tillhörde, om korna mjölkades med robot eller ”på vanligt sätt” samt om korna hölls i lösdrift eller i ett uppbundet system). Data hämtades från Kokontrollen för alla kor som kalvade mellan 1 mars 2010 och 28 februari 2011 i besättningar som hade fler än 60 mjölkande kor. Dessutom skickades en enkät till landets husdjursföreningar för att samla information om besättningarna blandade ensilage och kraftfoder helt och hållet (fullfoder), blandade till viss del (blandfoder) eller om de fodrade ensilage och kraftfoder helt skilt från varandra (individuell utfodring). Totalt ingick 759 besättningar och 63561 kor i studien. Sambandet mellan de olika riskfaktorerna och sannolikheten att kon blir dräktig på första inseminationen eller antalet inseminationer per ko utvärderades med olika statistiska metoder.

I korthet hade kor av den svenska röda rasen bättre fruktsamhet än svenska Holsteinkor (svart-vita). Kor som hölls i lösdrift och utfodrades med fullfoder eller blandfoder hade inte bättre fruktsamhet jämfört med kor som hölls i uppbundna stall och utfodrades individuellt. Kor som mjölkades med robot hade inte sämre fruktsamhet än kor som mjölkades på annat sätt. Dock hade ekologiska KRAV-anslutna besättningar något sämre fruktsamhet än kor i icke KRAV-anslutna besättningar. Kor som kalvade och seminerades under en period då gården förändrades, dvs bytte system från uppbundet till lösdrift, från konventionell mjölkning till robot eller från konventionell produktion till KRAV-anslutning, hade alla sämre fruktsamhet under den aktuella perioden. Slutligen, kor med kraftiga klövskador eller kor som gått från lågt till högt celltal under tiden efter kalvning hade kraftigt nedsatt fruktsamhet.

8.3 Slutsatser och rekommendationer

Tillskottsutfodring med glycerol eller propylenglykol kan öka mjölkavkastningen utan att korna samtidigt får en sänkt energibalans eller sänkt fruktsamhet. Det är dock tveksamt om den ökade mjölkmängden medför några ekonomiska vinster då kostnaden för själva tillskottet samt det ökade arbetet med att ge preparatet jämförs med den ökade mjölkinkomsten.

Energibalansen varierar mer mellan enskilda kor än mellan besättningar. Detta tyder på att förebyggande åtgärder, till exempel tillskottsutfodring, kan få större effekt om man ger det till kor med särskilda behov istället för att som här ge det till alla kor i en besättning.

Man kan inte med ett enskilt blodprov taget tidigt i laktation förutsäga kons risk för nedsatt fruktsamhet vilket gör att ett sådant test har låg användbarhet. Däremot är det inte uteslutet att flera prover som tas i följd och tolkas tillsammans kan användas för testning av kor i besättningar med stora problem. Om kons ålder dessutom tas i beaktande kan användbarheten på testet öka.

Det är viktigt att förebygga klövskador genom regelbunden verkning och att förebygga juverinflammation efter kalvningen. Förekomsten av dessa sjukdomar har stor inverkan på fruktsamheten hos kon och åtgärder för att minska dessa störningar minskar både djurlidande och mjölkproduktionen samt förbättrar fruktsamheten.

Generellt störs inte fruktsamheten i besättningarna av nya tekniker och inhysningssystem när de nya rutinerna väl ”har satt sig”. Det är däremot mycket viktigt att djurägare är beredda att investera extra tid för att hinna med att både sköta djurhälsa och fruktsamhet förutom det extra arbete som det innebär att bygga om på gården.

References

Adewuyi, A.A., Gruys, E. & Eerdenburg, F.J.C.M.v. (2005). Non esterified fatty acids (NEFA) in dairy cattle. A review *Veterinary Quarterly* 27(3), 117-126.

Agenäs, A., Burstedt, E. & Holtenius, K. (2003a). Effects of feeding intensity during the dry period. 1. Feed intake, body weight, and milk production. *Journal of Dairy Science* 86, 870-882.

Agenäs, S., Dahlborn, K. & Holtenius, K. (2003b). Changes in metabolism and milk production during and after feed deprivation in primiparous cows selected for different milk fat content. *Livestock Production Science* 83(2-3), 153-164.

Andersson, L. & Lundström, K. (1984). Milk and blood ketone bodies, blood isopropanol and plasma glucose in dairy cows; methodological studies and dirunal variations. *Zentralblatt fu Veterinärmedizin Rehie A* 31, 340-349.

Aschenbach, J.R., Kristensen, N.B., Donkin, S.S., Hammon, H.M. & Penner, G.B. (2010). Gluconeogenesis in dairy cows: The secret of making sweet milk from sour dough. *IUBMB Life* 62(12), 869-877.

Barkema, H.W., Westrik, J.D., van Keulen, K.A.S., Schukken, Y.H. & Brand, A. (1994). The effects of lameness on reproductive performance, milk production and culling in Dutch dairy farms. *Preventive Veterinary Medicine* 20(4), 249-259.

Baumann, D. (2000). Regulation of nutrient partitioning during lactation: Homeostatis and Homeorhesis. In: Cronjé, P. (Ed.) *Ruminant Physiology. Digestion, metabolism, growth and reproduction.* Wallingford: CABI publishing.

Bell, A.W. (1995). Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *Journal of Animal Science* 73(2804-2819).

Berglund, B. (2008). Genetic improvment of dairy cow reproductive performance. *Reproduction of domestic animals* 43(2), 89-95.

Bergsten, C. & Herlin, A.H. (1996). Sole haemorrhages and heel horn erosion in dairy cows: The influence of housing system on their prevalence and severity. *Acta Veterinaria Scandinavica* 37(4), 395-408.

Björnhag, G. (1997). De svenska husdjursrasernas historia. In: Larsson, B.*, et al.* (Eds.) *Agrarhistoria*. Stockholm: LTs förlag.

Britt, J.H. (1992). Impacts of early postpartum metabolism on follicular development and fertility. *The bovine proceedings* 24, 39-43.

Butler, S.T., Pelton, S.H. & Butler, W.R. (2006). Energy balance, metabolic status, and the first postpartum ovarian follicle wave in cows administered propylene glycol. *Journal of Dairy Science* 89, 2938-2951.

Butler, W.R. (2003). Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. *Livestock Production Science* 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. *Journal of Animal Science* 53(3), 742-748.

Butler, W. R. & Smith, R. H. (1989). Interrelationships between energy balance and postpartum reproductive function in dairy cattle. *Journal of Dairy Science* 72, 767-783.

Canfield, R.W. & Butler, W.R. (1989). Energy balance and pulsatile LH secretion in early postpartum dairy cattle. *Domestic Animal Endocrinology* 7, 323-330.

Carvalho, E.R., Schmelz-Roberts, N.S., White, H.M., Doane, P.H. & Donkin, S.S. (2011). Replacing corn with glycerol in diets for transition dairy cows. *Journal of Dairy Science* 94(2), 908-916.

Chagas, L.M., Bass, J.J., Blache, D., Burke, C.R., Kay, J.K., Lindsay, D.R., Lucy, M.C., Martin, G.B., Meier, S., Rhodes, F.M., Roche, J.R., Thatcher, W.W. & Webb, R. (2007a). Invited review: New perspectives on the roles of nutrition and metabolic priorities in the subfertility of high-producing dairy cows. *Journal of Dairy Science* 90(9), 4022-4032.

Chagas, L.M., Gore, P.J.S., Meier, S., Macdonald, K.A. & Verkerk, G.A. (2007b). Effect of monopropylene glycol on luteinizing hormone, metabolites, and postpartum anovulatory intervals in primiparous dairy cows. *Journal of Dairy Science* 90(3), 1168-1175.

Chapinal, N., Carson, M., Duffield, T.F., Capel, M., Godden, S., Overton, M., Santos, J.E.P. & LeBlanc, S.J. (2011). The association of serum metabolites with clinical disease during the transition period. *Journal of Dairy Science* 94(10), 4897-4903.

Christensen, J.O., Grummer, R.R., Rasmussen, F.E. & Bertics, S.J. (1997). Effect of Method of Delivery of Propylene Glycol on Plasma Metabolites of Feed-Restricted Cattle. *Journal of Dairy Science* 80(3), 563-568.

Chung, Y.H., Girard, I.D. & Varga, G.A. (2009a). Effects of feeding dry propylene glycol to early postpartum Holstein dairy cows on production and blood parameters. *Animal* 3(10), 1368-1377.

Chung, Y.H., Martinez, C.M., Brown, N.E., Cassidy, T.W. & Varga, G.A. (2009b). Ruminal and blood responses to propylene glycol during frequent feeding. *Journal of Dairy Science* 92(9), 4555-4564.

Coughlin, S., Trock, B., Criqui, M., Pickle, L., Browner, D. & Tefft, M. (1992). The logistic modeling of sensitivity, specificity, and predictive value of a diangostic test. *Journal of Clinical Epidemiology* 45, 1-7.

DeFrain, J.M., Hippen, A.R., Kalscheur, K.F. & Jardon, P.W. (2004). Feeding glycerol to transition dairy cows: Effects on blood metabolites and lactation performance. *Journal of Dairy Science* 87, 4195-4206.

De Rensis, F. & Scaramuzzi, R. J. (2003). Heat stress and seasonal effects on reproduction in the dairy cow – A review. *Theriogenology* 60, 1139 – 1151.

Dohoo, I., Martin, W. & Stryhn, H. (2009a). Screening and diagnostic tests. In: Dohoo, I.*, et al.* (Eds.) *Veterinary epidemiologic research*. Carlottown: VER inc.

Dohoo, I., Martin, W. & Stryhn, H. (2009b). Modelling survival data. In: Dohoo, I., et al. (Eds.) *Veterinary epidemiologic research*. Charlottetown: VER inc.

Dohoo, I.R., Tillard, E., Stryhn, H. & Faye, B. (2001). The use of multilevel models to evaluate sources of variation in reproductive performance in dairy cattle in Reunion Island. *Preventive Veterinary Medicine* 50, 127-144.

Donkin, S. & Doane, P. (2007) Glycerol as a feed ingredient for dairy cows. In: *Proceedings of Tri-state Dairy Nutrition Conference*, Fort Wayne, Indiana.

Donkin, S.S., Koser, S.L., White, H.M., Doane, P.H. & Cecava, M.J. (2009). Feeding value of glycerol as a replacement for corn grain in rations fed to lactating dairy cows. *Journal of Dairy Science* 92(10), 5111-5119.

Drackley, J. (1999). Biology of dairy cows during the transition period: the final frontier? *Journal of dairy science* 82(11), 2259 – 2273.

Drackley, J. & Andersen, J. (2006). Splanchnic metabolism of long-chain fatty acids in ruminants. In: Sejrsen, K*., et al.* (Eds.) *Ruminant physiology. Digestion, metabolism and impact of nutrition on gene expression, immunology and stress*. Wageningen: Wageningen Academic Publishers

Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T. & Webster, G. (1989). A body condition scoring chart for Holstein dairy cows. *Journal of Dairy Science* 72, 68-78.

Fall, N. & Emanuelsson, U. (2009). Milk yield, udeer health and reproductive performance in Swedish organic and conventional dairy herds. *Journal of Dairy Research* 76 (04), 402 – 410.

Fall, N., Grohn, Y.T., Forslund, K., Essen-Gustafsson, B., Niskanen, R. & Emanuelson, U. (2008). An observational study on early-lactation metabolic profiles in Swedish organically and conventionally managed dairy cows. *Journal of Dairy Science* 91(10), 3983-3992.

Fisher, L.J., Erfle, J.D., Lodge, G.A. & Sauer, F.D. (1973). Effects of propylene glycol or glycerol supplementation of the diet of dairy cows on feed intake, milk yield and composition, and incidence of ketosis. *Canadian Journal of Animal Science* 53, 289-296.

Forde, N., Beltman, M.E., Lonergan, P., Diskin, M., Roche, J.F. & Crowe, M.A. (2011). Oestrous cycles in Bos taurus cattle. *Animal Reproduction Science* 124(3–4), 163-169.

Friggens, N., Bjerring, M., Ridder, C., Höjsgaard, S. & Larsen, T. (2008). Improved detection of reproductive status in dairy cows using milk progesterone measurements. *Reproduction in Domestic Animals* 43, 113-121.

Friggens, N.C. (2003). Body lipid reserves and the reproductive cycle: towards a better understanding. *Livestock Production Science* 83, 219-236.

Friggens, N.C., Berg, P., Theilgaard, P., Korsgaard, I.R., Ingvartsen, K.L., Løvendahl, P. & Jensen, J. (2007). Breed and parity effects on energy balance profiles through lactation: Evidence of genetically driven body energy change. *Journal of Dairy Science* 90(11), 5291-5305.

Frössling, J., Nødtvedt, A., Lindberg, A. & Björkman, C. (2008). Spatial analysis of Neospora caninum distribution in dairy cattle from Sweden. *Geospatial Health* 3 (1), 39 – 45.

Garnsworthy, P.C., Fouladi-Nashta, A.A., Mann, G.E., Sinclair, K.D. & Webb, R. (2009). Effect of dietary-induced changes in plasma insulin concentrations during the early post partum period on pregnancy rate in dairy cows. *Reproduction* 137(4), 759-768.

Goldhawk, C., Chapinal, N., Veira, D.M., Weary, D.M. & von Keyserlingk, M.A.G. (2009). Prepartum feeding behavior is an early indicator of subclinical ketosis. *Journal of Dairy Science* 92(10), 4971-4977.

Gong, J.G., Lee, W.J., Garnsworthy, P.C. & 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(3), 419-427.

Greiner, M. & Gardner, I.A. (2000). Epidemiologic issues in the validation of veterinary diagnostic tests. *Preventive Veterinary Medicine* 45(1–2), 3-22.

Greiner, M., Pfeiffer, D. & Smith, R.D. (2000). Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. *Preventive Veterinary Medicine* (45), 23-41.

Greiner, M., Sohr, D. & Göbel, P. (1995). A modified ROC analysis for the selection of cut-off values and the definition of intermediate results of serodiagnostic tests. *Journal of Immunological Methods* 185(1), 123-132.

Grummer, R.R., Mashek, D.G. & Hayirli, A. (2004). Dry matter intake and energy balance in the transition period. *Veterinary Clinics of North America. Food Animal Practice* 20(3), 447-70.

Gröhn, Y.T. & Rajala-Schultz, P.J. (2000). Epidemiology of reproductive performance in dairy cows. *Animal Reproduction Science* 60–61(0), 605-614.

Gustafsson, H. (1987). Reproduktionsfysiologi, hondjur. In: Swensson, T. (Ed.) *Artificiell insemination och reproduktion*. Eskilstuna: Svensk Husdjursskötsel ek. för.

Hachenberg, S., Weinkauf, C., Hiss, S. & Sauerwein, H. (2007). Evaluation of classification modes potentially suitable to identify metabolic stress in healthy dairy cows during the peripartal period. *Journal of Animal Science* 85(8), 1923-1932.

Heuer, C., Van Straalen, W.M., Schukken, Y.H., Dirkzwager, A. & Noordhuizen, J.P.T.M. (2000). Prediction of energy balance in a high yielding dairy herd in early lactation: Model development and precision. *Livestock Production Science* 65(1-2), 91-105.

Hockett, M.E., Almeida, R.A., Rohrbach, N.R., Oliver, S.P., Dowlen, H.H. & Schrick, F.N. (2005). Effects of induced clinical mastitis during preovulation on endocrine and follicular function. *Journal of Dairy Science* 88(7), 2422-2431.

Hoedemaker, M., Prange, D., Zerbe, H., Frank, J., Daxenberger, A. & Meyer, H.H.D. (2004). Peripartal propylene glycol supplementation and metabolism, animal health, fertility, and production in dairy cows. *Journal of Dairy Science* 87, 2136-2145.

Holtenius, K., Agenäs, S., Delavaud, C. & Chilliard, Y. (2003). Effects of feeding density during the dry period. 2. Metabolic and hormonal responses. *Journal of Dairy Science* 86(883-891).

Holtenius, K., Waller, K.P., Essen-Gustavsson, B., Holtenius, P. & Sandgren, C. H. (2004). Metabolic parameters and blood leukocyte profiles in cows from herds with or low mastitis incidence. *Veterinary Journal* 168, 65 – 73.

Hosmer, D.V. & Lemeshow, S. (2000). *Applied logistic regression*. 2nd edition. ed. New York: John Wiley & Sons Inc.

Hove, K. (1978). Insulin secretion in lactating cows: responses to glucose infused intravenously in normal, ketonemic, and starved animals. *Journal of Dairy Science* 61(10), 1407-1413.

Hovinen, M., Rasmussen, M.D. & Pyörälä, S.P. (2009). Udder health of cows changing from tie stalls or free stalls with conventional milking to free stalls with either conventional or automatic milking. *Journal of Dairy Science* 92(8), 3696-3703.

Hudson, C.D., Bradley, A.J., Breen, J.E. & Green, M.J. (2012). Associations between udder health and reproductive performance in United Kingdom dairy cows. *Journal of Dairy Science* 95(7), 3683-3697.

Hultgren, J., Manske, T. & Bergsten, C. (2004). Associations of sole ulcer at claw trimming with reproductive performance, udder health, milk yield, and culling in Swedish dairy cattle. *Preventive Veterinary Medicine* 62(4), 233-251.

Huzzey, J.M., Veira, D.M., Weary, D.M. & Von Keyserlingk, M.A.G. (2007). Prepartum behavior and dry matter intake identify dairy cows at risk for metritis. *Journal of Dairy Science* 90(7), 3220-3233.

Inchaisri, C., Jorritsma, R., Vos, P.L.A.M., van der Weijden, G.C. & Hogeveen, H. (2010). Economic consequences of reproductive performance in dairy cattle. *Theriogenology* 74(5), 835-846.

Inchaisri, C., Jorritsma, R., Vos, P.L.A.M., van der Weijden, G.C. & Hogeveen, H. (2011). Analysis of the economically optimal voluntary waiting period for first insemination. *Journal of Dairy Science* 94(8), 3811-3823.

Ingvartsen, K.L. (2006). Feeding- and management-related diseases in the transition cow. Physiological adaptations around calving and strategies to reduce feeding-related diseases. *Animal Feed Science and Technology* 126, 175-213.

Ingvartsen, K.L. & Andersen, J.B. (2000). Integration of metabolism and intake regulation: A review focusing on periparturient animals. *Journal of Dairy Science* 83(7), 1573-1597.

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. *Livestock Production Science* 83, 277-308.

Jacobs, J.A. & Siegford, J.M. (2012). Invited review: The impact of automatic milking systems on dairy cow management, behavior, health, and welfare. *Journal of Dairy Science* 95(5), 2227-2247.

Jenness, R. (1985). Biochemical and nutritional aspects of milk and colostrum. In: Larson, B. (Ed.) *Lactation*. Ames: The Iowa state university press.

Jensen, P. (1995). *Djurens beteenden och orsakerna till det*. Stockholm: LTs förlag.

Johnson, R. (1954). The treatment of ketosis with glycerol and propylene glycol. *The Cornell Veterinarian* 44, 6-21.

Jorritsma, R., Groot, M.W.d., Vos, P.L.A.M., Kruip, T.A.M., Wensing, T. & Noordhuizen, J.P.T.M. (2003). Acute fasting in heifers as a model for assessing the relationship between plasma and follicular fluid NEFA concentrations. *Theriogenology* 60(1), 151-161.

Juchem, S.O., Santos, F.A.P., Imaizumi, H., Pires, A.V. & Barnabe, E.C. (2004). Production and blood parameters of Holstein cows treated prepartum with sodium monensin or propylene glycol. *Journal of Dairy Science* 87(3), 680-689.

Kessel, S., Stroehl, M., Meyer, H.H.D., Hiss, S., Sauerwein, H., Schwarz, F.J. & Bruckmaier, R.M. (2008). Individual variability in physiological adaptation to metabolic stress during early lactation in dairy cows kept under equal conditions. *Journal of Animal Science* 86(11), 2903-2912.

Kristensen, N.B., Danfaer, A., Rojen, B.A., Raun, B.M.L., Weisbjerg, M.R. & Hvelplund, T. (2002). Metabolism of propionate and 1,2-propanediol absorbed from the washed reticulorumen of lactating cows. *Journal of Animal Science* 80(8), 2168-2175.

Kristensen, N.B. & Raun, B.M.L. (2007). Ruminal and Intermediary Metabolism of Propylene Glycol in Lactating Holstein Cows. *Journal of Dairy Science* 90(10), 4707-4717.

Kullberg, K. (2008). *Glycerol till mjölkkor-effekter på våmmetabolismen*. Uppsala: Department of Animal Nutrition and Management. (Examensarbete; 254).

Lakic, B. (2011). *Effects of a single proplonged milking interval in cows. Study of indicators and mediators of inflammation, milk composition and yield.* Diss. Uppsala: Swedish university of agricultural sciences.

Larsson, B. & Berglund, B. (2000). Reproductive performance in cows with extended calving interval. *Reproduction in Domestic Animals* 35(6), 277-280.

Lavon, Y., Ezra, E., Leitner, G. & Wolfenson, D. (2011). Association of conception rate with pattern and level of somatic cell count elevation relative to time of insemination in dairy cows. *Journal of Dairy Science* 94, 4538-4545.

LeBlanc, S. (2010). Monitoring metabolic health of dairy cattle in the transition period. *Journal of Reproduction and Development* 56 Suppl, S29-35.

Leroy, J., Soom, A.V., Opsomer, G., Goovaerts, I. & Bols, P. (2008). Reduced fertility in high-yielding dairy cows: Are the oocyte and embryo in danger? Part II. Mechanisms linking nutrition and reduced oocyte and embryo quality in high-yielding dairy cows. *Reproduction in Domestic Animals* 43, 623 – 632.

Leroy, J.L.M.R., Bossaert, P., Opsomer, G. & Bols, P.E.J. (2011). The effect of animal handling procedures on the blood non-esterified fatty acid and glucose concentrations of lactating dairy cows. *Veterinary Journal* 187(1), 81-84.

Leroy, J.L.M.R., Vanholder, T., Opsomer, G., Van Soom, A. & de Kruif, A. (2006). The In Vitro development of bovine oocytes after maturation in glucose and β-hydroxybutyrate concentrations associated with negative energy balance in dairy cows. *Reproduction in Domestic Animals* 41(2), 119-123.

Leroy, J.L.M.R., Vanholder, T., Delanghe, J.R., G.O., Soom, A.V., Bols, P.E.J., Dewulf, J. & Kruif, A.d. (2004). Metabolic changes in follicular fluid of the dominant follicle in high-yielding dairy cows early post partum. *Theriogenology* 62, 1131-1143.

Leroy, J.L.M.R., Vanholder, T., Mateusen, B., Christophe, A., Opsomer, G., de Kruif, A., Genicot, G. & Van Soom, A. (2005). Non-esterified fatty acids in follicular fluid of dairy cows and their effect on developmental capacity of bovine oocytes in vitro. *Reproduction* 130(4), 485-495.

Linke, P.L., DeFrain, J.M., Hippen, A.R. & Jardon, P.W. (2004). Ruminal and plasma responses in dairy cows to drenching or feeding glycerol. *Journal of Dairy Science* 87(suppl. 1), 383.

Liu, Q., Wang, C., Yang, W.Z., Zhang, W.W., Yang, X.M., C., D., He, D.C., Dong, K.H. & Huang, Y.X. (2009). Effects of feeding propylene glycol on dry matter intake, lactation performance, energy balance and blood metabolites in early lactation dairy cows. *Animal* 3(10), 1420-1427.

Lopez, H., Satter, L.D. & Wiltbank, M.C. (2004). Relationship between level of milk production and estrous behavior of lactating dairy cows. *Animal Reproduction Science* 81(3-4), 209-223.
Lucy, M.C. (2001). Reproductive Loss in High-Producing Dairy Cattle: Where Will It End? *Journal of Dairy Science* 84(6), 1277-1293.
Löf, E. (2012). *Epidemiological studies of reproductive performance indicators in Swedish dairy cows.* Diss. Uppsala:Swedish university of agricultural sciences.
Löf, E., Emanuelsson, U. & Gustafsson, H. (2007a). Data management affects reproductive performance indicators in Swedsih dairy herds. *Acta Agriculturae Scandinavica A: Animal Sciences* 57, 73-80.
Löf, E., Gustafsson, H. & Emanuelsson, U. (2007b). Associations between herd characteristics and reproductive effieciency in dairy herds. *Journal of Dairy Science* 90, 4897-4907.
Löf, E., Gustafsson, H. & Emanuelson, U. (2012). Evaluation of two dairy herd reproductive performance indicators that are adjusted for voluntary waiting period. *Acta Veterinaria Scandinavica* 54(1), 5.
Løvendahl, P. & Purup, H.M. (2001). Technical note: time-resolved fluoro-immunometric assay for intact insulin in livestock species. *Journal of Animal Science* 80, 191-195.
McArt, J.A.A., Nydam, D.V. & Oetzel, G.R. (2012). A field trial on the effect of propylene glycol on displaced abomasum, removal from herd, and reproduction in fresh cows diagnosed with subclinical ketosis. *Journal of Dairy Science* 95(5), 2505-2512.
McDonald, P., Edwards, R., Greenhalgh, J. & Morgan, C. (2002). *Animal nutrition*. 6. ed. Harlow: Pearson Education Limited.
Miyamoto, A., Shirasuna, K. & Sasahara, K. (2009). Local regulation of corpus luteum development and regression in the cow: Impact of angiogenic and vasoactive factors. *Domestic Animal Endocrinology* 37(3), 159-169.
Miyoshi, S., Pate, J.L. & Palmqquist, D.L. (2001). Effects of propylene glycol drenching on energy balance, plasma glucose, plasma insulin, ovarian function and conception in dairy cows. *Animal Reproduction Science* 68, 29-43.
Moallem, U., Katz, M., Lehrer, H., Livshitz, L. & Yakoby, S. (2007). Role of peripartum dietary propylene glycol or protected fats on metabolism and early postpartum ovarian follicles. *Journal of Dairy Science* 90(3), 1243-1254.
Morris, M.J, Kaneko, K., Walker, S.L., Jones, D.N., Routly, J.E., Smith, R.F. & Dobson, H. (2011). Influence of lameness on follicular growth, ovulation, reproductive hormone concentrations and estrus behavior in dairy cows. *Theriogenology* 76(4), 658 – 668.
National Veterinary Institute. (2012). Sjukdomsrapportering 2011. Available from: http://www.sva.se/upload/Redesign2011/Pdf/Om%20SVA/publikationer/Sjukd_rapp2011_LOW.pdf

Nielsen, N.I., Friggens, N.C., Chagunda, M.G.G. & Ingvartsen, K. L. (2005). Predicting risk of ketosis in dairy cows using in-line measurements of β-hydroxybutyrate: A biological model. *Journal of Dairy Science* 88, 2441 – 2453.

Nielsen, N.I. & Ingvartsen, K.L. (2004). Propylene glycol for dairy cows. A review of the metabolism of propylene glycol and its effects on physiological parameters, feed intake, milk production and risk of ketosis. *Animal Feed Science and Technology* 115, 191-213.

Noakes, D. (2001). Endogenous and exogenous control of ovarian cyklicity. In: Noakes, D*., et al.* (Eds.) *Arthur's Veterinary reproduction and obstetrics*. London: W.B Saunders.

Nordlund, K.V. (2006). Transition cow index™. In: *39th Proceedings American Association Bovine Practitioners 2006.* St Paul, Minnesota, USA.

Oetzel, G.R. (2004). Monitoring and testing dairy herds for metabolic disease. *Veterinary clinics of North America: Food animal practice* 20(3), 651-674.

Oltenacu, P.A. & Algers, B. (2005). Selection for Increased Production and the Welfare of Dairy Cows: Are New Breeding Goals Needed? *Ambio* 34(4/5), 311-315.

Omazic, A.W., Bertilsson, J., Tråvén, M. & Holtenius, K. (2011). Crude vs. refined glycerol supplementation to dairy cows in early lactation - effects on dry matter intake, lactation performance and metabolism. In: *Proceedings of the 8th international symposium on the nutrition of herbivores.* Aberystwyth, Wales, UK.

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

Osman, M.A., Allen, P.S., Mehyar, N.A., Bobe, G., Coetzee, J.F., Koehler, K.J. & Beitz, D.C. (2008). Acute metabolic responses of postpartal dairy cows to subcutaneous glucagon injections, oral glycerol or both. *Journal of Dairy Science* 91, 3311-3322.

Ospina, P.A., Nydam, D.V., Stokol, T. & Overton, T.R. (2010a). Associations of elevated nonesterified fatty acids and [beta]-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. *Journal of Dairy Science* 93(4), 1596-1603.

Ospina, P.A., Nydam, D.V., Stokol, T. & Overton, T.R. (2010b). Evaluation of nonesterified fatty acids and [beta]-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. *Journal of Dairy Science* 93(2), 546-554.

Overton, T.R. & Waldron, M.R. (2004). Nutritional management of transition dairy cows: Strategies to optimize metabolic health. *Journal of Dairy Science* 87(13_suppl), E105-119.

Palmer, M.A., Law, R. & O'Connell, N.E. (2012). Relationships between lameness and feeding behaviour in cubicle-housed Holstein-Friesian dairy cows. *Applied Animal Behaviour Science*.

Parkinson, T. (2001). Infertility in the cow: Structural and functional abnormalities, management deficienses and non-specific infections. In: Noakes, D*., et al.* (Eds.) *Arthur's veterinary reproduction and obstetrics*. 8. ed. London: WB Saunders.

Pehrson, B., Forshell, K.P. & Carlsson, J. (1991). The effect of additional feeding on the fertility of high-yielding dairy cows. *Journal of Veterinary Medicine series A* 39, 187-192.

Petersson, K.-J., Strandberg, E., Gustafsson, H. & Berglund, B. (2006a). Environmental effects on progesterone profile measures of dairy cow fertility. *Animal Reproduction Science* 91, 201-214.

Petersson, K.-J., Strandberg, E., Gustafsson, H., Royal, M.D. & Berglund, B. (2008). Detection of delayed cyclicity in dairy cows based on progesterone content in monthly milk samples. *Preventive Veterinary Medicine* 86, 153-163.

Petersson, K.J., Gustafsson, H., Strandberg, E. & Berglund, B. (2006b). Atypical Progesterone Profiles and Fertility in Swedish Dairy Cows. *Journal of Dairy Science* 89(7), 2529-2538.

Philipsson, J. & Lindhé, B. (2003). Experiences of including reproduction and health traits in Scandinavian dairy cattle breeding programmes. *Livestock Production Science* 83(2-3), 99-112.

Quiroz-Rocha, G.F., LeBlanc, S.J., Duffield, T.F., Jefferson, B., Wood, D., Leslie, K.E. & Jacobs, R.M. (2010). Short communication: Effect of sampling time relative to the first daily feeding on interpretation of serum fatty acid and β-hydroxybutyrate concentrations in dairy cattle. *Journal of Dairy Science* 93(5), 2030-2033.

Rabe-Hesketh, S. & Skrondal, A. (2005). *Multilevel and longitudinal modeling using Stata*. ISBN 978-1-597 18-008-5.

Reksen, O., Havrevoll, Ø., Gröhn, Y.T., Bolstad, T., Waldmann, A. & Ropstad, E. (2002). Relationships among body condition score, milk constituents, and post partum luteal function in Norweigan dairy cows. *Journal of Dairy Science* 85, 1406 – 1415.

Rémond, B., Souday, E. & Jouany, J.P. (1993). In Vitro and in vivio fermentation of glycerol by rumen microbes. *Animal Feed Science and Technology* 41, 121-132.

Rizos, D., Kenny, D.A., Griffin, W., Quinn, K.M., Duffy, P., Mulligan, F.J., Roche, J.F., Boland, M.P. & Lonergan, P. (2008). The effect of feeding propylene glycol to dairy cows during the early postpartum period on follicular dynamics and on metabolic parameters related to fertility. *Theriogenology* 69(6), 688-699.

Roche, J.R., Friggens, N.C., Kay, J.K., Fisher, M.W., Stafford, K.J. & Berry, D.P. (2009). Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. *Journal of Dairy Science* 92(12), 5769-5801.

Rodriguez-Martinez, H., Hultgren, J., Båge, R., Bergqvist, A.-S., Svensson, C., Bergsten, C., Lidfors, L., Gunnarsson, S., Algers, B., Emanuelsson, U., Berglund, B., Andersson, G., Håård, M., Lindhé, B., Stålhammar, H. & Gustafsson, H. (2008). Reproductive performance in high-yielding dairy cows: Can we sustain it under current practice? *International Veterianary Information Service.* Available at: www.ivis.org.

Salasel, B., Mokhtari, A. & Taktaz, T. (2010). Prevalence, risk factors for and impact of subclinical endometritis in repeat breeder dairy cows. *Theriogenology* 74(7), 1271-1278.

Sandholm, M., Honkanen-Buzalski, T., Kaartinen, L. & Pyörälä, S. (Eds.) (1995). *The bovine udder and mastitis*. Helsinki: University of Helsinki.

Sauer, F.D., Erfle, J.D. & Fisher, L.J. (1973). Propylene glycol and glycerol as a feed additive for lactating dairy cows: An evaluation of blood metabolite parameters. *Canadian Journal of Animal Science* 53, 265-271.

Schams, D., Graf, F., Graule, B., Abele, M. & Prokopp, S. (1991). Hormonal changes during lactation in cows of three different breeds. *Livestock Production Science* 27(4), 285-296.

Schirmann, K., Chapinal, N., Weary, D.M., Heuwieser, W. & von Keyserlingk, M.A.G. (2011). Short-term effects of regrouping on behavior of prepartum dairy cows. *Journal of Dairy Science* 94(5), 2312-2319.

Spörndly, R. (2003). *Fodertabeller för idisslare 2003*. Vol 257, Institutionen för husdjurens utfodring och vård.Uppsala, Sweden

Spörndly, R. (2005). Fullfoder/Blandfoder. Får vi det att fungera i praktiken? In: *Djurhälso och utfodringskonferens 2005.* Jönköping: Svensk mjölk.

Swedish Board of Agriculture (2011). *Yerbook of Agricultural Statistics 2011 including food statistics* [online] Jönköping. Available from: http://www.jordbruksverket.se/omjordbruksverket/statistik/jordbruksstatistiskarsbok/jordbruksstatistiskarsbok2011.4.4b2051c513030542a92800012165.html.

Swedish Dairy Association. (2012). *Cattle statistics*. Stockholm: Swedish Dairy Association.

Swedish Dairy Association. (2004). *Utdata från Kodatabasen*. Stockholm: Swedish Dairy Association.

Sørensen, A., Muir, D.D. & Knight, C.H. (2008). Extended lactation in dairy cows: Effects of milking frequency, calving season and nutrition on lactation persistency and milk quality. *Journal of Dairy Research* 75(1), 90-97.

Sørensen, J.T. & Østergaard, S. (2003). Economic consequences of postponed first insemination of cows in a dairy cattle herd. *Livestock Production Science* 79(2-3), 145-153.

Taylor, V.J., Cheng, Z., Pushpakumara, P.G.A., 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. *Veterinary Record* 155(19), 583-588.

Tucker, C. (2009). Behaviour of cattle. In: Jensen, P. (Ed.) *The ethology of domestic animals*. Wallingford: CABI.

Vanholder, T., Leroy, J., Van Soom, A., Opsomer, G., Maes, D., Coryn, M. & de Kruif, A. (2005). Effect of non-esterified fatty acids on bovine granulosa cell steroidogenesis and proliferation in vitro. *Animal Reproduction Science* 87(1-2), 33-44.

Velazquez, M.A., Spicer, L.J. & Wathes, D.C. (2008). The role of endocrine insulin-like growth factor-I (IGF-I) in female bovine reproduction. *Domestic Animal Endocrinology* 35(4), 325-342.

Veerkamp, R.F., Beerda, B. & Lende, T.v.d. (2003). Effects of genetic selection for milk yield on energy balance, level of hormones, and metabolites in lactating cattle, and possible links to reduced fertility. *Livestock Production Science* 83, 257-275.

Walker, S.L., Smith, R.F., Jones, D.N., Routly, J. E., Morris, M. J. & Dobson, H. (2010). The effect of a chronic stressor, lameness, on detailed sexual behaviour and hormonal profiles in milk and plasma of dairy cattle. *Reproduction in Domestic Animals* 45, 109 – 117.

Walker, S.L., Smith, R.F., Routly, J.E., Jones, D.N., Morris, M.J. & Dobson, H. (2008). Lameness, activity time-budgets, and estrus expression in dairy cattle. *Journal of Dairy Science* 91(12), 4552-4559.

Wang, C., Liu, Q., Yang, W.Z., Huo, W.J., Dong, K.H., Huang, Y.X., Yang, X.M. & He, D.C. (2009). Effects of glycerol on lactation performance, energy balance and metabolites in early lactation Holstein dairy cows. *Animal Feed Science and Technology* 151(1-2), 12-20.

Wathes, D.C., Bourne, N., Cheng, Z., Mann, G.E., Taylor, V.J. & Coffey, M.P. (2007a). Multiple correlation analyses of metabolic and endocrine profiles with fertility in primiparous and multiparous cows. *Journal of Dairy Science* 90(3), 1310-1325.

Wathes, D.C., Fenwick, M., Cheng, Z., Bourne, N., Llewellyn, S., Morris, D.G., Kenny, D., Murphy, J. & Fitzpatrick, R. (2007b). Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow. *Theriogenology* 68(Supplement 1), S232-S241.

Webb, R., Gong, J.G., Law, A.S. & Rusbridge, S.M. (1992). Control of ovarian function in cattle. *Journal of Reproduction and Fertility. Supplement* 45, 141-156.

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