Animal (2014), **8:s1**, pp 82–90 © The Animal Consortium 2014
doi:10.1017/S1751731114000482

animal

Endocrine and metabolic mechanisms linking *postpartum* glucose with early embryonic and foetal development in dairy cows

M. C. Lucy[1†], S. T. Butler[2] and H. A. Garverick[1]

[1]*Division of Animal Sciences, University of Missouri, Columbia, MO, USA;* [2]*Animal & Grasslands Research and Innovation Centre, Teagasc Moorepark, Fermoy Co Cork, Ireland*

(Received 6 November 2013; Accepted 7 February 2014; First published online 28 March 2014)

Milk and milk solids production per cow is increasing annually in dairy systems. Peak milk production is in early lactation when the uterus and ovary are recovering from the previous pregnancy. The competing processes of milk production and restoration of reproductive function can be at odds, particularly if unique homeorhetic mechanisms that typify early lactation become imbalanced and cows experience metabolic disease. Homeorhesis leads to an increase in the synthesis of glucose that is irreversibly lost to milk lactose. Irreversible loss of glucose during lactation can invoke an endocrine and metabolic state that impinges upon postpartum *uterine health, oestrous cyclicity and subsequent establishment of pregnancy. The first 30 days* postpartum *may be most critical in terms of the impact that metabolites and metabolic hormones have on reproduction. Depressed immune function caused in part by the* postpartum *metabolic profile leads to a failure in uterine involution and uterine disease. Oestrous cyclicity (interval to first ovulation and subsequent periodicity) is affected by the same hormones and metabolites that control* postpartum *immune function. Slower growth of the embryo or foetus perhaps explained by the unique metabolic profile during lactation may predispose cows to pregnancy loss. Understanding homeorhetic mechanisms that involve glucose and collectively affect* postpartum *uterine health, oestrous cyclicity and the establishment of pregnancy should lead to methods to improve* postpartum *fertility in dairy cows.*

Keywords: glucose, IGF1, insulin, pregnancy, dairy cow

Implications

Competing processes of milk production and reproduction can be at odds in dairy cows, particularly if the unique metabolic processes that typify early lactation become imbalanced. A potential end result is that cows do not become pregnant during the breeding period. The need to replace infertile cows adds to the cost of production. Understanding basic mechanisms that link the first 60 days of lactation with the subsequent success or failure of establishing pregnancy during the breeding period, therefore, is an important area of research for the dairy industry.

Introduction

Within most dairy production systems, milk or milk solids production per cow is increasing annually. The average United States dairy cow, for example, produces in excess of 9500 kg of milk annually. Peak milk production is in early lactation (within 30 to 60 days after calving) when the uterus is involuting and the ovary is restoring the cyclic processes that lead to oestrus, ovulation, and the formation of a corpus luteum. Competing processes of milk production, uterine involution, and the restoration of ovarian activity can be at odds, particularly if the unique homeorhetic processes that typify early lactation become imbalanced and there is metabolic disease. A potential end result of the competition between high milk production and the reproductive system is that cows do not become pregnant during the breeding period which is typically initiated at ~60 days after calving. Pregnancy failure causes inefficiency in dairy systems because non-pregnant cows do not produce a calf, do not have a subsequent lactation and are replaced with a new (typically 2-year-old) cow. The need to replace infertile cows adds to the cost of production (De Vries, 2006). Understanding basic mechanisms that link the first 60 days of lactation with the subsequent success or failure of establishing pregnancy during the breeding period, therefore, is an important area of research for the dairy industry.

A large number of reviews have been written over the past decade on metabolic processes that link the restoration of ovarian activity with early lactation milk yield (Wathes *et al.*, 2007; Lucy, 2008; Walsh *et al.*, 2011). There is

† E-mail: lucym@missouri.edu

https://doi.org/10.1017/S1751731114000482 Published online by Cambridge University Press

general agreement that restoration of LH pulsatility via release of gonadotropin-releasing hormone (GnRH) from the hypothalamus is a key driver of the process. Hormones such as insulin and IGF1 that hypothetically control GnRH activity at the level of the hypothalamus act synergistically with LH at the level of the ovary. The purpose of this review is to primarily examine factors affecting the establishment of pregnancy during the breeding period via mechanisms that involve the uterus and placenta. This review will specifically focus on glucose because of its dual purpose as a major component of cow's milk and a molecule that coordinates homeorhetic mechanisms that could possibly impinge upon *postpartum* uterine health and the subsequent establishment of pregnancy.

Glucose: a central molecule involved in lactation and reproduction

Glucose metabolism presents an interesting challenge for lactating cows (Aschenbach *et al.*, 2010). The digestive tract of ruminants is specifically adapted to the digestion of forages. Microorganisms in the rumen ferment carbohydrates to volatile fatty acids (VFA) that can be oxidized for energy. In addition to VFA, protein and fat passing into the lower digestive tract are absorbed and used for the synthesis of milk protein and fat. Seventy-two grams of glucose are required for each kg of milk produced (Bell, 1995). Most of this glucose is converted directly into lactose (milk sugar). Although glucose is a major product of carbohydrate digestion in the rumen it is rapidly fermented to VFA. Glucose, therefore, must be resynthesized in the liver of *postpartum* cows via gluconeogenesis (Figure 1). A typical early lactation cow in the United States will produce 50 to 100 kg of milk per day. This equates to a glucose requirement for milk production alone of 3.6 to 7.2 kg/day. Requirements for glucose place a substantial strain on the physiology of dairy cows. Available VFA can only meet about 85% of glucose requirements during early lactation, leaving a 500 g daily deficit for glucose (Bell, 1995). Cows undergo a series of homeorhetic mechanisms that are aimed towards elevating blood glucose. In addition to a large increase in hepatic gluconeogenesis shortly after calving, cows assume a state of insulin resistance that redirects glucose to non-insulin-dependent tissues like mammary gland. In spite of these mechanisms, *postpartum* cows have chronically low blood concentrations of glucose because of failure to meet metabolic requirements for glucose.

Glucose may coordinate whole animal metabolism through its capacity to orchestrate changes in circulating concentrations of insulin and IGF1 (Lucy, 2008). Glucose causes insulin release and insulin partitions nutrients towards adipose tissue and muscle (tissue-promoting (anabolic effect) of insulin). Insulin stimulates the liver to release IGF1 into the circulation (Butler *et al.*, 2003). As long as glucose remains low, insulin and IGF1 remain low, and cows remain in a catabolic (tissue-losing) state during lactation. Glucose deficiency is only corrected when the mammary gland produces less milk in later lactation, blood insulin and IGF1 increase, and cows partition glucose towards adipose tissue and muscle (an anabolic state). The switch from a catabolic state to an anabolic state is a key regulator of the reproductive axis (Kawashima *et al.*, 2012).

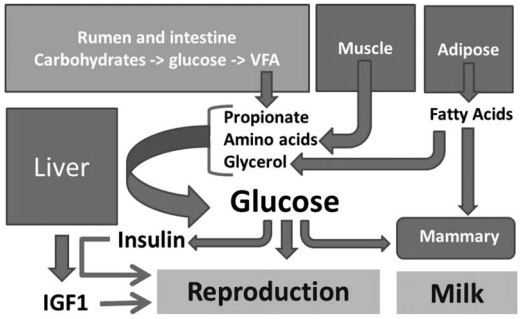


Figure 1 Metabolic processes in early *postpartum* cows that link glucose to the reproductive system. Glucose is synthesized in the liver via gluconeogenesis from substrates arising from rumen fermentation and the catabolism of muscle and adipose tissue. Glucose may ultimately control both circulating insulin (directly) and liver IGF1 production (via insulin-stimulated IGF1 synthesis and secretion). Glucose is required for milk synthesis. Low circulating glucose may impair reproductive processes that are needed to re-establish pregnancy during early lactation.

Associations between early postpartum *glucose and subsequent reproduction*

Blood concentrations of glucose decrease in dairy cows after calving. The decrease in blood glucose is hypothetically caused by the initiation of lactation and the associated glucose requirement for milk production. Blood concentrations of glucose were examined in early *postpartum* cows that either became pregnant or failed to become pregnant later *postpartum* (Garverick *et al.*, 2013). Cows that became pregnant after first artificial insemination (AI) had greater blood concentrations of glucose when compared with cows that did not become pregnant. Significant differences in blood glucose occurred on the day of calving and at 3 days *postpartum*. Greater blood concentrations of glucose on day 3 *postpartum* were associated with greater probability of pregnancy at first AI (Garverick *et al.*, 2013). A relationship between serum concentrations of non-esterified fatty acid (NEFA) and subsequent pregnancy was detected (cows that became pregnant at first AI had lesser *postpartum* NEFA during the early *postpartum* period when compared with cows that did not become pregnant at first AI).

Additional studies published recently have established a link between early *postpartum* glucose and subsequent reproduction. Effects of lactation on early *postpartum* nutrient partitioning and the establishment of pregnancy were tested by using a lactating/non-lactating cow model (Green *et al.*, 2012). Non-lactating cows had greater plasma concentrations of glucose (~ +10 mg/dl) compared with lactating cows. Cows that did not get pregnant after first AI at ~60 days *postpartum* had lesser plasma concentrations of glucose during the first 30 days *postpartum* when compared

https://doi.org/10.1017/S1751731114000482 Published online by Cambridge University Press

with pregnant cows. Later *postpartum* (30 to 60 days *postpartum*) there was no relationship between plasma concentrations of glucose and pregnancy.

Green *et al.* (2012) and Garverick *et al.* (2013) examined association between *postpartum* blood glucose and pregnancy at first AI. In separate studies performed in Ireland (Cummins *et al.*, 2012; Moore *et al.*, 2014), blood concentrations of glucose were examined in dairy cows with similar genetic merit for milk production but with good or poor genetic merit for fertility (Fert+ or Fert−, respectively). Cummins *et al.* (2012) failed to demonstrate differences in circulating glucose *postpartum* (although early *postpartum* insulin and IGF1 were greater in Fert+ cows). Subsequent studies of Fert+ and Fert− cows, however, demonstrated greater glucose in Fert+ cows on the day of calving and 1 week later (Moore *et al.*, 2014). Later *postpartum*, Fert+ and Fert− cows were similar for blood concentrations of glucose. Finally, in a recent pooled analysis of studies of *prepartum* nutrition and subsequent *postpartum* reproduction, Cardoso *et al.* (2013) determined that greater blood concentrations of glucose at weeks 3 and 4 *postpartum* were associated with shorter days to pregnancy.

Studies described in the previous three paragraphs were associative and do not establish cause and effect. Described relationships between blood glucose and pregnancy were for early *postpartum* blood concentrations of glucose (generally within the 1st month of lactation) and the establishment of pregnancy (several months after the glucose measurements were made). The suggestion is that the early *postpartum* metabolic profile that includes blood concentrations of glucose is predictive of subsequent *postpartum* fertility. A key question is how the metabolic profile of early *postpartum* cows controls reproductive processes leading to pregnancy that occur several months after the early *postpartum* period.

Confounding of glucose with other metabolites postpartum
Glucose may be a mediator of *postpartum* reproduction because glucose acts as a substrate for milk production and is an essential molecule for reproductive processes. An obvious limitation to associations involving glucose and reproduction presented in the previous section is that circulating metabolites of early *postpartum* cows are controlled by endocrine pathways that coordinate several metabolic processes. Uncoupling of the somatotropic axis at calving leads to a decrease in circulating concentrations of IGF1 that exists for at least 60 days *postpartum*. Demand for glucose during lactation leads to lesser blood concentrations of glucose and insulin. Lesser insulin theoretically prevents (or slows) recoupling of the somatotropic axis through its effects on GH receptor 1A (GHR1A; Butler *et al.*, 2003; Lucy, 2008). If a cow is not milked *postpartum* then blood concentrations of glucose normalize within a few days (Green *et al.*, 2012; Maillo *et al.*, 2012; Thompson *et al.*, 2012). These same cows with normalized blood glucose, however, have normalized blood concentrations of insulin and IGF1 and a reversal of the metabolic profile that typifies early *postpartum* cows. It is impossible to say, therefore, whether any change in reproductive function is a consequence of any one hormone/metabolite alone or the collective action of several.

In attempt to address the possibility that glucose is the primary metabolic driver of the entire system, glucose was infused into early *postpartum* cows in a physiologically relevant manner. Increasing daily doses from 500 to 1500 g/day glucose were administered via jugular infusion by using a constant rate of glucose infusion (Lucy *et al.*, 2013). Glucose infusion increased blood concentrations of insulin and caused a marked decrease in both NEFA and β-hydroxy butyric acid (BHBA). In addition to changes in insulin and circulating metabolites, glucose infusion increased circulating concentrations of IGF1 (Lucy *et al.*, 2013). Insulin may have mediated stimulatory effects of glucose on *postpartum* IGF1 through its capacity to recouple the somatotropic axis. The magnitude of the IGF1 increase in response to glucose that was observed (10 to 15 ng/ml) approximated the magnitude of IGF1 difference reported elsewhere for cows that have or have not commenced oestrous cycles (Beam and Butler, 1998) or *postpartum* pregnant *v.* non-pregnant cows (Taylor *et al.*, 2004; Green *et al.*, 2012). The infusion studies demonstrated that a single molecule (glucose) could rapidly reverse the metabolic profile that typifies early lactation. Based on these results, a well-coordinated homeorhetic system that supplies adequate glucose relative to demand in early lactation may be impacting the reproductive systems that are undergoing restoration during the same time period.

Linking the metabolic profile to reproductive processes during the early *postpartum* period

The state of lowered blood glucose during lactation theoretically compromises the function of tissues that depend on glucose. Associated metabolites such as NEFA and BHBA as well as insulin and IGF1 play a role in controlling tissue function. Based on the data presented above, the first 30 days *postpartum* may be the most-critical in terms of the impact that metabolites and metabolic hormones have on reproduction. Within this review, two essential processes that occur during the first 30 days *postpartum* will be examined with respect to metabolites and metabolic hormones – the restoration of ovarian cyclicity and uterine involution.

Restoration of ovarian cyclicity postpartum
With respect to *postpartum* reproduction, most of the research performed on metabolites and metabolic hormones has focused on the re-initiation of ovarian cyclicity. Cows that have not commenced oestrous cyclicity are by definition infertile because they have not ovulated. Furthermore, fertility generally improves with each successive oestrous cycle before breeding (Peter *et al.*, 2009). There has been, therefore, a traditional focus on understanding mechanisms that control the restoration of ovarian activity before breeding with earlier restoration viewed as favourable.

https://doi.org/10.1017/S1751731114000482 Published online by Cambridge University Press

A frequently addressed topic is the positive association between insulin, IGF1, and the day *postpartum* that cows begin to cycle (Velazquez *et al.*, 2008).

The capacity of a variety of metabolites and metabolic signals to act on the hypothalamus and pituitary to increase GnRH and LH pulsatility has been reviewed (Chagas *et al.*, 2007; LeRoy *et al.*, 2008). At the ovary, both insulin and IGF1 promote proliferation, differentiation and survival of follicular cells (Lucy, 2008). Perhaps the most important actions of insulin and IGF1 are observed when either hormone acts synergistically with gonadotropins (either FSH or LH). The synergistic relationship between insulin, IGF1 and gonadotropins is seen for a variety of cellular functions including mitogenesis, cell survival, and steroidogenesis. Glucose controls insulin secretion and ultimately controls hepatic IGF1 secretion via insulin release. Circulating glucose and the insulin/IGF1 systems, therefore, are functionally linked in the whole animal. There are several reviews that have been published recently on the relationships between insulin, IGF1 and *postpartum* ovarian function (Lucy, 2008; Kawashima *et al.*, 2012).

Effects of hormones and metabolites on ovarian cyclicity in *postpartum* cows are generally thought to be coincident meaning that changes in circulating insulin, IGF1, glucose, etc. exert their effects in real time. The 'Britt hypothesis' states that early *postpartum* hormones and metabolites can exert carry-over effects on the somatic and germ cells (Britt, 1991). The Britt hypothesis is based on the fact that ovulatory follicles require ~60 days to fully develop. The oocyte that ovulates around the time of first insemination and somatic cells within the same follicle are initiating their development at calving (or shortly thereafter). Associations between *postpartum* hormones and metabolites and subsequent reproduction are found early *postpartum* when the most-extreme homeorhetic adjustments are known to occur. The early *postpartum* metabolic profile, therefore, may have the capacity to imprint ovarian tissue either through permanent effects on the genome (epigenetic mechanisms) or by changing the chemical composition of the cells themselves.

Perhaps the best-studied example of a metabolic imprint is for *postpartum* NEFA and its effect on composition of the oocyte and function of follicular cells (Leroy *et al.*, 2011; Van Hoeck *et al.*, 2011; Wathes, 2012). The possibility that there are permanent epigenetic modifications to the genome during the early *postpartum* period that affect long-term developmental competence of follicular cells has not been demonstrated at this time. According to the Britt hypothesis, the only way to correct the metabolic imprint is to allow the imprinted cells (in this case the oocyte, granulosa and theca cells) to exhaust their physiologically relevant lifespan.

Uterine health and immune function

Under normal circumstances, involution (process through which the uterus returns to a normal non-pregnant state) is completed during the 1st month *postpartum*. During involution, the uterus shrinks in size, re-establishes the luminal epithelium, and immune cells (primarily polymorphonuclear neutrophils (PMN)) infiltrate the uterus to clear residual placental tissue as well as infectious microorganisms (Gilbert *et al.*, 2011; LeBlanc, 2012; Wathes *et al.*, 2012). *Postpartum* cows have a depressed immune system particularly during the 1st month after calving. With respect to uterine involution and disease, the current theory is that the metabolic environment in *postpartum* cows suppresses the innate immune system through effects on PMN function (Graugnard *et al.*, 2012; LeBlanc, 2012; Ingvartsen and Moyes, 2013; Figure 2). In most cases, changes in circulating concentrations of nutrients and metabolites that occur in *postpartum* cows are exactly opposite to those that would benefit the function of PMN (Table 1). Inhibitory effects of the *postpartum* metabolic profile can affect traditional aspects of PMN function (migration, oxidative burst, phagocytosis, etc.). There is good agreement between *in vitro* analyses of PMN function and epidemiological evidence that indicates that an abnormal periparturient metabolic profile predisposes cows to uterine disease during the early *postpartum* period and infertility later *postpartum* (Chapinal *et al.*, 2012).

Glucose is the primary metabolic fuel that PMN use to generate the oxidative burst that leads to killing activity. Glucose is stored as glycogen within the PMN. Concentrations of glycogen in PMN decrease in a manner that is similar to the decrease in blood glucose *postpartum* with a lag period of ~7 days (Galvão *et al.*, 2010). The lag in the decrease in PMN glycogen may represent the time required for glycogen loading during maturation of PMN. Galvão *et al.* (2010) observed that cows developing uterine disease had lesser concentrations of glycogen in their PMN. Their conclusion was that lesser glycogen reserve led to a reduced capacity for oxidative burst in PMN and uterine disease.

Most of the available data indicate that the metabolic profile of *prepartum* cows is equally important to that of

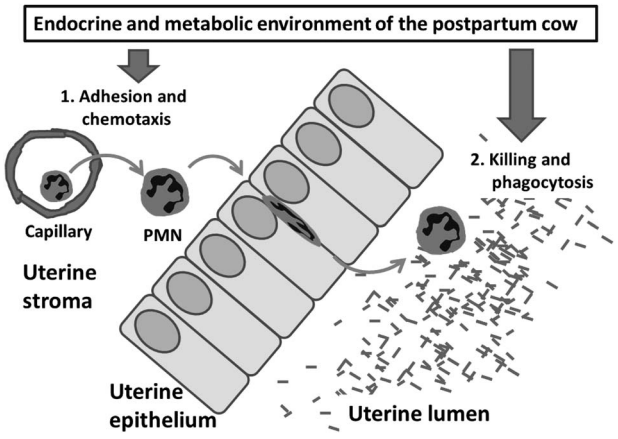


Figure 2 Model for uterine polymorphonuclear neutrophils (PMN) function in *postpartum* cows. The PMN migrate into the uterine lumen *postpartum* where they kill bacteria and clear debris. The endocrine and metabolic environment of *postpartum* cows may compromise PMN. Cows are predisposed to uterine disease because the PMN fail to efficiently migrate into the uterus or have reduced capacity for phagocytosis and killing.

https://doi.org/10.1017/S1751731114000482 Published online by Cambridge University Press

Table 1 *Hormones and metabolites affected by milk production in* postpartum *dairy cows, their known effects on polymorphonuclear neutrophil (PMN) function, and relative concentrations (*postpartum v. *later lactation)*

Item	Known effects on PMN function (reference)	*Postpartum*
Insulin	Activates growth factor pathways; ↑ cytokine production; ↑ chemotaxis; ↑ phagocytic activity (Sunahara *et al.*, 2012)	Low insulin
IGF1	Cell-survival factor; activation of PI3K (Himpe *et al.*, 2013)	Low IGF1
Glucose	↑ respiratory burst; ↑ chemotaxis; ↑ phagocytosis (Chou *et al.*, 2010)	Low glucose
BHBA	Inhibits formation of extracellular traps and inhibits bactericidal activity against *Escherichia coli* (Grinberg *et al.*, 2008)	High BHBA
NEFA	↓ production of reactive oxygen species (Ster *et al.*, 2012)	High NEFA

BHBA = β-hydroxy butyric acid; NEFA = non-esterified fatty acid.

postpartum cows for subsequent uterine health and (or) the establishment of pregnancy (Castro *et al.*, 2012). In their work in which an index for physiological imbalance was created, Moyes *et al.* (2013) concluded that an index that included NEFA, BHBA and glucose was predictive of *postpartum* uterine disease especially when the *prepartum* index was used. In *postpartum* cows, blood concentrations of NEFA were the best predictor of *postpartum* disease. Their finding is consistent with the general notion that NEFA can negatively affect immune cell function in *postpartum* cows. Cardoso *et al.* (2013) also concluded that NEFA was the key molecule associated with *postpartum* disease.

In all likelihood the metabolic profile associated with uterine disease is initiated before calving or shortly thereafter. Perhaps this is not surprising given the relatively acute nature of physiological events at calving and the homeorhetic mechanisms at the initiation of lactation. A cow's homeorhetic capacity (i.e. capacity for gluconeogenesis, lipid mobilization, etc.) and her inherent resistance to disease are largely manifested after calving but the underlying biology is theoretically in place before calving.

Linking the early postpartum *metabolic profile with subsequent pregnancy*

Metabolic or uterine disease *postpartum* extends the interval from calving to pregnancy and increases pregnancy loss (Ribeiro *et al.*, 2013). Arguments presented in the previous paragraph are that the metabolic profile that typifies *postpartum* cows (elevated NEFA and BHBA and reduced blood glucose) leads to reduced immune cell function that predisposes cows to uterine disease. What is the signal, however, that leads to long-term reproductive failure in cows with relatively short-term *postpartum* uterine disease? Presumably the disease state of cows is resolved at some stage *postpartum* but nonetheless there appear to be carry-over effects of the early *postpartum* period on the establishment of pregnancy. The Britt hypothesis (typically applied to the ovarian follicle) should perhaps be expanded to include the uterus of *postpartum* cows. The uterus is repaired during uterine involution that occurs primarily within the first 30 days after calving. The process is largely completed before breeding but nonetheless the uterus appears to have a relatively long-term memory (imprinting) for metabolic and disease states of the early *postpartum* period.

The nature of the metabolic or disease-related imprinting that occurs within the uterus is completely unknown. It is possible that cows with *postpartum* uterine disease have not fully resolved the infection at the time of first *postpartum* breeding. Primary methods of assessing uterine health during the breeding period (presence of pus in vaginal mucous or existence of an enlarged uterus) would only detect more extreme cases of prolonged uterine disease. It is possible, therefore, that there are remnants of uterine inflammation in a seemingly normal uterus and that these remnants in some way affect the capacity for cows to become pregnant. Turner *et al.* (2012) described aggregates of uterine immune cells in *postpartum* cows. We have noticed these unique structures as well (Figure 3). We believe that they are analogous to mucosa-associated lymphoid tissue (MALT). Uterine MALT appear as clusters of immune cells within the uterine stroma and uterine caruncular areas. The MALT may be deep within stroma or near the surface epithelium. In unpublished work, MALT were observed within the endometrium of pregnant cows (~100 days *postpartum*) that had been previously diagnosed with a *postpartum* uterine infection (Figure 3). Whether the presence of MALT has any implications for pregnancy establishment, maintenance or loss is unknown.

In addition to potential physical imprinting of the uterus (via resident immune cells), it is possible that proliferation and differentiation of the uterine epithelium is permanently affected by the early *postpartum* metabolic profile or the disease state of the *postpartum* uterus. Uterine epithelium, and to a certain extent stroma, is regenerated during uterine involution presumably from stem cells that reside within the uterus. It is possible that the uterine environment that theoretically includes pathogens, PMN, metabolites, and hormones leaves an epigenetic mark on uterine epithelial and stromal cells as they go through the process of differentiating from the stem cell pool. These epigenetic marks would be inherited by daughter cells as they proliferate to occupy uterine epithelium and stroma. This altered epigenome of mature uterine epithelial cells and stroma may in some manner affect the capacity of the uterus to establish pregnancy during the breeding period that occurs several months after the uterine epithelial lining has been established.

Mechanisms linking lactation to pregnancy during the breeding period

Assuming that uterine involution is complete and cows have commenced oestrous cycles then what are the implications of

https://doi.org/10.1017/S1751731114000482 Published online by Cambridge University Press

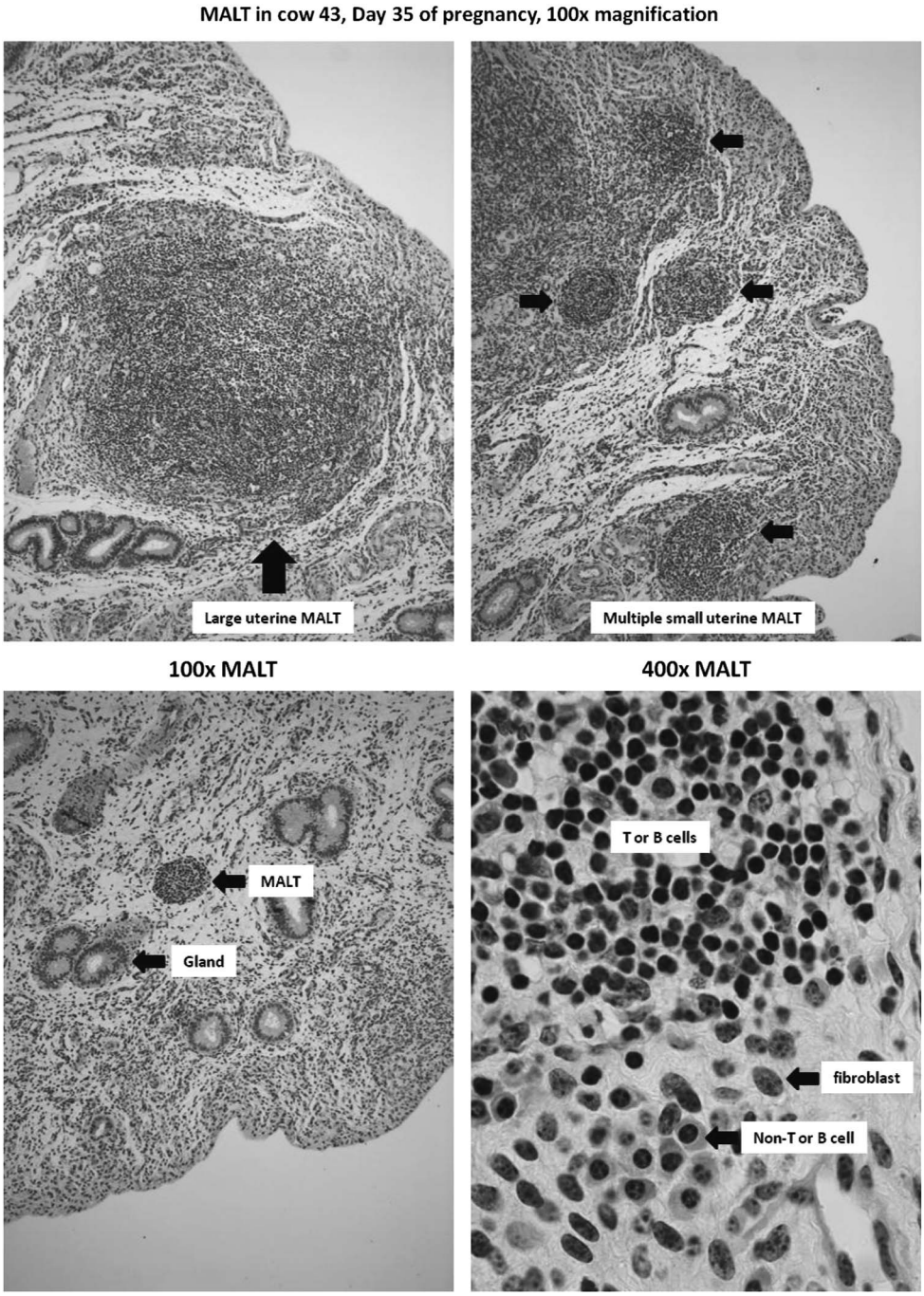


Figure 3 Uterine mucosa-associated lymphoid tissue (MALT) in cow 43 (top panel) and low power and high power magnification of a uterine MALT (bottom panel). Herd records noted a *postpartum* uterine infection after calving in cow 43. Histological examination of the uterus of the same cow at slaughter (100 days *postpartum*, top panel) revealed a large number of MALT perhaps implying an immune-cell based carry-over effect of the *postpartum* uterine infection on uterine morphology of the pregnant uterus. Based on morphology, the MALT appear to be comprised of lymphocytes (either T or B cells, bottom panel).

lactation for cows during the breeding period? Several authors have recently addressed potential relationships between normal oestrous cycles and the metabolic profile of cows during the breeding period (Wathes *et al.*, 2007; López-Gatius, 2012). As detailed above, the metabolic profile of later *postpartum* cows (>30 days *postpartum*) still involves relatively low concentrations of glucose, insulin and IGF1 although concentrations of NEFA and BHBA have typically normalized.

Oestrous cyclicity during the breeding period

Patterns of oestrous cyclicity for lactating cows are different (appear to be less regular) when compared with oestrous cycle of nulliparous heifers. An existing hypothesis is that the same hormones that control when cows begin to cycle (insulin, IGF1 and LH) have an effect on the periodicity of cyclicity which relates to the functionality of follicles and corpora lutea (Figure 4). As described for early lactation

https://doi.org/10.1017/S1751731114000482 Published online by Cambridge University Press

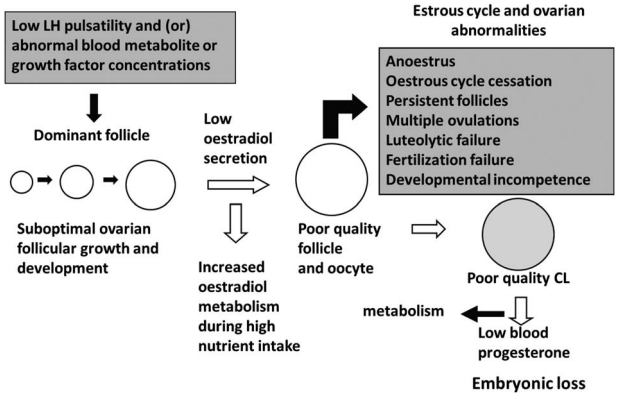


Figure 4 Mechanisms that link metabolism, metabolic hormones and metabolites to oestrous cycle abnormalities, infertility, and embryonic loss in *postpartum* cows. Follicles and corpora lutea (CL) respond to changing concentrations of gonadotropins, growth factors, and blood metabolites in *postpartum* cows. Steroids produced by follicles and CL are metabolized by the liver of lactating cows resulting in reduced circulating concentrations. Endocrine failure can lead to oestrous cycle and ovarian abnormalities as well as a poor quality oocyte that may not develop after fertilization. If corpora lutea are compromised and steroid metabolism is greater then blood concentrations of progesterone are low and there is potential for embryonic loss.

cows, the hormonal environment created by lactation (in this example low blood concentrations of glucose, insulin and IGF1) may potentially affect the capacity of ovarian cells to respond to gonadotropins. In cows that have commenced oestrous cycles, this could potentially affect oestradiol production by follicles as well as progesterone production by corpora lutea. Low blood glucose could potentially compromise a variety of essential metabolic processes in ovarian cells including oocytes that depend on glucose for energy (Clark *et al.*, 2011; Berlinguer *et al.*, 2012). In their recent study of bovine follicles, Walsh *et al.* (2012) concluded that STAR gene expression (the rate limiting enzyme in steroidogenesis) was specifically down-regulated by the metabolic profile found in early lactation (low glucose, insulin and IGF1).

Work cited in the previous paragraph indicates that follicles in lactating cows are compromized because lesser glucose, insulin and IGF1 affect the capacity of follicles to respond to LH and synthesize oestradiol. There is the potential for greater steroid metabolism in lactating compared with non-lactating cows that can be explained by greater dry matter intake in cows that are lactating (Wiltbank *et al.*, 2011). Lesser circulating oestradiol from preovulatory follicles has been implicated in abnormal patterns of follicular growth leading to anovulatory conditions, multiple ovulation and reduced oestrous expression in lactating cows (Figure 4). Likewise, greater steroid metabolism has been implicated as a mechanism leading to low circulating progesterone in lactating cows that could potentially affect early embryonic and foetal development (see subsequent paragraph).

Subnormal luteal function

Most of the work relating early embryonic development to lactation addresses the possibility that low progesterone during the 1st weeks after insemination leads to slower embryonic development that predisposes cows to embryonic loss (Diskin and Morris, 2008; Lonergan, 2011). Progesterone stimulates uterine histotroph secretion and lesser uterine histotroph secretion (caused by low progresteorne) leads to slower embryonic development. The theory is that slowly developing embryos fail to reach adequate size to generate an adequate interferon-tau (IFNT) signal to the mother (Robinson *et al.*, 2008). Pregnancies are lost because the mother fails to recognize the pregnancy and undergoes luteal regression as if not pregnant. Low progesterone during the first week after AI is believed to occur more frequently in lactating cows because of abnormal luteal function or greater steroid metabolism assoicated with lactation. Several authors have recently reviewed mechanisms assoicated with subnormal luteal development and early embryonic loss (Pursley and Martins, 2011; Wiltbank *et al.*, 2011; Bridges *et al.*, 2013).

Glucose as a substrate for the developing embryo and foetus

What other mechanisms in addition to reduced blood concentrations of progesterone potentially explain the rate at which the embryo or foetus grows as well as pregnancy loss is lactating dairy cows? Glucose is typically thought of as a key energy source for ATP production through mitochondrial oxidative phosphorylation. Glucose is not used primarily for metabolic fuel production, however, by either the mammary gland or the pregnancy. In mammary gland, most of the glucose is used to produce lactose (milk sugar). Likewise, in the uterus and placenta most of the glucose is used to supply carbons for the synthesis of cellular components (nucleotides, amino acids, lipids, etc.). This latter phenomenon is known as the 'Warburg effect' and typifies proliferating cells (Vander Heiden *et al.*, 2009).

Green *et al.* (2012) either milked cows normally or dried them off (not milked) immediately after calving. Cows were inseminated at ~60 days *postpartum* and the foetus and placenta were collected on day 28, 35 or 42. One major conclusion was that for a given day of pregnancy, the foetus and placenta from a lactating cow were smaller (weighed less) than the foetus and placenta from a non-lactating cow. In subsequent work, concentrations of glucose were less in foetal fluids collected from lactating compared with non-lactating cows, perhaps because maternal concentrations of glucose were less during lactation (Lucy *et al.*, 2012). This latter observation agreed with the general notion that glucose transport to the foetus primarily depends on Glut 1 and Glut 3 transporters with glucose moving down its concentration gradient through facilitative diffusion (Wooding *et al.*, 2005). Lesser maternal glucose leads to lesser foetal glucose because the foetus and placenta cannot sequester glucose against a concentration gradient. Furthermore, the foetus and placenta have a limited capacity for gluconeogenesis (Battaglia and Meschia, 1978). The reduction in glucose reaching the uterus and conceptus can potentially affect how the pregnancy develops because the pregnancy depends on glucose as a substrate for tissue synthesis and metabolic energy (Battaglia and Meschia, 1978).

Growth of the foetus and placenta, therefore, depends on the metabolic milieu of cows. Low concentrations of glucose

https://doi.org/10.1017/S1751731114000482 Published online by Cambridge University Press

in *postpartum* cows may predispose cows to pregnancy loss because the foetus/placenta may not have adequate substrate for the creation of new cells. Neither the mammary gland nor the placenta can concentrate glucose via a glucose transporter. Greater blood flow to the mammary gland facilitates greater capacity to extract glucose from the circulation, and sets up the metabolic scenario where the mammay gland has priority over the pregnancy for glucose.

Does slower embryonic or foetal growth lead to pregnancy loss?

What is the evidence that that slower embryonic or foetal growth caused by either low progesterone or low glucose growth actually leads to pregnancy loss? The slower developing embryo or foetus may simply be responding to its environment and adjusting to lesser histotroph secretion (low progesterone environment) or lesser glucose availability (low glucose environment) by growing more slowly. This is, of course, inevitable in as much as a tissue cannot grow faster than its capacity to sequester and use substrates required for growth. Perhaps slower growth is 'normal' when substrates for growth are limiting and slower growth may not predispose cows to pregnancy to loss.

Few published studies in cattle have asked the question 'does slower growth of the foetal/placental unit lead to pregnancy loss?' Pregnant cows that undergo pregnancy loss have lesser blood concentrations of pregnancy-associated glycoprotein (PAG) leading up to the time that the pregnancy is aborted (Gábor *et al*., 2007; Thompson *et al*., 2010; Pohler *et al*., 2013). Lesser blood concentrations of PAG may indicate that cows are pregnant with a small embryo or foetus. These data showing a relationship between smaller embryos or foetuses and pregnancy loss raise the question of cause and effect. Does slow growth eventually lead to death of the embryo/foetus or does an embryo/foetus destine to undergo loss have slower growth.

Conclusions

The endocrine and metabolic environment of lactating cows affects the capacity of cows to become pregnant *postpartum*. There is ample evidence that the hormones that coordinate homeorhetic adjustments that support lactation can act on the uterus and ovary before and during the breeding period. In addition to the hormonal environment, the metabolic environment created by lactation that includes low blood glucose and elevated NEFA and BHBA impinges upon the ovary as well as the immune system that plays a critical role in restoring uterine health in *postpartum* cows. Specific mechanisms through which the metabolic environment of early lactation deposits a lasting imprint on uterine and ovarian function is less clear. Also less clear are the mechanisms that link lactation to a predisposition for pregnancy loss in lactating cows. The slow rate of embryonic or foetal growth in lactating cows with low blood progesterone and low blood concentrations of glucose may be an important mechanism explaining pregnancy loss.

Acknowledgements

The authors would like to thank the Fulbright Commission/ Education USA, the University of Missouri, and Teagasc, Animal & Grassland Research and Innovation Programme, Moorepark for supporting the research leave granted to MCL during which time this manuscript was prepared and presented.

References

Aschenbach JR, Kristensen NB, Donkin SS, Hammon HM and Penner GB 2010. Gluconeogenesis in dairy cows: the secret of making sweet milk from sour dough. IUBMB Life 62, 869–877.

Battaglia FC and Meschia G 1978. Principal substrates of fetal metabolism. Physiology Review 58, 499–527.

Beam SW and Butler WR 1998. Energy balance, metabolic hormones, and early postpartum follicular development in dairy cows fed prilled lipid. Journal of Dairy Science 81, 121–131.

Bell AW 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. Journal of Animal Science 73, 2804–2819.

Berlinguer F, Gonzalez-Bulnes A, Contreras-Solis I, Spezzigu A, Torres-Rovira L, Succu S, Naitana S and Leoni GG 2012. Glucogenic supply increases oocyte developmental competence in sheep. Reproduction, Fertility and Development 24, 1055–1062.

Bridges GA, Day ML, Geary TW and Cruppe LH 2013. Triennial Reproduction Symposium: deficiencies in the uterine environment and failure to support embryonic development. Journal of Animal Science 91, 3002–3013.

Britt JH 1991. Impacts of early postpartum metabolism on follicular development and fertility. The Bovine Practitioner Proceedings 24, 39–43.

Butler ST, Bork AL, Pelton SH, Radcliff RP, Lucy MC and Butler WR 2003. Insulin restores hepatic growth hormone (GH) responsiveness during lactation-induced negative energy balance in dairy cattle: effects on expression of insulin-like growth factor-I and GH receptor 1A. Journal of Endocrinology 176, 205–217.

Cardoso FC, LeBlanc SJ, Murphy MR and Drackley JK 2013. Prepartum nutritional strategy affects reproductive performance in dairy cows. Journal of Dairy Science 96, 5859–5871.

Castro N, Kawashima C, van Dorland HA, Morel I, Miyamoto A and Bruckmaier RM 2012. Metabolic and energy status during the dry period is crucial for the resumption of ovarian activity postpartum in dairy cows. Journal of Dairy Science 95, 5804–5812.

Chagas LM, Bass JJ, Blache D, Burke CR, Kay JK, Lindsay DR, Lucy MC, Martin GB, Meier S, Rhodes FM, Roche JR, Thatcher WW and Webb R 2007. 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, 4022–4032.

Chapinal N, Leblanc SJ, Carson ME, Leslie KE, Godden S, Capel M, Santos JE, Overton MW and Duffield TF 2012. Herd-level association of serum metabolites in the transition period with disease, milk production, and early lactation reproductive performance. Journal of Dairy Science 95, 5676–5682.

Chou JY, Jun HS and Mansfield BC 2010. Glycogen storage disease type I and G6Pase-β deficiency: etiology and therapy. Nature Reviews in Endocrinology 6, 676–688.

Clark AR, Stokes YM and Thompson JG 2011. Estimation of glucose uptake by ovarian follicular cells. Annals of Biomedical Engineering 39, 2654–2667.

Cummins SB, Lonergan P, Evans AC, Berry DP, Evans RD and Butler ST 2012. Genetic merit for fertility traits in Holstein cows: I. Production characteristics and reproductive efficiency in a pasture-based system. Journal of Dairy Science 95, 1310–1322.

De Vries A 2006. Economic value of pregnancy in dairy cattle. Journal of Dairy Science 89, 3876–3885.

Diskin MG and Morris DG 2008. Embryonic and early foetal losses in cattle and other ruminants. Reproduction in Domestic Animals 43 (suppl. 2), 260–267.

Gábor G, Tóth C, Ozsvári L, Abonyi-Tóth Z and Sasser RG 2007. Early detection of pregnancy and embryonic loss in dairy cattle by ELISA tests. Reproduction in Domestic Animals 42, 633–636.

https://doi.org/10.1017/S1751731114000482 Published online by Cambridge University Press

Galvão KN, Flaminio MJ, Brittin SB, Sper R, Fraga M, Caixeta L, Ricci A, Guard CL, Butler WR and Gilbert RO 2010. Association between uterine disease and indicators of neutrophil and systemic energy status in lactating Holstein cows. Journal of Dairy Science 93, 2926–2937.

Garverick HA, Harris MN, Vogel-Bluel R, Sampson JD, Bader J, Lamberson WR, Spain JN, Lucy MC and Youngquist RS 2013. Concentrations of nonesterified fatty acids and glucose in blood of periparturient dairy cows are indicative of pregnancy success at first insemination. Journal Dairy Science 96, 181–188.

Gilbert RO 2011. The effects of endometritis on the establishment of pregnancy in cattle. Reproduction, Fertility, and Development 24, 252–257.

Graugnard DE, Bionaz M, Trevisi E, Moyes KM, Salak-Johnson JL, Wallace RL, Drackley JK, Bertoni G and Loor JJ 2012. Blood immunometabolic indices and polymorphonuclear neutrophil function in peripartum dairy cows are altered by level of dietary energy prepartum. Journal of Dairy Science 95, 1749–1758.

Green JC, Meyer JP, Williams AM, Newsom EM, Keisler DH and Lucy MC 2012. Pregnancy development from day 28 to 42 of gestation in postpartum Holstein cows that were either milked (lactating) or not milked (not lactating) after calving. Reproduction 143, 699–711.

Grinberg N, Elazar S, Rosenshine I and Shpigel NY 2008. Beta-hydroxybutyrate abrogates formation of bovine neutrophil extracellular traps and bactericidal activity against mammary pathogenic Escherichia coli. Infection and Immunity 76, 2802–2807.

Himpe E, Abdul Rahim SA, Verdood P, Mano H and Kooijman R 2013. Tec kinase stimulates cell survival in transfected Hek293T cells and is regulated by the anti-apoptotic growth factor IGF-I in human neutrophils. Cell Signalling 25, 666–673.

Ingvartsen KL and Moyes K 2013. Nutrition, immune function and health of dairy cattle. Animal 7 (suppl. 1), 112–122.

Kawashima C, Matsui M, Shimizu T, Kida K and Miyamoto A 2012. Nutritional factors that regulate ovulation of the dominant follicle during the first follicular wave postpartum in high-producing dairy cows. Journal of Reproduction and Development 58, 10–16.

Kwintkiewicz J and Giudice LC 2009. The interplay of insulin-like growth factors, gonadotropins, and endocrine disruptors in ovarian follicular development and function. Seminar in Reproductive Medicine 27, 43–51.

LeBlanc SJ 2012. Interactions of metabolism, inflammation, and reproductive tract health in the postpartum period in dairy cattle. Reproduction in Domestic Animals 47 (suppl. 5), 18–30.

Leroy JL, Vanholder T, Van Knegsel AT, Garcia-Ispierto I and Bols PE 2008. Nutrient prioritization in dairy cows early postpartum: mismatch between metabolism and fertility? Reproduction in Domestic Animals 43 (suppl. 2), 96–103.

Leroy JL, Rizos D, Sturmey R, Bossaert P, Gutierrez-Adan A, Van Hoeck V, Valckx S and Bols PE 2011. Intrafollicular conditions as a major link between maternal metabolism and oocyte quality: a focus on dairy cow fertility. Reproduction Fertility and Development 24, 1–12.

Lonergan P 2011. Influence of progesterone on oocyte quality and embryo development in cows. Theriogenology 76, 1594–1601.

López-Gatius F 2012. Factors of a noninfectious nature affecting fertility after artificial insemination in lactating dairy cows. A review. Theriogenology 77, 1029–1041.

Lucy MC 2008. Functional differences in the growth hormone and insulin-like growth factor axis in cattle and pigs: implications for post-partum nutrition and reproduction. Reproduction in Domestic Animals 43, 31–39.

Lucy MC, Escalante RC, Keisler DH, Lamberson WR and Mathew DJ 2013. Short communication: glucose infusion into early postpartum cows defines an upper physiological set point for blood glucose and causes rapid and reversible changes in blood hormones and metabolites. Journal of Dairy Science 96, 5762–5768.

Lucy MC, Green JC, Meyer JP, Williams AM, Newsom EM and Keisler DH 2012. Short communication: glucose and fructose concentrations and expression of glucose transporters in 4 to 6 week pregnancies collected from Holstein cows that were either lactating or not lactating. Journal of Dairy Science 95, 5095–5101.

Maillo V, Rizos D, Besenfelder U, Havlicek V, Kelly AK, Garrett M and Lonergan P 2012. Influence of lactation on metabolic characteristics and embryo development in postpartum Holstein dairy cows. Journal of Dairy Science 95, 3865–3876.

Moore SG, Fair T, Lonergan P and Butler ST 2014. Genetic merit for fertility traits in Holstein cows: IV. Transition period, uterine health and resumption of cyclicity. Journal of Dairy Science 97, doi:10.3168/jds.2013-7278.

Moyes KM, Larsen T and Ingvartsen KL 2013. Generation of an index for physiological imbalance and its use as a predictor of primary disease in dairy cows during early lactation. Journal of Dairy Science 96, 2161–2170.

Peter AT, Vos PL and Ambrose DJ 2009. Postpartum anoestrus in dairy cattle. Theriogenology 71, 1333–1342.

Pohler KG, Geary TW, Johnson CL, Atkins JA, Jinks EM, Busch DC, Green JA, MacNeil MD and Smith MF 2013. Circulating bovine pregnancy associated glycoproteins are associated with late embryonic/fetal survival but not ovulatory follicle size in suckled beef cows. Journal of Animal Science 91, 4158–4167.

Pursley JR and Martins JP 2011. Impact of circulating concentrations of progesterone and antral age of the ovulatory follicle on fertility of high-producing lactating dairy cows. Reproduction, Fertility, and Development 24, 267–271.

Ribeiro ES, Lima FS, Greco LF, Bisinotto RS, Monteiro AP, Favoreto M, Ayres H, Marsola RS, Martinez N, Thatcher WW and Santos JE 2013. Prevalence of periparturient diseases and effects on fertility of seasonally calving grazing dairy cows supplemented with concentrates. Journal of Dairy Science 96, 5682–5697.

Robinson RS, Hammond AJ, Wathes DC, Hunter MG and Mann GE 2008. Corpus luteum-endometrium-embryo interactions in the dairy cow: underlying mechanisms and clinical relevance. Reproduction in Domestic Animals 43 (suppl. 2), 104–112.

Ster C, Loiselle MC and Lacasse P 2012. Effect of postcalving serum non-esterified fatty acids concentration on the functionality of bovine immune cells. Journal of Dairy Science 95, 708–717.

Sunahara KK, Sannomiya P and Martins JO 2012. Briefs on insulin and innate immune response. Cellular Physiology and Biochemistry 29, 1–8.

Taylor VJ, Cheng Z, Pushpakumara PG, Beever DE and Wathes DC 2004. Relationships between the plasma concentrations of insulin-like growth factor-I in dairy cows and their fertility and milk yield. Veterinary Record 155, 583–588.

Thompson IM, Cerri RL, Kim IH, Green JA, Santos JE and Thatcher WW 2010. Effects of resynchronization programs on pregnancy per artificial insemination, progesterone, and pregnancy-associated glycoproteins in plasma of lactating dairy cows. Journal of Dairy Science 93, 4006–4018.

Thompson IM, Cerri RL, Kim IH, Ealy AD, Hansen PJ, Staples CR and Thatcher WW 2012. Effects of lactation and pregnancy on metabolic and hormonal responses and expression of selected conceptus and endometrial genes of Holstein dairy cattle. Journal of Dairy Science 95, 5645–5656.

Turner ML, Healey GD and Sheldon IM 2012. Immunity and inflammation in the uterus. Reproduction in Domestic Animals 47 (suppl. 4), 402–409.

Vander Heiden MG, Cantley LC and Thompson CB 2009. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324, 1029–1033.

Van Hoeck V, Sturmey RG, Bermejo-Alvarez P, Rizos D, Gutierrez-Adan A, Leese HJ, Bols PE and Leroy JL 2011. Elevated non-esterified fatty acid concentrations during bovine oocyte maturation compromise early embryo physiology. PLoS One 6, e23183.

Velazquez MA, Spicer LJ and Wathes DC 2008. The role of endocrine insulin-like growth factor-I (IGF-I) in female bovine reproduction. Domestic Animal Endocrinology 35, 325–342.

Walsh SW, Williams EJ and Evans AC 2011. A review of the causes of poor fertility in high milk producing dairy cows. Animal Reproduction Science 123, 127–138.

Walsh SW, Mehta JP, McGettigan PA, Browne JA, Forde N, Alibrahim RM, Mulligan FJ, Loftus B, Crowe MA, Matthews D, Diskin M, Mihm M and Evans AC 2012. Effect of the metabolic environment at key stages of follicle development in cattle: focus on steroid biosynthesis. Physiological Genomics 44, 504–517.

Wathes DC 2012. Mechanisms linking metabolic status and disease with reproductive outcome in the dairy cow. Reproduction in Domestic Animals 47 (suppl. 4), 304–312.

Wathes DC, Clempson AM and Pollott GE 2012. Associations between lipid metabolism and fertility in the dairy cow. Reproduction, Fertility, and Development 25, 48–61.

Wathes DC, Fenwick M, Cheng Z, Bourne N, Llewellyn S, Morris DG, Kenny D, Murphy J and Fitzpatrick R 2007. Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow. Theriogenology 68, S232–S241.

Wiltbank MC, Souza AH, Carvalho PD, Bender RW and Nascimento AB 2011. Improving fertility to timed artificial insemination by manipulation of circulating progesterone concentrations in lactating dairy cattle. Reproduction, Fertility, and Development 24, 238–243.

Wooding FB, Fowden AL, Bell AW, Ehrhardt RA, Limesand SW and Hay WW 2005. Localisation of glucose transport in the ruminant placenta: implications for sequential use of transporter isoforms. Placenta 26, 626–640.

https://doi.org/10.1017/S1751731114000482 Published online by Cambridge University Press