Reprod Dom Anim **43** (Suppl. 2), 96–103 (2008); doi: 10.1111/j.1439-0531.2008.01148.x
ISSN 0936-6768

Nutrient Prioritization in Dairy Cows Early Postpartum: Mismatch Between Metabolism and Fertility?

JLMR Leroy[1], T Vanholder[1], ATM Van Knegsel[2], I Garcia-Ispierto[3] and PEJ Bols[1]

[1]*Veterinary Centre Someren, Someren;* [2]*Adaptation Physiology Group and Animal Nutrition Group, Wageningen Institute of Animal Sciences, Wageningen University, Wageningen, The Netherlands;* [3]*Department of Animal Health and Anatomy, Autonomous University of Barcelona, Barcelona, Spain*

Contents

For several decades, researchers worldwide report a decrease in fertility in high-yielding dairy cows, most probably based on conflicting metabolic and reproductive needs. The dairy herd manager's success at improving milk production has been accompanied by a negative trend for the most visible reproductive parameters such as calving intervals, number of days open and number of inseminations needed per pregnancy. In parallel, many research groups studied the metabolic and endocrine factors that influence follicular growth and the developmental competence of oocytes and embryos. In the past, herd managers and reproductive biologists each tried to tackle the same problems with limited consultation. More recently, the situation has improved significantly and theriogenologists, nutritionists and veterinarians now conduct research in multidisciplinary teams. This review paper starts in a general way by discussing nutrient prioritization towards the udder to guarantee milk production and by describing interactions between the somatotropic and gonadotropic axis. It then focuses on the consequences of the negative energy balance on follicular growth and environment, oocyte and embryo quality, not only by summarizing the currently accepted hypotheses but also based on clear scientific evidence at the follicular level. All this, with one question in mind: is there a mismatch between metabolism and fertility and what can the dairy manager learn from research to tackle the problem of reduced fertility?

Introduction

Disappointing reproductive performance in high-producing dairy herds is a global problem, characterized as multifactorial and urged a multidisciplinary approach in which animal scientists, veterinarians and molecular biologists were required to unravel the complex pathogenesis of this 'subfertility syndrome'. After all, producing a calf at regular intervals is considered a prerequisite for profitable lactational performance (Royal et al. 2000; Huirne et al. 2002). After giving birth, the process of becoming pregnant again in dairy cows starts with clearance and involution of the uterus followed by resumption of ovarian activity. This should result in the completion of the growth of a healthy follicle, enclosing a competent oocyte, and ultimately in oestrus, ovulation, fertilization and uterine attachment by a viable embryo. Any upset of these balanced and fine-tuned biological and mechanical events leads to failing reproduction – and this is exactly where the shoe pinches in our modern dairy herds.

The subfertility syndrome can be divided into two major sub-problems. First of all, up to 50% of modern dairy cows display abnormal oestrus cycles postpartum leading to prolonged calving to first insemination intervals (Opsomer et al. 1998). In this context, especially instability within the hypothalamo–pituitary–ovarian–uterine axis has been studied thoroughly (Lucy 2001; Butler 2003). The concomitant reduced oestrus expression or even anoestrus, cyst formation and delayed first ovulation have been extensively documented (Beam and Butler 1997; de Vries and Veerkamp 2000; Lopez et al. 2004; Vanholder et al. 2006a). Secondly, attention was focussed on disappointing conception rates (Bousquet et al. 2004) and the increasingly high incidence of early embryonic mortality (Dunne et al. 1999; Mann and Lamming 2001; Bilodeau-Goeseels and Kastelic 2003). Fertilization of oocytes from high-genetic merit cows resulted in significantly lower blastocyst yields *in vitro*, irrespective of milk production as such (Snijders et al. 2000). Embryo quality was also reduced in high-producing dairy cows compared with non-lactating counterparts (Wiltbank et al. 2001; Leroy et al. 2005a). A high proportion of non-viable embryos were found in lactating cows compared with non-lactating cows (Sartori et al. 2002). Approximately 70–80% of the total embryonic and foetal losses typically occur during the early embryonic, pre-attachment period (Santos et al. 2004a) (for review, see Leroy et al. 2007).

Modern dairy cows, albeit sub-fertile, produce vast amounts of milk mainly because of significant genetic improvements, combined with nutritional management optimized towards lactation. Based on almost unchanged heifer fertility, we can conclude that the reproductive processes of modern dairy cattle are essentially normal when lactation demands are not imposed (Lucy 2007). Why do modern dairy cows prioritize milk production at the expense of sustained reproductive efficiency? In this review, we aim to answer this question. Are high milk yields and good fertility outcomes conflicting interests metabolically speaking?

From Phylogenetically Driven to Genetically Enforced Nutrient Prioritization: The Consequences on Metabolism

From a biological point of view, it makes sense for mammals in early lactation to favour milk production over fertility: this we can refer to as nutrient prioritization (Lucy 2003). As nutrition becomes scarce, the lactating dam will preferentially invest the limited resources in the survival of living offspring rather than gambling on the oocyte that is yet to be ovulated,

fertilized and cared for during an entire gestation. This maternal catabolic mechanism, also genetically programmed, should maximize the chance of survival of the newborn offspring (Silvia 2003). Over the past 40 years, the focus of dairy industry has been on maximizing milk yield, thereby creating a 'nutrient highway' from the daily ration and body reserves (estimated on 74% body fat and 6% body protein: Tamminga et al. 1997) directly to the udder to sustain milk production.

Nutrient requirements of the gravid uterus late in gestation impose a catabolic status on the dairy cow. Following parturition, an additional demand for glucose, fatty acids and protein is established as milk production starts. During this transition period, cows are unable to compensate for such increased energy demands by increasing feed intake, and this results in negative energy balance (NEB). Drastically reduced insulin concentrations bring approximate energy mobilization and partitioning of energy to the udder. Hypoinsulinaemia promotes gluconeogenesis in the liver (up to 4 kg glucose each day) and acts as a massive lipolytic trigger. The mobilized non-esterified fatty acids (NEFAs) serve as an alternative energy source for other tissues to preserve glucose, which is preferentially used by the mammary gland to form lactose (Vernon 2002). NEFAs are predominantly transported to the liver where they are oxidized to provide energy or transformed into ketone bodies, again an alternative energy source elsewhere in the body. An aberrant over-load of the liver by NEFAs can induce steatosis and disturbed liver function (Herdt 2000). Hormone-sensitive lipases in adipose tissue of high-yielding dairy cows have an increased sensitivity to lipolytic stimuli (such as low insulin, and high catecholamines or glucocorticoids concentrations). In other words, high-yielding dairy cows have been genetically selected to partition even more energy reserves into milk production (Coffey et al. 2004). A higher dietary energy intake will therefore result in greater milk production, but a similar energy imbalance remains, with no beneficial effects on body condition score (BCS) at all (Patton et al. 2006).

A series of biological mechanisms bring an approximate prioritization for milk production at the cost of body reserves in early postpartum dairy cows. First of all, the udder benefits because it does not need insulin to facilitate glucose uptake into cells by the glucose-transport molecules, GLUT 1 and 3, while most other tissues predominantly express insulin-dependent GLUT 4 (Zhao et al. 1996). Secondly, using repeatedly intravenous glucose-tolerance tests, we recently found a temporary suppression of pancreatic function in early postpartum high-yielding dairy cows and this was correlated with elevated NEFA concentrations (Bossaert et al. 2007). *In vitro*, high NEFA levels have toxic effects on pancreatic cells (Cnop et al. 2001; Maedler et al. 2001). Thirdly, in the early postpartum period, low insulin concentrations uncouple the growth hormone (GH)–insulin like growth factor 1 (IGF-I) axis in the liver because of down-regulation of GH 1A receptors and this can be restored by increasing insulin (Butler et al. 2003). As IGF-I production in the liver is suppressed, the negative feedback of IGF-I is removed at the level of the hypothalamus/pituitary, and GH concentrations increase. High GH concentrations not only stimulate milk production but also provoke liver gluconeogenesis and lipolysis in adipocytes. The resulting high blood NEFA and GH concentrations antagonize insulin action and create a further state of peripheral insulin resistance (Lucy 2007; Pires et al. 2007). In this way even more glucose is conserved to be available for lactose synthesis.

Fatter cows tend to mobilize more body fat because of reduced appetite (Garnsworthy and Topps 1982). It is broadly accepted that genetic selection for milk production results in greater BCS loss, further suggesting that energy is partitioned towards the udder (Roche et al. 2006). An excessive BCS loss during the transition period is a major risk factor for health and fertility disorders (Roche et al. 2007), which stresses the importance of BCS monitoring early postpartum as a management tool (Chagas et al. 2007).

Interactions Between the Somatotropic and the Gonadotropic Axis

Extensive scientific research has shown that mechanisms that regulate energy and nutrient distribution in the somatotropic system may affect the reproductive system at different levels of the hypothalamo–pituitary–ovarian axis (Roche 2006; Chagas et al. 2007). Within the hypothalamus, interactions between the gonadotropic and somatotropic systems may occur in the pre-optic area (Blache et al. 2006, 2007). This region produces the releasing hormones that control the secretion of both gonadotropins and somatotropin (Kacsoh 2000). In addition, it plays a crucial role in integrating appetite (Wynne et al. 2005), oestrus behaviour (Pfaff 2005) and sensing of the nutritional status (Wade and Jones 2004). Consequently, metabolic inputs in the hypothalamus may have divergent effects on the gonadotropic and somatotropic axis, i.e. stimulation of GH production may be accompanied by inhibition of GnRH secretion (Zieba et al. 2005). The hormones/metabolites that are most likely to exert a signalling function are glucose and insulin. Low postpartum insulin and glucose concentrations suppress hypothalamic GnRH secretion and subsequent pituitary LH release (Diskin et al. 2003; Ohkura et al. 2004). By activation of specific neurons in the forebrain, peptides such as neuropeptide Y and catecholamines are released, which suppress the hypothalamic GnRH pulse generator (Ichimaru et al. 2001; Diskin et al. 2003; Wade and Jones 2004). Other metabolic signals may involve leptin and NEFA, although their role currently remains unclear (Liefers et al. 2003; Wade and Jones 2004; Amstalden et al. 2005).

At ovarian level, follicular growth and development seems to be directly influenced by altered insulin, IGF-I, leptin and NEFA levels. Because insulin locally stimulates follicular growth, maturation and steroidogenesis, reduced postpartum concentrations are linked to ovarian dysfunction (Gutierrez-Aguilar 1997; Landau et al. 2000; Armstrong et al. 2002a; Butler et al. 2004; Vanholder et al. 2005a; Kawashima et al. 2007). Long-term treatment with exogenous bovine somatotropin clearly increased the number of small follicles (Bols et al. 1998).

Gong (2002) showed that the beneficial effects of insulin on ovarian functions are independent of changes in GnRH/LH release. In ovarian cells, insulin-independent GLUT-1 and GLUT-3 are the major glucose transporters, while the insulin-dependent GLUT-4 only plays a supportive role (Nishimoto et al. 2006). Hence, insulin may exert its effects through mechanisms other than mediating glucose uptake.

Together with insulin, the IGF system plays an important role in follicle growth and development by acting directly on ovarian cells (Spicer and Echternkamp 1995; Gutierrez-Aguilar 1997; Beam and Butler 1999; Webb et al. 1999; Gong 2002). Consequently, low circulating IGF-1 concentrations negatively influence the onset of postpartum ovarian activity and seem involved in the development of cystic ovarian follicles (Beam and Butler 1997; Kawashima et al. 2007; Ortega et al. 2007). The effects of leptin on steroidogenesis and cell proliferation are, yet, dependent on the circulating concentrations of IGF-1, LH and insulin (Spicer and Francisco 1997, 1998; Spicer et al. 2000).

At ovarian level, NEFAs may affect follicular growth and development by acting directly on follicle cells. Adding NEFAs *in vitro*, at concentrations measured in follicular fluid (FF) during NEB has detrimental effects on follicle cell viability and function (Leroy et al. 2005b; Vanholder et al. 2005b, 2006b).

In conclusion, metabolic changes induced by the somatotropic system to sustain a high milk yield also affect the reproductive system. By acting at different levels of the hypothalamo–pituitary–ovarian axis, altered hormone and metabolite levels exert a negative effect on follicle growth, development and probably ovulation.

Consequences for Oocyte Quality

Researchers assume the existence of a carry-over effect of the adverse metabolic conditions during primary follicle growth early postpartum on the health of the pre-ovulatory follicle 2–3 months later (Britt 1992). Such follicles may be less capable of producing adequate amounts of oestrogens and progesterone (following ovulation) and might be doomed to contain an oocyte of inferior quality (Britt 1992; Roth et al. 2001a). The developmental capacity of the oocyte is intrinsically linked to the growth phase and health of the developing follicle (Bilodeau-Goeseels and Panich 2002; Sutton et al. 2003; Lequarre et al. 2005). Last but not least, diet composition can also alter the endocrine and metabolic micro-environment of the developing oocyte (Boland et al. 2001; McEvoy et al. 2001; Kenny et al. 2002).

A deviant endocrine environment, because of or as a consequence of a NEB can alter oocyte quality through various mechanisms such as spindle formation, prolonged follicular growth and resumption of meiotic progression, all of which has been extensively reviewed earlier (Leroy et al. 2007). Only a few studies have examined possible effects of NEB-associated low glucose, elevated β-hydroxybutyrate (β-OHB) or NEFA concentrations on oocyte quality. Apart from indirect effects of hypoglycaemia in early postpartum dairy cows (through an effect on LH secretion or ovarian responsiveness to gonadotrophins), hypoglycaemic conditions (e.g. clinical ketosis) are reflected in the microenvironment of the pre-ovulatory oocyte, and can compromise the oocyte's developmental capacity, because glucose is an indispensable molecule for proper oocyte maturation (Bilodeau-Goeseels 2006; for review see Sutton et al. 2003; Leroy et al. 2004, 2006). Kruip and Kemp (1999) suggested possible direct toxic effects of high NEFA concentrations at the ovarian level of the ovary. Indeed, in an *in vitro* maturation model, saturated long-chain fatty acids, reduced rates of maturation, fertilization, cleavage and blastocyst formation. Apoptosis, and even cumulus cell necrosis, during maturation could explain these observations (Leroy et al. 2005b). Finally, elevated ammonia and urea concentrations in the FF, because of an unbalanced diet and protein catabolism were toxic for the oocyte (Sinclair et al. 2000; De Wit et al. 2001; Leroy et al. 2004).

Early Pregnancy in High-producing Dairy Cows: Embryo Quality

Early embryonic death is a major cause of reproductive failure in dairy cows accounting for up to a total 80% pregnancy losses (Santos et al. 2004a). There are four major factors impinging on embryo quality in the specific case of high-producing dairy cows: gamete quality, corpus luteum quality combined with the circulating progesterone concentration, uterine involution and nutrition. Yet, only those that are related to NEB will be discussed.

Adverse pre-ovulatory conditions, such as NEB, may have carry-over effects on embryo metabolism and viability resulting in early embryonic mortality (Yaakub et al. 1999; Lozano et al. 2003; Rhoads et al. 2006). Distinguishing oocyte effects on embryo quality from post-fertilization influences is extremely difficult. Only at least a 6-day-old embryo can be transferred to a recipient to assess the impact of uterine environment on embryo quality. Lucy (2007) supports the concept that fertility could be improved in dairy cows by using embryo transfer and thus circumventing the period of oocyte and early embryonic development.

Well-timed and balanced post-mating progesterone concentrations are vital for zygote viability as progesterone modulates the endometrial secretions, and thus optimal uterine receptivity (McEvoy et al. 1995). It has been suggested that disappointing pregnancy results in modern dairying are partially caused by the retarded onset of the progesterone rise and suboptimal progesterone concentrations during the luteal phase (Mann and Lamming 2001). Furthermore, the typical NEB observed early postpartum can reduce the number of ovulatory oestrous cycles preceding AI which may hamper adequate uterus preparation (Butler 2003). Villa-Godoy et al. (1988) showed that cows in NEB postpartum had lower progesterone concentrations during the first three ovarian cycles following calving. Despite larger volumes of luteal tissue, compared with non-lactating heifers, maximal progesterone concentrations in lactating cows are lower, possibly because of a higher rate of degradation in the liver (Sangsritavong et al. 2002; Sartori et al. 2004; Wiltbank et al. 2006).

Good postpartum uterine involution, comprising endometrium repair and evacuation of bacterially contaminated contents, is of critical importance for reproductive performance (for review, see Roche et al. 2006). Because of a reduced immune response, negative energy status can dramatically delay this process, jeopardizing future fertility (Wathes et al. 2007).

Apart from interest in the consequences of NEB and associated endocrine and metabolic imbalances, there is a growing focus towards the effect of milk yield-promoting diets that are rich in energy and protein (for review, see Leroy et al. 2007). From these studies we can learn that optimum nutritional conditions for follicle growth and ovulation are not compatible with embryo survival and maintenance of pregnancy (O'Callaghan and Boland 1999).

Finally, heat stress can cause a reduction in dry matter intake which prolongs the period of NEB, decreasing plasma concentrations of insulin, glucose and IGF-I, while increasing GH and NEFA (Drew 1999; Butler 2001). In addition, there is a direct effect of heat stress on FSH (increased) and oestradiol (decreased) plasma concentrations (Wolfenson et al. 1997; Wilson et al. 1998). This causes not only poor expression of oestrus, but also delayed follicle selection and thus has potentially adverse effects on oocyte quality (Roth et al. 2001a,b). Heat stress may furthermore increase uterine temperature by decreasing blood flow to the uterus. These changes inhibit embryonic development (Rivera and Hansen 2001), increase early embryonic loss and reduce the proportion of successful inseminations (Garcia-Ispierto et al. 2007). The impact of this heat effect decreases as the embryo develops (Paula-Lopes et al. 2003).

All the factors described above, potentially affecting fertility, are diagrammatically presented in Fig. 1.

Some Clues to Modify the Somatotropic Axis in Order to Generate Acceptable Fertility Results

Tackling the multifactorial problem of subfertility in dairy cows is a real challenge as the 'skewed somatotropic axis' described above is not the only reason for the decline in fertility. Evolving farm systems, growing herd sizes, increased managerial demands combined with a reduced labour input per animal and increased susceptibility to diseases, all interfere with the ability of the dairy cow to be successfully bred (Mallard et al. 1998). All these factors should be carefully addressed when formulating management advice to the dairy farm manager, but this is beyond the scope of the present paper.

Metabolic disorders (hypocalcaemia, ketosis and acidosis) and infectious diseases during the puerperium are all key risk factors for efficient reproductive performance (Grohn and Rajala-Schultz 2000; Santos et al. 2004b). Balanced and sophisticated birth management combined with strict follow-up of cow health status early postpartum is vital to prevent a drop in the animal's appetite. Accurate and repeated assessment of BCS to estimate changes in body reserves is critical. Minimizing BCS changes and thus the 'exhaustion of the energy reserves' early postpartum requires an optimal dietary strategy by reducing energy intake during the first weeks of the dry period then an increased energy supply (carbohydrates) shortly pre-partum (for review, see Overton and Waldron 2004). Yet, the beneficial effect of extra pre-partum energy on postpartum energy balance is a matter of debate (Grummer 2007). Furthermore, adequate dietary modulations during the demanding early postpartum period are a promising approach although difficult to achieve (Grummer 2007). Therefore, Van Knegsel et al. (2007a) fed on an isocaloric and isonitrogenous basis, a mainly glucogenic (by-pass starch) or a mainly lipogenic diet (beetpulp, MEGALAC and palm oil) and showed that the glucogenic (or 'insulinogenic') diet, stimulates energy partitioning towards body reserves in early lactation. Cows fed the glucogenic diet had lower NEB and reduced body fat mobilization, which led to milk fat depression and less energy partitioned to milk (Van Knegsel et al. 2007a). In a follow-up study (Van Knegsel et al. 2007b), multiparous cows fed with glucogenic diet also had higher plasma insulin concentrations and tended to resume ovarian activity earlier (20.4 ± 2.1 days) compared to cows fed with more lipogenic diet (26.1 ± 2.1 days).

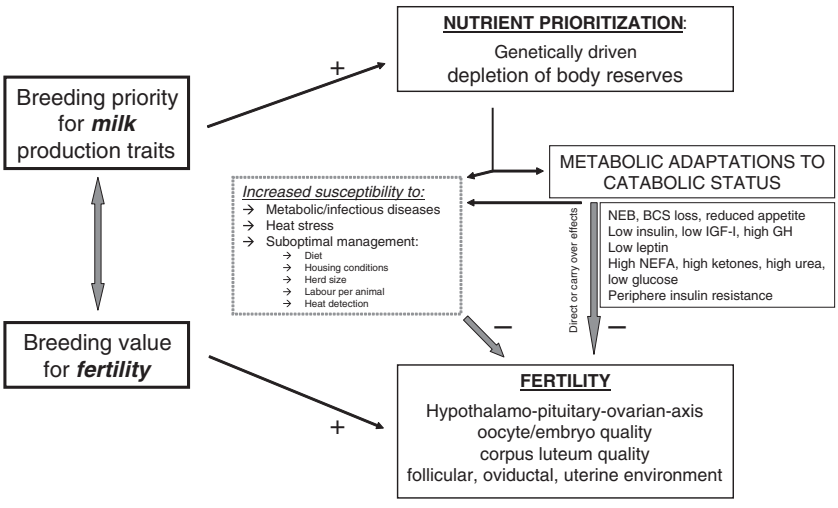

Fig. 1. Interaction between genetic selection for milk production and fertility. The imposed metabolic and endocrine changes sustaining milk production in combination with the increased susceptibility to heat stress, diseases and suboptimal management conditions all negatively affect reproductive performance of the high-producing dairy cow

It is speculated that changes in management are more likely to have a positive effect on EB. Shortening or even skipping the dry period improves dry matter intake peripartum, reduces milk production in early lactation, improves energy balance and reduces the number of days postpartum till resumption of ovarian activity (Gumen et al. 2005; Rastani et al. 2005).

Besides this, growing attention is being paid to dietary fatty acid content and composition provided by supplemented by-pass fats during the early postpartum period. Not the effect on energy balance as such but improved steroid secretion and alteration of the fatty acid profile (more ω-3 poly-unsaturated fatty acids), resulting in modified prostaglandin metabolism (Thatcher et al. 2006). Suppression of milk fat synthesis by supplementation of rumen-protected conjugated linoleic acids (*trans*-10, *cis*-12) has been suggested to restrict energy loss through milk (Castaneda-Gutierrez et al. 2005). Yet, in spite of several recent interesting papers, the outcome on energy balance and fertility are equivocal. An extensive description and clear overview of nutritional strategies supporting the metabolic demands during the transition period are given by Overton and Waldron (2004) and are beyond the scope of the present paper.

Finally, genetic selection programmes in the dairy industry have emphasized milk production traits by unintended mobilization of cow body reserves. This loss in BCS is not only dependent on the available mass of adipose tissue but also on a genetically determined set-point for BCS. This set-point is correlated with reproductive outcome (Lucy 2007). Therefore, not only fertility traits as such (Royal et al. 2000), but also variables comprising changes in BCS early postpartum should be included in genetic selection criteria.

Conclusions

Intense selection for milk production has resulted in an immense priority for the high-producing dairy cow to partition energy to milk, at the cost of body reserves. This has resulted in excessive NEB and poor reproductive performance. Thus, milk production and reproductive performance have conflicting interests in high-producing dairy cows. Metabolites and metabolic hormones associated with energy prioritizing for milk production (NEFA, insulin, glucose, IGF-1, β-OH) influence fertility, indirectly by modulating the somatotropic/gonadotropic axis, as well as directly at the ovary, follicle or uterine environment. Strict follow-up peripartum to monitor health and BCS loss and direct treatment of (infectious or metabolic) disorders in early lactation will limit fertility disorders postpartum. Furthermore, a series of promising management, genetic selection and nutritional strategies have been proposed, which have the potential to shift the somatotropic axis prioritizing energy partitioning of milk to a somatotropic axis with an increased priority for body reserves to improve fertility. Yet, research in this area is limited. Exploring such strategies, comparing their benefit or even combining two or more strategies is an extremely interesting area of research, and essential to improve health and welfare of the modern dairy cow.

References

Amstalden M, Harms PG, Welsh TH Jr, Randel RD, Williams GL, 2005: Effects of leptin on gonadotropin-releasing hormone release from hypothalamic-infundibular explants and gonadotropin release from adenohypophyseal primary cell cultures: further evidence that fully nourished cattle are resistant to leptin. Anim Reprod Sci **85,** 41–52.

Armstrong DG, Baxter G, Hogg CO, Woad KJ, 2002a: Insulin-like growth factor (IGF) system in the oocyte and somatic cells of bovine preantral follicles. Reproduction **123,** 789–797.

Beam SW, Butler WR, 1997: Energy balance and ovarian follicle development prior to first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol Reprod **56,** 133–142.

Beam SW, Butler WR, 1999: Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. J Reprod Fertil Suppl **54,** 411–424.

Bilodeau-Goeseels S, 2006: Effect of culture media and energy sources on the inhibition of nuclear maturation in bovine oocytes. Theriogenology **66,** 297–306.

Bilodeau-Goeseels S, Kastelic JP, 2003: Factors affecting embryo survival and strategies to reduce embryonic mortality in cattle. Can J Anim Sci **83,** 659–671.

Bilodeau-Goeseels S, Panich P, 2002: Effects of quality on development and transcriptional activity in early bovine embryos. Anim Reprod Sci **71,** 143–155.

Blache D, Zhang S, Martin GB, 2006: Dynamic and integrative aspects of the regulation of reproduction by metabolic status in male sheep. Reprod Nutr Dev **46,** 379–390.

Blache D, Chagas LM, Martin GB, 2007: Nutritional inputs into the reproductive neuroendocrine control system – a multidimensional perspective. Reproduction, Supplement **64,** 124–139.

Boland MP, Lonergan P, O'Callaghan D, 2001: Effect of nutrition on endocrine parameters, ovarian physiology, and oocyte and embryo development. Theriogenology **55,** 1323–1340.

Bols PEJ, Ysebaert MT, Lein A, Coryn M, Van Soom A, de Kruif A, 1998: Effects of long-term treatment with bovine somatotropin on follicular dynamics and subsequent oocyte and blastocyst yield in an OPU-IVF program. Theriogenology **49,** 983–995.

Bossaert P, Leroy JLMR, Cools S, Opsomer G, 2007: Impact of metabolic and endocrine parameters on ovulation in high-yielding dairy cows (abstract). Reprod Domest Anim **42,** 118.

Bousquet D, Bouchard E, Du Tremblay D, 2004: Decreasing fertility in dairy cows: myth or reality? Le Médecin Vétérinaire **34,** 59–61.

Britt JH, 1992: Impacts of early postpartum metabolism on follicular development and fertility. Proc Annu Convention Am Assoc Bovine Pract **24,** 39–43.

Butler WR, 2001: Nutritional effects on resumption of ovarian cyclicity and conception rate in postpartum dairy cows. Anim Sci Occas Publ **26,** 133–145.

Butler WR, 2003: Energy balance relationships with follicular development, ovulation and fertility in pp dairy cows. Livestock Prod Sci **83,** 211–218.

Butler ST, Marr AL, Pelton SH, Radcliff RP, Lucy MC, Butler WR, 2003: Insulin restores GH responsiveness during lactation-induced negative energy balance in dairy cattle: effects on expression of IGF-I and GH receptor 1A. J Endocrinol **176,** 205–217.

Butler ST, Pelton SH, Butler WR, 2004: Insulin increases 17β-estradiol production by the dominant follicle of the first postpartum follicle wave in dairy cows. Reproduction **127,** 537–545.

Castaneda-Gutierrez E, Overton TR, Butler WR, Bauman DE, 2005: Dietary supplements of two doses of calcium salts of conjugated linoleic acid during the transition period and early lactation. J Dairy Sci **88,** 1078–1089.

Chagas LM, Bass JJ, Blache D, Burke CR, Kay JK, Lindsay DR, Lucy MC, Martin GB, Meier S, Rhodes FM, Roche JR, Thatcher WW, Webb R, 2007: New perspectives on the roles of nutrition and metabolic priorities in the subfertility of high-producing dairy cows. J Dairy Sci **90,** 4022–4032.

Cnop M, Hannaert JC, Hoorens A, Eizirik DL, Pipeleers DG, 2001: Inverse relationship between cytotoxicity of free fatty acids in pancreatic islet cells and cellular triglyceride accumulation. Diabetes **50,** 1771–1777.

Coffey MP, Simm G, Oldham JD, Hill WG, Brotherstone S, 2004: Genotype and diet effects on energy balance in the first three lactations of dairy cows. J Dairy Sci **87,** 4318–4326.

De Wit AAC, Cesar MLF, Kruip TAM, 2001: Effect of urea during *in vitro* maturation on nuclear maturation and embryo development of bovine cumulus-oocyte-complexes. J Dairy Sci **84,** 1800–1804.

Diskin MG, Mackey DR, Roche JF, Sreenan JM, 2003: Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle. Anim Reprod Sci **78,** 345–370.

Drew B, 1999: Practical nutrition and management of heifers and high yielding dairy cows for optimal fertility. Cattle Pract **7,** 243–248.

Dunne LD, Diskin MG, Boland MP, O'Farrell KJ, Sreenan JM, 1999: The effect of pre- and post-insemination plane of nutrition on embryo survival in beef heifers. Anim Sci **69,** 411–417.

Garcia-Ispierto I, Lopez-Gatius F, Bech-Sabat G, Santolaria P, Yaniz JL, Nogareda C, De Rensis F, Lopez-Bejar M, 2007: Climate factors affecting conception rate of high producing dairy cows in north-eastern Spain. Theriogenology **67,** 1379–1385.

Garnsworthy PC, Topps JH, 1982: The effect of body condition score at calving on their food intake and performance when given complete diets. Anim Prod **35,** 113–119.

Gong JG, 2002: Influence of metabolic hormones and nutrition on ovarian follicle development in cattle: practical implications. Domest Anim Endocrinol **23,** 229–241.

Grohn YT, Rajala-Schultz PJ, 2000: Epidemiology of reproductive performance in dairy cows. Anim Reprod Sci **60,** 605–614.

Grummer RR, 2007: Strategies to improve fertility of high yielding dairy farms: management of the dry period. Theriogenology **68,** S281–S288.

Gumen A, Rastani RR, Grummer RR, Wiltbank MC, 2005: Reduced dry periods and varying prepartum diets alter postpartum ovulation and reproductive measures. J Dairy Sci **88,** 2401–2411.

Gutierrez-Aguilar CG, 1997: The effect of nutrition and metabolic hormones on follicular development in cattle. PhD Dissertation, University of Edinburgh, pp. 146–166.

Herdt TH, 2000: Ruminant adaptation to negative energy balance. Vet Clin North Am Food Anim Pract **16,** 215–230.

Huirne RBM, Saatkamp HW, Bergevoet RHM, 2002: Economic analysis of common health problems in dairy cattle. In: Kaske M, Scholz H, Höltershinken M(eds), Twelfth World Buiatrics Congress, Hannover, Germany, 18–23 August 2002, pp. 420–431.

Ichimaru T, Mori Y, Okamura H, 2001: A possible role of neuropeptide Y as a mediator of undernutrition to the hypothalamic gonadotropin-releasing hormone pulse generator in goats. Endocrinology **142,** 2389–2498.

Kacsoh B, 2000: Endocrine Physiology. McGraw Hill Book Co., New York, NY.

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

Kenny DA, Humpherson PG, Leese HJ, Morris DG, Tomos AD, Diskin MG, Sreenan JM, 2002: Effect of elevated systemic concentrations of ammonia and urea on the metabolite and ionic composition of oviductal fluid in cattle. Biol Reprod **66,** 1797–1804.

Kruip TAM, Kemp B, 1999: Voeding en vruchtbaarheid bij landbouwhuisdieren. Tijdschr Diergeneeskd **124,** 462–467.

Landau S, Braw-Tal R, Kaim M, Bor A, Bruckental I, 2000: Preovulatory follicular status and diet affect the insulin and glucose content of follicles in high-yielding dairy cows. Anim Reprod Sci **64,** 181–197.

Lequarre AS, Vigneron C, Ribaucour F, Holm P, Donnay I, Dalbies-Tran R, Callesen H, Mermillod P, 2005: Influence of antral follicle size on oocyte characteristics and embryo development in the bovine. Theriogenology **63,** 841–859.

Leroy JLMR, Vanholder T, Delange JR, Opsomer G, Van Soom A, Bols PEJ, Dewulf J, de Kruif A, 2004: Metabolic changes in follicular fluid of the dominant follicle in high-yielding dairy cows early post partum. Theriogenology **62,** 1131–1143.

Leroy JLMR, Opsomer G, De Vliegher S, Vanholder T, Goossens L, Geldhof A, Bols PEJ, de Kruif A, Van Soom A, 2005a: Comparison of embryo quality in high-yielding dairy cows, in dairy heifers and in beef cows. Theriogenology **64,** 2022–2036.

Leroy JLMR, Vanholder T, Mateusen B, Christophe A, Opsomer G, de Kruif A, Genicot G, Van Soom A, 2005b: Non-esterified fatty acids in follicular fluid of dairy cows and their effect on developmental capacity of bovine oocytes in vitro. Reproduction **130,** 485–495.

Leroy JLMR, Vanholder T, Opsomer G, Van Soom A, de Kruif A, 2006: The in vitro development of bovine oocytes after maturation in glucose and beta-hydroxybutyrate concentrations associated with negative energy balance in dairy cows. Reprod Domest Anim **41,** 119–123.

Leroy JLMR, Opsomer G, Van Soom A, Goovaerts IGF, Bols PEJ, 2007: Reduced fertility in high yielding dairy cows: are the oocyte and embryo in danger? Part I: The importance of negative energy balance and altered corpus luteum function to the reduction of oocyte and embryo quality in high yielding dairy cows (review). Reprod Domest Anim, in press. DOI:10.1111/j.1439–0531.2007.00960.

Liefers SC, Veerkamp RF, te Pas MFW, Delavaud C, Chilliard Y, van der Lende T, 2003: Leptin concentrations in relation to energy balance, milk yield, intake, live weight and estrus in dairy cows. J Dairy Sci **86,** 799–807.

Lopez H, Satter LD, Wiltbank MC, 2004: Relationship between level of milk production and oestrus behavior of lactating dairy cows. Anim Reprod Sci **81,** 209–223.

Lozano JM, Lonergan P, Boland MP, O'Callaghan D, 2003: Influence of nutrition on the effectiveness of superovulation programmes in ewes: effect on oocyte quality and post-fertilization development. Reproduction **125,** 543–553.

Lucy MC, 2001: Reproductive loss in high-producing dairy cattle: where will it end? J Dairy Sci **84,** 1277–1293.

Lucy MC, 2003: Mechanisms linking nutrition and reproduction in postpartum cows. Reprod Suppl. **61,** 415–427.

Lucy MC, 2007: Fertility in high-producing dairy cows: reasons for decline and corrective strategies for sustainable improvement. Reprod Suppl. **64,** 237–254.

Maedler K, Spinas GA, Dyntar D, Moritz W, Kaiser N, Donath MY, 2001: Distinct effects of saturated and mono-

unsaturated fatty acids on beta-cell turnover and function. Diabetes **50,** 69–76.

Mallard BA, Dekkers JC, Ireland MJ, Leslie KE, Sharif S, Vankampen CL, Wagter L, Wilkie BN, 1998: Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. J Dairy Sci **81,** 585–595.

Mann GE, Lamming GE, 2001: Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. Reproduction **121,** 175–180.

McEvoy TG, Robinson JJ, Aitken RP, Findlay PA, Palmer RM, Robertson IS, 1995: Dietary-induced suppression of pre-ovulatory progesterone concentrations in superovulated ewes impairs the subsequent in vivo and in vitro development of their ova. Anim Reprod Sci **39,** 89–107.

McEvoy TG, Robinson JJ, Ashworth CJ, Rooke JA, Sinclair KD, 2001: Feed and forage toxicants affecting embryo survival and fetal development. Theriogenology **55,** 113–129.

Nishimoto H, Matsutani R, Yamamoto S, Takahashi T, Hayashi KG, Miyamoto A, Hamano S, Tetsuka M, 2006: Gene expression of glucose transporter (GLUT) 1, 3 and 4 in bovine follicle and corpus luteum. J Endocrinol **188,** 111–119.

O'Callaghan D, Boland MP, 1999: Nutritional effects on ovulation, embryo development and the establishment of pregnancy in ruminants. Anim Sci **68,** 299–314.

Ohkura S, Ichimaru T, Itoh F, Matsuyama S, Okamura H, 2004: Further evidence for the role of glucose as a metabolic regulator of hypothalamic gonadotropin-releasing hormone pulse generator activity in goats. Endocrinology **145,** 3239–3246.

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

Ortega HH, Palomar MM, Acosta JC, Salvetti NR, Dallard BE, Lorente JA, Barbeito CG, Gimeno EJ, 2008: Insulin-like growth factor I in sera, ovarian follicles and follicular fluid of cows with spontaneous or induced cystic ovarian disease. Res Vet Sci **84,** 419–427.

Overton TR, Waldron MR, 2004: Nutrtional management of transition dairy cows: strategies to optimize metabolic health. J Dairy Sci **87,** E105–E119.

Patton J, Kenny DA, Mee JF, O'Mara FP, Wathes DC, Cook M, Murphy JJ, 2006: Effect of milking frequency and diet on milk production, energy balance and reproduction in dairy cows. J Dairy Sci **89,** 1478–1487.

Paula-Lopes FF, Al-Katanani YM, Majewski AC, McDowell LR, Hansen PJ, 2003: Manipulation of antioxidant status fails to improve fertility of lactating cows or survival of heat-shocked embryos. J Dairy Sci **86,** 2343–2351.

Pfaff D, 2005: Hormone-driven mechanisms in the central nervous system facilitate the analysis of mammalian behaviours. J Endocrinol **184,** 447–453.

Pires JAA, Souza AH, Grummer RR, 2007: Induction of hyperlipidemia by intravenous infusion of Tallow emulsion causes insulin resistance in Holstein cows. J Dairy Sci **90,** 2735–2744.

Rastani RR, Grummer RR, Bertics SJ, Gumen A, Wiltbank MC, Mashek DG, Schwab MC, 2005: Reducing dry period length to simplify feeding transition cows: milk production, energy balance, and metabolic profiles. J Dairy Sci **88,** 1004–1014.

Rhoads ML, Rhoads RP, Gilbert RO, Toole R, Butler WR, 2006: Detrimental effects of high plasma urea nitrogen levels on viability of embryos from lactating dairy cows. Anim Reprod Sci **91,** 1–10.

Rivera RM, Hansen PJ, 2001: Development of cultured bovine embryos after exposure to high temperatures in the physiological range. Reproduction **121,** 107–115.

Roche JF, 2006: The effect of nutritional management of the dairy cow on reproductive efficiency. Anim Reprod Sci **96,** 282–296.

Roche JR, Berry DP, Kolver ES, 2006: Holstein-Friesian strains and feed effects on milk production, bodyweight and body condition score profiles in grazing dairy cows. J Dairy Sci **89,** 3532–3543.

Roche JR, Macdonald KA, Burke CR, Lee JM, Berry DP, 2007: Associations among body condition score, body weight and reproductive performance in seasonal-calving dairy cattle. J Dairy Sci **90,** 376–391.

Roth Z, Meweidan R, Shaham-Albalancy A, Braw-Tal R, Wolfenson D, 2001a: Delayed effect of heat stress on steroid production in medium-size and preovulatory bovine follicles. Reproduction **121,** 745–751.

Roth Z, Arav A, Bor A, Zeron Y, Braw-Tal R, Wolfenson D, 2001b: Improvement of quality of oocytes collected in the autumn by enhanced removal of impaired follicles from previously heat-stressed cows. Reproduction **122,** 737–744.

Royal M, Mann GE, Flint APF, 2000: Strategies for reversing the trend towards subfertility in dairy cattle. Vet J **160,** 53–60.

Sangsritavong S, Combs DK, Sartori R, Armentano LE, Wiltbank MC, 2002: High feed intake increase liver blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. J Dairy Sci **85,** 2831–2842.

Santos JEP, Thatcher WW, Chebel RC, Cerri RLA, Galvao KN, 2004a: The effect of embryonic death rates in cattle on the efficacity of estrus synchronization programs. Anim Reprod Sci **82/83,** 513–535.

Santos JE, Cerri RL, Ballou MA, Higginbotham GE, Kirk JH, 2004b: Effect of timing of first clinical mastitis occurrence on lactational and reproductive performance of Holstein dairy cows. Anim Reprod Sci **80,** 31–45.

Sartori R, Sartor-Bergfelt R, Mertens SA, Guenther JN, Parrish JJ, Wiltbank MC, 2002: Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. J Dairy Sci **85,** 2803–2812.

Sartori R, Haughian JM, Shaver RD, Rosa GJM, Wiltbank MC, 2004: Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. J Dairy Sci **87,** 905–920.

Silvia WJ, 2003: Addressing the decline in reproductive performance of lactating dairy cows: a researcher's perspective. Vet Sci Tomorrow **3,** 1–5.

Sinclair KD, Kuran M, Gebbie FE, Webb R, McEvoy TG, 2000: Nitrogen metabolism and fertility in cattle: II. Development of oocytes recovered from heifers offered diets differing in their rate of nitrogen release in the rumen. J Anim Sci **78,** 2670–2680.

Snijders SE, Dillon P, O'Callaghan D, Boland MP, 2000: Effect of genetic merit, milk yield, body condition and lactation number on *in vitro* oocyte development in dairy cows. Theriogenology **53,** 981–989.

Spicer LJ, Echternkamp SE, 1995: The ovarian insulin and insulin-like growth factor system with an emphasis on domestic animals. Domest Anim Endocrinol **12,** 223–245.

Spicer LJ, Francisco CC, 1997: The adipose obese gene product, leptin: evidence of a direct inhibitory role in ovarian function. Endocrinology **138,** 3374–3379.

Spicer LJ, Francisco CC, 1998: Adipose obese gene product, leptin, inhibits bovine ovarian thecal cell steroidogenesis. Biol Reprod **58,** 207–212.

Spicer LJ, Chamberlain CS, Francisco CC, 2000: Ovarian action of leptin: effects on insulin-like growth factor-I-

stimulated function of granulosa and thecal cells. Endocr **12,** 53–59.
Sutton ML, Gilchrist RB, Thompson JG, 2003: Effects of in vivo and in vitro environments on the metabolism of the cumulus-oocyte complex and its influence on oocyte developmental capacity. Hum Reprod Update **9,** 35–48.
Tamminga S, Luteijn PA, Meijer RGM, 1997: Changes in composition and energy content of liveweight loss in dairy cows with time after parturition. Livestock Prod Sci **52,** 129–155.
Thatcher WW, Bilby TR, Bartolome JA, Silvestre F, Staples CR, Santos JEP, 2006: Strategies for improving fertility in the modern dairy cow. Theriogenology **65,** 30–44.
Van Knegsel ATM, Van den Brand H, Dijkstra J, van Straalen WM, Heetkamp MJW, Tamminga S, Kemp B, 2007a: Dietary energy source in dairy cows in early lactation: energy partitioning and milk composition. J Dairy Sci **90,** 1467–1476.
Van Knegsel ATM, Van den Brand H, Dijkstra J, van Straalen WM, Jorritsma R, Tamminga S, Kemp B, 2007b: Effect of glucogenic vs. lipogenic diets on energy balance, blood metabolites, and reproduction in primiparous and multiparous dairy cows in early lactation. J Dairy Sci **90,** 3397–3409.
Vanholder T, Leroy JLMR, Dewulf J, Duchateau L, Coryn M, de Kruif A, 2005a: Hormonal and metabolic profiles of high-yielding dairy cows prior to ovarian cyst formation or first ovulation post partum. Reprod Domest Anim **40,** 460–469.
Vanholder T, Leroy JLMR, Van Soom A, Opsomer G, Maes D, Coryn M, de Kruif A, 2005b: Effect of non-esterified fatty acids on bovine granulosa cell steroidogenesis and proliferation in vitro. Anim Reprod Sci **87,** 33–44.
Vanholder T, Opsomer G, de Kruif A, 2006a: Aetiology and pathogenesis of cystic ovarian follicles in dairy cattle: a review. Reprod Nutr Dev **46,** 105–119.
Vanholder T, Leroy JLMR, Van Soom A, Maes D, Coryn M, Fiers T, de Kruif A, Opsomer G, 2006b: Effect of non-esterified fatty acids on bovine theca cell steroidogenesis and proliferation in vitro. Anim Reprod Sci **92,** 51–63.
Vernon RG, 2002: Nutrient partitioning, lipid metabolism and relevant imbalances. Proceedings of the 12th World Buiatrics Congress, Hannover, Germany, 18–23 August, 2002.
Villa-Godoy A, Hughes TL, Emery RS, Chapin LT, Fogwell RL, 1988: Association between energy balance and luteal function in lactating dairy cows. J Dairy Sci **71,** 1063–1072.
de Vries MJ, Veerkamp RF, 2000: Energy balance of dairy cattle in relation to milk production variables and fertility. J Dairy Sci **83,** 62–69.
Wade GN, Jones JE, 2004: Neuroendocrinology of nutritional infertility. Am J Physiol. Regul, Integrative Comp Physiol **287,** 1277–1296.
Wathes DC, Fenwick M, Cheng Z, Bourne N, Llewellyn S, Morris DG, Kenny D, Murphy J, Fitzpatrick R, 2007: Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow. Theriogenology **68,** S232–S241.
Webb R, Garnsworthy PC, Gong JG, Robinson RS, Wathes DC, 1999: Consequences for reproductive function of metabolic adaptation to load. Anim Sci Occasion Publ **24,** 99–112.
Wilson SJ, Marion RS, Spain JN, Spiers DE, Keisler DH, Lucy MC, 1998: Effect of controlled heat stress on ovarian function in dairy cattle: I. Lactating cows. J Dairy Sci **1,** 2124–2131.
Wiltbank MC, Sartori R, Sangsritavong S, Lopez H, Haughian JM, Fricke PM, Gumen A, 2001: Novel effects of nutrition on reproduction in lactating dairy cows. J Dairy Sci Suppl **84,** 32 (abstract).
Wiltbank M, Lopez H, Sartori R, Sangsritavong S, Gümen A, 2006: Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology **65,** 17–29.
Wolfenson D, Lew BJ, Thatcher WW, Graber Y, Meidan R, 1997: Seasonal and acute heat stress effects on steroid production by dominant follicles in cow. Anim Reprod Sci **47,** 9–19.
Wynne K, Stanley S, McGowan B, Bloom S, 2005: Appetite control. J Endocrinol **184,** 291–318.
Yaakub H, O'Callaghan D, Boland MP, 1999: Effect of roughage type and concentrate supplementation on follicle numbers and in vitro fertilisation on follicle numbers and in vitro fertilisation and development of oocytes recovered from beef heifers. Anim Reprod Sci **44,** 1–12.
Zhao F, Dixon WT, Kennelly JJ, 1996: Localization and gene expression of glucose transporters in bovine mammary gland. Comprehens Biochem Physiol **115,** 127–134.
Zieba DA, Amstalden M, Williams GL, 2005: Regulatory roles of leptin in reproduction and metabolism: a comparative review. Domest Anim Endocrinol **29,** 166–185.

Author's address (for correspondence): J Leroy, Laboratory for Veterinary Physiology, Department of Veterinary Sciences, Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Universiteitsplein 1, Gebouw U, B-2610 Wilrijk, Belgium. E-mail: jo.leroy@ua.ac.be

Conflict of interests: All authors declare no conflict of interests.