animal

# Integration of physiological mechanisms that influence fertility in dairy cows[*]

P. C. Garnsworthy[†], K. D. Sinclair and R. Webb

*University of Nottingham School of Biosciences, Sutton Bonington Campus, Loughborough LE12 5RD, UK*

*Fertility in dairy cows has been declining for the past three decades. Genetic selection for increased milk production has been associated with changes in key metabolic hormones (growth hormone, insulin, IGF and leptin) that regulate metabolism by homoeostasis and homeorhesis. These metabolic hormones, particularly insulin, provide signals to the reproductive system so that regulation of ovarian function is coordinated with changes in metabolic status. Studies have shown, for example, that increasing circulating insulin concentrations during the early* post partum *period can advance the resumption of oestrous cycles by enhancing follicular growth. However, high concentrations of insulin can be detrimental to the developmental competence of oocytes, which is also influenced by the supply of fatty acids at the systemic level and at the ovarian level. Insulin status is also associated with the incidence and characteristics of abnormal ovarian cycles. These changes can occur without significant variation in circulating gonadotrophin concentrations. This suggests that additional factors, such as peripheral metabolites, metabolic hormones and locally produced growth factors, may have a modulating role. Recent evidence has demonstrated that ovarian responses to metabolic signals and nutrient profile vary according to the stage of the reproductive cycle. Improved understanding of this multifactorial process enables nutrition to be matched to genotype and milk production, with a positive impact on pregnancy rate.*

## Introduction

Genetic selection of dairy cows has focussed primarily on increased milk production over the past three decades, and this has been associated with declining fertility in many countries (Butler, 2000; Royal *et al.*, 2000; Evans *et al.*, 2006; Petersson *et al.*, 2006; Garcia-Ispierto *et al.*, 2007). Components of reduced fertility include delayed resumption of oestrous cycles *post partum*, greater incidence of abnormal oestrous cycles and poorer conception rates to first and subsequent inseminations (Royal *et al.*, 2002). The combined effects of these components are longer calving intervals and higher replacement rates, leading to reduced lifetime performance. High replacement rates are of particular concern because when average herd life of cows drops below three lactations (replacement rate 33%), insufficient female replacements can be produced to maintain herd size (Garnsworthy, 2005).

Several management factors that affect fertility have changed in parallel with increasing genetic merit for milk production. For example, average herd size has increased and labour input per cow has decreased (Defra, 2007), both of which can result in less attention to detail, increased disease incidence and less time for oestrous detection. For the purposes of this review, however, we will concentrate on biological factors related to fertility.

Poor fertility has negative consequences for the economics of milk production; Stott *et al.* (1999) calculated that poor fertility could reduce gross margins by approximately 20%; Evans *et al.* (2006) calculated that, of the potential increase in farm profitability resulting from improved genetic merit between 1990 and 2003, 50% had been lost due to poor fertility. Poor fertility also increases the environmental impact of dairy systems; involuntary culling for failure to conceive means that more cows are required per unit of milk produced and more non-productive replacements are required per milking cow in the herd. These extra animals increase resource use (feed, fertiliser, fossil fuel) and emissions of pollutants (methane, ammonia, nitrate, nitrous oxide). It has been estimated that if fertility

---

could be restored to levels achieved in the 1980s, total emissions of methane by dairy herds would be reduced by 24% and emissions of ammonia by 17% (Garnsworthy, 2004).
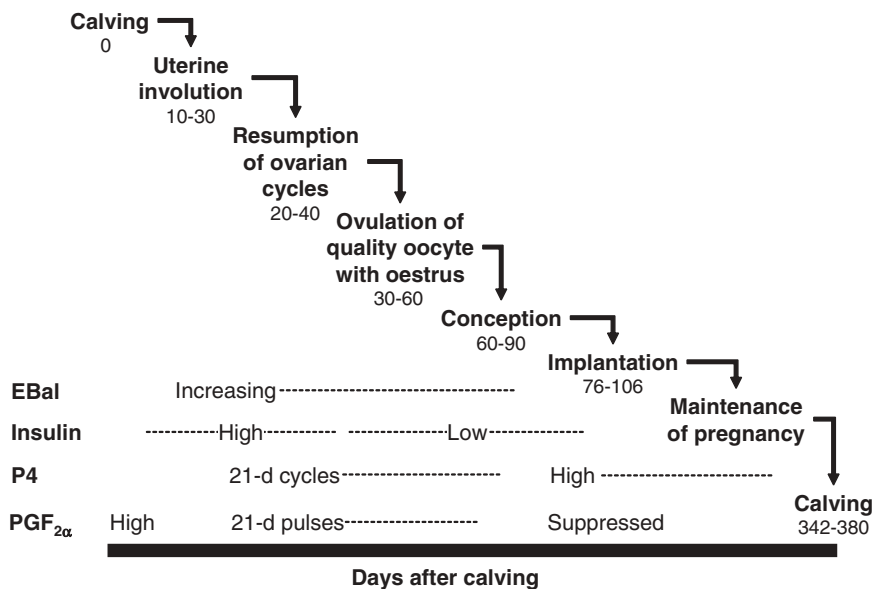
## Reproductive events

In dairy cows, successful reproduction is the result of a chain of events including resumption of oestrous cycles *post partum*, development and ovulation of a healthy oocyte, fertilisation, embryo development, implantation in the uterus, maintenance of pregnancy and parturition (Figure 1). Failure at any stage results in failure of the whole process. The ovaries play a central role in the reproduction chain, so compromised ovarian function, manifested as anoestrus or failure to conceive and maintain pregnancy, is often a major cause of poor fertility. Ovarian function is regulated by a complex interaction of factors and feedback mechanisms at the cell (intra-follicle), organ (ovary) and animal (e.g. pituitary, pancreas, liver, adipose tissue) levels (Figure 2). These ovarian regulatory mechanisms must operate in concert with the animal's overall homoeostatic and homeorhetic regulatory mechanisms, with the systems being linked by signals from metabolic hormones and metabolites (see Garnsworthy and Webb, 1999; Webb *et al.*, 2004; Webb *et al.*, 2007).

## Regulation of blood glucose and body fatness

Metabolic regulation of nutrient partitioning (Figure 2a) is important for successful lactation and represents the physiological basis for improvements in productive efficiency of high-merit dairy cows (Bauman, 2000). Homoeostatic

controls of blood glucose are of primary importance in dairy cows because up to 80% of glucose turnover is used for mammary synthesis of lactose, which is the major osmotic component of milk that determines milk yield (Bauman and Currie, 1980). Insulin and glucagon play major complementary roles in glucose homoeostasis: insulin stimulates glucose uptake by tissues, promotes protein synthesis and increases lipogenesis; glucagon stimulates gluconeogenesis and glycogenolysis (Vernon, 1988). Both insulin and glucagon respond rapidly to changes in nutrient absorption and utilisation throughout the day. Long-term regulation of nutrient partitioning is by homeorhetic mechanisms, which attenuate responses of specific tissues to homoeostatic signals (Bauman, 2000). Thus, for example, when growth hormone is increased (at the onset of lactation, by bovine somatotrophin (BST) administration, or by genetic selection for high milk yield), less nutrients are directed to body fat reserves following a meal because of altered responses to insulin, and more nutrients are taken up by the mammary gland to support milk synthesis (Bauman, 2000).

Some of the effects of insulin and growth hormone are mediated through the IGF system. IGF-I is synthesised primarily in the liver, in response to growth hormone, and IGF receptors are found in most tissues (Baumrucker, 2000). IGF is transported in the bloodstream by six high-affinity binding proteins (IGFBP), whose specific characteristics allow local regulation of IGF availability in target tissues (Jones and Clemmons, 1995). During early lactation, growth hormone receptors in the liver are down-regulated and circulating concentrations of IGF-I are dramatically reduced despite elevated growth hormone concentrations (Butler



**Figure 1** Sequence of reproductive events in the dairy cow. Each event depends on the success of preceding events. Numbers indicate optimum range (days after calving) to achieve an average calving interval of 365 days. Major temporal factors that influence success are: energy balance (EBal), which should start to increase early in lactation; insulin, which stimulates resumption of oestrous cycles, but may reduce oocyte quality; progesterone (P4), which is low during anoestrus, high during luteal phases of cycles, and low during follicular phases of cycles; and prostaglandin-F$_{2\alpha}$ (PGF$_{2\alpha}$), which stimulates uterine involution and corpus luteum regression, but has to be suppressed for successful implantation and maintenance of pregnancy. For detailed explanation, see text.
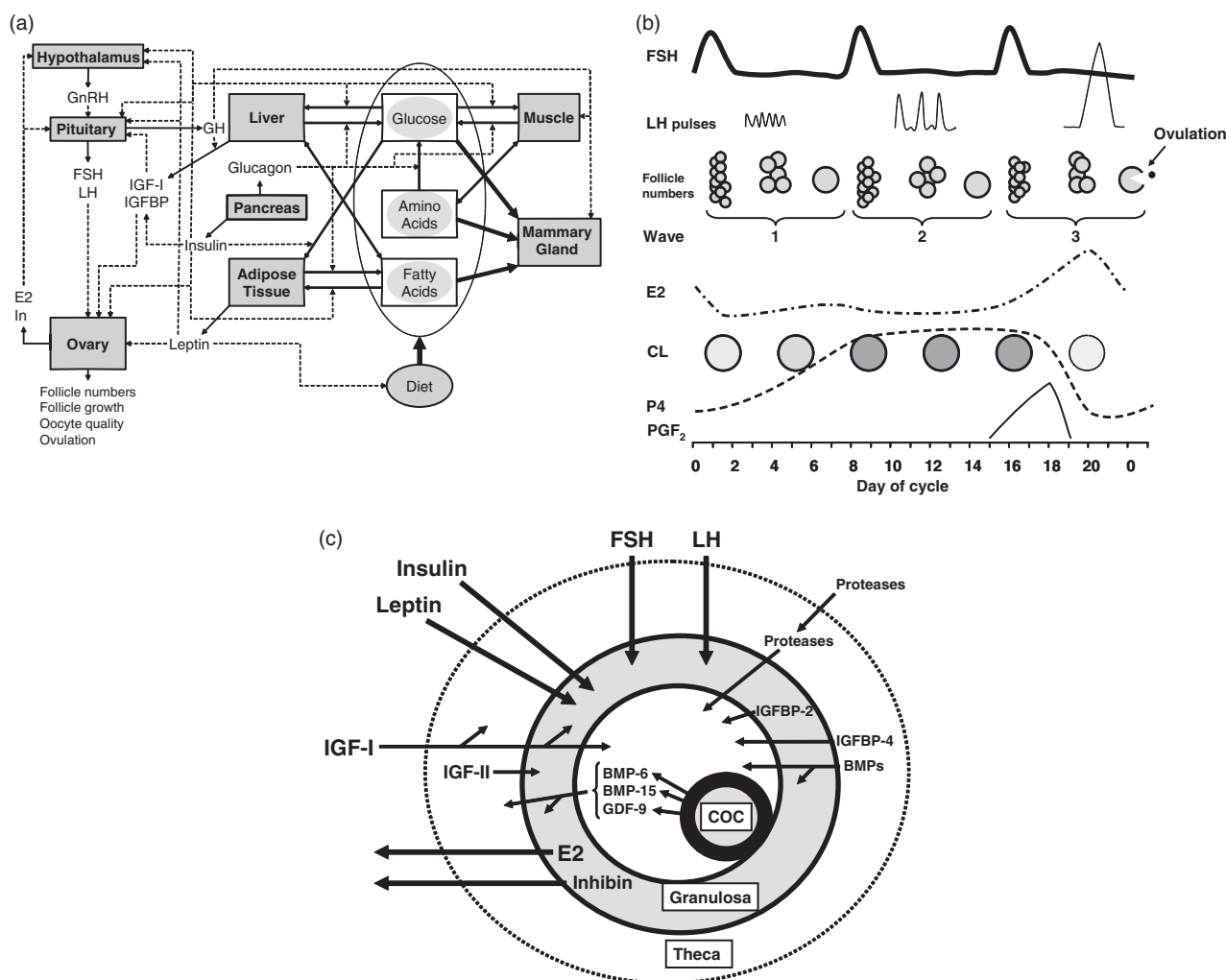
**Figure 2** Interactions among physiological regulatory mechanisms at (a) animal, (b) ovarian and (c) follicular levels. At the animal level (a), plasma concentrations of glucose, amino acids and fatty acids are determined primarily by dietary intake and mammary uptake. When plasma glucose is high, insulin stimulates the uptake of glucose by the liver, muscles and adipose tissue, and stimulates lipogenesis. When plasma glucose is low, glucagon stimulates gluconeogeneisis from amino acids, release of glycogen from liver and muscle, and lipolysis. Growth hormone regulates muscle deposition and milk yield, and also regulates IGF and IGF binding protein (IGFBP) synthesis in the liver. Leptin provides feedback from adipose tissue to regulate feed intake. All of these metabolic hormones and metabolites interact with the hypothalamus–pituitary–ovarian axis. The hypothalamus releases GnRH to regulate FSH and LH release from the pituitary gland, with feedback from ovarian oestradiol (E2) and inhibin (In). At the ovarian level (b), FSH stimulates follicle recruitment and early growth in two or three waves per cycle. Pulsatile LH is then required for continued growth and development of the dominant ovulatory follicle, which secretes E2. The corpus luteum (CL), which develops after ovulation, secretes progesterone (P4) until prostaglandin-F$_{2\alpha}$ is released by the non-pregnant uterus to cause CL regression. At the follicular level (c), gonadotrophins and metabolic hormones work in conjunction with locally produced growth factors (e.g. IGFBP, bone morphogenic proteins (BMP) and growth differentiation factor-9 (GDF-9)) released by granulosa and theca cells, and the oocyte, to regulate development of the follicle and cumulus-oocyte complex (COC). For detailed explanation, see text and also: Garnsworthy and Webb (1999); Webb et al. (2004); Webb and Campbell (2007); Webb et al. (2007).

et al., 2003). This down-regulation can be overcome, however, and circulating IGF-I concentrations restored, by elevated plasma insulin concentrations during early lactation (Butler et al., 2003).

Leptin also has a homeorhetic role by acting as an indicator of body fatness and by providing a feedback mechanism to control feed intake, although the overall regulatory system is complex (Vernon et al., 2001). In addition to its effects on feed intake, leptin has been found to modulate nutrient transfer and partitioning by interaction with other hormones including insulin, glucagon, glucocorticoids, growth hormone, IGF-I, cytokines and thyroid

hormones (Hill, 2004). It appears that both insulin and IGF-I are involved in regulating leptin responses to nutrition (Armstrong et al., 2003). Other factors secreted by adipose tissue (tumour necrosis factor α and resistin) have also been shown to interact with leptin in the regulation of body fatness (Vernon et al., 2001).

Regulation of body fatness has important implications for energy balance in early lactation. During the first few weeks of lactation, high-yielding dairy cows are usually in negative energy balance because energy output in milk exceeds energy intake from the diet, implying that body fat reserves are being mobilised to make up the energy deficit. This

mobilisation can be energetically equivalent to one-third of the milk produced (Bauman and Currie, 1980). Body fat mobilisation is not, however, a passive response to high milk yield and low feed intake. As reviewed by Garnsworthy (2007), there is much evidence to indicate that dairy cows adjust their feed intake to regulate body fatness over the first 3 months of lactation.

The concept that dairy cows regulate their feed intake to achieve a target level of body fatness in early lactation was proposed by Garnsworthy and Topps (1982), based on observations of cows fed to achieve different levels of body condition score (BCS) at calving. Cows with a BCS above their target had lower feed intakes and lost BCS throughout the study; cows with a BCS below their target had higher feed intakes and gained BCS throughout the study. Consequently, BCS of all cows converged to an average of 2.5 (1 to 4 scale) by week 12 of lactation. Importantly, there was no difference in milk yield among treatment groups, indicating that homeorhetic mechanisms were actively regulating body fatness through the negative feedback effect of body fat on feed intake.

Numerous studies in the 1980s and 1990s (reviewed by Broster and Broster, 1998; Garnsworthy, 1988; Stockdale, 2001) confirmed the strong negative relationship between BCS at calving and change in BCS during early lactation. More recently, Garnsworthy (2007) compared responses published between 1980 and 1993 with responses published between 2000 and 2006. The BCS at calving resulting in no net change in BCS over the first 12 weeks of lactation has decreased from 2.5 (1 to 5 scale) in older studies to 2.1 in recent studies. This suggests that the average biological target BCS has decreased with increasing genetic merit for milk production, so modern dairy cows are genetically 'thinner'.

It is important to distinguish between phenotypic and genetic body fatness. Dechow et al. (2002) examined correlations between BCS and BCS loss in 310 000 lactation records. Phenotypically, an increase in BCS at calving was associated with greater BCS loss in early lactation, as expected if cows are attempting to reach biological targets for BCS. Genetically, however, higher BCS at calving was correlated with less BCS loss during early lactation. In other words, management conditions that increase BCS at calving result in greater BCS loss, but genetically fat cows maintain BCS in early lactation.

## Negative energy balance and fertility

Several studies have shown that high genetic merit, negative energy balance, body fat mobilisation and low plasma insulin are all associated with delayed first ovulation *post partum* and reduced pregnancy rates (see reviews by Garnsworthy and Webb, 1999; Butler, 2003; Pryce *et al.*, 2004; Butler, 2005; Garnsworthy, 2007). Lopez-Gatius *et al.* (2003) performed a meta-analysis of 15 papers corresponding to nearly 8000 cows to examine the relationships between BCS and reproductive performance. Compared

with cows losing 0 to 0.5 BCS, cows losing 0.5 to 1.0 BCS took 3.5 days longer to conceive, and cows losing >1.0 BCS took 10.6 days longer to conceive; cows gaining BCS took 3.7 days less to conceive. Similarly, Butler (2005) reported that cows losing less than 0.5 BCS over the first 30 days *post partum* took an average of 30 days from calving to first ovulation; cows losing 0.5 to 1.0 BCS took 36 days; cows losing more than 1.0 BCS took 50 days. In addition, Butler (2005) concluded from several studies that conception rate decreases by 10% per 0.5 unit BCS loss. It is clear, therefore, that reducing the extent and duration of negative energy balance and BCS loss should be beneficial for fertility.

Three basic strategies are available to reduce the extent and duration of negative energy balance and BCS loss in early lactation. The first strategy is to reduce BCS at calving so that energy intake is not limited by the negative feedback effect of BCS, as discussed above; a recent study showed that high-genetic merit cows can maintain a BCS of 2.5 throughout lactation (Yan *et al.*, 2006). The second strategy is to feed low-protein diets that reduce body fat mobilisation (Garnsworthy and Jones 1987; Westwood *et al.*, 2000; Schei *et al.*, 2005). The third and the commonest strategy is to increase dietary energy concentration by increasing the starch or fat components of the diet at the expense of forage components. Such changes in carbohydrate source have implications for rumen function, milk composition, nutrient partitioning and metabolic hormones, particularly insulin (Sutton, 1989; Sutton *et al.*, 2003; Reynolds, 2006). These implications are discussed in subsequent sections.

## Resumption of oestrous cycles

Metabolic hormones and reproductive function were measured in dairy cows selected for high and low genetic merit for milk yield (Gutierrez *et al.*, 2006). Resumption of normal oestrous cycles *post partum* occurred approximately 8 days later in the high-merit cows, and this was associated with lower plasma insulin concentrations. Interestingly, these differences occurred despite no difference in milk yield between the two selection lines during this period, suggesting other underlying mechanisms linked to selection for total milk yield. A further study investigated whether feeding diets designed to increase circulating insulin concentrations during the early *post partum* period can overcome the delay in first ovulation *post partum* shown by cows selected for increased milk yield (Gong *et al.*, 2002a). Two isoenergetic diets were formulated to either stimulate or depress plasma insulin concentrations, and these were fed to cows of high or low genetic merit. Again, first ovulation and resumption of normal oestrous cycles *post partum* were delayed in cows of high genetic merit. This was associated with lower circulating insulin concentrations, but did not involve an alteration in basal plasma gonadotrophin concentrations or patterns of ovarian follicular development during the early *post partum* period.

Feeding the diet designed to increase circulating insulin concentrations advanced first ovulation *post partum* so that 100% of low-merit cows and 80% of high-merit cows ovulated within the first 50 days of lactation when fed on the high-insulin diet, compared with 60% of low-merit cows and 50% of high-merit cows fed on the control diet.

These data suggest that genetic influences on reproductive performance can be partially overcome through dietary manipulation because the responses observed by Gong *et al.* (2002a) were independent of milk yield and energy balance, as were the differences between cows of high and low genetic merit in the study of Gutierrez, *et al.* (2006). It appears that insulin acts as a metabolic signal to the reproductive system, signalling that nutritional status is adequate and conception can proceed. The exact mechanism for this signalling is still unclear, but it probably involves interactions between insulin, IGFs and gonadotrophins (Figure 2a).

### Regulation of follicle development

Ovarian follicular growth is a developmental process during which follicles leave the primordial pool, increase in diameter and complexity, and differentiate into the three cell types (oocyte, granulosa and theca) that ultimately constitute the preovulatory follicle (Webb *et al.*, 2007). Functional changes include changes in the steroidogenic capability of follicles, particularly during the antral stages of development, the acquisition of granulosa cell FSH receptors during the preantral stage of development and the acquisition of granulosa cell LH receptors around the time of selection of the dominant follicle (see Webb *et al.*, 2003 and 2004). These later stages of antral follicle development are characterised by two or three waves of follicular growth (Figure 2b) during each oestrous cycle (Adams, 1999). Each wave of follicle growth involves recruitment of a group of follicles that grow to approximately 6 to 8 mm in diameter. One follicle is then selected for continued growth and becomes dominant. The remaining follicles become atretic and regress. The precise mechanism for selection of dominant follicles remains to be fully elucidated, but does involve the action of gonadotrophins as well as locally produced factors (Webb *et al.*, 2004). The very early stages of folliculogenesis can occur without gonadotrophins but FSH, along with other extra-ovarian and locally produced growth factors, may affect the rate of preantral follicle growth (Hulshof *et al.*, 1995; Gutierrez *et al.*, 2000; Webb *et al.*, 2004; Webb and Campbell, 2007). Both FSH and LH exert their effects on follicular somatic cells via specific membrane-bound receptors that exhibit alternate patterns of expression. Granulosa cells of large oestrogenic (dominant) antral follicles develop LH receptors (Bao and Garverick, 1998; Webb *et al.*, 1999a), and this event is critical to the process of follicle selection. In addition, changes in the expression of mRNAs for both gonadotrophin receptors and steroidogenic enzymes (e.g. P450arom) (Bao and Garverick, 1998; Webb *et al.*, 1999a; Webb and Campbell,

2007) occur at this stage of development. Thus the presence of LH receptors on granulosa cells enables the dominant follicle to switch its gonadotrophic dependence from FSH to LH and attain dominance over other antral follicles, which remain FSH-dependent (Webb *et al.*, 2007).

Development of follicles depends also on interactions with a range of local and circulating growth factors. For example, treatment with (BST), through increasing circulating insulin and IGF concentrations, can increase both the number of gonadotrophin-responsive follicles and their quality (Gong *et al.*, 1991 and 1993). Nutrition also influences follicle development (Garnsworthy and Webb, 1999), with short-term changes in plane of nutrition affecting small antral follicle (1 to 4 mm) recruitment, without affecting circulating concentrations of FSH (Gutierrez *et al.*, 1997b; Gong *et al.*, 2002a), resulting in a larger number of ovulations after a superovulatory gonadotrophin challenge (Gong *et al.*, 2002b). Increased dietary energy decreases steady-state concentrations of mRNAs encoding IGFBP-2 and -4 in small antral follicles, which in turn increase the bioavailability of locally produced IGF-II and systemically derived IGF-I in these follicles (Armstrong *et al.*, 2003; Webb *et al.*, 2003). Hence this work provides important evidence that diet can have direct effects at the ovarian level, as well as the whole animal level, to influence follicular development and oocyte quality.

### Regulation of oestrous cycles

Oestrous cycles have a follicular phase, at the end of which there is a surge release of gonadotrophins (LH and FSH) from the anterior pituitary gland accompanied by ovulation, and a luteal phase with the secretion of progesterone from the newly formed corpus luteum (Figure 2a and b). The oestrous cycle is driven primarily by GnRH, which is secreted into the hypophyseal portal system in a pulsatile fashion from the terminals of neurons originating around the preoptic area and mediobasal hypothalamus at the base of the brain. GnRH neurons are regulated by feedback from oestradiol and progesterone (Clarke and Pompolo, 2005). Both FSH and LH are released from the anterior pituitary in response to GnRH (Webb *et al.*, 1992; Wolfenson *et al.*, 2004). Each pulse of GnRH is followed by a pulse of LH that is released into the peripheral circulation. In contrast, there is a less well-defined association between GnRH and FSH release, which is thought to be dependent on the rate of FSH synthesis. Both ovarian-derived oestradiol and inhibin feedback to inhibit FSH release by suppressing FSH gene expression (Pawson and McNeilly, 2005).

A number of growth factors (e.g. insulin, IGF-I and leptin) and metabolites (e.g. glucose, free fatty acids and ammonium) influence GnRH release from hypothalamic neurons (Sinclair and Webb, 2005). Leptin and IGF-I receptors are expressed within the anterior pituitary, and both leptin and IGF-I increase LH secretion in primary cultures of anterior pituitary cells (Daftary and Gore, 2005; Zieba *et al.*, 2005).

Extra-ovarian factors, such as metabolic hormones and nutrients, interact with growth factors produced locally within the ovary (Figure 2c) to induce changes in follicle dynamics and oocyte quality (Webb et al., 2004). A large number of in vitro studies have demonstrated the direct action of metabolic factors on granulosa and theca cells (Webb et al., 1999a and 1999b; Lucy, 2000; Spicer et al., 2002; Armstrong et al., 2003). Some cell culture studies have shown bovine granulosa cells to be critically dependent on the presence of physiological concentrations of insulin (Gutierrez et al., 1997a). Moreover, diet-induced increases in circulating concentrations of insulin are correlated with increased oestradiol production in cultured granulosa cells from small antral (1 to 4 mm) follicles (Armstrong et al., 2002), demonstrating a direct action of metabolic hormones on follicle function.

## Oocyte quality

At the follicle level, cell proliferation and development are controlled largely by endocrine and paracrine growth factors and nutrient supply (Figure 2c). Collectively, these determine maturation and developmental competence of the oocyte. Studies have highlighted the link between nutrient intake and oocyte developmental competence in cattle (reviewed by O'Callaghan and Boland, 1999; Boland et al., 2001). From our own studies in cattle we know that diets containing high levels of rumen-degradable nitrogen can increase concentrations of ammonium and urea in plasma and follicular fluid, which can lead to significantly reduced cleavage rates after in vitro maturation (IVM) and fertilisation (IVF), and reduced blastocyst yields during in vitro culture (IVC) (Sinclair et al., 2000a). Such diets also depress plasma insulin concentrations significantly (Sinclair et al., 2000b), which may contribute to this phenomenon. In heifers, high-energy (1.6 times maintenance ME requirements) and high-protein (27 g/MJ ME) diets, although enhancing ovarian follicle development, led to a significant reduction in oocyte quality, determined by the ability of oocytes to mature, fertilise and develop to the blastocyst stage during IVC (Armstrong et al., 2001). The ovarian IGF system has the potential to interact directly with the oocyte through the Type-I IGF receptor, so that the reduced levels of IGFBP-2 and -4, together with increased concentrations of both IGF-I and insulin reported by Armstrong et al. (2001), may have led to the oocyte being 'overstimulated.' Indeed, we (Adamiak et al., 2005) have subsequently demonstrated that the effects of high levels of feeding on oocyte quality in cattle are cumulative, are linked to insulin metabolism and are dependent on the initial BCS of the animal. Although feeding a high-energy (2.0 times maintenance ME requirements) diet to thin heifers (BCS 2.0) improved early embryo development following IVM, IVF and IVC, the same diet offered to fat heifers (BCS 3.75) reduced embryo development significantly. Furthermore, the effects were cumulative, with embryo development for the fat heifers on twice maintenance deteriorating over the 9-week

experimental period. A significant proportion of these animals were hyperinsulinaemic (plasma insulin >1.66 ng/ml), and this condition was associated directly with impaired oocyte quality. As observed in the study of Armstrong et al. (2001), high-energy diets enhanced ovarian follicular development. Therefore, from studies with heifers it appears that diets that promote ovarian follicular development may be detrimental for oocyte quality.

Results of our subsequent studies with lactating dairy cows are consistent with results from heifer studies, although insulin concentrations are lower in lactating dairy cows (e.g. 0.32 ng/ml for a high-insulin diet v. 0.21 ng/ml for a low-insulin diet in the study of Gong et al., 2002b). These dairy cow studies indicate that high-insulin status may encourage early resumption of oestrous cycles, but high insulin might not be beneficial for oocyte quality. In one experiment (Fouladi-Nashta et al., 2005), cows were fed diets with either low or high starch content in order to induce differences in plasma insulin. Oocytes were collected for IVF and cultured to examine developmental potential to the blastocyst stage. For the high-insulin diet, 26% of cleaved embryos developed to blastocysts, compared with 41% for the low-insulin diet. Furthermore, the high-insulin diet produced a significantly higher number of grade 4 (poor quality) oocytes (high insulin 28%, low insulin 13%). In another experiment, addition of fat to a high-starch diet improved blastocyst yield from 29% to 38% (Fouladi-Nashta et al., 2007).

## Embryo development and implantation

Progesterone secreted by the developing corpus luteum during the post-ovulatory period plays a critical role in supporting uterine function and numerous studies have linked poor progesterone secretion at this time to poor embryo development and early embryo loss (e.g. Mann and Lamming, 1999 and 2001). Progesterone secretion is related directly to the size of the corpus luteum, which can be increased by feeding supplemental dietary fat (Staples and Thatcher, 2005). Metabolic hormones might also play a role in embryo development; for example, we found a lower proportion of elongated embryos (>10 cm at 16 days after insemination) in heifers with a low insulin to glucagon ratio, although progesterone concentrations were not affected by treatment (Mann et al., 2003).

Embryo mortality, during the pre-implantation period, is a key contributor to reduced fertility in cattle, with up to 40% of total embryo losses occurring between days 8 and 17 of pregnancy (Thatcher et al., 2001). This stage of embryonic loss coincides with the inhibitory effects of interferon-$\tau$, produced by trophectoderm cells of embryos, on prostaglandin release from the uterus (Flint, 1995). This suggests that a proportion of embryos are unable to inhibit prostaglandin-$F_{2\alpha}$ release, leading to regression of the corpus luteum, reduction in progesterone production and termination of pregnancy (Flint, 1995; Thatcher et al., 1997). Hence the quality of the pre-implantation embryo

and its ability to communicate with the cells of the uterus are critical for the establishment and continuation of pregnancy.

## Integration of physiological mechanisms

From the previous sections, it is clear that several physiological mechanisms influence fertility in dairy cows. As illustrated in Figures 1 and 2, these mechanisms operate at different levels (cell, ovary, animal) and on different timescales (minutes, hours, days, weeks). The interactions between regulatory systems require an integrated approach when addressing fertility issues because changes in one system can have consequences for other systems. It is necessary to consider general homoeostatic and homeorhetic mechanisms simultaneously with regulation of ovarian cycles, folliculogenesis and oocyte development.

In reviewing reproductive mechanisms affected by energy balance, Butler (2003) found that negative energy balance is strongly associated with attenuation of LH pulse frequency and low levels of blood glucose, insulin and IGF-I that collectively limit oestrogen production by dominant follicles. Negative energy balance was also associated with a diminished quality of oocytes, reduced embryo development and lower serum progesterone concentrations. Thus, it would seem that the primary goal when addressing poor fertility is to reduce the extent and duration of negative energy balance. As discussed previously, however, strategies designed to reduce negative energy balance will alter circulating concentrations of metabolites and hormones.

Circulating concentrations of metabolites and hormones are determined by the balance between nutrient intake, milk production level and body tissue reserves, relative to the animal's genetic potential. As outlined above, these factors are regulated by homoeostatic and homeorhetic mechanisms, but they can be manipulated by altering diet composition. Insulin appears to be the most important hormone that links metabolic status to the regulation of ovarian function. After addressing the issue of negative energy balance, the next most promising strategy for improving fertility is to promote resumption of oestrous cycles by ensuring that insulin status is adequate.

There is a potential conflict between strategies designed to start cows cycling and production of good-quality oocytes. High plasma insulin concentrations stimulate resumption of oestrous cycles, but have detrimental effects on oocyte developmental competence. This presents an interesting challenge for researchers and producers, and might explain why the fertility problem is so widespread in dairy cows. Our recent studies addressed these differential responses by nutritional manipulation of insulin at different stages of the reproductive cycle. In an initial experiment (Garnsworthy, Sinclair, Webb *et al.*: unpublished), increasing insulin status immediately *post partum*, and then reducing insulin status during the mating period, doubled the pregnancy rate at 120 days in milk. This suggests that progress towards restoring fertility can be made by an integrated approach that allows for interactions between physiological mechanisms that regulate metabolism and reproduction.

## References

Adamiak SJ, Mackie K, Watt RG, Webb R and Sinclair KD 2005. Impact of nutrition on oocyte quality: cumulative effects of body composition and diet leading to hyperinsulinemia in cattle. Biology of Reproduction 73, 918–926.

Adams GP 1999. Comparative patterns of follicle development and selection in ruminants. Reproduction in domestic ruminants IV. Journal of Reproduction and Fertility Supplement 54, 17–32.

Armstrong DG, McEvoy TG, Baxter G, Robinson JJ, Hogg CO, Woad K, Webb R and Sinclair KD 2001. The effect of dietary energy and protein on bovine follicular dynamics and embryo production in vitro; associations with the ovarian insulin-like growth factor system. Biology of Reproduction 64, 1624–1632.

Armstrong DG, Gong JG, Gardner JO, Baxter G, Hogg CO and Webb R 2002. Steroidogenesis in bovine granulosa cells: the effect of short-term changes in dietary intake. Reproduction 123, 371–378.

Armstrong DG, Gong JG and Webb R 2003. Interactions between nutrition and ovarian activity in cattle: physiological, cellular and molecular mechanisms. Reproduction in Domestic Ruminants V. Reproduction 61 (Suppl.), 403–414.

Bao B and Garverick HA 1998. Expression of steroidogenic enzyme and gonadotropin receptor genes in bovine follicles during ovarian follicular waves: a review. Journal of Animal Science 76, 1903–1921.

Bauman DE 2000. Regulation of nutrient partitioning during lactation: homeostasis and homeorhesis revisited. In Ruminant physiology: digestion, metabolism, growth and reproduction (ed. PB Cronje), pp. 311–328. CABI Publishing, Wallingford, UK.

Bauman DE and Currie WB 1980. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and home-orhesis. Journal of Dairy Science 63, 1514–1529.

Baumrucker CR 2000. The insulin-like growth factor (IGF) system in the mammary gland: role of IGFBP-3 binding protein. In Ruminant physiology: digestion, metabolism, growth and reproduction (ed. PB Cronje), pp. 329–351. CABI Publishing, Wallingford, UK.

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

Broster WH and Broster VJ 1998. Body score of dairy cows. Journal of Dairy Research 65, 155–173.

Butler WR 2000. Nutritional interactions with reproductive performance in dairy cattle. Animal Reproduction Science 60–61, 449–457.

Butler WR 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livestock Production Science 83, 211–218.

Butler WR 2005. Nutrition, negative energy balance and fertility in the postpartum dairy cow. Cattle Practice 13, 13–18.

Butler ST, Marr AL, Pelton SH, Radcliff RP, Lucy MC and 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. Journal of Endocrinology 176, 205–217.

Clarke IJ and Pompolo S 2005. Synthesis and secretion of GnRH. Animal Reproduction Science 88, 29–55.

Daftary SS and Gore AC 2005. IGF-1 in the brain as a regulator of reproductive neuroendocrine function. Experimental Biology and Medicine 230, 292–306.

Dechow CD, Rogers GW and Clay JS 2002. Heritability and correlations among body condition score loss, body condition score, production traits and reproductive performance. Journal of Dairy Science 85, 3062–3070.

Defra 2007. Defra Economics and Statistics website. Retrieved October 11, 2007, from http://statistics.defra.gov.uk/esg/

Evans RD, Wallace M, Shalloo L, Garrick DJ and Dillon P 2006. Financial implications of recent declines in reproduction and survival of Holstein-Friesian cows in spring-calving Irish dairy herds. Agricultural Systems 89, 165–183.

Flint APF 1995. Interferon, the oxytocin receptor and the maternal recognition of pregnancy in ruminants and nonruminants – a comparative approach. Reproduction, Fertility and Development 7, 313–318.

Fouladi-Nashta AA, Gutierrez CG, Garnsworthy PC and Webb R 2005. Effects of dietary carbohydrate source on oocyte/embryo quality and development in high-yielding, lactating dairy cattle. Biology of Reproduction (Special issue), 135–136.

Fouladi-Nashta AA, Gutierrez CG, Gong JG, Garnsworthy PC and Webb R 2007. Impact of dietary fatty acids on oocyte quality and development in lactating dairy cows. Biology of Reproduction 77, 9–17.

Garcia-Ispierto I, Lopez-Gatius F, Santolaria P, Yaniz JL, Nogareda C and Lopez-Bejar M 2007. Factors affecting the fertility of high producing dairy herds in northeastern Spain. Theriogenology 67, 632–638.

Garnsworthy PC 1988. The effect of energy reserves at calving on performance of dairy cows. In Nutrition and lactation in the dairy cow (ed. PC Garnsworthy), pp. 157–170. Butterworths, London, UK.

Garnsworthy PC 2004. The environmental impact of fertility in dairy cows: a modelling approach to predict methane and ammonia emissions. Animal Feed Science and Technology 112, 211–223.

Garnsworthy PC 2005. Modern calves and heifers: challenges for rearing systems. In Calf and heifer rearing: principles of rearing the modern dairy heifer from calf to calving (ed. PC Garnsworthy), pp. 1–11. Nottingham University Press, Nottingham, UK.

Garnsworthy PC 2007. Body condition score in dairy cows: targets for production and fertility. In Recent advances in animal nutrition – 2006 (ed. PC Garnsworthy and J Wiseman), pp. 61–86. Nottingham University Press, Nottingham, UK.

Garnsworthy PC and Jones GP 1987. The influence of body condition at calving and dietary protein supply on voluntary food intake and performance in dairy cows. Animal Production 44, 347–353.

Garnsworthy PC and Topps JH 1982. The effect of body condition of dairy cows at calving on their food intake and performance when given complete diets. Animal Production 35, 113–119.

Garnsworthy PC and Webb R 1999. The Influence of nutrition on fertility in dairy cows. In Recent advances in animal nutrition – 1999 (ed. PC Garnsworthy and J Wiseman), pp. 39–57. Nottingham University Press, Nottingham, UK.

Gong JG, Bramley T and Webb R 1991. The effect of recombinant bovine somatotropin on ovarian-function in heifers – follicular populations and peripheral hormones. Biology of Reproduction 45, 941–949.

Gong JG, Bramley T and Webb R 1993. The effect of recombinant bovine somatotropin on ovarian follicular-growth and development in heifers. Biology of Reproduction and Fertility 97, 247–254.

Gong JG, Armstrong DG, Baxter G, Hogg CO, Garnsworthy PC and Webb R 2002a. The effect of increased dietary intake on superovulatory response to FSH in heifers. Theriogenology 57, 1591–1602.

Gong JG, Lee WJ, Garnsworthy PC and Webb R 2002b. Effect of dietary-induced increases in circulating insulin concentrations during the early postpartum period on reproductive function in dairy cows. Reproduction 123, 419–427.

Gutierrez CG, Campbell BK and Webb R 1997a. Development of a long-term bovine granulosa cell culture system: induction and maintenance of estradiol production, response to follicle-stimulating hormone, and morphological characteristics. Biology of Reproduction 56, 608–616.

Gutierrez CG, Oldham J, Bramley TA, Gong JG, Campbell BK and Webb R 1997b. The recruitment of ovarian follicles is enhanced by increased dietary intake in heifers. Journal of Animal Science 75, 1876–1884.

Gutierrez CG, Ralph JH, Telfer EE, Wilmut I and Webb R 2000. Growth and antrum formation of bovine preantral follicles in long-term culture in vitro. Biology of Reproduction 62, 1322–1328.

Gutierrez CG, Gong JG, Bramley TA and Webb R 2006. Selection on predicted breeding value for milk production delays ovulation independently of changes in follicular development, milk production and body weight. Animal Reproduction Science 95, 193–205.

Hill RA 2004. The role of the leptin axis in modulating energy partitioning and nutrient utilisation in livestock species. In Recent advances in animal nutrition – 2004 (ed. PC Garnsworthy and J Wiseman), pp. 149–184. Nottingham University Press, Nottingham, UK.

Hulshof SCJ, Figueiredo JR, Beckers JF, Bevers MM, van der Donk JA and van den Hurk R 1995. Effects of fetal bovine serum, FSH and 17β-estradiol on the culture of bovine preantral follicles. Theriogenology 44, 217–226.

Jones JI and Clemmons DR 1995. Insulin-like growth factors and their binding proteins: biological actions. Endocrine Reviews 16, 3–34.

Lopez-Gatius F, Yaniz J and Madriles-Helm D 2003. Effects of body condition score and score change on the reproductive performance of dairy cows: a meta-analysis. Theriogenology 59, 801–812.

Lucy MC 2000. Regulation of ovarian follicular growth by somatotropins and insulin-like growth factors in cattle. Journal of Dairy Science 83, 1635–1647.

Mann GE and Lamming GE 1999. The influence of progesterone during early pregnancy in cattle. Reproduction in Domestic Animals 34, 269–274.

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

Mann GE, Green MP, Sinclair KD, Demmers KJ, Fray MD, Gutierrez CG, Garnsworthy PC and Webb R 2003. Effects of circulating progesterone and insulin on early embryo development in beef heifers. Animal Reproduction Science 79, 71–79.

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

Pawson AJ and McNeilly AS 2005. The pituitary effects of GnRH. Animal Reproduction Science 88, 75–94.

Petersson KJ, Gustafsson H, Strandberg E and Berglund B 2006. Atypical progesterone profiles and fertility in Swedish dairy cows. Journal of Dairy Science 89, 2529–2538.

Pryce JE, Royal MD, Garnsworthy PC and Mao IL 2004. Fertility in the high producing dairy cow. Livestock Production Science 86, 125–135.

Reynolds CK 2006. Production and metabolic effects of site of starch digestion in dairy cattle. Animal Feed Science and Technology 130, 78–94.

Royal MD, Darwash AO, Flint APF, Webb R, Woolliams JA and Lamming GE 2000. Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. Animal Science 70, 487–501.

Royal MD, Woolliams JA and Flint APF 2002. Genetic and phenotypic relationships among endocrine and traditional fertility traits and production traits in Holstein-Friesian dairy cows. Journal of Dairy Science 85, 958–967.

Schei I, Volden H and Baevre L 2005. Effects of energy balance and metabolizable protein level on tissue mobilization and milk performance of dairy cows in early lactation. Livestock Production Science 95, 35–47.

Sinclair KD and Webb R 2005. Fertility in the modern dairy heifer. In Calf and heifer rearing: principles of rearing the modern dairy heifer from calf to calving (ed. PC Garnsworthy), pp. 277–306. Nottingham University Press, Nottingham, UK.

Sinclair KD, Kuran M, Gebbie FE, Webb R and McEvoy TG 2000a. 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. Journal of Animal Science 78, 2670–2680.

Sinclair KD, Sinclair LA and Robinson JJ 2000b. Nitrogen metabolism and fertility in cattle: I. Adaptive changes in intake and metabolism to diets differing in their rate of energy and nitrogen release in the rumen. Journal of Animal Science 78, 2659–2669.

Spicer LJ, Chamberlain CS and Maciel SM 2002. Influence of gonadotropins on insulin and insulin-like growth factor-I (IGF-I)-induced steroid production by bovine granulosa cells. Domestic Animal Endocrinology 22, 237–254.

Staples CR and Thatcher WW 2005. Effects of fatty acids on reproduction of dairy cows. In Recent advances in animal nutrition – 2005 (ed. PC Garnsworthy and J Wiseman), pp. 229–256. Nottingham University Press, Nottingham, UK.

Stockdale CR 2001. Body condition at calving and the performance of dairy cows in early lactation under Australian conditions: a review. Australian Journal of Experimental Agriculture 41, 823–839.

Stott AW, Veerkamp RF and Wassell TR 1999. The economics of fertility in the dairy herd. Animal Science 68, 49–58.

Sutton JD 1989. Altering milk composition by feeding. Journal of Dairy Science 72, 2801–2814.

Sutton JD, Dhanoa MS, Morant SV, France J, Napper DJ and Schuller E 2003. Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. Journal of Dairy Science 86, 3620–3633.

Thatcher WW, Binelli M, Burke J, Staples CR, Ambrose JD and Coelho S 1997. Antiluteolytic signals between the conceptus and endometrium. Theriogenology 47, 131–140.

Thatcher WW, Guzeloglu A, Mattos R, Binelli M, Hansen TR and Pru JK 2001. Uterine-conceptus interactions and reproductive failure in cattle. Theriogenology 56, 1435–1450.

Vernon RG 1988. The partition of nutrients during the lactation cycle. In Nutrition and lactation in the dairy cow (ed. PC Garnsworthy), pp. 32–52. Butterworths, London, UK.

Vernon RG, Denis RGP and Sørensen A 2001. Signals of adiposity. Domestic Animal Endocrinology 21, 197–214.

Webb R and Campbell BK 2007. Development of the dominant follicle: mechanisms of selection and maintenance of oocyte quality. In Reproduction in Domestic Ruminants VI (ed. JL Juengel, JF Murray and MF Smith), pp. 140–163. Nottingham University Press, Nottingham, UK.

Webb R, Gong JG, Law AS and Rusbridge SM 1992. Control of ovarian-function in cattle. Journal of Reproduction and Fertility Supplement 45, 141–156.

Webb R, Campbell BK, Garverick HA, Gong JG, Gutierrez CG and Armstrong DG 1999a. Molecular mechanisms regulating follicular recruitment and selection. Journal of Reproduction and Fertility Supplement 54, 33–48.

Webb R, Gosden RG, Telfer EE and Moor RM 1999b. Factors affecting folliculogenesis in ruminants. Animal Science 68, 257–284.

Webb R, Nicholas B, Gong JG, Campbell BK, Gutierrez CG, Garverick HA and Armstrong DG 2003. Mechanisms regulating follicular development and selection of the dominant follicle. Reproduction Supplement 61, 71–90.

Webb R, Garnsworthy PC, Gong JG and Armstrong DG 2004. Control of follicular growth: local interactions and nutritional influences. Journal of Animal Science 82, E63–E74.

Webb R, Garnsworthy PC, Campbell BK and Hunter MG 2007. Intra-ovarian regulation of follicular development and oocyte competence in farm animals. Theriogenology 68S, S22–S29.

Westwood CT, Lean IJ, Garvin JK and Wynn PC 2000. Effects of genetic merit and varying dietary protein degradability on lactating dairy cows. Journal of Dairy Science 83, 2926–2940.

Wolfenson D, Inbar G, Roth Z, Kaim M, Bloch A and Braw-Tal R 2004. Follicular dynamics and concentrations of steroids and gonadotropins in lactating cows and nulliparous heifers. Theriogenology 62, 1042–1055.

Yan T, Mayne CS, Keady TWJ and Agnew RE 2006. Effects of dairy cow genotype with two planes of nutrition on energy partitioning between milk and body tissue. Journal of Dairy Science 89, 1031–1042.

Zieba DA, Amstalden M and Williams GL 2005. Regulatory roles of leptin in reproduction and metabolism: a comparative review. Domestic Animal Endocrinology 29, 166–185.