Milking reproduction

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Abstract

Reproductive function is influenced by metabolic status and is compromised in conditions of negative energy balance. In the recent past, significant advances have been made in our understanding of how the brain regulates appetite and energy expenditure, largely due to the identification of blood borne factors that act on the neurons in the hypothalamus that produce the so-called 'appetite regulating peptides'. This review provides an overview of the control of energy balance and the interface between 'appetite regulating' systems and gonadotropin releasing hormone secretion and action. This is placed in the context of the lactating dairy cow in an effort to understand what might cause poor reproductive performance. It is apparent that we know significantly more about the relationship between energy balance and reproduction in non-bovine species. This review indicates areas which deserve further attention in the dairy cow.

Keywords: gonadotropin releasing hormone; leptin; insulin; energy balance; lactation

Introduction

Any consideration of the fertility of dairy cows is generally predicated by the fact that, due to selection of high producing cows, milk production has increased substantially in the last 50 years. Associated with this, there has been a decline in the fertility of high-producing cows (Hansen 2000), which is most likely caused by a number of environmental, physiological and genetic factors. The reduced fertility may be due to factors that involve the brain, pituitary gland, ovaries and uterus. This can lead to poor quality oocytes and an unfavourable uterine environment (Santos et al. 2010). These latter factors have been studied and documented quite extensively, but there is relatively little information on the hypothalamo-pituitary factors that contribute to anovulation. This is in spite of the indication that combined use of prostaglandin and gonadotropin releasing hormone (GnRH) can induce ovulations and procure pregnancies in high-producing dairy cows. This technique was first developed in 1995 (Pursley et al. 1995) and refined until the present day (Gumen et al. 2012; Yilmazbas-Mecitoglu et al. 2012).

GnRH is the neuropeptide that drives the reproductive system, but its synthesis and secretion is controlled by higher neural centres within the brain. Factors such as stress, act on the brain to inhibit reproduction (Clarke et al. 2009). It is well known that the appetite regulating peptides (ARP) also influence the secretion of GnRH from the brain (Clarke et al. 2009). This is important because, as a general rule, orexigenic peptides inhibit GnRH and gonadotropin secretion. There may, however be some exceptions, as detailed later. This review is focussed on energy balance and the factors that control GnRH secretion, including those which have been recognised quite recently, namely kisspeptin and gonadotropin inhibitory hormone (GnIH). Much of the data are derived from another ruminant, the sheep, in the hope that what has been learned from this species may be applicable to the dairy cow. After consideration of our state of knowledge in relation to the sheep, some recommendations for further research on the dairy cow will be made.

It is also well documented that negative energy balance has a deleterious effect on the reproductive axis. Changes in metabolic state are 'sensed' by the brain to varying degrees, depending upon species. A range of circulating hormones and metabolic substrates signal to the brain. Several of these have been manipulated to restore reproductive function in animals that are in a 'lean' condition. In this review, I will consider such models as surrogates to the dairy cow; an animal in which energy stores are used extensively for milk production.

Brain sensing of metabolic state

Our knowledge of the control of food intake and energy expenditure was rudimentary until 30 years ago. Historical landmark studies showed that lesions in the ventromedial nucleus of the hypothalamus cause hyperphagia and obesity (Hetherington et al. 1939) and lesions in the lateral hypothalamus caused hypophagia (Anand et al. 1951). However, the appetite regulating neurons and regulatory peptides found in these regions of the hypothalamus were not identified for another 30 years. In relation to farm animals, Dukes (1955) mentioned that 'deglutition' or swallowing, was under the control of a centre in the medulla oblongata, but had no knowledge of the control of energy balance. By the 1980s it was generally recognised that there were vagovagal connections between the gastrointestinal tract and the brain (Blessing 1997). The fact that choleocystokinin (CCK) (Williams 1981) was found in the small intestine, and in the brain, was registered by Rehfeld (1978). By 1981, Williams (1981) stated that there had been reports that CCK inhibited food intake, but merely as a passing reference. This was in spite of the fact that others (Gibbs et al. 1973) had shown that CCK powerfully reduced food intake in rats. As late as

1983, text books acknowledged that the brain controlled food intake and energy expenditure by sensing circulating factors, but none were mentioned. In a chapter on obesity (Bierman et al. 1981), it was stated that "Since neural regulation of adipose mass appears likely .... future developments of drugs altering such mechanisms holds promise". This proved to be a prescient statement!

Meanwhile, work had been done in farm animals and the prodigious output of Baile and associates on the control of food intake and energy balance was reviewed in 1974 (Baile et al. 1974). It was recognised that sex steroids, glucocorticoids and growth hormone played a role in energy balance and the role of the central nervous system was recognised. In this regard, the following quotation from their review is salient. "Some factors may affect gastrointestinal function and thus perhaps the satiety-hunger signals. Some may influence lipid metabolism and may possibly affect feeding via the feedback system from lipid depots. Others almost certainly affect CNS (central nervous system) function so as to impinge on the action of the centres controlling feeding". This was 20 years prior to the discovery of leptin, so work in domestic animals was certainly keeping pace!

The modern era of the science of energy balance may be thought of as beginning with the identification of a number of ARP in the brain, as well as the discovery of a range of factors produced in the stomach, fat and other organs that are secreted into the bloodstream and act on the brain to regulate food intake and energy expenditure. Neuropeptide Y (NPY) was discovered in 1981 (Tatemoto et al. 1982). By 1984, this was shown to be a potent orexigen (Clark et al. 1984; Levine et al. 1984). Following this discovery, a range of neuropeptides were discovered including orexins (ORX), melanin concentrating hormone (MCH), cocaine and amphetamine-related transcript (CART) and agouti related peptide (AGRP), all of which regulate metabolic function (Langhans et al. 2009). Much of this was aided by the use of molecular biology. In addition, the discovery of pro-opiomelanocortin (POMC) as a pre-hormone that encodes for a number of peptides, including the opioids and melanocortins, eventually led to the recognition that neuropeptides regulating various functions also act as orexigens/anorexigens (Langhans et al. 2009). Actually, POMC was purified earlier (Roberts et al. 1977). It was also known that β-endorphin (β-end) was encoded by POMC, but it was not until much later that the role β-endorphin and α-melanocyte stimulating hormone (α-MSH) play in the regulation of metabolic function was recognised. In 1990 it was noted by Brady et al. (1990) that food restriction or food deprivation, increased expression of the NPY gene and reduced the expression of the POMC gene in rats, but it was not until 1997 that the role of melanocortins in the control of feeding was fully appreciated (Fan et al. 1997).

This explosion of new factors on the scene changed the way that we view central regulation of food intake. It also became apparent that these factors regulate energy expenditure. Whilst the notion of adaptive thermogenesis had been understood for many years (Rothe 1975), it took some time to recognise that the so called ARP also control this. It was demonstrated that NPY promoted white fat lipid storage and reduced brown fat activity (Billington et al. 1991). The opposite effect was shown for melanocortins, derived from POMC (Hayes et al. 1999).

While it was recognised many years earlier, that brown adipose tissue was responsible for non-shivering thermogenesis (Haywards et al. 1967) and this discrete tissue bed became known as the hibernation gland, the mechanism for this important means of dissipating energy was not identified until 1980 (Klingenberg et al. 1980). These authors described the uncoupling process within mitochondria, and the entity responsible was, for a number of years, known as thermogenin (Jacobsson et al. 1994). It later became known universally as uncoupling protein-1 (UCP-1) (Matthias et al. 1999). The function of UCP-1 in brown adipose tissue is controlled by the sympathetic nervous system through the β-adrenergic system, particularly via β3 receptors. UCP-1 essentially 'steals' protons diverting energy away from the production of ATP to the generation and dissipation of heat (Susulic et al. 1995). We now know that a poly-synaptic pathway exists from the ARP neurons of the hypothalamus to white (Adler et al. 2012) and brown (Oldfield et al. 2002) fat. The only domestic species in which similar studies have been done is the pig, where it was shown that polysynaptic pathways exist between the leptin receptor expressing cells of the hypothalamus and the peri-renal fat (Czaja et al. 2003). This allows central regulation of peripheral thermogenesis and co-ordination of the control of food intake and energy expenditure. Recent data indicate that skeletal muscle has 'thermogenic' properties, also under the control of the sympathetic nervous system (Henry et al. 2011).

Not only do we now appreciate that food intake and energy expenditure are controlled by various centres in the hypothalamus, through the function of ARP, but it is also understood that other systems in the brain, such as those involved in reward and stress responses, also impact upon energy balance. In fact, it seems likely that every neurotransmitter and every neuropeptide in the brain has some effect on food intake and/or energy expenditure! Of course this means that an alteration in the function of any of these factors may lead to correction through another factor. A general rule is that brain peptides and/or transmitters that increase food intake also reduce energy expenditure and vice versa. This has not, however, been tested in ruminant species.

Peripheral regulators of food intake and energy expenditure

As indicated above, early studies implicated a number of hormones as regulators of food intake (Baile et al. 1974). Prominent amongst these was insulin, but the story is not simple, as outlined by (Morley 1987). The acute effect of insulin is to enhance feeding, whereas the chronic effect is to reduce eating. Clearly such effects also involve changes in circulating glucose levels. A classic study (Coleman et al 1969), using parabiosis of mice, revealed that unidentified circulating factors existed. Thus, by joining the circulation of animals that were obese (*db/db* mutants) to normal animals, the latter died of starvation. It was concluded that this was due to high levels of a circulating satiety factor in the *db/db* mutants. Pairing of *ob/ob* obese mice with *db/db* mice showed that the former reduced their food intake and became lean, again leading to the conclusion that there was a satiety factor that was present in the *db/db* mice that was absent in the *ob/ob* mice. The *ob* gene was eventually cloned in 1994, with the encoded protein being named leptin (Zhang et al. 1994). Although the *ob* gene had been recognised for many years, it was not until this classic finding that obesity in *ob/ob* mice was identified as a mutation in the leptin gene. The obesity phenotype of *db/db* mice was revealed as a mutation in the leptin receptor (Tartaglia et al. 1995).

Another important milestone was the discovery of ghrelin, which was achieved through screening for ligands which activated the receptor for growth hormone releasing peptide (Kojima et al. 1999). Although this receptor had been identified earlier (Howard et al. 1996), it was not until later that it became apparent that ghrelin is a circulating orexigen, potently stimulating feeding by action on this receptor (Tschop et al. 2000; Wren et al. 2000).

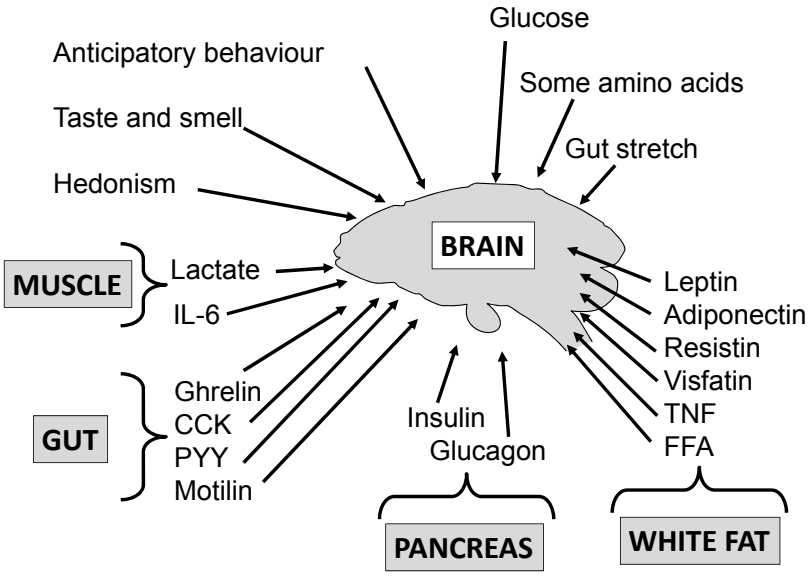
Leptin, ghrelin and insulin act on ARP neurons in the hypothalamus to regulate food intake and energy expenditure. These and other factors, such as sugar, fatty acids, cytokines and other circulating entities act in a co-ordinated manner, as well described in recent reviews (Langhans et al. 2009; Reichenbach et al. 2012). An outline of this system is seen in Figure 1. It is obvious that the control of food intake and energy expenditure is multifaceted and complicated. In order to understand this, and to manipulate it in any way, one needs to adopt an approach that takes account of as many factors as possible. Within the brain, these factors act on a multitude of orexigenic or anorexigenic ARP.

Changes during lactation of circulating factors that regulate appetite and energy expenditure

Physiological changes that occur in early lactation reflect negative energy balance verging on starvation! Leptin levels are lowered in lactation in various species (Smith et al. 2002), including sheep (Sorensen et al. 2002) and cows (Laeger et al. 2013; Wathes et al. 2007a). Plasma insulin and glucose levels are lower in early lactation than in late lactation and the post-prandial insulin response, which is marked in late lactation, is virtually non-existent in early lactation (Bradford et al. 2008; Laeger et al. 2013). Early lactating cows have markedly higher non-esterified fatty acid concentrations in plasma, with a further increase upon withdrawal from feeding, indicating mobilisation of body fat (Bradford et al. 2008; Laeger et al. 2013). Cortisol levels rose in response to a ghrelin injection in lactating cows, but not in non-lactating cows (Itoh et al. 2006).

The gut-derived metabolic regulators, glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide 1-(7-36) amide (GLP-1) and cholecystokinin (CCK) were measured in the plasma of dairy cows from 11 days before calving until 19 days after calving (Relling et al. 2007). It was found that the generalised rise in levels of all these peptides was associated with lower levels of insulin and glucose in early lactation. The consequence of this increase in secretion of gut peptides is certainly not to reduce food intake (Relling et al. 2007). Another gut peptide, ghrelin, is the only known peripheral stimulator of appetite. In one study, plasma ghrelin levels were seen to be markedly increased during early lactation in the dairy cow (Bradford et al 2008), but this was not

Figure 1 Factors that influence food intake and energy expenditure by action within the brain. IL-6 = Interleukin 6; CCK = Choleocystokinin; PYY = Peptide YY; TNF = Tumor necrosis factor; FFA = Free fatty acids.



seen in another study (Laeger et al. 2013). The secretion of ghrelin in dairy cows is suppressed by glucose challenge (Roche et al. 2008).

The very recent study of Laeger et al. (2013) has provided useful information on the transport of metabolic substrates and circulating regulatory factors into the central nervous system. In this study, cerebrospinal fluid (CSF) and plasma samples were analysed. The CSF was taken by sampling at the level of the 6th lumbar vertebra and the sacrum by needle biopsy, so the extent to which this represents levels in the brain is open to some question. If one accepts that levels of such factors are uniform throughout the CSF, then the data are important in terms of feedback signalling to the brain. CSF glucose levels fell, in parallel with the fall in plasma levels during early lactation and this may cause greater hunger drive. CSF concentrations of fatty acids were much lower than those in plasma and the changes in plasma were not mirrored in CSF, casting doubt on whether feedback is exerted on ARP neurons by these metabolic factors. Complex changes in amino acid levels were also reported, but the relevance of these to control of energy balance is not clear at this stage. Resistin, which is produced by fat, may also regulate metabolic function by central action, but levels did not change in early lactation (Laeger et al. 2013). Apart from these relatively sparse data on the metabolic indicators in dairy cows, there is no information on other regulators of metabolic function, appetite and energy expenditure, such as adiponectin or interleukins.

Impact of negative energy balance, including lactation, on hypothalamic appetite regulating peptides

The effect of alteration in live weight, especially negative energy balance, on the expression of genes for ARP has been studied extensively in at least one model ruminant, the sheep. Early work showed that reduction in live weight, by food restriction, increased expression of the gene for NPY in the arcuate nucleus (McShane et al. 1993), as well as immunoreactive NPY peptide (Barker-Gibb et al. 1996), expression of the POMC gene in the arcuate nucleus is lowered (McShane et al. 1993). The result for NPY has been replicated in other studies (Adam et al. 1997). Agouti-related peptide (AGRP) gene expression, which is co-localised to NPY neurons, was also increased in lean animals. The result for POMC gene expression with altered live weight is equivocal (Henry et al. 2000). We found no difference in expression of pre-pro-orexin or dynorphin gene expression in ovariectomised ewes which were either fat or lean because of dietary manipulation (Iqbal et al. 2003), but there were differences that were region-specific in the levels of enkephalin and CART gene expression (Henry et al. 2001b; Iqbal et al. 2003). Most certainly, the growth hormone axis is upregulated in the lean condition, due to increased expression of the gene for growth hormone releasing hormone neurons in the arcuate nucleus and reduced somatostatin gene expression (Henry et al. 2001c).

Interestingly, in mice at least, relatively few changes are seen in the production of ARP in the transition from a normal to an overweight condition (Enriori et al. 2007). This presumably indicates that there is little difference in the sensing of adequate or excess body stores, through leptin or insulin. Perhaps of greater relevance, is the effect on ARP in the hypothalamus of a dynamic change in the plane of nutrition. This was addressed in an elegant experiment conducted in sheep (Miller et al. 2007). Here, lean sheep were placed on an increasing plane of nutrition and fat sheep were given a decreasing plane of nutrition while Control animals were held static. The increasing plane led to a reduction in NPY/AgRP expression and an increase in POMC expression in the arcuate nucleus, relative to those on a declining plane. Those on the declining plane of nutrition showed the opposite effect. Plasma and CSF insulin and leptin levels increased in the animals on increasing plane of nutrition. The authors reasoned that these circulating factors drove the changes in ARP gene expression. Mean plasma luteinizing hormone (LH) levels and LH pulse frequency were higher in animals in an increasing plane of nutrition and lower in those on a declining plane of nutrition (Miller et al. 2007). It is possible that the dynamic period of negative energy balance that exists in the dairy cow in early lactation could cause similar changes to those seen in sheep on a declining plane of nutrition.

Lactation can be regarded as a state of negative energy balance. As such, the function of ARP at this time has been well studied in the rat (Smith et al. 2002). A profound drain of energy that is brought about by lactation causes cessation of reproduction (Brogan et al. 1999; Tsukamura et al. 2001). The effect in rats may be due to suckling, since pup-removal can restore LH levels (Smith et al. 2002; Tsukamura et al. 2001). Energy expenditure during lactation exceeds energy intake, so the body stores accumulated during pregnancy are lost (Naismith et al 1982). This could be the cause of reduced gonadotropin secretion. On the other hand, changes in circulating hormones, such as prolactin, and effects of altered levels of circulating metabolites, could be a factor in the lowered gonadotropin secretion in suckling animals. As reviewed (Smith et al. 2002), expression of genes for orexigenic peptides is upregulated during lactation and that for anorectic peptides is downregulated, consistent with the state of negative energy balance. Thus, in rats, the expression of the gene for the orexigenic peptide NPY is upregulated and POMC gene expression is down-regulated (Smith 2002). Levels of leptin receptor expression are also reduced in the hypothalamus (Brogan et al. 2000). The extent to which the situation in rats pertains to the dairy cow is debatable, especially since there has been systematic selection for production of milk in the latter. Recent studies comparing fertility in Holstein-Friesian cows in different parts of the world indicate that there are

inherent breed influences on the timing of resumption of reproductive function and fertility after calving. These involve factors other than body condition score or energy balance (Piccand et al. 2011). Whatever the cause, the fact that dairy cows can be successfully bred following GnRH treatment, points to a fundamental deficit in GnRH at the level of the hypothalamus.

An early study in sheep (Broad et al. 1993) showed a slight elevation in the level of gene expression in the hypothalamus for POMC during lactation, compared with late pregnancy. However, later work indicated that there was an increase in hypothalamic expression of the NPY and AGRP genes and reduction in POMC and CART gene expression (Sorensen et al. 2002). In addition, and contrary to the observations in rats, leptin receptor levels were increased in lactating ewes (Sorensen et al. 2002). This points to an important difference between rodents and ruminants and suggests that data from the former cannot necessarily be extrapolated to the latter.

Impact of negative energy balance, low live weight and lactation on reproductive function

Whereas marked changes in the expression of genes for ARP are seen with alteration in live weight, especially negative energy expenditure, the same is not true for GnRH gene expression or synthesis (Clarke et al. 2005). Nevertheless, reproduction is compromised in low energy states, so the point at which this is manifest is probably upstream of the GnRH neurons.

In terms of regulatory factors, insulin and leptin could play a significant role (Miller et al. 2007), but so could ghrelin, as discussed below. Wathes and colleagues (Wathes 2012), have presented a case for the growth hormone axis impacting upon the reproductive axis. This again could be effected via the neurons upstream of GnRH cells in the hypothalamus. In essence, the levels of insulin like growth factor (IGF)-1 fall after calving and those of IGF binding protein 2 rise after calving, so reduced IGF signalling could be important with respect to fertility. Whatever the case, the reduction in gonadotropin secretion in animals with reduced dietary energy can be overridden by administration of GnRH (Kile et al. 1991).

In terms of lactation and the associated negative energy balance in relation to reproduction, it is interesting that no effect of lactation was seen on the pulsatile secretion of LH in dairy cows (Canfield et al. 1991). Nevertheless, as indicated in the Introduction, the Ovsynch method, employing GnRH, clearly causes ovulation (Ayres et al. 2013), so there may well be a defect in the ovulatory mechanism in the early post-partum period. Studies in sheep showed that there is an enhanced negative feedback of estrogen on LH secretion in the early post-partum period (Wright et al. 1981) and failure, in more than 50% of ewes up to 28 days post-partum, of the positive feedback effect of estrogen, that causes the preovulatory LH surge (Wright et al. 1980).

Metabolic hormones, appetite regulating peptides and reproduction

Hormones

Leptin treatment of sheep that were hypo-gonadotropic, due to lean condition induced by dietary restriction, restored pulsatile LH secretion to normal (Henry et al. 2001a). Similarly, in human females, short-term starvation virtually eliminates pulsatile LH secretion that can be restored by leptin treatment (Chan et al. 2006). In the case of the hypogonadotropic, lean ewe, leptin treatment did not suppress appetite drive (Henry et al. 2001a). In animals of normal live weight, the effect of leptin was to suppress food intake but there was no effect on free running LH secretion, perhaps because endogenous leptin levels were signalling adequacy of body stores (Henry et al. 2001a). Intravenous leptin injections stimulated plasma LH levels in estrogen-treated, ovariectomised cows (Zieba et al. 2003), which may be due to direct effects at the level of the pituitary gland (Amstalden et al. 2003). Leptin treatment can also prevent the fasting-induced reduction in plasma LH levels in heifers, enhancing the pituitary response to GnRH (Maciel et al. 2004). Overall, these studies suggest that an adequate level of circulating leptin is required for normal function of the hypothalamo-pituitary axis that drives reproduction. A later study showed that pulsatile LH secretion could be restored in lean hypogonadotropic animals by refeeding, but this was not associated with an increase in leptin levels, calling into question the relevance of an acute regulatory effect of leptin (Szymanski et al. 2007). Changes in insulin levels and the circulating levels of metabolic fuels could be responsible for this acute effect on the reproductive axis (Szymanski et al. 2007). Indeed, infusion of propionate into the mesenteric artery has a short-term, but not a long-term, effect to restore LH levels in hypogonadotropic, lean ewes (Szymanski et al. 2011). Other studies, in rats, also indicate that 'physiological' levels of leptin do not restore gonadotropin levels in animals subjected to caloric restriction or a 48 hour fast (True et al. 2011). This was associated with a lack of effect of leptin to restore low levels of kisspeptin gene expression (True et al. 2011). As these authors point out, this goes against a large body of work showing that leptin is essential for reproduction and stimulates the gonadotropic axis in rodents. Regarding the latter, they point out that many studies have used supraphysiological doses of leptin. In any event, the ruminant may well be different to the rodent, since leptin treatment can partially restore kisspeptin levels in the arcuate nucleus of lean hypogonadotropic ewes as well as restoring gonadotropin secretion (Backholer et al. 2010a).

Insulin is another circulating hormone that appears to be essential for appropriate function of the reproductive axis. Central infusion of insulin stimulates LH secretion in sheep and insulin levels fall

in early lactation (Wathes et al. 2007a, b), which could contribute to low fertility at this time.

As mentioned above, ghrelin is a gut-derived hormone that acts centrally to stimulate appetite, but it also has a potent effect to suppress pulsatile LH secretion, so this could also play a role in suppression of reproductive function during lacatation (Iqbal et al. 2006).

Appetite regulating peptides

A general rule for the role of ARP in reproduction is that those which stimulate food intake also suppress reproduction and vice versa. Thus, NPY suppresses reproduction (Barker-Gibb et al. 1996), whilst melanocortins are stimulatory (Backholer et al. 2010a). AgRP, an orexigen, inhibits reproduction (Wu et al. 2012). Orexin inhibits GnRH function (Furuta et al. 2002; Tamura et al. 1999), although there are also indications of the opposite effect (Small et al. 2003). Melanin concentrating hormone, which is considered to be orexigenic, appears to stimulate reproductive function (Murray et al. 2006), so it is an exception to the general rule.

Hypothalamic peptides regulating reproduction

In recent years, there has been substantial revision of the central regulation of reproduction. In particular, the recognition that kisspeptin is a potent stimulator of GnRH secretion and kisspeptin cells mediate the feedback effects of sex steroids is now a central tenant of neuroendocrine theory (Clarke 2011). In cows, kisspeptin appears to stimulate the secretion of growth hormone as well as gonadotropins (Kadokawa et al. 2008). In contrast to the situation in the sheep, where kisspeptin regulates GnRH secretion (Smith et al. 2008), it appears to act on the pituitary gonadotropes in the cow (Ezzat et al. 2010). In sheep, kisspeptin gene expression is reduced in animals that are hypogonadotropic because of lean condition. This is partly corrected by leptin administration (Backholer et al. 2010b). Whether kisspeptin levels are reduced in the high-producing dairy cow, under conditions of negative energy balance, is unknown.

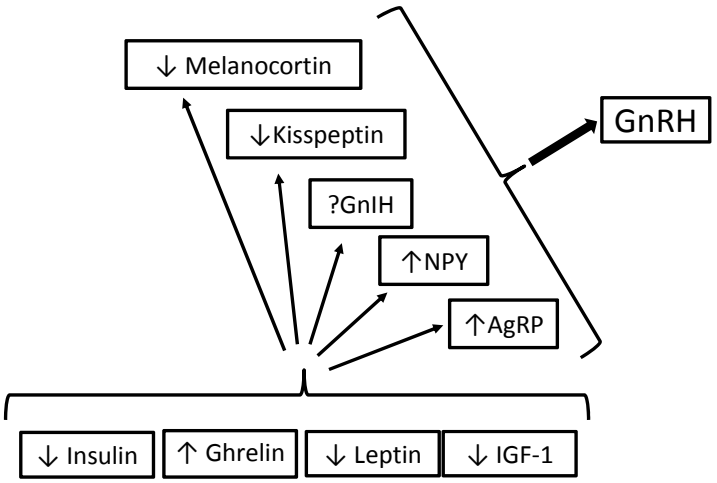
Another factor that has emerged as a regulator of reproduction is GnIH. This peptide, produced in the dorsomedial nucleus of the hypothalamus, is secreted into the hypophysial portal system and acts on pituitary gonadotropes to counter-act the effects of GnRH (Sari et al. 2009; Smith et al. 2008). Whereas secretion is higher in seasonal anestrous (Smith et al 2008), it is not known whether it is affected by negative energy balance. It is apparent, that chronic upregulation of cortisol levels in sheep leads to a doubling of GnIH gene expression (IJ Clarke, Unpublished data). RF9 is an antagonist of GnIH (Caraty et al. 2012), which is effective in sheep and may well be so in cows.

What about the dairy cow?

In the last 30 years, we have progressed from the point where appetite/energy expenditure barely rated mention in the physiology text books to a position where it is apparent that many factors may be integrated into a closed-circuit diagram for energy balance. Lessons from lactation in species other than cows, and information on the circuit-diagram in sheep under various metabolic states, might be applied to the dairy cow, for which there is limited information. The dairy cow may however, present a special case as has been argued above. Nevertheless, there is minimal information on central control of appetite/energy expenditure and virtually no knowledge of the integration of the energy and reproductive systems in the dairy cow. On one hand there are data to indicate that post-partum anestrus may be extended in the high producing dairy cow (Peter et al. 2009) and that this may be associated with perturbation within the neuroendocrine hypothalamus. On the other hand, comparisons between dairy cows in different countries such as between USA and New Zealand, and comparisons between beef and dairy cows, indicate high milk production does not necessarily equate with infertility.

In this review, I have summarised our knowledge of how reproduction is affected by circulating hormones and hypothalamic peptides that are also involved in energy balance. It is clear that we have significant knowledge of the interface in rodent species and sheep. Since negative energy balance can influence levels of ghrelin, insulin, leptin and

Figure 2 The relationship between metabolic hormones and hypothalamic peptides and the control of reproduction – impact of negative energy balance. Changes in circulating levels of insulin, ghrelin, leptin and IGF-1 act centrally to alter levels of melanocortins, kisspeptin, NPY and AgRP. In turn, these changes negatively impact on the function of GnRH neurons, leading to reduced reproductive performance.



metabolic substrates, this leads to altered levels of ARP and peptides in the hypothalamus that control reproductive function. The combined changes may have significant impact on the high producing dairy cow. Drawing on the information reviewed above, one can develop a working hypothesis for the lactating dairy cow. The impact of negative energy balance is summarised in Figure 2.

In order to achieve a better understanding of the special physiological state that exists in these uniquely selected animals, we need to obtain more information on the hypothalamic cells that have dual function in the regulation of energy balance and reproduction.

References

Adam CL, Findlay PA, Kyle CE, Young P, Mercer JG 1997. Effect of chronic food restriction on pulsatile luteinizing hormone secretion and hypothalamic neuropeptide Y gene expression in castrate male sheep. Journal of Endocrinology 152: 329–337.

Adler ES, Hollis JH, Clarke IJ, Grattan DR, Oldfield BJ 2012. Neurochemical characterization and sexual dimorphism of projections from the brain to abdominal and subcutaneous white adipose tissue in the rat. Journal of Neuroscience 32: 15913–15921.

Amstalden M, Zieba DA, Edwards JF, Harms PG, Welsh TH, Jr., Stanko RL, Williams GL 2003. Leptin acts at the bovine adenohypophysis to enhance basal and gonadotropin-releasing hormone-mediated release of luteinizing hormone: differential effects are dependent upon nutritional history. Biology of Reproduction 69: 1539–1544.

Anand BK, Brobeck JR 1951. Hypothalamic control of food intake in rats and cats. The Yale Journal of Biology and Medicine 24: 123–140.

Ayres H, Ferreira RM, Cunha AP, Araujo RR, Wiltbank MC 2013. Double-Ovsynch in high-producing dairy cows: effects on progesterone concentrations and ovulation to GnRH treatments. Theriogenology 79: 159–164.

Backholer K, Bowden M, Gamber K, Bjorbaek C, Iqbal J, Clarke IJ 2010a. Melanocortins mimic the effects of leptin to restore reproductive function in lean hypogonadotropic ewes. Neuroendocrinology 91: 27–40.

Backholer K, Smith JT, Rao A, Pereira A, Iqbal J, Ogawa S, Li Q, Clarke IJ 2010b. Kisspeptin cells in the ewe brain respond to leptin and communicate with neuropeptide Y and proopiomelanocortin cells. Endocrinology 151: 2233–2243.

Baile CA, Forbes JM 1974. Control of feed intake and regulation of energy balance in ruminants. Physiological Reviews 54: 160–214.

Barker-Gibb ML, Clarke IJ 1996. Increased galanin and neuropeptide-Y immunoreactivity within the hypothalamus of ovariectomised ewes following a prolonged period of reduced body weight is associated with changes in plasma growth hormone but not gonadotropin levels. Neuroendocrinology 64: 194–207.

Barker-Gibb ML, Scott CJ, Boublik JH, Clarke IJ 1995. The role of neuropeptide Y (NPY) in the control of LH secretion in the ewe with respect to season, NPY receptor subtype and the site of action in the hypothalamus. Journal of Endocrinology 147: 565–579.

Berne RM, Levy MN 1983. Physiology. St Louis, USA: CV Mosby & Co. 165p.

Bierman EL, Hirsch J 1981. Obesity. In: Williams RH ed. Textbook of endocrinology. Philadelphia, USA: W.B.Saunders & Co. Pg. 907–921.

Billington CJ, Briggs JE, Grace M, Levine AS 1991. Effects of intracerebroventricular injection of neuropeptide Y on energy metabolism. American Journal of Physiology 260: R321–327.

Blessing WW 1997. The lower brainstem and bodily homeostasis. New York, USA: Oxford University Press. 579p.

Bradford BJ, Allen MS 2008. Negative energy balance increases periprandial ghrelin and growth hormone concentrations in lactating dairy cows. Domestic Animal Endocrinology 34: 196–203.

Brady LS, Smith MA, Gold PW, Herkenham M 1990. Altered expression of hypothalamic neuropeptide mRNAs in food-restricted and food-deprived rats. Neuroendocrinology 52: 441–447.

Broad KD, Kendrick KM, Sirinathsinghji DJ, Keverne EB 1993. Changes in pro-opiomelanocortin and pre-proenkephalin mRNA levels in the ovine brain during pregnancy, parturition and lactation and in response to oestrogen and progesterone. Journal of Neuroendocrinology 5: 711–719.

Brogan RS, Grove KL, Smith MS 2000. Differential regulation of leptin receptor but not orexin in the hypothalamus of the lactating rat. Journal of Neuroendocrinology 12: 1077–1086.

Brogan RS, Mitchell SE, Trayhurn P, Smith MS 1999. Suppression of leptin during lactation: contribution of the suckling stimulus versus milk production. Endocrinology 140: 2621–2627.

Canfield RW, Butler WR 1991. Energy balance, first ovulation and the effects of naloxone on LH secretion in early postpartum dairy cows. Journal of Animal Science 69: 740–746.

Caraty A, Blomenrohr M, Vogel GM, Lomet D, Briant C, Beltramo M 2012. RF9 powerfully stimulates gonadotrophin secretion in the ewe: Evidence for a seasonal threshold of sensitivity. Journal of Neuroendocrinology 24: 725–736.

Chan JL, Matarese G, Shetty GK, Raciti P, Kelesidis I, Aufiero D, De Rosa V, Perna F, Fontana S, Mantzoros CS 2006. Differential regulation of metabolic, neuroendocrine, and immune function by leptin in humans. Proceedings of the National Academy of Sciences of the United States of America 103: 8481–8486.

Clark JT, Kalra PS, Crowley WR, Kalra SP 1984. Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. Endocrinology 115: 427–429.

Clarke IJ 2011. Control of GnRH secretion: one step back. Frontiers in Neuroendocrinology 32: 367–375.

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

Clarke IJ, Tilbrook AJ 2009. Gonadotropin, neural and hormonal control. In: Squire L ed. New

encyclopedia of neuroscience. Amsterdam: Elsevier Press. Pg. 959-965.

Coleman DL, Hummel KP 1969. Effects of parabiosis of normal with genetically diabetic mice. American Journal of Physiology 217: 1298–1304.

Czaja K, Kraeling RR, Barb CR 2003. Are hypothalamic neurons transsynaptically connected to porcine adipose tissue? Biochemical and Biophysical Research Communications 311: 482–485.

Dukes HH 1955. The physiology of domestic animals. London, UK: Bailliere, Tindall and Cox. 1020p.

Enriori PJ, Evans AE, Sinnayah P, Jobst EE, Tonelli-Lemos L, Billes SK, Glavas MM, Grayson BE, Perello M, Nillni EA, Grove KL, Cowley MA 2007. Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons. Cell Metabolism 5: 181–194.

Ezzat AA, Saito H, Sawada T, Yaegashi T, Goto Y, Nakajima Y, Jin J, Yamashita T, Sawai K, Hashizume T 2010. The role of sexual steroid hormones in the direct stimulation by Kisspeptin-10 of the secretion of luteinizing hormone, follicle-stimulating hormone and prolactin from bovine anterior pituitary cells. Animal Reproduction Science 121: 267–272.

Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD 1997. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature 385: 165–168.

Furuta M, Funabashi T, Kimura F 2002. Suppressive action of orexin A on pulsatile luteinizing hormone secretion is potentiated by a low dose of estrogen in ovariectomized rats. Neuroendocrinology 75: 151–157.

Gibbs J, Young RC, Smith GP 1973. Cholecystokinin decreases food intake in rats. Journal of Comparative and Physiological Psychology 84: 488–495.

Gumen A, Keskin A, Yilmazbas-Mecitoglu G, Karakaya E, Alkan A, Okut H, Wiltbank MC 2012. Effect of presynchronization strategy before Ovsynch on fertility at first service in lactating dairy cows. Theriogenology 78: 1830–1838.

Hansen LB 2000. Consequences of selection for milk yield from a geneticist's viewpoint. Journal of Dairy Science 83: 1145–1150.

Haynes WG, Morgan DA, Djalali A, Sivitz WI, Mark AL 1999. Interactions between the melanocortin system and leptin in control of sympathetic nerve traffic. Hypertension 33: 542–547.

Hayward JS, Lyman CP 1967. Nonshivering heat production during arousal from hibernation and evidence for the contribution of brown fat. In: Fisher KC, Dawe AR, Lyman CP, Schonbaum E, South FE eds. Mammalian hibernation III. Ediburgh, Scotland: Oliver & Boyd. Pg. 346-355.

Henry BA, Andrews ZB, Rao A, Clarke IJ 2011. Central leptin activates mitochondrial function and increases heat production in skeletal muscle. Endocrinology 152: 2609–2618.

Henry BA, Goding JW, Tilbrook AJ, Dunshea FR, Clarke IJ 2001a. Intracerebroventricular infusion of leptin elevates the secretion of luteinising hormone without affecting food intake in long-term food-restricted sheep, but increases growth hormone irrespective of bodyweight. Journal of Endocrinology 168: 67–77.

Henry BA, Rao A, Ikenasio BA, Mountjoy KG, Tilbrook AJ, Clarke IJ 2001b. Differential expression of cocaine- and amphetamine-regulated transcript and agouti related-protein in chronically food-restricted sheep. Brain Research 918: 40–50.

Henry BA, Rao A, Tilbrook AJ, Clarke IJ 2001c. Chronic food-restriction alters the expression of somatostatin and growth hormone-releasing hormone in the ovariectomised ewe. Journal of Endocrinology 170: R1–5.

Henry BA, Tilbrook AJ, Dunshea FR, Rao A, Blache D, Martin GB, Clarke IJ 2000. Long-term alterations in adiposity affect the expression of melanin-concentrating hormone and enkephalin but not proopiomelanocortin in the hypothalamus of ovariectomized ewes. Endocrinology 141: 1506–1514.

Hetherington AW, Ranson SW 1939. Experimental hypothalamo-hypophyseal obesity in the rat. Proceedings of the Royal Society for Experimental Biology and Medicine 41: 465–466.

Howard AD, Feighner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI, Hamelin M, Hreniuk DL, Palyha OC, Anderson J, Paress PS, Diaz C, Chou M, Liu KK, McKee KK, Pong SS, Chaung LY, Elbrecht A, Dashkevicz M, Heavens R, Rigby M, Sirinathsinghji DJ, Dean DC, Melillo DG, Patchett AA, Nargund R, Griffin PR, DeMartino JA, Gupta SK, Schaeffer JM, Smith RG, Van der Ploeg LH 1996. A receptor in pituitary and hypothalamus that functions in growth hormone release. Science 273: 974–977.

Iqbal J, Henry BA, Pompolo S, Rao A, Clarke IJ 2003. Long-term alteration in bodyweight and food restriction does not affect the gene expression of either preproorexin or prodynorphin in the sheep. Neuroscience 118: 217–226.

Iqbal J, Kurose Y, Canny B, Clarke IJ 2006. Effects of central infusion of ghrelin on food intake and plasma levels of growth hormone, luteinizing hormone, prolactin, and cortisol secretion in sheep. Endocrinology 147: 510–519.

Itoh F, Komatsu T, Kushibiki S, Hodate K 2006. Effects of ghrelin injection on plasma concentrations of glucose, pancreatic hormones and cortisol in Holstein dairy cattle. Comparative Biochemistry and Physiology Part A, Molecular and Integrative Physiology 143: 97–102.

Jacobsson A, Muhleisen M, Cannon B, Nedergaard J 1994. The uncoupling protein thermogenin during acclimation: indications for pretranslational control. American Journal of Physiology 267: R999–1007.

Kadokawa H, Matsui M, Hayashi K, Matsunaga N, Kawashima C, Shimizu T, Kida K, Miyamoto A 2008. Peripheral administration of kisspeptin-10 increases plasma concentrations of GH as well as LH in prepubertal Holstein heifers. Journal of Endocrinology 196: 331–334.

Kile JP, Alexander BM, Moss GE, Hallford DM, Nett TM 1991. Gonadotropin-releasing hormone overrides the negative effect of reduced dietary

energy on gonadotropin synthesis and secretion in ewes. Endocrinology 128: 843–849.

Klingenberg M, Hackenberg H, Kramer R, Lin CS, Aquila H 1980. Two transport proteins from mitochondria: I. Mechanistic aspects of asymmetry of the ADP, ATP translocator, II. The uncoupling protein of brown adipose tissue mitochondria. Annals of the New York Academy of Sciences 358: 83–95.

Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402: 656–660.

Laeger T, Sauerwein H, Tuchscherer A, Bellmann O, Metges CC, Kuhla B 2013. Concentrations of hormones and metabolites in cerebrospinal fluid and plasma of dairy cows during the periparturient period. Journal of Dairy Science. 96: 2883–2893.

Langhans W, Harrold J, Williams G, Geary N 2009. Control of eating. In: Williams G, Fruhbeck G eds. Obesity: Science to Practice. Chichester, UK: J. Wiley & Sons. Pg. 127-163.

Levine AS, Morley JE 1984. Neuropeptide Y: a potent inducer of consummatory behavior in rats. Peptides 5: 1025–1029.

Maciel MN, Zieba DA, Amstalden M, Keisler DH, Neves JP, Williams GL 2004. Leptin prevents fasting-mediated reductions in pulsatile secretion of luteinizing hormone and enhances its gonadotropin-releasing hormone-mediated release in heifers. Biology of Reproduction 70: 229–235.

Matthias A, Jacobsson A, Cannon B, Nedergaard J 1999. The bioenergetics of brown fat mitochondria from UCP1-ablated mice. Ucp1 is not involved in fatty acid-induced de-energization ("uncoupling"). Journal of Biological Chemistry 274: 28150–28160.

McShane TM, Petersen SL, McCrone S, Keisler DH 1993. Influence of food restriction on neuropeptide-Y, proopiomelanocortin, and luteinizing hormone-releasing hormone gene expression in sheep hypothalami. Biology of Reproduction 49: 831–839.

Miller DW, Harrison JL, Bennett EJ, Findlay PA, Adam CL 2007. Nutritional influences on reproductive neuroendocrine output: insulin, leptin, and orexigenic neuropeptide signaling in the ovine hypothalamus. Endocrinology 148: 5313–5322.

Morley JE 1987. Neuropeptide regulation of appetite and weight. Endocrine Reviews 8: 256–287.

Murray JF, Hahn JD, Kennedy AR, Small CJ, Bloom SR, Haskell-Luevano C, Coen CW, Wilson CA 2006. Evidence for a stimulatory action of melanin-concentrating hormone on luteinising hormone release involving MCH1 and melanocortin-5 receptors. Journal of Neuroendocrinology 18: 157–167.

Naismith DJ, Richardson DP, Pritchard AE 1982. The utilization of protein and energy during lactation in the rat, with particular regard to the use of fat accumulated in pregnancy. British Journal of Nutrition 48: 433–441.

Oldfield BJ, Giles ME, Watson A, Anderson C, Colvill LM, McKinley MJ 2002. The neurochemical characterisation of hypothalamic pathways projecting polysynaptically to brown adipose tissue in the rat. Neuroscience 110: 515–526.

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

Piccand V, Meier S, Cutullic E, Weilenmann S, Thomet P, Schori F, Burke CR, Weiss D, Roche JR, Kunz PL 2011. Ovarian activity in Fleckvieh, Brown Swiss and two strains of Holstein-Friesian cows in pasture-based, seasonal calving dairy systems. Journal of Dairy Research 78: 464–470.

Pursley JR, Mee MO, Wiltbank MC 1995. Synchronization of ovulation in dairy cows using PGF2alpha and GnRH. Theriogenology 44: 915–923.

Rehfeld JF 1978. Immunochemical studies on cholecystokinin. I. Development of sequence-specific radioimmunoassays for porcine triacontatriapeptide cholecystokinin. Journal of Biological Chemistry 253: 4016–4021.

Reichenbach A, Stark R, Andrews ZB 2012. Hypothalamic control of appetite and energy metabolism. In: Dudas B ed. The human hypothalamus: anatomy, functions and disease: Hauppauge, New York, USA: NOVA Publishers. Pg. 247–282.

Relling AE, Reynolds CK 2007. Plasma concentrations of gut peptides in dairy cattle increase after calving. Journal of Dairy Science 90: 325–330.

Roberts JL, Herbert E 1977. Characterization of a common precursor to corticotropin and beta-lipotropin: identification of beta-lipotropin peptides and their arrangement relative to corticotropin in the precursor synthesized in a cell-free system. Proceedings of the National Academy of Sciences of the United States of America 74: 5300–5304.

Roche JR, Sheahan AJ, Chagas LM, Boston RC 2008. Short communication: change in plasma ghrelin in dairy cows following an intravenous glucose challenge. Journal of Dairy Science 91: 1005–1010.

Rothe CF 1975. Regulation of visceral function B. Homeostasis and negative feedback control. In: Selkurk EE ed. Physiology. Boston, USA: Little, Brown & Co. Pg. 201–207.

Santos JEP, Bisinotto RS, Ribeiro ES, Lima FS, Greco LF, Staples CR, Thatcher WW 2010. Reproduction in domestic ruminants VII. In: Lucy MC, Pate JL, Smith MF, Spencer TE eds. Nottingham, UK: Nottingham University Press. Pg. 387–403.

Sari IP, Rao A, Smith JT, Tilbrook AJ, Clarke IJ 2009. Effect of RF-amide-related peptide-3 on luteinizing hormone and follicle-stimulating hormone synthesis and secretion in ovine pituitary gonadotropes. Endocrinology 150: 5549–5556.

Small CJ, Goubillon ML, Murray JF, Siddiqui A, Grimshaw SE, Young H, Sivanesan V, Kalamatianos T, Kennedy AR, Coen CW, Bloom SR, Wilson CA 2003. Central orexin A has site-specific effects on luteinizing hormone release in female rats. Endocrinology 144: 3225–3236.

Smith JT, Rao A, Pereira A, Caraty A, Millar RP, Clarke IJ 2008. Kisspeptin is present in ovine hypophysial portal blood but does not increase during the preovulatory luteinizing hormone surge: evidence that gonadotropes are not direct targets of kisspeptin in vivo. Endocrinology 149: 1951–1959.

Smith MS 1993. Lactation alters neuropeptide-Y and proopiomelanocortin gene expression in the arcuate nucleus of the rat. Endocrinology 133: 1258–1265.

Smith MS, Grove KL 2002. Integration of the regulation of reproductive function and energy balance: lactation as a model. Frontiers in Neuroendocrinology 23: 225–256.

Sorensen A, Adam CL, Findlay PA, Marie M, Thomas L, Travers MT, Vernon RG 2002. Leptin secretion and hypothalamic neuropeptide and receptor gene expression in sheep. American Journal of Physiology – Regulatory, Integrative and Comparatie Physiology 282: R1227–1235.

Susulic VS, Frederich RC, Lawitts J, Tozzo E, Kahn BB, Harper ME, Himms-Hagen J, Flier JS, Lowell BB 1995. Targeted disruption of the beta 3-adrenergic receptor gene. Journal of Biological Chemistry 270: 29483–29492.

Szymanski LA, Schneider JE, Friedman MI, Ji H, Kurose Y, Blache D, Rao A, Dunshea FR, Clarke IJ 2007. Changes in insulin, glucose and ketone bodies, but not leptin or body fat content precede restoration of luteinising hormone secretion in ewes. Journal of Neuroendocrinology 19: 449–460.

Szymanski LA, Schneider JE, Satragno A, Dunshea FR, Clarke IJ 2011. Mesenteric infusion of a volatile fatty acid prevents body weight loss and transiently restores luteinising hormone pulse frequency in ovariectomised, food-restricted ewes. Journal of Neuroendocrinology 23: 699–710.

Tamura T, Irahara M, Tezuka M, Kiyokawa M, Aono T 1999. Orexins, orexigenic hypothalamic neuropeptides, suppress the pulsatile secretion of luteinizing hormone in ovariectomized female rats. Biochemical and Biophysical Research Communications 264: 759–762.

Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Wool EA, Monroe CA, Tepper RI 1995. Identification and expression cloning of a leptin receptor, OB-R. Cell 83: 1263–1271.

Tatemoto K, Carlquist M, Mutt V 1982. Neuropeptide Y--a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature 296: 659–660.

True C, Kirigiti M, Ciofi P, Grove KL, Smith MS 2011. Characterisation of arcuate nucleus kisspeptin/neurokinin B neuronal projections and regulation during lactation in the rat. Journal of Neuroendocrinology 23: 52–64.

Tschop M, Smiley DL, Heiman ML 2000. Ghrelin induces adiposity in rodents. Nature 407: 908–913.

Tsukamura H, Maeda K 2001. Non-metabolic and metabolic factors causing lactational anestrus: rat models uncovering the neuroendocrine mechanism underlying the suckling-induced changes in the mother. Progress in Brain Research 133: 187–205.

Wathes DC 2012. Mechanisms linking metabolic status and disease with reproductive outcome in the dairy cow. Reproduction in Domestic Animals = Zuchthygiene 47 Supplement 4: 304–312.

Wathes DC, Bourne N, Cheng Z, Mann GE, Taylor VJ, Coffey MP 2007a. Multiple correlation analyses of metabolic and endocrine profiles with fertility in primiparous and multiparous cows. Journal of Dairy Science 90: 1310–1325.

Wathes DC, Cheng Z, Bourne N, Taylor VJ, Coffey MP, Brotherstone S 2007b. Differences between primiparous and multiparous dairy cows in the inter-relationships between metabolic traits, milk yield and body condition score in the periparturient period. Domestic Animal Endocrinology 33: 203–225.

Williams RH 1981. Gastrointestinal hormones. In: Williams RH ed. Textbook of Endocrinology: Philadelphia, USA: W.B. Saunders. Pg. 685-715.

Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DG, Ghatei MA, Bloom SR 2000. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology 141: 4325–4328.

Wright PJ, Geytenbeek PE, Clarke IJ, Findlay JK 1980. Pitutary responsiveness to LH-RH, the occurrence of oestradiol- 17 beta-induced LH-positive feedback and the resumption of oestrous cycles in ewes post partum. Journal of Reproduction and Fertility 60: 171–176.

Wright PJ, Geytenbeek PE, Clarke IJ, Findlay JK 1981. Evidence for a change in oestradiol negative feedback and LH pulse frequency in post-partum ewes. Journal of Reproduction and Fertility 61: 97–102.

Wu Q, Whiddon BB, Palmiter RD 2012. Ablation of neurons expressing agouti-related protein, but not melanin concentrating hormone, in leptin-deficient mice restores metabolic functions and fertility. Proceedings of the National Academy of Sciences of the United States of America 109: 3155–3160.

Yilmazbas-Mecitoglu G, Karakaya E, Keskin A, Alkan A, Okut H, Gumen A 2012. Effects of presynchronization with gonadotropin-releasing hormone-prostaglandin F2alpha or progesterone before Ovsynch in noncyclic dairy cows. Journal of Dairy Science 95: 7186–7194.

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM 1994. Positional cloning of the mouse obese gene and its human homologue. Nature 372: 425–432.

Zieba DA, Amstalden M, Maciel MN, Keisler DH, Raver N, Gertler A, Williams GL 2003. Divergent effects of leptin on luteinizing hormone and insulin secretion are dose dependent. Experimental Biology and Medicine 228: 325–330.