Potential signals mediating the maintenance of reproductive activity during the non-breeding season of the mare

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The seasonal nature of reproductive activity in mares is widely accepted and considerable attention has focused on the mechanisms that lead to the initiation of the breeding season. In contrast, considerably less information is available about the termination of the breeding season. It is interesting to note that each winter a sub-population of mares continues to undergo oestrous cyclicity during the non-breeding season. Continuation of reproductive activity during the winter occurs most frequently in mares that maintain a non-pregnant condition in successive years. The maintenance of a non-pregnant condition in successive years leads to an increase in the percentage of total body fat and it has been proposed that the degree of adiposity may be a determinant of reproductive activity during the winter months. To investigate this hypothesis we have manipulated fat stores by either pharmacological treatments or feed restriction. The studies described in this review demonstrate that manipulation of body fat during the autumn months fails to modify the mechanisms that lead to anoestrus or the proportion of mares that continue to show oestrous cyclicity during the winter months. On the basis of these and related studies two hypotheses are presented that may serve as a template for future work. The first hypothesis proposes that one aspect of the long-term regulation of seasonal reproductive rhythms in mares, specifically anoestrus, may reflect recognition of the availability of metabolic fuels before perception of a change in photoperiod. Alternatively, energy availability may need to reach a critical value before a presumptive inhibitory daylength signal initiates termination of the breeding season. This review describes previous and current studies that have led to development of these proposals.

Introduction

The majority of mammals living in the temperate zones show a seasonal pattern of reproductive activity ensuring that offspring are born at a time of year that favours their
survival. In the Northern Hemisphere, the natural breeding season for mares is from April to late September (Hughes et al., 1975). Short daylengths during the winter months are generally considered to be a period of reproductive inactivity, characterized by reduced follicular development and the absence of ovulation. However, each winter, a proportion of mares fail to cease ovarian cyclicity. Thus, the neuroendocrine events that lead to a decrease in GnRH and gonadotrophin secretion, and ultimately anoestrous, are disrupted in this sub-population of mares. The mechanisms that underlie this phenomenon are not completely understood.

In many breeding associations, including Thoroughbreds, the designated birthdate for an foals born in the same season is 1 January. This regulation provides an incentive for breeders to produce foals early in the year, thus maximizing traits associated with increased foal maturity, compared with those born later in the year. An understanding of the mechanisms that lead a proportion of mares to show reproductive activity continuously throughout the winter months might allow the development of new methods to manipulate the timing of reproductive activity to coincide more closely with the timing of the designated breeding season. In the Northern Hemisphere, this designated period encompasses the months between February and early July. In view of these observations, several laboratories are engaged in studies to elucidate the mechanisms that control continuation of oestrous cyclicity throughout the winter months. This review describes preliminary data from several ongoing studies that focus primarily on the role of adipose-related hormones and their potential role as signals permitting continuation of oestrous cyclicity during the non-breeding season.

Seasonal anoestrous: photoperiodic versus metabolic signals

In many species of seasonal breeding mammals, the importance of photoperiod as a modulator of seasonal reproductive rhythms is well established. Investigation of the relationship between changes in daylength and reproductive activity in mares has also received considerable attention and several reviews summarize current understanding (Irvine, 1996; Nagy et al., 2000). The importance of the timing of onset of the breeding season for the Thoroughbred breeding industry and the associated development of methods to change its timing have provided a major impetus for an accumulation of information concerning the mechanisms controlling the onset of the breeding season. In contrast, there is a paucity of information with respect to our understanding of the mechanisms controlling the onset of anoestrous. Undoubtedly, this lack of information reflects not only the bias of investigators to focus on the onset of the breeding season, but also because concepts derived from studies on the onset of the breeding season have been extrapolated to account for the cessation of the breeding season. For example, increasing daylength is associated with the onset of reproductive activity and the timing of the onset can be advanced by an abrupt, artificial increase in daylength. A logical supposition of this finding is that a decrease in daylength is responsible for timing the cessation of reproductive cycles. However, evidence to support this concept is confounded by variable responses of mares to either a natural or an artificial reduction in daylength (Kooistra and Ginther, 1975; Scraba and Ginther, 1985; Guillaume and Palmer, 1992). Furthermore, several studies have demonstrated that mares may become reproductively inactive during the winter months when daylength is increasing in January and February ( Fitzgerald and Schmidt, 1995; Nagy et al., 1998). Similar variable responses in reproductive activity have been observed in mares treated with exogenous melatonin to provide a presumptive inhibitory signal to the hypothalamus–pituitary axis (Guillaume et al., 1995; Fitzgerald and McManus, 2000). The concept that similar mechanisms underlie the onset or cessation of seasonal reproductive activity may be challenged further by numerous studies in other seasonally breeding mammals that demonstrate that the onset and the
Control of seasonal anoestrous in mares

The finding that a decrease in daylength, or a presumptive inhibitory melatonin cue, is associated with a variable reproductive response indicates that factors in addition to daylength may interact to control the onset of anoestrus. Support for this proposal is forthcoming from several observations. Firstly, the proportion of young mares (<5 years of age) that enter anoestrus during the winter months is considerably greater than that which occurs in older mares (Palmer and Driancourt, 1983). Secondly, the degree of adiposity may determine the response of the hypothalamus-pituitary-ovarian axis to photoperiod, as a greater proportion of lean mares than fat mares enter anoestrus (Fitzgerald and McManus, 2000). As aging and increasing body fat appear to be related temporally (Hintz, 1993), published studies have not distinguished the relative importance of each variable. However, several unpublished observations indicate that age *per se* may not be an important factor. For example, regardless of age, pregnancy and lactation are highly energy demanding events and invariably lead to cessation of reproductive activity during the winter months (Palmer and Driancourt, 1983). In addition, the propensity of a proportion of mares to show oestrous cycles during the winter months is not associated with age-related deficiencies in melatonin secretion (B. P. Fitzgerald, unpublished). Finally, continuation of oestrous cycles during the winter months may in fact occur relatively early in life, within 5 years of age, in mares that maintain a non-pregnant condition in successive years (Guillaume et al., 2001). Collectively, the available data indicate, albeit by association, that the changes in the availability of metabolic fuels with energy expenditure (for example, pregnancy and lactation) and storage (increased fat deposition in mature mares) play a role in the manifestation of oestrous cycles during the winter months. In an effort to unravel the complex mechanisms controlling the disruption of seasonal anoestrus, our initial studies have focused on the role of metabolic hormones and their role in regulating the availability of metabolic fuel.

**Adipogenic hormones: their potential role in the long-term regulation of seasonal reproductive activity in mares**

Increased adipose stores are associated with increased circulating concentrations of several hormones that may affect the mechanisms that allow the continuation of reproductive activity in mares during the winter months. However, caution should be stressed regarding the interpretation of associations between fatness, adipogenic hormones and reproductive activity, as these factors probably only provide a signal to the central nervous system regarding nutrient status and, thus, should be considered as intermediaries of the recognition of energy status. The change in adipogenic hormones occurs in response to the primary sensory signal relating to body fat mass. With this reservation in mind, we have attempted to modify seasonal reproductive activity by pharmacological or physiological manipulations of two adipogenic hormones, namely leptin and insulin, that may be intermediary signals of energy status to the brain.

**Leptin**

The discovery of leptin (Zhang et al., 1994) and its secretion by adipose tissue promptly stimulated a plethora of studies into the role of this hormone in the regulation of appetite regulation and obesity (Hirschberg, 1998; Casanueva and Dieguez, 1999; Clarke and Henry, 1999; Williams et al., 2000; Clarke et al., 2001). Recently, leptin synthesis and secretion have been demonstrated in a variety of tissues and diverse biological effects have been identified (Ahima and Flier, 2000; Ahima et al., 2000). Interest in the role of leptin in reproduction was
initiated after the demonstration that infertile ob/ob mutant mice which lack the ability to produce leptin could be made fertile by injection of leptin (Chehab et al., 1996; Chehab, 1997).

An apparent association between leptin and reproductive activity in the mare was inferred from the observation that, in mature mares, higher amounts of body fat were associated with high circulating concentrations of leptin during the summer and autumn months when mares are reproductively active. Furthermore, mature, fat mares were more likely to show continued oestrous cycles during the winter than were young, lean mares (Fitzgerald and McManus, 2000). In the absence of the availability of equine leptin protein to test directly the role of leptin in the mechanisms controlling the onset of anoestrus, we have investigated the effect of pharmacological manipulation of circulating leptin concentrations.

Adipose tissue is innervated by the sympathetic nervous system and drugs known to exert \( \beta \)-adrenergic activity have been shown to modify leptin secretion, both in vitro and in vivo (Li et al., 1997, 1998; Kumar et al., 1999; Sivitz et al., 1999; Scriba et al., 2000). Clenbuterol, a \( \beta \)-adrenergic agonist, has been used in animal studies as a repartitioning agent (Ricks et al., 1984; Peters, 1989). Significant increases in skeletal muscle protein, as well as decreases in body fat, characterize the repartitioning action of clenbuterol. The occurrence of this tissue redistribution is dissociated from any increase in food consumption, as shown in studies using laboratory animals (Reeds et al., 1988). Thus, clenbuterol administration should provide a method to manipulate adipose tissue stores indirectly affecting circulating concentrations of leptin. This possibility and the role of leptin in the onset of anoestrus in the mare was investigated by administering clenbuterol (3.2 \( \mu \)g kg\(^{-1}\) i.v.) each day to eight mares for 103 days, beginning in the late breeding season (mid-October) and ending in late January (McManus and Fitzgerald, 1999). As predicted from rodent studies, administration of clenbuterol reduced the percentage of body fat compared with that of untreated controls and this reduction was accompanied by a decrease in circulating leptin concentrations (Fig. 1). However, despite significant reductions in the estimated percentage of body fat and leptin, the timing of anoestrus was not different between clenbuterol-treated and untreated mares.

In addition, the use of clenbuterol to manipulate adipose tissue mass and circulating concentrations of leptin may evoke significant changes in other hormones that may affect reproduction. For example, recent studies in the obese Zucker rat demonstrated that administration of clenbuterol markedly reduced insulin resistance in this experimental model (Pan et al., 2001). In a study in mares (Fitzgerald and McManus, 2000), insulin concentrations were modified by administration of clenbuterol and were clearly suppressed during treatment and for several weeks after treatment compared with those of untreated controls (Fig. 2). The suppression of insulin that accompanies treatment with clenbuterol may reflect an indirect effect associated with the reduction of adipose tissue. This explanation may similarly account for the lower circulating concentrations observed after treatment. Alternatively, the suppression of insulin during treatment may reflect a direct action on insulin release from the pancreas via sympathetic stimulation. However, this latter suggestion may not account adequately for the continued suppression of insulin after cessation of treatment.

Although equine leptin protein is not currently available for experimental investigation, the reported protein structure shares about 80% similarity with human leptin. In several species, administration of recombinant human leptin (rh-leptin) has biological effects (for example, suppression of appetite; Henry et al., 1999). Furthermore, in sheep, the feed-restriction-induced suppression of LH secretion has been partially restored as demonstrated in some studies by central or peripheral administration of rh-leptin (Nagatani et al., 2000; Henry et al., 2001). However, there is no general agreement between reports, as similar studies using central administration of recombinant ovine leptin failed to modify LH secretion (Morrison et al., 2001). Despite these limitations, we investigated the effect of peripheral administration of rh-leptin...
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![Graph showing changes in serum leptin concentrations](image)

**Fig. 1.** Changes in mean (± SEM) serum leptin concentrations in mares (n = 8) treated with clenbuterol (3.2 μg kg⁻¹, i.e.; •) each day and in untreated mares (n = 6; ○). The horizontal bar indicates the duration of clenbuterol administration. From a preliminary report (McManus and Fitzgerald, 1999).

![Graph showing changes in circulating insulin](image)

**Fig. 2.** Changes in mean (± SEM) circulating concentrations of insulin in mares (n = 8) treated with clenbuterol (3.2 μg kg⁻¹, i.e.; •) each day or in untreated mares (n = 6; ○) during the experimental period between October and April. The horizontal bar indicates the duration of clenbuterol administration. For unpublished observations from a preliminary report see McManus and Fitzgerald (1999).

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Fig. 3. Changes in mean (± SEM) serum concentrations of (a) FSH and (b) leptin in untreated mares (n = 4, ○) and in mares (n = 4) treated with rh-leptin (50 µg kg⁻¹, s.c.; ●) at 0 and 8 h. Note that despite supraphysiological concentrations of leptin in mares that received treatment, mean concentrations of FSH were unaltered. In untreated and leptin-treated mares, FSH peaked and 16 h after treatment was administered. The role of some species in suppression of FSH (Williams et al., 2000) was further confirmed by the reduced FSH release in animals with high leptin concentrations (Morgan et al., 2000). Therefore, it is suggested that leptin plays a role in the regulation of FSH secretion in mares. Increased leptin concentrations in the brain, consistent with the control of the hypothalamo-pituitary axis, are consistent with the compelling evidence that leptin is a key regulator of energy balance and reproduction in mares.
secretion to determine the pattern of secretion for eight anoestrous mares during the 8 h before and 16 h after s.c. administration of rh-leptin (50 μg kg⁻¹, n = 4) or saline (n = 4) at 8 h intervals. Supraphysiological concentrations of leptin were sustained during the 16 h treatment period, whereas leptin concentrations remained low (< 5 ng ml⁻¹) in saline-treated mares (Fig. 3b). A similar dose of rh-leptin in feed-restricted, steroid-treated wethers was associated with a marked increase in pulsatile gonadotrophin secretion (Nagatani et al., 2000). However, in anoestrous mares this same dose was not accompanied by changes in FSH secretion (Fig. 3a).

**Insulin**

The role of insulin in the mechanisms controlling reproductive function is controversial. In some species, a deficiency of insulin resulting from chemically induced diabetes leads to a suppression of reproductive function, whereas in other species, reproduction is not affected (Williams et al., 1996; Bucholtz et al., 2000; Tanaka et al., 2000). However, a growing consensus of information, particularly that derived from gene knockout mice, provides compelling evidence that the disruption of insulin signalling or central insulin receptors modifies reproductive activity (Bruning et al., 2000; Burks et al., 2000).

Increased adiposity leads to higher circulating concentrations of insulin, which in turn is associated with a disruption of glucose metabolism. As glucose is the primary fuel used by the brain, considerable research has focused on the role of insulin as a potential metabolic signal linking reproduction and nutrition. To date, there has been no research on the role of insulin in the control of reproduction in mares. In view of this consideration, concentrations of insulin were compared between blood samples collected in mature, fat mares and young, lean mares (Fitzgerald and McManus, 2000). Insulin concentrations in mature, fat mares were significantly higher (P < 0.05) than in young, lean mares (Fig. 4). As insulin modulates GnRH secretion (Miller et al., 1998; Blache et al., 2000), these findings might implicate a role for this hormone in the mechanisms controlling the continuation of reproductive activity during the winter months. Such a postulated action of insulin on GnRH secretion may reflect a central action of insulin, as insulin can cross the blood–brain barrier and insulin receptors have been observed in several brain areas, including the hypothalamus (Bruning et al., 2000; Burks et al., 2000). Alternatively, high circulating concentrations of insulin might indicate insulin resistance in fat mares. Insulin resistance is generally characterized by increased circulating concentrations of insulin and reduced glucose uptake in muscle and adipose tissue, the latter resulting from reduced recruitment of the insulin-dependent glucose transporter, Glut4. Therefore, it is possible that reduced uptake of glucose peripherally is somehow sensed by a central mechanism, thereby leading to continuation of reproductive function during the winter months.

The role of insulin and insulin resistance in the control of oestrous cycles during the non-breeding season was investigated by performing an experiment to compare the timing of the cessation of the breeding season in fat and lean mares. Insulin sensitivity was determined by using a euglycaemic hyperinsulinaemic clamp and this approach was used to construct three experimental groups as follows: (i) Group 1: obese, insulin-resistant, mature mares; (ii) Group 2: lean, insulin-sensitive, mature mares; and (iii) Group 3: lean, insulin-sensitive young mares. Group 1 mares were selected from a pool of mature mares identified previously to be

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*FSH* pulse frequency was on average one pulse per 16 h (data not shown; M. I. L. Brown, D. M. Powell, S. E. Reedy and B. P. Fitzgerald, unpublished). (c) Changes in circulating concentrations of FSH in a representative mare that was treated with rh-leptin (50 μg kg⁻¹, s.c.; ●) at 0 and 8 h.
predisposed to show oestrous cycles during the winter months and the mares were kept on pasture to maintain body weight. Group 2 mares were selected from the same pool, but were subjected to feed restriction during the summer months (July–September) to achieve a lean body condition. Feed restriction consisted of maintaining mares on a dry-lot with provision of hay to achieve a thin body condition score of 3–4 on a 10 point scale (Henneke et al., 1983). The final group of mares (Group 3) were young, lean mares and included animals used in an earlier study (Fitzgerald and McManus, 2000). Therefore, it was anticipated that this group of mares would show cessation of oestrous cycles during the winter months. For each group, insulin sensitivity was established using a euglycaemic hyperinsulinaemic clamp consisting of an overnight fast and followed by a bolus injection of insulin (0.4 μU kg$^{-1}$) and subsequent continuous infusion (1.2 μU kg$^{-1}$ min$^{-1}$) for 2 h. A solution of 50% (w/v) dextrose was infused simultaneously and peripheral glucose was monitored using a portable blood glucose monitor. The rate of dextrose infusion was adjusted as appropriate to achieve a relatively constant circulating glucose concentration. Obesity was associated with insulin resistance, characterized by reduced glucose uptake in peripheral tissues (Figs 5b and 6). In contrast, the remaining mares were considered to be insulin sensitive. Despite these differences in insulin sensitivity and percentage body fat, the proportion of mares that ceased oestrous cycles was not different between groups. An additional observation of interest was that at the termination of data collection to establish reproductive status, a second euglycaemic hyperinsulinaemic clamp procedure performed in late February indicated that insulin sensitivity was clearly different from that performed in November. Each group showed a shift in the glucose infusion rate curve, indicating increased insulin resistance (Fig. 5b). This shift was particularly marked for the food-restricted mares in Group 2. It is well known that under conditions of long-term food deprivation, or malnutrition associated with trauma, insulin resistance develops as a homeostatic mechanism to repartition the availability of glucose preferentially to the brain and other resistance to tissues. All associatio

On the insulin resistance during the amount of Manning, accompany species in restriction and sense an isolate confirms a approxim:
and other insulin-sensitive tissues (Chiolero et al., 1997). The development of insulin resistance under these conditions is thought to result from continual mobilization of free fatty acids from adipose tissue for use as a metabolic fuel, thereby sparing glucose for neural tissues. Although mares in Group 2 were not emaciated, they developed insulin resistance in association with a lean body weight, but continued to ovulate during the winter months.

On the basis of these preliminary studies, the degree of body fat stores and peripheral insulin sensitivity do not appear to be factors that control the continuation of oestrous cycles during the winter months. This result is in general agreement with current dogma that the amount of stored body fat per se is not a determinant of reproductive activity (Bronson and Manning, 1991). However, the observation that feed restriction was not accompanied by a cessation of reproductive activity is in marked contrast to studies in other species in which feed restriction abruptly terminates reproductive activity. Indeed, feed restriction is frequently used as an experimental model to investigate the identity of signals and sensors that link nutrition and reproduction. Although unexpected, our observation is not an isolated finding for this species, as a recent preliminary report of a study in pony mares confirms and extends our observations (Guillaume et al., 2001). In this regard, pony mares of approximately 2.5 years of age were put either on a maintenance diet or on a diet consisting of
50% of maintenance requirement for 19 months. In the first winter, approximately 50–60% of mares from each group continued to show oestrous cycles. However, in the second winter, a higher proportion of feed-restricted pony mares ceased reproductive activity compared with mares on a maintenance diet. In addition, beginning in late December, a sub-group of each treatment group was exposed to an abrupt artificial increase in daylength to advance the onset of the breeding season. Whereas the timing of onset of the oestrous cycle was advanced mares on a maintenance diet, feed-restricted mares failed to respond to the change in daylength.

The continuation of oestrous cycles, and by inference LH secretion, during the winter months in this large mammal, despite feed restriction, is an observation without parallel in small, short-lived mammals. However, Hileman et al. (1999) reported that in a high proportion of ewe lambs 7 weeks of feed restriction was unaccompanied by a change in LH secretion, compared with lambs fed ad libitum. Thus, in some conditions and for some small mammalian species, the availability of metabolic fuels may be sufficient for the continuation of reproductive activity, despite feed restriction. In a study by Guillaume et al. (2001), long-term feed restriction (19 months) led to the cessation of oestrous cycles in the second winter period, but not in the first. Although such a long-term feed restriction was accompanied by cessation of reproductive activity during the non-breeding season, oestrous cycles during the next breeding season apparently were not interrupted. This finding clearly indicates that the signals associated with feed restriction or energy intake somehow interact with changes in daylength to terminate the breeding season in this species. How is this interaction integrated into a change in reproductive activity?

In consideration of the finding that feed restriction apparently must be of long-term duration to affect a change in reproductive activity during the winter months, it is possible to develop two working hypotheses to account for these findings. Firstly, energy availability may need to reach a critical level before a presumptive inhibitory daylength signal can initiate the termination of the breeding season. This hypothesis excludes the possibility that energy availability per se is the primary signal leading to the termination of the breeding season.

An alternative hypothesis is that metabolic signals limiting reproductive activity are recognized before perception of a change in photoperiod that leads to the cessation of breeding season. This proposal raises the possibility that the reproductive response to photoperiod is gated by the availability of energy at a specific time of year. Extending this reasoning to our studies and those of Guillaume et al. (2001), it is conceivable that the availability of metabolic fuels during the spring but not autumn months determines the subsequent response to short daylengths in the autumn and winter. This proposal might account for an absence of anoestrus in mares that were feed restricted only during the autumn months.

From the foregoing discussion, we currently have no conclusive evidence that in mares the degree of adiposity is the primary signal that underlies the continuation of oestrous cycles during the winter months. Our studies have clearly demonstrated that manipulation of metabolic hormones during the autumn does not affect the timing of the cessation of reproductive activity during the winter months. Future studies might consider the time of year as an interacting factor in the mechanisms controlling the continuation of reproductive activity during the winter months. Finally, an additional consideration that has received only limited attention in this large mammal is the availability of oxidizable metabolic fuels.

**Metabolic signals as determinants of seasonal anoestrus**

The concept that in mammals ovulation is blocked by reduced availability of metabolic fuels is firmly established. Several reviews have documented our current understanding of potential...
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signals and sensors involved in this phenomenon (Schneider and Wade, 1989; l’Anson et al.,
1991; Bronson and Heideman, 1992; Wade and Schneider, 1992; Bucholtz et al., 1996;
Wade et al., 1996). However, much of the available information has focused on short-term
changes in reproductive activity in response to abrupt changes in food availability by enforced
fasting or the availability of metabolic fuels after pharmacological modification. To date,
efforts to investigate the role of metabolic fuel availability in the mare by pharmacological
blockade have been impractical and unsuccessful. For example, in several species peripheral
administration of 2-deoxy-D-glucose (2DG) inhibits cellular glucose utilization and this
pharmacologic effect results in suppression of gonadotrophin secretion and interruption of
oestrous cycles (Schneider et al., 1997a). In mares, systemic administration of 2DG, at a dose
approaching that used in some rodent studies (100 mg kg⁻¹), was associated with a marked
reduction in experimental subjects, probably reflecting increased secretion of adrenalin, as
described in rats and other small mammals (Hokfelt and Bydgoszcz, 1961; Scheurink and
Kitter, 1993). It has been proposed that the increased adrenal medullary secretion is a
homeostatic mechanism that facilitates delivery of glucose to the brain by stimulation of
hepatic glycogenolysis and inhibition of insulin secretion (Froman et al., 1993; Scheurink and
Kitter, 1993; Ritter et al., 1998). In some instances, the excitation observed in individual mares
precluded frequent blood sample collection. Despite a change in behaviour and increased
oscillating concentrations of glucose induced by 2DG, circulating gonadotrophin concen-
trations were not affected (Fig. 7). The absence of a change in gonadotrophin secretion
associated with the 2DG blockade of cellular glucose uptake was unexpected, but may reflect an
insufficient dose to block glucose uptake. However, the aberrant behaviour associated with
inhibition of intracellular
fatty acid oxidation (Schneider et al., 1997b), which in turn may disrupt reproductive activity when combined with 2DG. We have not explored this approach in mares because of the aberrant behaviours associated with 2DG.

**Conclusions**

On the basis of current studies, we have developed a model of interest for investigation of the long-range regulation and integration of metabolic signals, daylength and seasonal reproductive activity. Whereas small mammal models provide compelling information relating to signals, sensors and neuroendocrine pathways in the short-term regulation of reproduction by nutrition, their use as a model for long-lived species appears somewhat questionable. Further-
more, it now seems likely that fat mass is regulated quite differently in small, compared with large mammals (Mercer and Speakman, 2001; Morgan and Mercer, 2001). This difference implies differences in homeostatic mechanisms in the control of body mass, availability of metabolic fuels and possibly reproduction.

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Reference

Bronson FH and Heideman PD (1992) Lack of reproductive photoresponsiveness and correlative failure to respond to melatonin in a tropical rodent, the Cane mouse Biology of Reproduction 46:246–250
Bruning JC, Gautam D, Burks DJ et al. (2000) Role of brain insulin receptor in control of body weight and reproduction Science 289:2122–2125
Chehab FF, Lim ME and Lu R (1996) Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin Nature Genetics 12:318–320


Hokfelt S and Bydgenman S (1964) Increased adrenaline production following administration of 2-deoxy-D-glucose in the rat. Proceedings of the Society for Experimental Biology and Medicine 106 537-539


Irvine CHG (1979) The non pregnant mare: a review of some current research and of the last 25 years of endocrinology. Biology of Reproduction Monograph Series 1 343-360


Schneider JE and Ware GN (1989) Availability of metabolic fuels controls estrus cyclicity of Syrian hamsters. Science 244 1326-1328


Schneider JE, Hall AJ and Ware GN (1997b) Central vs. peripheral metabolic control of estrous cycles in Syrian hamsters. I. Lipopolysaccharide American Journal of Physiology 272 R406-R405


Wade GN, Schneider JE and Li HW (1996) Control of fertility
Interactions of ovarian activity

Ivy JL (2001) in conscious mares: a review


JL (1984) Use of steers

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ol of fertility

by metabolic cues American Journal of Physiology 270 E1–E10


Williams NI, Lancas MJ and Cameron JL (1996) Stimulation of luteinizing hormone secretion by food intake: evidence against a role for insulin Endocrinology 137 2565–2573