REPRODUCTION SYMPOSIUM: Hypothalamic neuropeptides and the nutritional programming of puberty in heifers

Article *in* Journal of Animal Science · June 2014

DOI: 10.2527/jas.2014-7808 · Source: PubMed

4 authors:

Marcel Amstalden
Texas A&M University
67 PUBLICATIONS **1,666** CITATIONS
SEE PROFILE

Rodolfo C Cardoso
Texas A&M University
35 PUBLICATIONS **218** CITATIONS
SEE PROFILE

Bruna Rios Coelho Alves
University of Nevada, Reno
21 PUBLICATIONS **111** CITATIONS
SEE PROFILE

Gary L Williams
Texas A&M University
141 PUBLICATIONS **3,729** CITATIONS
SEE PROFILE

REPRODUCTION SYMPOSIUM: Hypothalamic neuropeptides and the nutritional programming of puberty in heifers

M. Amstalden, R. C. Cardoso, B. R. C. Alves and G. L. Williams

J ANIM SCI 2014, 92:3211-3222.
doi: 10.2527/jas.2014-7808 originally published online June 3, 2014

REPRODUCTION SYMPOSIUM: Hypothalamic neuropeptides and the nutritional programming of puberty in heifers[1,2]

M. Amstalden,*[3] R. C. Cardoso,*† B. R. C. Alves,* and G. L. Williams*†

*Department of Animal Science, Texas A&M University, College Station 77843;
and †Animal Reproduction Laboratory, Texas A&M AgriLife Research, Beeville 78102

ABSTRACT: Nutrition during the juvenile period has a major impact on timing reproductive maturity in heifers. Restricted growth delays puberty, whereas elevated BW gain advances the onset of puberty. The initiation of high-frequency episodic release of GnRH and, consequently, LH during the peripubertal period is crucial for maturation of the reproductive axis and establishment of normal estrous cycles. Nutritional signals are perceived by metabolic-sensing cells in the hypothalamus, which interact with estradiol-receptive neurons to regulate the secretory activity of GnRH neurons. The orexigenic peptide, neuropeptide Y (NPY), and the anorexigenic peptide derived from the proopiomelanocortin (*POMC*) gene, melanocyte-stimulating hormone α (αMSH), are believed to be major afferent pathways that transmit inhibitory (NPY) and excitatory (αMSH) inputs to GnRH neurons. The neuropeptide kisspeptin is considered a major stimulator of GnRH secretion and has been shown to mediate estradiol's effect on GnRH neuronal activity. Kisspeptin may also integrate the neuronal pathways mediating the metabolic and gonadal steroid hormone control of gonadotropin secretion. Recent studies in our laboratories indicate that functional and structural changes in the pathways involving NPY, POMC, and kisspeptin neurons occur in response to high rates of BW gain during the juvenile period in heifers. Changes include regulation of expression in *NPY*, *POMC*, and *KISS1* and plasticity in the neuronal projections to GnRH neurons and within the neuronal network comprising these cells. Moreover, an intricate pattern of differential gene expression in the arcuate nucleus of the hypothalamus occurs in response to feeding high concentrate diets that promote elevated BW gain. Genes involved include those controlling feeding intake and cell metabolism, neuronal growth and remodeling, and synaptic transmission. Characterizing the cellular pathways and molecular networks involved in the mechanisms that control the timing of pubertal onset will assist in improving existing strategies and facilitate the development of novel approaches to program puberty in heifers. These include the use of diets that elevate BW gain during strategic periods of prepubertal development.

Key words: kisspeptin, neuropeptide Y, nutrition, programming, proopiomelanocortin, puberty

 J. Anim. Sci. 2014.92:3211–3222
doi:10.2527/jas2014-7808

INTRODUCTION

The lifetime productivity of beef cows is influenced by their ability to reach reproductive maturity, conceive, sustain pregnancy, and calve for the first time by approximately 24 mo of age (Lesmeister et al., 1973). Therefore, improved strategies and novel approaches to maximize replacement heifer development to optimally time the onset of puberty can have a significant impact in cattle production systems. A major constraint in events leading to maturation of the reproductive neuroendocrine axis is the initiation of high-frequency release of GnRH and consequently LH (Day et al., 1984). The requisite increase in pulsatile LH release is believed to occur when changes in the net output of excitatory signals that control GnRH secretion exceed inhibitory inputs. Developmental alterations in the sensitivity to estradiol negative feedback and the permissive effects

[1]Based on a presentation at the Reproduction Symposium titled "External Influences on Reproductive Neuroendocrinology" at the Joint Annual Meeting, July 8–12, 2013, Indianapolis, IN.

[2]Supported by Agriculture and Food Research Initiative Competitive Grant no. 2009-65203-05678 from the USDA National Institute of Food and Agriculture.

[3]Corresponding author: m.amstalden@tamu.edu

Received March 3, 2014.
Accepted March 8, 2014.

of molecules signaling nutrient sufficiency are involved in this process. The integration of these signals occurs through metabolic- and estradiol-sensing pathways in the hypothalamus. Neuropeptide Y (**NPY**), proopiomelanocortin (**POMC**), and kisspeptin neurons are critical components of the neuronal network that control the pulsatile release of GnRH. In heifers, increased rates of BW gain during the juvenile period can advance puberty (Gasser et al., 2006a,b,c,d; Cardoso et al., 2014), and functional changes in the NPY and POMC neuronal pathways appear to contribute to the process (Allen et al., 2012). In this paper, we present an overview of the neuroendocrine control of pubertal development in females, with emphasis on ruminants. Recent research conducted in our laboratories investigating the involvement of key hypothalamic neuropeptides in the mechanisms through which nutrition advances pubertal onset in heifers and potential strategies for nutritionally programming puberty are discussed.

PHYSIOLOGICAL MECHANISMS UNDERLYING REPRODUCTIVE MATURATION IN HEIFERS

Puberty in heifers can be defined as the first ovulation followed by an estrous cycle of normal duration (Kinder et al., 1987). However, maturation of the reproductive axis in heifers involves a transitional period that includes a gradual rise in the episodic release of LH (Gonzalez-Padilla et al., 1975). Increased stimulation of the ovaries by gonadotropins supports enhanced steroidogenesis in growing follicles leading to elevated circulating concentrations of estradiol, which, in turn, stimulates a surge release of LH that causes ovulation. Peripubertal ovulations are often followed by 1 to 3 limited increases in circulating concentrations of progesterone (short luteal phases; Gonzalez-Padilla et al., 1975; Berardinelli et al., 1979). Full expression of estrous behavior and high fertility is usually achieved only after the second or third ovulation and complete maturation of the reproductive tract (Byerley et al., 1987).

Neuroendocrine Control of LH Secretion during Pubertal Development

The development of a high-frequency pattern of LH release is a critical neuroendocrine event leading to the onset of puberty in females. It begins approximately 2 mo before first ovulation in heifers (Day et al., 1986a) and, based on studies in sheep, it occurs in response to an increase in the episodic release of GnRH (I'Anson et al., 2000). The physiological mechanisms involved in the regulation of GnRH secretion include signaling from gonadal and metabolic hormones as well as factors secreted locally within the hypothalamus that modulate neuronal and glial cell function (reviewed by Prevot et al., 2010). During most of the juvenile period, the episodic release of LH is maintained at a low frequency, due mainly to the inhibitory effects of estradiol. Ovariectomy in prepubertal heifers (Day et al., 1984) leads to an increase in the frequency of LH release. Replacement with estradiol maintains the low frequency of LH release, similar to intact, prepubertal females. The escape from enhanced sensitivity to estradiol negative feedback is a critical event for pubertal progression. Mechanisms by which estradiol exerts its inhibitory effects are unclear but may involve estradiol actions through both genomic and nongenomic pathways via nuclear and membrane estrogen receptors (Stormshak and Bishop, 2008). However, studies using knockout mice have indicated that the estrogen receptor-α (**ESR1**) is the primary mediator of estradiol signaling in the control of episodic gonadotropin release (Dorling et al., 2003). Because, at least in sheep, GnRH neurons do not contain ESR1 (Lehman and Karsch, 1993), estradiol negative feedback is believed to be mediated by estradiol-sensitive afferent pathways to GnRH neurons. Kisspeptin neurons have been characterized as a major contributor to the cellular pathway by which estradiol regulates the secretion of GnRH.

Kisspeptin as a Mediator of Estradiol Negative Feedback

Kisspeptin is a peptide with potent stimulatory effects on the secretion of gonadotropins in various species, including cattle (Kadokawa et al., 2008). The role of kisspeptin in the control of reproductive function was first characterized in studies in which mutations in the gene encoding the kisspeptin receptor (***KISS1R***) identified in humans were associated with disruption of normal pubertal development (de Roux et al., 2003; Seminara et al., 2003). A genetic mutation in *KISS1R* produced in mice leads to a phenotype similar to that in humans and includes immature reproductive organs, low circulating concentrations of gonadotropins, and lack of normal pubertal development (Seminara et al., 2003). Studies in rats have indicated that kisspeptin can advance the onset of puberty (Navarro et al., 2004b), at least in part, by increasing the circulating concentrations of LH. The stimulatory actions of kisspeptin on gonadotropin secretion occur primarily through its effects on GnRH secretion. This is supported by the observations that kisspeptin neurons project to GnRH neurons (Smith et al., 2008), GnRH neurons express the kisspeptin receptor (Smith et al., 2011), and kisspeptin increases the firing rate of GnRH neurons (Han et al., 2005). In addition, concentrations of GnRH in the cerebrospinal fluid collected from the third ventricle increase concurrently with elevations of LH after central administration of kisspeptin in ewes (Messager et al., 2005). The ability of kisspeptin to induced the release of LH in ewes is blocked by pas-

sive immunization against GnRH (Arreguin-Arevalo et al., 2007), indicating the need for GnRH signaling for kisspeptin actions. Stimulation of LH release by direct effects of kisspeptin in the adenohypophysis has been demonstrated (Suzuki et al., 2008), although the physiological relevance for direct effects of kisspeptin on gonadotropes is unclear.

Kisspeptin neurons in the mammalian brain are distributed in 2 major groups located in the preoptic area (**POA**)–periventricular (**PeV**) region and in the arcuate nucleus (**ARC**). Kisspeptin neurons in these populations express ESR1 (Franceschini et al., 2006); thus, they are direct targets for estradiol. Indeed, estradiol regulates *KISS1* expression in both populations but appears to do so in a distinct manner. In mice (Smith et al., 2005), rats (Navarro et al., 2004a), and pigs (Tomikawa et al., 2010), estradiol increases *KISS1* mRNA in the POA-PeV region. Increased *KISS1* mRNA in the POA-PeV region is also observed in ewes during the late-follicular phase, a period during which circulating concentrations of estradiol are expected to be elevated (Smith et al., 2009). In contrast, estradiol has been observed to inhibit *KISS1* expression in the ARC of mature ewes (Smith et al., 2007), and estradiol withdrawal in ovariectomized, estradiol-replaced ewes is associated with an increase in the number of kisspeptin-immunoreactive neurons in the ARC (Merkley et al., 2012).

As discussed previously, GnRH neurons do not contain ESR1, the primary estrogen receptor type mediating the actions of estradiol in the control of gonadotropin release. Therefore, kisspeptin neurons may serve as intermediary effectors of estradiol feedback control of gonadotropin secretion and be involved in the mechanisms through which changes in the sensitivity to estradiol negative feedback occur, permitting pubertal progression. Support for the latter exists in studies in laboratory rodents and sheep. The abundance of *KISS1* mRNA in the ARC increases during postnatal development in rats (Desroziers et al., 2012) and the number of immunoreactive kisspeptin neurons in the ARC is greater in postpubertal than in prepubertal ewe lambs (Nestor et al., 2012). More specifically, the activation of *KISS1* expression in the ARC is associated with the degree of maturity of the reproductive neuroendocrine axis. In ovariectomized, estradiol-replaced ewe lambs, the number of *KISS1* cells in the ARC increases with an increase in the frequency of LH release (Redmond et al., 2011). In contrast, the increase in the number of *KISS1* cells in the POA-PeV region is associated with age but less with changes in LH pulsatility (Redmond et al., 2011). These observations indicate that decreased estradiol inhibition of *KISS1* expression in the ARC may be critical for the onset of puberty in females. This hypothesis is further supported by the observations that mice carrying conditional *ESR1* knockout in kisspeptin neurons exhibit early onset of puberty (Mayer et al., 2010).

NUTRITIONAL INFLUENCES ON PUBERTY THROUGH METABOLIC SIGNALING PEPTIDES

The role of nutrition in the regulation of the reproductive neuroendocrine axis and control of pubertal development of heifers has been well established. Chronic nutrient restriction delays the onset of puberty by suppressing the release of LH (Day et al., 1986b). Acute nutrient restriction also suppresses LH secretion in prepubertal heifers (McCann and Hansel, 1986; Amstalden et al., 2000), an effect that is not observed in mature cows (Amstalden et al., 2002). Although feed restriction can influence gonadotrope function directly (Amstalden et al., 2003), studies in sheep have indicated that the inhibitory effects of undernutrition occur primarily through inhibition of pulsatile release of GnRH (I'Anson et al., 2000). A decrease in the magnitude of GnRH pulses may also play a role because GnRH pulses of low amplitude are not always associated with concurrent pulses of LH (Gazal et al., 1998; I'Anson et al., 2000). The exquisite ability of short-term fasting to inhibit LH secretion in prepubertal heifers may be associated with an increased sensitivity to estradiol negative feedback. The inhibition of LH release associated with feed restriction is enhanced in ovariectomized ewe lambs containing estradiol implants compared with those without implants (Foster and Olster, 1985).

Elevated BW gain during the infantile and juvenile periods influences the timing of the pubertal onset in heifers. In a series of studies in which heifer calves were weaned at approximately 3 to 4 mo of age and fed high-concentrate diets to promote high rates of BW gain, Gasser et al. (2006a,b,c,d) demonstrated that a high proportion of heifers exhibited precocious puberty (defined as puberty by 300 d of age). Although these studies were conducted using heifers of British and Continental breeds, early puberty was also observed in heifers of *Bos indicus*-influenced breeds weaned early and fed high-concentrate diets (Garcia et al., 2003). This effect appears particularly effective when BW gain is enhanced between 4 and 6.5 mo of age (Gasser et al., 2006d; Cardoso et al., 2014). Although the mechanisms and pathways involved in the nutritional acceleration of puberty are not fully understood, it appears that NPY and POMC neurons are involved (Alves et al., 2011; R. C. Cardoso, B. R. C. Alves, G. L. Williams, and M. Amstalden, Texas A&M University, College Station, personal communication). In addition, change in the sensitivity to estradiol negative feedback is probably involved because attenuation of estradiol inhibition of LH release occurs in heifers growing at high rates and becoming pubertal earlier (Gasser et al., 2006b). Therefore, metabolic- and gonadal hormone-

sensitive pathways in the hypothalamus interact to control the pattern of pulsatile GnRH release during maturation of the reproductive neuroendocrine axis.

Nutritional effects on GnRH secretory activity are believed to be mediated through metabolic signaling molecules that act by altering afferent inputs to GnRH neurons. These include metabolic hormones (e.g., insulin, IGF1, leptin, and ghrelin) and nutritional factors (e.g., glucose, amino acids, and fatty acids) that act in metabolic-sensing hypothalamic neurons (reviewed by Schneider, 2004; Crown et al., 2007). The leptin–NPY and leptin–POMC pathways will be emphasized in this review because of evidence supporting their relevance in the nutritional control of reproductive function in heifers.

Leptin as a Metabolic Hormone Signaling Nutritional Status for Timing the Onset of Puberty

Leptin is a protein hormone secreted primarily by adipose tissue (Zhang et al., 1994). The expression of the leptin gene in adipose tissue and concentrations of leptin in circulation increase with increasing adiposity (Garcia et al., 2002). A major endocrine effect of leptin is to regulate food intake and energy expenditure (Friedman and Halaas, 1998). Mice carrying mutations in the leptin gene (*ob*/*ob*) are obese and exhibit impaired reproductive function (Barash et al., 1996), and leptin supplementation rescues infertility in *ob*/*ob* mice (Chehab et al., 1996). Although initial studies indicated that exogenous administration of leptin could advance the onset of puberty, subsequent studies with rats (Cheung et al., 2001) and heifers (Maciel et al., 2004a; Zieba et al., 2004) showed that leptin alone is insufficient to trigger puberty. Therefore, the current hypothesis is that leptin acts as a permissive factor for pubertal progression in heifers. Short-term fasting reduces leptin expression in adipose tissue and circulating concentrations of leptin concurrent with decreases in LH pulsatility in prepubertal heifers (Amstalden et al., 2000). Leptin treatment prevents the decrease in the frequency of LH pulses associated with fasting in prepubertal heifers (Maciel et al., 2004b). Although leptin stimulates basal release of LH from adenohypophyseal tissue explants obtained from fasted cows (Amstalden et al., 2003), the actions of leptin on preventing the fasting-induced inhibition of LH pulsatility is likely mediated primarily at the hypothalamus. The observation that leptin administration in fasted cows increases the magnitude of GnRH pulses and concentrations of GnRH in the cerebrospinal fluid collected from the third ventricle (Zieba et al., 2004) supports this premise. Therefore, elevated BW gain facilitates adipose tissue accretion during juvenile development and enhances synthesis and release of leptin. Increased circulating leptin is proposed to signal the central nervous system the availability of sufficient nutritional reserves to support pubertal transition.

The GnRH neuron does not appear to contain the leptin receptor and conditional deletion of the leptin receptor in GnRH neurons does not impair reproductive function in mice (Quennell et al., 2009). Therefore, the permissive effect of leptin on the development of high-frequency release of GnRH during pubertal development is probably mediated by interneurons. It is believed that the ARC is a primary area for the actions of leptin in the control of neuroendocrine functions (Satoh et al., 1997). Recent studies in mice have indicated that the premammillary region of the hypothalamus may also be an important area in which leptin acts to permit pubertal progression (Donato et al., 2011). However, whether or not the premammillary hypothalamus has a similar function in other mammals, including cattle, remains to be determined. Nevertheless, NPY and POMC neurons in the ARC are major targets for leptin. These cell types contain the leptin receptor and are regulated by leptin (Håkansson et al., 1998).

Hypothalamic Neurocircuitry Linking Metabolism and Reproduction

Neuropeptide Y/Agouti-Related Protein Pathway. Neurons that express *NPY* in the ARC co-express *neuropeptide Y/agouti-related protein* (***AGRP***; Hahn et al., 1998) and leptin receptor (Håkansson et al., 1998; Iqbal et al., 2001). Feed restriction increases *NPY* mRNA (McShane et al., 1992) and NPY immunoreactivity (Chaillou et al., 2002) in the ARC and concentrations of NPY in the cerebrospinal fluid of ewes (McShane et al., 1993). In cows, third ventricular administration of NPY reduces GnRH and LH secretion (Gazal et al., 1998). However, pretreatment with leptin does not prevent NPY inhibition of LH release (Garcia et al., 2004), indicating that the effects of leptin on GnRH and LH secretion occur upstream from NPY receptor activation (Fig. 1). As discussed previously, GnRH neurons do not appear to be direct targets of leptin. However, GnRH neurons are directly influenced by NPY because NPY receptors have been detected in GnRH neurons (Campbell et al., 2001), and NPY inhibits GnRH neuronal activity in preparations of mouse brain tissue (Roa and Herbison, 2012).

Pubertal development has been associated with functional changes in the NPY circuitry. Expression of *NPY* in the ARC is decreased in peripubertal compared with early juvenile ewe lambs (Tillet et al., 2010). In contrast, the area covered by NPY fibers was greater in the ARC of peripubertal lambs (Tillet et al., 2010). The latter may indicate decreased NPY release because peptide accumulation in nerve terminals leads to increased immunoreactivity (Chaillou et al., 2002) and enhanced

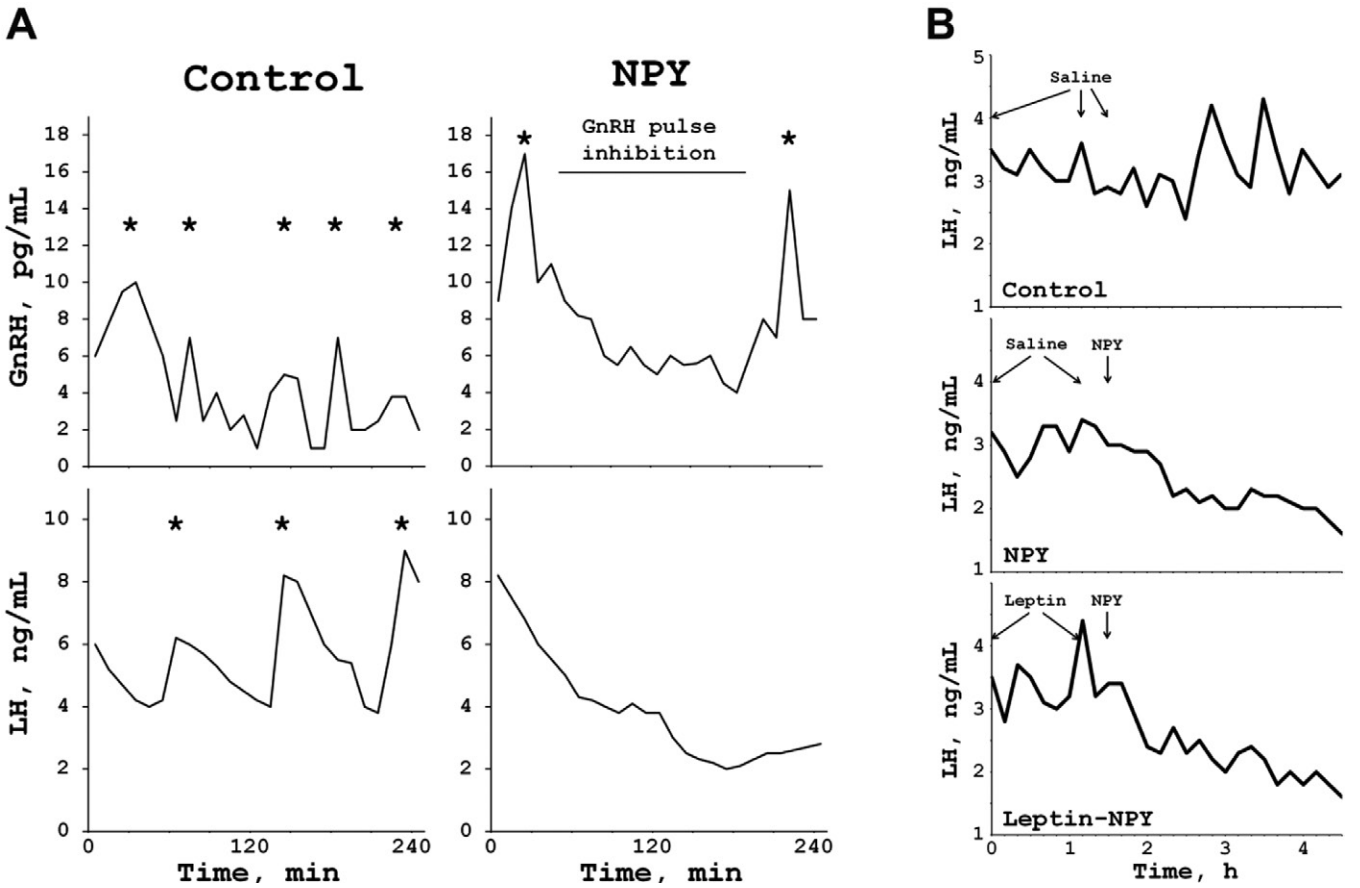

Figure 1. Neuropeptide Y (NPY) is a potent inhibitor of the episodic release of GnRH and LH in cows. Inhibition of pulsatile GnRH (top, panel A) and LH (bottom, panel A) release by third ventricular administration of 500 µg of NPY in ovariectomized, estradiol-implanted cows (adapted from Gazal et al. [1998], with permission of the publisher). Pretreatment with leptin (bottom, panel B) does not prevent NPY inhibition of LH release in ovariectomized cows (panel B; adapted from Garcia et al. [2004], with permission of the publisher).

detection of fibers. Maintenance of a high NPY inhibitory tone appears to suppress pubertal development. In female rats, central administration of NPY delays the onset of puberty (Pierroz et al., 1995), whereas administration of the NPY receptor Y1 antagonist BIBP 3226 increased concentrations of LH during the peripubertal period (Pralong et al., 2000). Moreover, deletion of NPY Y1 receptor in mice attenuates the delay in the onset of puberty induced by food restriction (Gonzales et al., 2004). These observations are consistent with the hypothesis that diminished inhibitory tone of NPY is critical for the initiation of high-frequency release of GnRH during pubertal transition.

Agouti-related protein is an endogenous antagonist of the melanocortin receptor (**MCR**) and acts synergistically with NPY in the control of metabolism by inhibiting the anorexigenic actions of melanocortins. Although not as potent of an orexigenic factor as NPY, the effect of AGRP persists longer (Hagan et al., 2000). The release of LH in monkeys (Vulliémoz et al., 2005) and the release of GnRH from rat hypothalamic explants (Lebrethon et al., 2007) is inhibited by AGRP. Similarly to NPY, AGRP may also act directly on GnRH neurons because direct actions of MCR agonists have been reported (Roa and Herbison, 2012).

The Proopiomelanocortin Pathway. The *POMC* gene encodes a number of peptides derived from the cleavage of the precursor protein. Melanocyte-stimulating hormone α (**αMSH**) is one of the products of POMC that has been demonstrated to be important for the control of energy balance (Seeley et al., 2004). Neurons expressing *POMC* have also been involved in the control of reproductive function. The MCR agonist melanotan II increases the release of LH in sheep (Backholer et al., 2010). This effect likely involves direct actions on GnRH neurons because αMSH, as well as the MCR3 and MCR4 agonists, stimulate firing activity in GnRH neurons (Roa and Herbison, 2012). Neurons expressing *POMC* in the ARC are considered, along with *NPY*, a primary neuronal population that mediates leptin actions in the hypothalamus. Leptin receptors are present in POMC neurons (Iqbal et al., 2001) and leptin stimulates the expression of *POMC* in the ARC (Backholer et al., 2010). Therefore, by inhibiting *NPY* and stimulating *POMC* expression, leptin influences the αMSH and NPY pathways in a manner

that is consistent with balancing nutrient uptake and utilization and the metabolic control of reproductive neuroendocrine function.

Limited information is available regarding the involvement of αMSH in the process of pubertal development. Nevertheless, it appears that increased αMSH stimulation of GnRH release during the peripubertal period is important for the onset of puberty. In prepubertal female rats, αMSH injected centrally on d 28 advances vaginal opening by 2 d compared with saline-treated rats (Durando et al., 1989). Administration of αMSH is also associated with increased circulating concentrations of LH and stimulation of GnRH release from hypothalamic explants collected from 25-d-old but not younger rats (Durando et al., 1989). Therefore, the juvenile development in the ability to respond to αMSH appears to also be a functional change that occurs during pubertal transition.

The Kisspeptin Pathway. Kisspeptin neurons are sensitive to changes in nutritional status. Acute feed restriction inhibits *KISS1* expression and enhances *KISS1R* mRNA in the hypothalamus of prepubertal rats, and central administration of kisspeptin prevents the fasting-mediated decrease in mean concentrations of LH (Castellano et al., 2005). The inhibitory effect of nutrient restriction on *KISS1* expression may be mediated by the decrease in leptin signaling. Leptin increases *KISS1* mRNA in leptin-signaling deficient mice (Smith et al., 2006) and rats (Castellano et al., 2006). Moreover, central administration of leptin for 3 d in lean ovariectomized ewes increased *KISS1* mRNA in both POA and ARC (Backholer et al., 2010). Although it is clear that kisspeptin neurons are sensitive to metabolic status, the relevance of the kisspeptin pathway for mediating leptin's control of reproduction remains to be resolved. Leptin receptor is found in a subpopulation of kisspeptin neurons (Smith et al., 2006; Backholer et al., 2010), but Quennell et al. (2011) demonstrated that kisspeptin neurons are not activated by exogenous leptin. In addition, Donato et al. (2011) reported that deletion of leptin receptor in kisspeptin neurons does not impair fertility in mice.

As discussed previously in this review, kisspeptin is considered to be a major secretagogue of GnRH. Similarly to αMSH, there is a developmental change in the ability of kisspeptin to stimulate GnRH and LH secretion (Navarro et al., 2004b; Han et al., 2005). However, this change does not appear to involve regulation of *KISS1R* in GnRH neurons during maturation of the reproductive neuroendocrine axis (Han et al., 2005). Additional observations in support of the involvement of kisspeptin circuitry in timing the onset of puberty include increased expression of *KISS1* during reproductive maturation (Takase et al., 2009; Redmond et al., 2011) and an increased number of kisspeptin projections in close proximity to GnRH neurons in postpubertal compared with prepubertal ewe lambs (Nestor et al., 2012).

Interactive Roles of Neuropeptide Y, Proopiomelanocortin, and Kisspeptin Pathways for Timing the Onset of Puberty

Studies investigating the neuronal pathways involved in the pubertal activation of pulsatile release of LH have supported the involvement of NPY, POMC, and kisspeptin neurons in this process. In addition, an intricate interaction of signals within these circuitries appears highly relevant for controlling the GnRH secretory output required for the onset of puberty. Functional and structural changes in these neurons, as well as in other neuronal and nonneuronal cells directly connected in these pathways, may be critical for the physiological events underlying pubertal development. Because reciprocal projections among NPY, POMC, and kisspeptin neurons are observed, it is possible that interactions among these cell types occur and are important for an integrative response to the nutritional and gonadal steroid control of pubertal development. Neuropeptide Y and POMC neuronal projections are observed in close proximity to kisspeptin neurons in the ARC and POA of sheep (Backholer et al., 2010), and NPY appositions appear to form synaptic inputs with kisspeptin neurons in ewes (Amstalden et al., 2011). Kisspeptin projections are also observed in close proximity to NPY and POMC neurons in the ARC (Backholer et al., 2010). The evidence for structural integration within these neuronal networks is supported by observations that kisspeptin injected into the third cerebroventricle of ewes enhances *NPY* and inhibits *POMC* mRNA abundance in the ARC (Backholer et al., 2010). Although these results are not aligned with the stimulatory effects of kisspeptin on the reproductive axis, they indicate a complex interaction with gonadal steroid hormones because these results were obtained in ovariectomized ewes without estradiol replacement. In intact male and female mice, kisspeptin has been observed to increase excitation of POMC neurons and inhibit depolarization in NPY neurons (Fu and van den Pol, 2010), which would be consistent with a net stimulation of GnRH neuronal activity. In addition, the MCR agonist melanotan II stimulates *KISS1* expression in the POA and inhibits *KISS1* expression in the ARC in luteal phase ewes (Backholer et al., 2009). Such observations reinforce the complexity of these neuronal networks and the relevance of the specific neuronal population that is influenced by them.

Nutritional Programming of Hypothalamic Pathways Controlling the Onset of Puberty

Cells in the NPY, POMC, and kisspeptin neuronal networks are highly sensitive to changes in nutritional status and regulatory actions of metabolic hormones such as leptin. Although these cells differentiate in the hypothalamus early during fetal development (Aujla et al., 2013), their projections and connections are expanded considerably during the postnatal period (Coupé et al., 2010). The establishment of hypothalamic innervations during the infantile period appears to be regulated by leptin. The infantile increase in circulating concentrations of leptin (Coupé et al., 2010; Long et al., 2011) may play a role in programming the circuitry involved in the control of neuroendocrine functions (reviewed by Bouret, 2010). In sheep, the postnatal elevation in circulating leptin is associated with nutritional status of the dam during gestation (Long et al., 2011) and postnatal nutrition of the offspring (Ehrhardt et al., 2003).

To better understand the role of these pathways in the nutritional control of pubertal development, we have conducted various studies using a nutritional model that has been demonstrated to markedly advance puberty in heifers (Gasser et al., 2006a,b,c,d). In this experimental model, heifers are weaned at approximately 3.5 mo of age and fed under controlled conditions to promote low (i.e., 0.45 to 0.5 kg/d) or high (i.e., 0.9 to 1.0 kg/d) rates of BW gain. The latter nutritional regimen consistently elevates circulating concentrations of leptin in high-gain heifers (Alves et al., 2011; Allen et al., 2012; Cardoso et al., 2014). In heifers in which brain tissue was collected at 6.5 mo of age, an intricate differential regulation in the expression of a number of genes in the ARC was observed and included a decrease in *NPY* mRNA (Fig. 2) and an increase in *POMC* mRNA in high-gain heifers (Allen et al., 2012). In addition, differential regulation of genes involved in the control of metabolism, neuronal remodeling, axonal growth, synapse vesicle transport, and synaptic transmission were also observed. In subsequent studies, changes in the expression of *NPY* and *POMC* genes were confirmed. Moreover, we observed a decrease in NPY innervation of GnRH neurons, particularly those located in the mediobasal hypothalamus (Alves et al., 2011), and an increase in αMSH innervation of kisspeptin neurons (R. C. Cardoso, B. R. C. Alves, G. L. Williams, and M. Amstalden, Texas A&M University, College Station, personal communication) in heifers that were fed to gain BW at high rates. These observations are consistent with a net increase in excitatory tone of GnRH neurons (Fig. 3). Importantly, the structural and functional alterations in the NPY–POMC–kisspeptin pathways appeared to be established beginning a few to several months before puberty was expected. Therefore, these changes may be an integral component of the physiological mechanisms by which elevated BW gain and increased adiposity facilitates early onset of puberty in heifers.

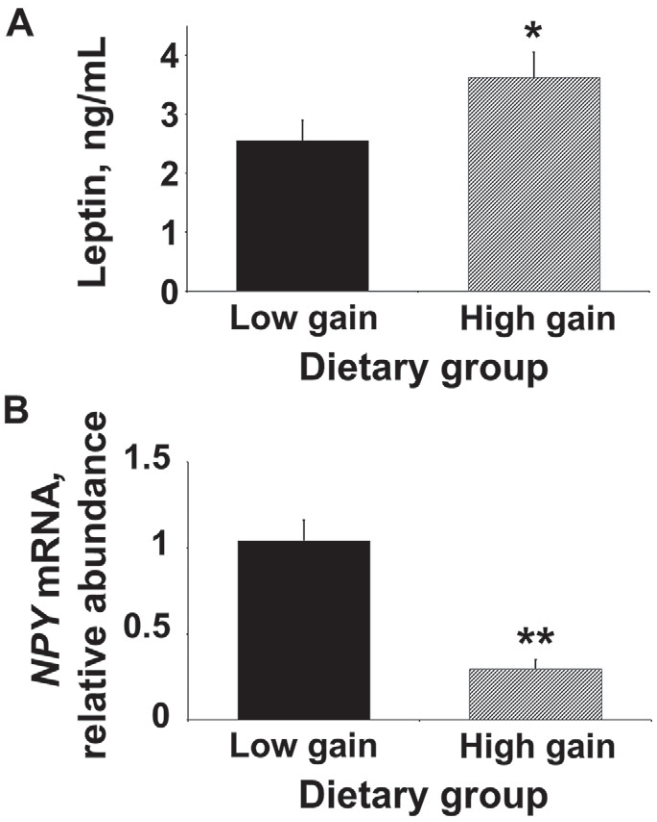

Figure 2. High rates of BW gain promote elevated metabolic status and differential gene expression in the hypothalamus of juvenile heifers that are consistent with facilitation of accelerated puberty. Heifers fed to gain BW at 0.9 kg/d exhibit increased circulating concentrations of leptin (panel A) and decreased *neuropeptide Y* (*NPY*) mRNA in the arcuate nucleus (panel B). Adapted from Allen et al. (2012). *$P < 0.1$; **$P < 0.001$.

NOVEL NUTRITIONAL STRATEGIES FOR PROGRAMMING THE ONSET OF PUBERTY IN HEIFERS

As noted previously, lifetime productivity of beef heifers is heavily dependent on their ability to calve early (Lesmeister et al., 1973). However, a significant proportion of beef heifers within existing U.S. production systems fail to reach the developmental end points necessary to achieve this objective (Patterson et al., 2005). This is particularly relevant for later-maturing breeds in which the skeletal size required to support a healthy and safe pregnancy is frequently attained well before puberty. Modifications in dietary energy status, both pre- and postweaning, have major impacts on this process. Feeding high-energy diets to promote a continuous high rate of gain after weaning is an option that can help assure the timely onset of puberty (Clanton et al., 1983; Lynch et al., 1997). Yet a relatively small percent-

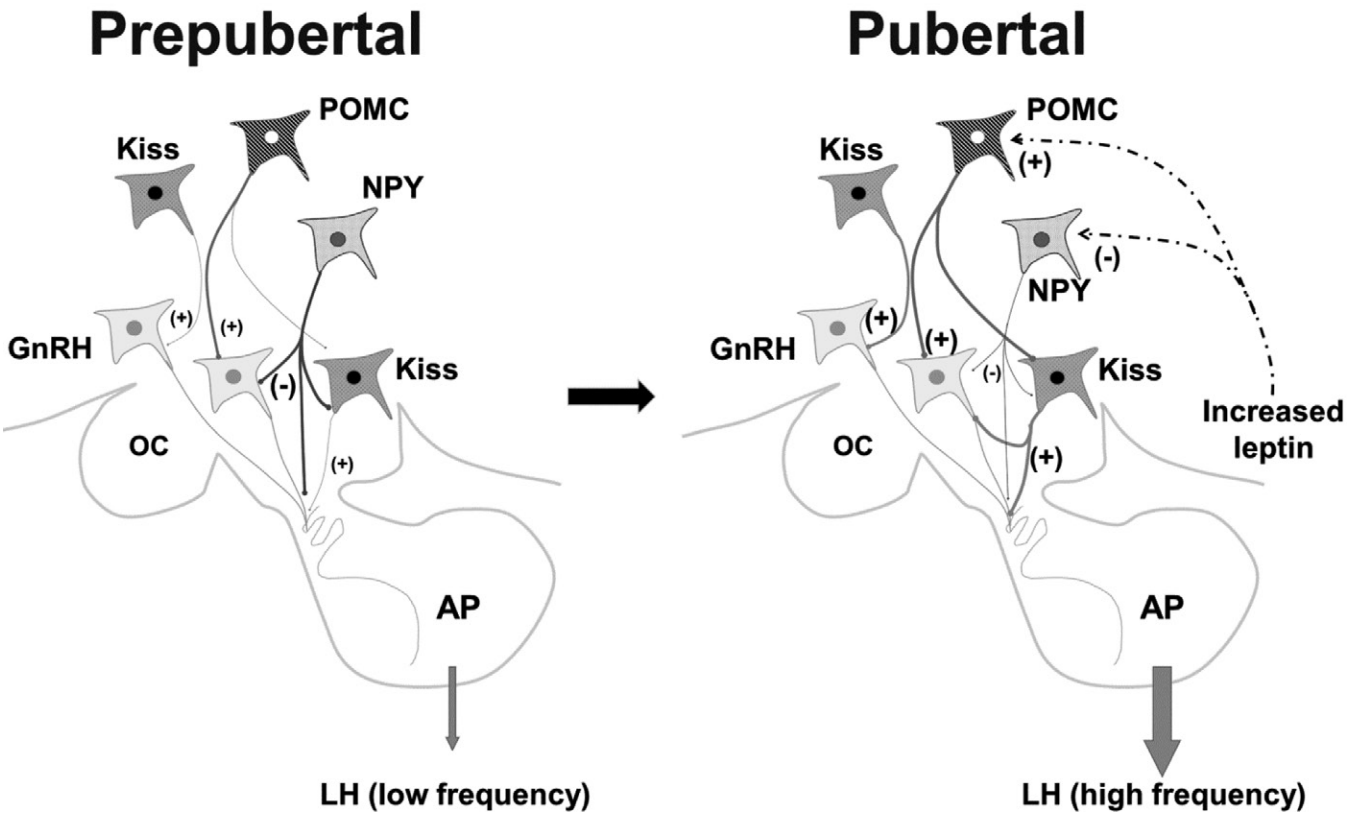

Figure 3. Illustration depicting a model for the involvement of neuropeptide Y (NPY), proopiomelanocortin (POMC), and kisspeptin (Kiss) neuronal pathways and their regulation of GnRH secretion during pubertal transition. Signals of nutrient sufficiency (e.g., leptin) are perceived by metabolic-sensing cells in the arcuate nucleus (e.g., NPY and POMC neurons) and transmitted to GnRH neurons through interactive pathways involving estradiol-sensitive cells (e.g., kisspeptin neurons). Increased BW gain during the juvenile period promotes differential gene expression and remodeling of neuronal projections that lead to decreased inhibitory (NPY) and enhanced excitatory (POMC and Kiss) afferents to the GnRH neurons. These functional and structural alterations, which include changes in synthesis of neuropeptides (illustrated by thickness of projections) and innervation of target neurons (proximity of projections), facilitate the acceleration in the frequency of episodic release of LH from the adenohypophysis (AP) and advance the onset of puberty. OC = optic chiasm.

age of cattlemen nutritionally preprogram their heifers as herd replacements (NAHMS, 1994). The cost associated with intensive nutritional management is one of the main factors limiting its adoption. Another major caveat is that uncontrolled, high rates of gain can result in excessive fattening, impair mammary development, and affect long-term productivity (Park et al., 1987). Thus, reproductive benefits derived from such strategies may be offset by deficits in lifetime efficiency.

The period between 4 and 7 mo of age appears critical for the nutritional programming of accelerated puberty in heifers and a high proportion of heifers fed high-concentrate diets during this period exhibits precocious puberty (Gasser et al., 2006a,b,c,d). Although extremely useful as a model to examine the physiological and cellular mechanisms underlying the process of pubertal development, precocious puberty is not a desirable phenomenon because of the increased risks associated with ill-timed pregnancies in young heifers that can compromise fetal and maternal health. With the intent of optimizing the timing of onset of puberty consistently to approximately 11 to 12 mo of age, we have examined nutritional approaches designed to promote increased growth during target periods of development. A stair-step nutritional regimen reported earlier by Park et al. (1987, 1998) emphasized alternating energy restriction and refeeding phases to potentiate compensatory weight gain and to modulate mammary gland development and productive capacity in dairy and beef heifers. We used a modified version of this approach to promote accelerated growth and adipose tissue accretion between 4 and 6.5 mo of age (early juvenile) or between 6.5 and 9 mo of age (mid juvenile; Cardoso et al., 2014). The objective was to promote biochemical and structural alterations in the hypothalamus that would sensitize the neuroendocrine system to signals of energy sufficiency for timed puberty. The period of accelerated BW gain (ad libitum consumption of a high-concentrate diet) was followed by a period of partial nutrient restriction to minimize the risks of excessive fattening and impaired mammary gland development and a second period of accelerated growth (Fig. 4). In this study, we observed that the majority of heifers that are fed ad libitum a high-concentrate diet beginning at 4.5 mo of age become pubertal by approximately 12 mo of age (Fig. 4; early juvenile). This was similar to heifers that are fed high concentrate

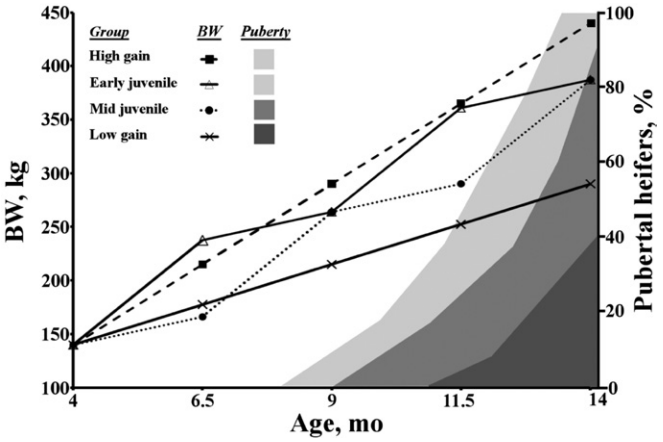

Figure 4. Model for a stair-step nutritional regimen applied to heifers during targeted periods of juvenile development to optimize growth and promote genomic, biochemical, and structural alterations in the hypothalamus that are permissive for early onset of puberty. Elevated BW gain during early- and mid-juvenile periods programs neuroendocrine functions and accelerates pubertal development in heifers. Heifers that are fed ad libitum a high-concentrate diet beginning at 4 mo of age (Early juvenile) become pubertal at a similar time as heifers gaining BW at high rates (High gain) throughout the prepubertal period, even though feed restriction is applied during the mid-juvenile period. The majority of heifers that are restricted during the early juvenile period but fed ad libitum between 6.5 and 9 mo of age (Mid juvenile) become pubertal by 14 mo of age, whereas only a small proportion of heifers gaining BW at low rates (Low gain) become pubertal by 14 mo of age. Adapted from Cardoso et al. (2014).

diets throughout the experimental period. In contrast, a considerably smaller proportion of heifers that were fed ad libitum beginning at 6.5 mo of age (Fig. 4; mid juvenile) became pubertal by 12 mo of age. However, by 14 mo of age, the proportion of heifers pubertal in this latter group did not differ from heifers gaining BW at high rates throughout the experimental period. These results indicate that timing of onset of puberty can be nutritionally programmed by promoting elevated BW gain during target periods of juvenile development. We believe that such strategy can optimize growth and reproductive maturation in the majority of replacement heifers and minimize the risks of high incidence of precocious puberty and ill-timed pregnancies.

SUMMARY AND CONCLUSIONS

Increased rates of BW gain during the juvenile period in heifers lead to differential expression of genes in the ARC, a major metabolic-sensing area of the hypothalamus. These genes are involved in cellular functions controlling cell–cell signaling, cellular structure, and responses to hormones and metabolites. Recent research supports a role for the NPY, POMC, and kisspeptin neuronal pathways in integrating signals that mediate the nutritional programming of puberty. Functional alterations in these pathways include decreased innervation of GnRH by NPY and increased innervation of kisspeptin neurons by αMSH. These alterations are consistent with the hypothesis that increased growth during the juvenile period leads to decreased inhibition and enhanced excitation of GnRH neurons and facilitates the early increase in GnRH pulsatility required for maturation of the reproductive neuroendocrine axis. Improving existing strategies and developing novel approaches to optimize growth during targeted periods of infantile and juvenile development may be useful practices to program puberty at a time compatible with maximal lifetime productivity.

LITERATURE CITED

Allen, C. C., B. R. C. Alves, X. Li, L. O. Tedeschi, H. Zhou, J. C. Paschal, P. K. Riggs, U. M. Braga-Neto, D. H. Keisler, G. L. Wiliams, and M. Amstalden. 2012. Gene expression in the arcuate nucleus of heifers is affected by controlled intake of high- and low-concentrate diets. J. Anim. Sci. 90:2222–2232.

Alves, B. R. C., S. Liu, E. Stevenson, J. Thorson, R. C. Cardoso, L. O. Tedeschi, D. H. Keisler, G. L. Williams, and M. Amstalden. 2011. Accelerated body weight gain during the juvenile period reduces neuropeptide Y close contacts with GnRH neurons in heifers. In: Proc. 44th Ann. Meeting Soc. Study Reprod., Portland, OR. p. 44 (Abstr.).

Amstalden, M., B. R. Alves, S. Liu, R. C. Cardoso, and G. L. Williams. 2011. Neuroendocrine pathways mediating nutritional acceleration of puberty: Insights from ruminant models. Front. Endocrinol. (Lausanne) 2:109.

Amstalden, M., M. R. Garcia, R. L. Stanko, S. E. Nizielski, C. D. Morrison, D. H. Keisler, and G. L. Williams. 2002. Central infusion of recombinant ovine leptin normalizes plasma insulin and stimulates a novel hypersecretion of luteinizing hormone after short-term fasting in mature beef cows. Biol. Reprod. 66:1555–1561.

Amstalden, M., M. R. Garcia, S. W. Williams, R. L. Stanko, S. E. Nizielski, C. D. Morrison, D. H. Keisler, and G. L. Williams. 2000. Leptin gene expression, circulating leptin, and luteinizing hormone pulsatility are acutely responsive to short-term fasting in prepubertal heifers: Relationships to circulating insulin and insulin-like growth factor I. Biol. Reprod. 63:127–133.

Amstalden, M., D. A. Zieba, J. F. Edwards, P. G. Harms, T. H. Welsh Jr., R. L. Stanko, and G. L. Williams. 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. Biol. Reprod. 69:1539–1544.

Arreguin-Arevalo, J. A., C. A. Lents, T. A. Farmerie, T. M. Nett, and C. M. Clay. 2007. KiSS-1 peptide induces release of LH by a direct effect on the hypothalamus of ovariectomized ewes. Anim. Reprod. Sci. 101:265–275.

Aujla, P. K., G. T. Naratadam, L. Xu, and L. T. Raetzman. 2013. Notch/Rbpjκ signaling regulates progenitor maintenance and differentiation of hypothalamic arcuate neurons. Development 140:3511–3521.

Backholer, K., J. Smith, and I. J. Clarke. 2009. Melanocortins may stimulate reproduction by activating orexin neurons in the dorsomedial hypothalamus and kisspeptin neurons in the preoptic area of the ewe. Endocrinology 150:5488–5497.

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

Barash, I. A., C. C. Cheung, D. S. Weigle, H. Ren, E. B. Kabigting, J. L. Kuijper, D. K. Clifton, and R. A. Steiner. 1996. Leptin is a metabolic signal to the reproductive system. Endocrinology 137:3144–3147.

Berardinelli, J. G., R. A. Dailey, R. L. Butcher, and E. K. Inskeep. 1979. Source of progesterone prior to puberty in beef heifers. J. Anim. Sci. 49:1276–1280.

Bouret, S. G. 2010. Role of early hormonal and nutritional experiences in shaping feeding behavior and hypothalamic development. J. Nutr. 140:653–657.

Byerley, D. J., R. B. Staigmiller, J. G. Berardinelli, and R. E. Short. 1987. Pregnancy rates of beef heifers bred either on puberal or 3rd estrus. J. Anim. Sci. 65:645–650.

Campbell, R. E., J. M. H. Ffrench-Mullen, M. A. Cowley, M. S. Smith, and K. L. Grove. 2001. Hypothalamic circuitry of neuropeptide Y regulation of neuroendocrine function and food intake via the Y5 receptor subtype. Neuroendocrinology 74:106–119.

Cardoso, R. C., B. R. C. Alves, L. D. Prezotto, J. F. Thorson, L. O. Tedeschi, D. H. Keisler, C. S. Park, M. Amstalden, and G. L. Williams. 2014. Use of a stair-step compensatory gain nutritional regimen to program the onset of puberty in beef heifers. J. Anim. Sci. (in press).

Castellano, J. M., V. M. Navarro, R. Fernandez-Fernandez, R. Nogueiras, S. Tovar, J. Roa, M. J. Vazquez, E. Vigo, F. F. Casanueva, E. Aguilar, L. Pinilla, C. Dieguez, and M. Tena-Sempere. 2005. Changes in hypothalamic KiSS-1 system and restoration of pubertal activation of the reproductive axis by kisspeptin in undernutrition. Endocrinology 146:3917–3925.

Castellano, J. M., V. M. Navarro, R. Fernandez-Fernandez, J. Roa, E. Vigo, R. Pineda, C. Dieguez, E. Aguilar, L. Pinilla, and M. Tena-Sempere. 2006. Expression of hypothalamic KiSS-1 system and rescue of defective gonadotropic responses by kisspeptin in streptozotocin-induced diabetic male rats. Diabetes 55:2602–2610.

Chaillou, E., R. Baumont, Y. Chilliard, and Y. Tillet. 2002. Several subpopulations of neuropeptide Y-containing neurons exist in the infundibular nucleus of sheep: An immunohistochemical study of animals on different diets. J. Comp. Neurol. 444:129–143.

Chehab, F. F., M. E. Lim, and R. Lu. 1996. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nat. Genet. 12:318–320.

Cheung, C. C., J. E. Thornton, S. D. Nurani, D. K. Clifton, and R. A. Steiner. 2001. A reassessment of leptin's role in triggering the onset of puberty in the rat and mouse. Neuroendocrinology 74:12–21.

Clanton, D. C., L. E. Jones, and M. E. England. 1983. Effect of rate and time of gain after weaning on the development of replacement beef heifers. J. Anim. Sci. 56:280–285.

Coupé, B., V. Amarger, I. Grit, A. Benani, and P. Parnet. 2010. Nutritional programming affects hypothalamic organization and early response to leptin. Endocrinology 151:702–713.

Crown, A., D. K. Clifton, and R. A. Steiner. 2007. Neuropeptide signaling in the integration of metabolism and reproduction. Neuroendocrinology 86:175–182.

Day, M. L., K. Imakawa, M. Garciawinder, D. D. Zalesky, B. D. Schanbacher, R. J. Kittok, and J. E. Kinder. 1984. Endocrine mechanisms of puberty in heifers – Estradiol negative feedback-regulation of luteinizing-hormone secretion. Biol. Reprod. 31:332–341.

Day, M. L., K. Imakawa, P. L. Pennel, D. D. Zalesky, A. C. Clutter, R. J. Kittok, and J. E. Kinder. 1986a. Influence of season and estradiol on secretion of luteinizing hormone in ovariectomized cows. Biol. Reprod. 35:549–553.

Day, M. L., K. Imakawa, D. D. Zalesky, R. J. Kittok, and J. E. Kinder. 1986b. Effects of restriction of dietary energy intake during the prepubertal period on secretion of luteinizing hormone and responsiveness of the pituitary to luteinizing hormone-releasing hormone in heifers. J. Anim. Sci. 62:1641–1648.

de Roux, N., E. Genin, J. C. Carel, F. Matsuda, J. L. Chaussain, and E. Milgrom. 2003. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc. Natl. Acad. Sci. USA 100:10972–10976.

Desroziers, E., J. D. Mikkelsen, A. Duittoz, and I. Franceschini. 2012. Kisspeptin-immunoreactivity changes in a sex- and hypothalamic-region-specific manner across rat postnatal development. J. Neuroendocrinol. 24:1154–1165.

Donato, J., Jr., R. M. Cravo, R. Frazao, L. Gautron, M. M. Scott, J. Lachey, I. A. Castro, L. O. Margatho, S. Lee, C. Lee, J. A. Richardson, J. Friedman, S. Chua Jr., R. Coppari, J. M. Zigman, J. K. Elmquist, and C. F. Elias. 2011. Leptin's effect on puberty in mice is relayed by the ventral premammillary nucleus and does not require signaling in Kiss1 neurons. J. Clin. Invest. 121:355–368.

Dorling, A. A., M. G. Todman, K. S. Korach, and A. E. Herbison. 2003. Critical role for estrogen receptor alpha in negative feedback regulation of gonadotropin-releasing hormone mRNA expression in the female mouse. Neuroendocrinology 78:204–209.

Durando, P., A. Ferreira, and M. E. Celis. 1989. Acute administration of alpha-melanotropin exerts a stimulatory control on puberty. Acta Endocrinol. (Copenh.) 120:661–666.

Ehrhardt, R. A., P. L. Greenwood, A. W. Bell, and Y. R. Boisclair. 2003. Plasma leptin is regulated predominantly by nutrition in preruminant lambs. J. Nutr. 133:4196–4201.

Foster, D. L., and D. H. Olster. 1985. Effect of restricted nutrition on puberty in the lamb: Patterns of tonic luteinizing hormone (LH) secretion and competency of the LH surge system. Endocrinology 116:375–381.

Franceschini, I., D. Lomet, M. Cateau, G. Delsol, Y. Tillet, and A. Caraty. 2006. Kisspeptin immunoreactive cells of the ovine preoptic area and arcuate nucleus co-express estrogen receptor alpha. Neurosci. Lett. 401:225–230.

Friedman, J. M., and J. L. Halaas. 1998. Leptin and the regulation of body weight in mammals. Nature 395:763–770.

Fu, L. Y., and A. N. van den Pol. 2010. Kisspeptin directly excites anorexigenic proopiomelanocortin neurons but inhibits orexigenic neuropeptide Y cells by an indirect synaptic mechanism. J. Neurosci. 30:10205–10219.

Garcia, M. R., M. Amstalden, D. H. Keisler, N. Raver, A. Gertler, and G. L. Williams. 2004. Leptin attenuates the acute effects of centrally administered neuropeptide Y on somatotropin but not gonadotropin secretion in ovariectomized cows. Domest. Anim. Endocrinol. 26:189–200.

Garcia, M. R., M. Amstalden, C. D. Morrison, D. H. Keisler, and G. L. Williams. 2003. Age at puberty, total fat and conjugated linoleic acid content of carcass, and circulating metabolic hormones in beef heifers fed a diet high in linoleic acid beginning at four months of age. J. Anim. Sci. 81:261–268.

Garcia, M. R., M. Amstalden, S. W. Williams, R. L. Stanko, C. D. Morrison, D. H. Keisler, S. E. Nizielski, and G. L. Williams. 2002. Serum leptin and its adipose gene expression during pubertal development, the estrous cycle, and different seasons in cattle. J. Anim. Sci. 80:2158–2167.

Gasser, C. L., E. J. Behlke, D. E. Grum, and M. L. Day. 2006a. Effect of timing of feeding a high-concentrate diet on growth and attainment of puberty in early-weaned heifers. J. Anim. Sci. 84:3118–3122.

Gasser, C. L., G. A. Bridges, M. L. Mussard, D. E. Grum, J. E. Kinder, and M. L. Day. 2006b. Induction of precocious puberty in heifers III: Hastened reduction of estradiol negative feedback on secretion of luteinizing hormone. J. Anim. Sci. 84:2050–2056.

Gasser, C. L., C. R. Burke, M. L. Mussard, E. J. Behlke, D. E. Grum, J. E. Kinder, and M. L. Day. 2006c. Induction of precocious puberty in heifers II: Advanced ovarian follicular development. J. Anim. Sci. 84:2042–2049.

Gasser, C. L., D. E. Grum, M. L. Mussard, F. L. Fluharty, J. E. Kinder, and M. L. Day. 2006d. Induction of precocious puberty in heifers I: Enhanced secretion of luteinizing hormone. J. Anim. Sci. 84:2035–2041.

Gazal, O. S., L. S. Leshin, R. L. Stanko, M. G. Thomas, D. H. Keisler, L. L. Anderson, and G. L. Williams. 1998. Gonadotropin-releasing hormone secretion into third-ventricle cerebrospinal fluid of cattle: Correspondence with the tonic and surge release of luteinizing hormone and its tonic inhibition by suckling and neuropeptide Y. Biol. Reprod. 59:676–683.

Gonzales, C., M. J. Voirol, M. Giacomini, R. C. Gaillard, T. Pedrazzini, and F. P. Pralong. 2004. The neuropeptide Y Y1 receptor mediates NPY-induced inhibition of the gonadotrope axis under poor metabolic conditions. FASEB J. 18:137–139.

Gonzalez-Padilla, E., R. Ruiz, D. LeFever, A. Denham, and J. N. Wiltbank. 1975. Puberty in beef heifers. III. Induction of fertile estrus. J. Anim. Sci. 40:1110–1118.

Hagan, M. M., P. A. Rushing, L. M. Pritchard, M. W. Schwartz, A. M. Strack, L. H. Van Der Ploeg, S. C. Woods, and R. J. Seeley. 2000. Long-term orexigenic effects of AgRP-(83—132) involve mechanisms other than melanocortin receptor blockade. Am. J. Physiol. 279:47–52.

Hahn, T. M., J. F. Breininger, D. G. Baskin, and M. W. Schwartz. 1998. Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. Nat. Neurosci. 1:271–272.

Håkansson, M. L., and B. Meister. 1998. Transcription factor STAT3 in leptin target neurons of the rat hypothalamus. Neuroendocrinology 68:420–427.

Han, S. K., M. L. Gottsch, K. J. Lee, S. M. Popa, J. T. Smith, S. K. Jakawich, D. K. Clifton, R. A. Steiner, and A. E. Herbison. 2005. Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. J. Neurosci. 25:11349–11356.

I'Anson, H., J. M. Manning, C. G. Herbosa, J. Pelt, C. R. Friedman, R. I. Wood, D. C. Bucholtz, and D. L. Foster. 2000. Central inhibition of gonadotropin-releasing hormone secretion in the growth-restricted hypogonadotropic female sheep. Endocrinology 141:520–527.

Iqbal, J., S. Pompolo, T. Murakami, E. Grouzmann, T. Sakurai, B. Meister, and I. J. Clarke. 2001. Immunohistochemical characterization of localization of long-form leptin receptor (OB-Rb) in neurochemically defined cells in the ovine hypothalamus. Brain Res. 920:55–64.

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

Kinder, J. E., M. L. Day, and R. J. Kittok. 1987. Endocrine regulation of puberty in cows and ewes. J. Reprod. Fertil. 34:167–186.

Lebrethon, M. C., A. Aganina, M. Fournier, A. Gerard, A. S. Parent, and J. P. Bourguignon. 2007. Effects of in vivo and in vitro administration of ghrelin, leptin and neuropeptide mediators on pulsatile gonadotrophin-releasing hormone secretion from male rat hypothalamus before and after puberty. J. Neuroendocrinol. 19:181–188.

Lehman, M. N., and F. J. Karsch. 1993. Do gonadotropin-releasing hormone, tyrosine hydroxylase-, and beta-endorphin-immunoreactive neurons contain estrogen receptors? A double-label immunocytochemical study in the Suffolk ewe. Endocrinology 133:887–895.

Lesmeister, J. L., P. J. Burfening, and R. L. Blackwell. 1973. Date of first calving in beef cows and subsequent calf production. J. Anim. Sci. 36:1–6.

Long, N. M., S. P. Ford, and P. W. Nathanielsz. 2011. Maternal obesity eliminates the neonatal lamb plasma leptin peak. J. Physiol. 589:1455–1462.

Lynch, J. M., G. C. Lamb, B. L. Miller, R. T. Brandt Jr., R. C. Cochran, and J. E. Minton. 1997. Influence of timing of gain on growth and reproductive performance of beef replacement heifers. J. Anim. Sci. 75:1715–1722.

Maciel, M. N., D. A. Zieba, M. Amstalden, D. H. Keisler, J. P. Neves, and G. L. Williams. 2004a. Chronic administration of recombinant ovine leptin in growing beef heifers: Effects on secretion of LH, metabolic hormones, and timing of puberty. J. Anim. Sci. 82:2930–2936.

Maciel, M. N., D. A. Zieba, M. Amstalden, D. H. Keisler, J. P. Neves, and G. L. Williams. 2004b. Leptin prevents fasting-mediated reductions in pulsatile secretion of luteinizing hormone and enhances its gonadotropin-releasing hormone-mediated release in heifers. Biol. Reprod. 70:229–235.

Mayer, C., M. Acosta-Martinez, S. L. Dubois, A. Wolfe, S. Radovick, U. Boehm, and J. E. Levine. 2010. Timing and completion of puberty in female mice depend on estrogen receptor alpha-signaling in kisspeptin neurons. Proc. Natl. Acad. Sci. USA 107:22693–22698.

McCann, J. P., and W. Hansel. 1986. Relationships between insulin and glucose metabolism and pituitary-ovarian functions in fasted heifers. Biol. Reprod. 34:630–641.

McShane, T. M., T. May, J. L. Miner, and D. H. Keisler. 1992. Central actions of neuropeptide-Y may provide a neuromodulatory link between nutrition and reproduction. Biol. Reprod. 46:1151–1157.

McShane, T. M., S. L. Petersen, S. McCrone, and D. H. Keisler. 1993. Influence of food restriction on neuropeptide-Y, proopiomelanocortin, and luteinizing hormone-releasing hormone gene expression in sheep hypothalami. Biol. Reprod. 49:831–839.

Merkley, C. M., K. L. Porter, L. M. Coolen, S. M. Hileman, H. J. Billings, S. Drews, R. L. Goodman, and M. N. Lehman. 2012. KNDy (kisspeptin/neurokinin B/dynorphin) neurons are activated during both pulsatile and surge secretion of LH in the ewe. Endocrinology 153:5406–5414.

Messager, S., E. E. Chatzidaki, D. Ma, A. G. Hendrick, D. Zahn, J. Dixon, R. R. Thresher, I. Malinge, D. Lomet, M. B. Carlton, W. H. Colledge, A. Caraty, and S. A. Aparicio. 2005. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. Proc. Natl. Acad. Sci. USA 102:1761–1766.

National Animal Health Monitoring System (NAHMS). 1994. Sparse use of reproductive management technology for beef heirs and cows. Centers for Epidemiology and Animal Health, United States Department of Agriculture, Animal and Plant Health Inspection Service, and Veterinary Services; Fort Collins, Colorado; www.aphis.usda.gov/animal_health/nahms/beefcowcalf/downloads/chapa/CHAPA_is_SparseRMT.pdf.

Navarro, V. M., J. M. Castellano, R. Fernandez-Fernandez, M. L. Barreiro, J. Roa, J. E. Sanchez-Criado, E. Aguilar, C. Dieguez, L. Pinilla, and M. Tena-Sempere. 2004a. Developmental and hormonally regulated messenger ribonucleic acid expression of KiSS-1 and its putative receptor, GPR54, in rat hypothalamus and potent luteinizing hormone-releasing activity of KiSS-1 peptide. Endocrinology 145:4565–4574.

Navarro, V. M., R. Fernandez-Fernandez, J. M. Castellano, J. Roa, A. Mayen, M. L. Barreiro, F. Gaytan, E. Aguilar, L. Pinilla, C. Dieguez, and M. Tena-Sempere. 2004b. Advanced vaginal opening and precocious activation of the reproductive axis by KiSS-1 peptide, the endogenous ligand of GPR54. J. Physiol. 561:379–386.

Nestor, C. C., A. M. Briscoe, S. M. Davis, M. Valent, R. L. Goodman, and S. M. Hileman. 2012. Evidence of a role for kisspeptin and neurokinin B in puberty of female sheep. Endocrinology 153:2756–2765.

Park, C. S., R. B. Danielson, B. S. Kreft, S. H. Kim, Y. S. Moon, and W. L. Keller. 1998. Nutritionally directed compensatory growth and effects on lactation potential of developing heifers. J. Dairy Sci. 81:243–249.

Park, C. S., G. M. Erickson, Y. J. Choi, and G. D. Marx. 1987. Effect of compensatory growth on regulation of growth and lactation: Response of dairy heifers to a stair-step growth pattern. J. Anim. Sci. 64:1751–1758.

Patterson, D. J., S. L. Wood, and R. F. Randle. 2005. Procedures that support reproductive management of replacement beef heifers. In: Proc. Appl. Reprod. Strat. Beef Cattle, November 12 and 13, College Station, TX. p. 271–292.

Pierroz, D. D., N. M. Gruaz, V. d'Alieves, and M. L. Aubert. 1995. Chronic administration of neuropeptide Y into the lateral ventricle starting at 30 days of life delays sexual maturation in the female rat. Neuroendocrinology 61:293–300.

Pralong, F. P., M. Voirol, M. Giacomini, R. C. Gaillard, and E. Grouzmann. 2000. Acceleration of pubertal development following central blockade of the Y1 subtype of neuropeptide Y receptors. Regul. Pept. 95:47–52.

Prevot, V., N. K. Hanchate, N. Bellefontaine, A. Sharif, J. Parkash, C. Estrella, C. Allet, S. de Seranno, C. Campagne, X. de Tassigny, and M. Baroncini. 2010. Function-related structural plasticity of the GnRH system: A role for neuronal-glial-endothelial interactions. Front. Neuroendocrinol. 31:241–258.

Quennell, J. H., C. S. Howell, J. Roa, R. A. Augustine, D. R. Grattan, and G. M. Anderson. 2011. Leptin deficiency and diet-induced obesity reduce hypothalamic kisspeptin expression in mice. Endocrinology 152:1541–1550.

Quennell, J. H., A. C. Mulligan, A. Tups, X. Liu, S. J. Phipps, C. J. Kemp, A. E. Herbison, D. R. Grattan, and G. M. Anderson. 2009. Leptin indirectly regulates gonadotropin-releasing hormone neuronal function. Endocrinology 150:2805–2812.

Redmond, J. S., G. M. Baez-Sandoval, K. M. Spell, T. E. Spencer, C. A. Lents, G. L. Williams, and M. Amstalden. 2011. Developmental changes in hypothalamic Kiss1 expression during activation of the pulsatile release of luteinising hormone in maturing ewe lambs. J. Neuroendocrinol. 23:815–822.

Roa, J., and A. E. Herbison. 2012. Direct regulation of GnRH neuron excitability by arcuate nucleus POMC and NPY neuron neuropeptides in female mice. Endocrinology 153:5587–5599.

Satoh, N., Y. Ogawa, G. Katsuura, M. Hayase, T. Tsuji, K. Imagawa, Y. Yoshimasa, S. Nishi, K. Hosoda, and K. Nakao. 1997. The arcuate nucleus as a primary site of satiety effect of leptin in rats. Neurosci. Lett. 224:149–152.

Schneider, J. E. 2004. Energy balance and reproduction. Physiol. Behav. 81:289–317.

Seeley, R. J., D. L. Drazen, and D. J. Clegg. 2004. The critical role of the melanocortin system in the control of energy balance. Annu. Rev. Nutr. 24:133–149.

Seminara, S. B., S. Messager, E. E. Chatzidaki, R. R. Thresher, J. S. Acierno Jr., J. K. Shagoury, Y. Bo-Abbas, W. Kuohung, K. M. Schwinof, A. G. Hendrick, D. Zahn, J. Dixon, U. B. Kaiser, S. A. Slaugenhaupt, J. F. Gusella, S. O'Rahilly, M. B. Carlton, W. F. Crowley Jr., S. A. Aparicio, and W. H. Colledge. 2003. The GPR54 gene as a regulator of puberty. N. Engl. J. Med. 349:1614–1627.

Smith, J. T., B. V. Acohido, D. K. Clifton, and R. A. Steiner. 2006. KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. J. Neuroendocrinol. 18:298–303.

Smith, J. T., C. M. Clay, A. Caraty, and I. J. Clarke. 2007. KiSS-1 messenger ribonucleic acid expression in the hypothalamus of the ewe is regulated by sex steroids and season. Endocrinology 148:1150–1157.

Smith, J. T., L. M. Coolen, L. J. Kriegsfeld, I. P. Sari, M. R. Jaafarzadehshirazi, M. Maltby, K. Bateman, R. L. Goodman, A. J. Tilbrook, T. Ubuka, G. E. Bentley, I. J. Clarke, and M. N. Lehman. 2008. Variation in kisspeptin and RFamide-related peptide (RFRP) expression and terminal connections to gonadotropin-releasing hormone neurons in the brain: A novel medium for seasonal breeding in the sheep. Endocrinology 149:5770–5782.

Smith, J. T., M. J. Cunningham, E. F. Rissman, D. K. Clifton, and R. A. Steiner. 2005. Regulation of Kiss1 gene expression in the brain of the female mouse. Endocrinology 146:3686–3692.

Smith, J. T., Q. Li, A. Pereira, and I. J. Clarke. 2009. Kisspeptin neurons in the ovine arcuate nucleus and preoptic area are involved in the preovulatory luteinizing hormone surge. Endocrinology 150:5530–5538.

Smith, J. T., Q. Li, K. S. Yap, M. Shahab, A. K. Roseweir, R. P. Millar, and I. J. Clarke. 2011. Kisspeptin is essential for the full preovulatory LH surge and stimulates GnRH release from the isolated ovine median eminence. Endocrinology 152:1001–1012.

Stormshak, F., and C. V. Bishop. 2008. Board-invited review: Estrogen and progesterone signaling: Genomic and nongenomic actions in domestic ruminants. J. Anim. Sci. 86:299–315.

Suzuki, S., H. Kadokawa, and T. Hashizume. 2008. Direct kisspeptin-10 stimulation on luteinizing hormone secretion from bovine and porcine anterior pituitary cells. Anim. Reprod. Sci. 103:360–365.

Takase, K., Y. Uenoyama, N. Inoue, H. Matsui, S. Yamada, M. Shimizu, T. Homma, J. Tomikawa, S. Kanda, H. Matsumoto, Y. Oka, H. Tsukamura, and K. I. Maeda. 2009. Possible role of oestrogen in pubertal increase of Kiss1/kisspeptin expression in discrete hypothalamic areas of female rats. J. Neuroendocrinol. 21:527–537.

Tillet, Y., S. Picard, G. Bruneau, P. Ciofi, M. Wankowska, A. Wojcik-Gladysz, and J. Polkowska. 2010. Hypothalamic arcuate neuropeptide Y-neurons decrease periventricular somatostatin-neuronal activity before puberty in the female lamb: Morphological arguments. J. Chem. Neuroanat. 40:265–271.

Tomikawa, J., T. Homma, S. Tajima, T. Shibata, Y. Inamoto, K. Takase, N. Inoue, S. Ohkura, Y. Uenoyama, K. Maeda, and H. Tsukamura. 2010. Molecular characterization and estrogen regulation of hypothalamic KISS1 gene in the pig. Biol. Reprod. 82:313–319.

Vulliémoz, N. R., E. Xiao, L. Xia-Zhang, S. L. Wardlaw, and M. Ferin. 2005. Central infusion of agouti-related peptide suppresses pulsatile luteinizing hormone release in the ovariectomized rhesus monkey. Endocrinology 146:784–789.

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

Zieba, D. A., M. Amstalden, S. Morton, M. N. Maciel, D. H. Keisler, and G. L. Williams. 2004. Regulatory roles of leptin at the hypothalamic-hypophyseal axis before and after sexual maturation in cattle. Biol. Reprod. 71:804–812.