SVU- International Journal of Veterinary Sciences, 3 (1): 10-26, 2020.
Print ISSN: 2535-1826 Online ISSN: 2535-1877

Review Article Open Access

Kisspeptin: A Central Regulator of Reproduction in Mammals

Nahoko Ieda[1,2#], Ahmed SA Hassaneen[3], Naoko Inoue[1], Yoshihisa Uenoyama[1] and Hiroko Tsukamura[1*]

[1]Laboratory of Reproductive Science, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, JAPAN, [2]Regional Office for Near East and North Africa, Food and Agricultural Organization of the United Nations, Cairo, EGYPT, [3]Department of Theriogenology, Obstetrics and Artificial Insemination, Faculty of Veterinary Medicine, South Valley University, Qena 83523, EGYPT

Abstract

The discovery of the role of kisspeptin neurons in the regulation of mammalian reproduction in 2003 was one of the biggest breakthroughs in reproductive endocrinology within the last few decades. Research during the past two decades since the discovery of kisspeptin has been unveiling the mechanism of how the hypothalamic kisspeptin neurons control reproductive functions through regulation of gonadotropin-releasing hormone (GnRH) secretion. This article aims to overview kisspeptin research, including the most recent studies from ours and other research groups, and to discuss the possibility of new strategies to control reproductive functions in mammals. In the first section, we introduce the critical role of kisspeptin neurons in puberty onset and reproductive functions in mammals, including the regulation of two modes of GnRH/gonadotropin secretion, namely pulsatile and surge modes. The next section focuses more on the mechanism of how the kisspeptin neurons in the arcuate nucleus in the hypothalamus precisely controls GnRH pulse using other two neuropeptides, neurokinin B and dynorphin A. The article also discusses the mechanism suppressing reproductive function during lactation and other stress conditions through inhibition of kisspeptin neurons and consequent GnRH/gonadotropin secretion, to provide insights on the possibility of new strategies to improve reproductive performance in mammals including domestic farm animals.

Keywords: Anteroventral Periventricular Nucleus, Arcuate Nucleus, Fertility, Gonadotropins, Pre-optic Area.

DOI: 10.21608/svu.2019.16569.1027
Received: September 03, 2019 Accepted: November 09, 2019 Published: November 11, 2019
*Corresponding Author: Hiroko Tsukamura E-mail: htsukamura@nagoya-u.jp

[#]This work was conducted at Laboratory of Reproductive Science, Graduate School of Bioagricultural Sciences, Nagoya University, JAPAN.

Citation: Ieda et al., Kisspeptin: A Central Regulator of Reproduction in Mammals. SVU-IJVS 2020, 3 (1): 10-26.

Competing interest: The authors have declared that no competing interest exists.

Introduction

The regulatory mechanism of gonadotropin-releasing hormone (GnRH) secretion, including positive and negative feedback mechanisms from ovarian steroids to the hypothalamic GnRH secretion, had been a "black box" since 1971 when GnRH was first identified in the hypothalamus of sheep (Burgus et al., 1972) and pigs (Schally et al., 1971) as a regulator of gonadotropins secretion from the pituitary. For the discovery of GnRH, the Nobel Prize was awarded to Dr. Guillemin and Schally in 1977. Forty years later, the discovery of the role of kisspeptin neurons in regulation of mammalian reproduction brought the biggest breakthrough in the study of reproductive neuroendocrinology (de Roux et al., 2003, Seminara et al., 2003). Recent research achievements on a regulatory role of kisspeptin on GnRH secretion are leading us to accumulate knowledge serving for the development of new strategies to control reproductive performance of livestock.

Kisspeptin is encoded by *Kiss1*, a gene first identified as a metastasis-suppressor gene, and was discovered as a natural ligand of an orphan receptor, GPR54 (Kotani et al., 2001, Ohtaki et al., 2001). Kisspeptin neuro-peptide was originally named "metastin" based on its metastasis-suppressing function of the gene (Ohtaki et al., 2001). These days, this neuro-peptide is often called "kisspeptin" in the field of reproduction. In 2003, two independent research groups in the United States and France demonstrated that loss-of-function mutations of *GPR54* cause severe hypogonadotropic hypogonadism and subsequent loss of reproductive function in humans and mice (de Roux et al., 2003, Seminara et al., 2003). These studies greatly contributed to highlight the function of the kisspeptin-GPR54 signaling as a master regulator of reproduction via direct stimulation of GnRH and subsequent gonadotropin release in mammals.

This article aims to overview kisspeptin research during the past two decades, including most recent studies from our research group, and to discuss the possibility of new strategies to control reproductive functions in domestic farm animals.

Control of pulse and surge modes of GnRH/gonadotropin secretion by kisspeptin neurons

The gonadal function is regulated by the hypothalamo-pituitary-gonadal axis (HPG axis) in mammals including rodents, ruminants, and primates. GnRH secretion from the hypothalamus stimulates the secretion of gonadotropins from the pituitary, subsequently enhancing gonadal activities. GnRH is secreted in two different modes, namely in a pulsatile mode at the basal level and a surge mode characterized by a transient and larger amount of secretion, in females (Fig. 1). The timing of each GnRH pulse precisely synchronizes with a luteinizing hormone (LH) pulse, as detected in the pituitary portal blood in ewes (Moenter et al., 1992). The pulsatile secretion of GnRH is critical to maintain the responsiveness of the pituitary to GnRH itself, because chronic exposure to GnRH causes reduction of the GnRH receptor in the gonadotrophs in the anterior pituitary (Nett et al., 1981). Indeed, the secretion of gonadotropins is attenuated after prolonged and constant exposure of GnRH (Belchetz et al., 1978). It is also well known that GnRH/LH

secretion is suppressed by negative feedback from ovarian estrogen while the follicles develop (Bronson, 1981, Evans et al., 1994). The GnRH/gonadotropin pulses at a physiological frequency enhance follicular development in the ovary, leading to an increased circulating estrogen level. The high level of circulating estrogen, in-turn, positively feedbacks to the hypothalamus and induces surge-mode secretion of GnRH/LH, and subsequently induces ovulation.

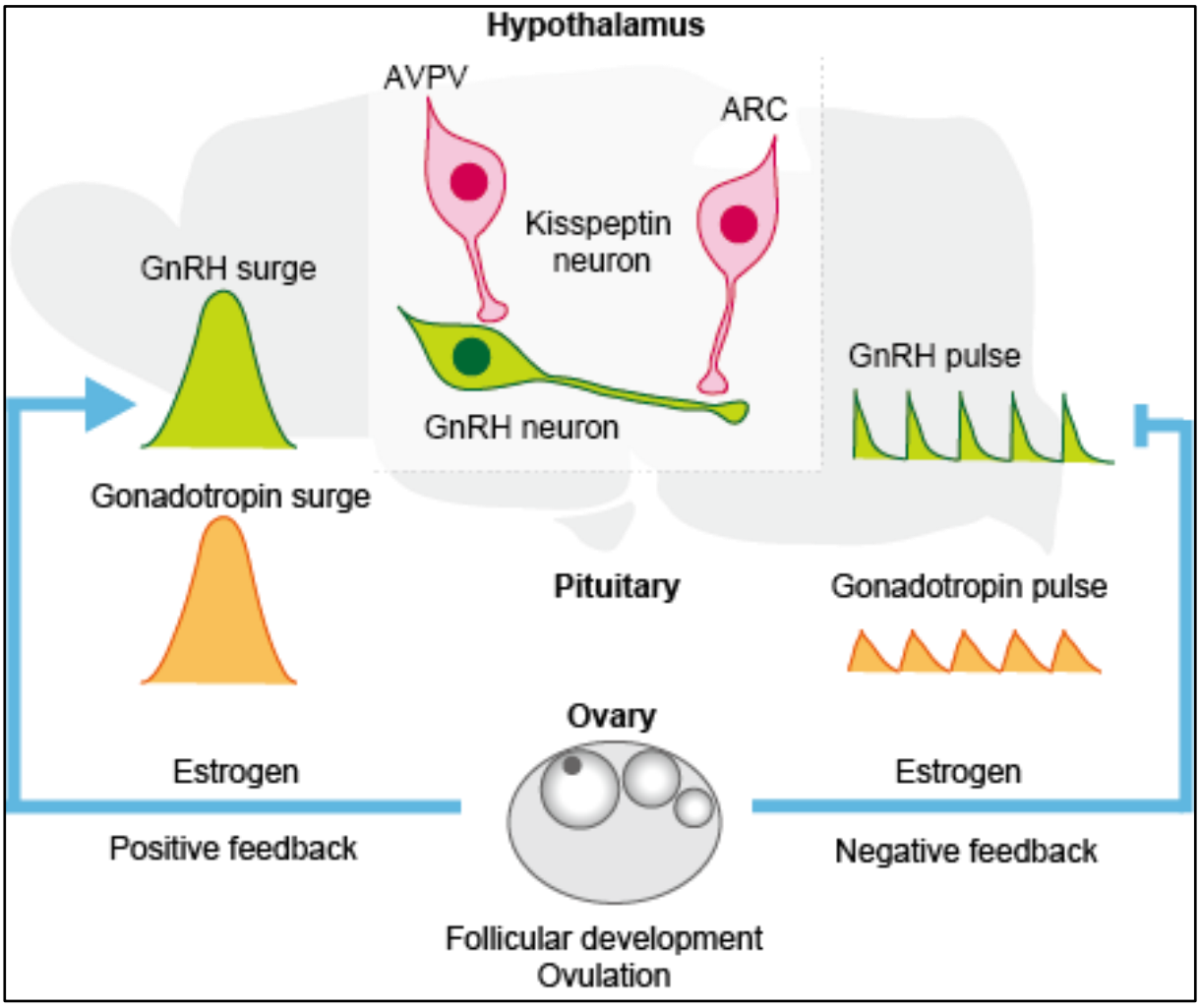

Fig. 1. Regulation of GnRH/gonadotropin surge and GnRH pulses by kisspeptin.

GnRH pulses from the hypothalamus enhance secretion from gonadotropins. Estrogen from the ovary at this stage negatively feedbacks to kisspeptin neurons in the ARC, so that GnRH/gonadotropins secretions are maintained at a low level. Continuous GnRH/gonadotropin pulses at the basal level are critically important for the follicular growth, which causes higher and higher concentration of estrogen in the blood circulation. When follicular growth reaches to the final stage, a high level of blood estrogen positively feedbacks to the kisspeptin neurons in the AVPV/POA that stimulate GnRH neurons. This leads to the substantial secretion of gonadotropins from the pituitary and subsequently induces ovulation.

G-protein-coupled receptor 54 (GPR54; a kisspeptin receptor) signaling is the fundamental regulator of the HPG axis in mammals as described above. To demonstrate that kisspeptin itself is essential for reproduction, we previously generated *Kiss1* knockout (KO) rats, of which the *Kiss1* locus was replaced with a red fluorescent protein reporter gene (Uenoyama et al., 2015). The *Kiss1* KO rats showed non-detectable levels of plasma gonadotropins, atrophic gonads, and therefore, the absence of spontaneous puberty in both sexes. Further, the lack of kisspeptin caused attenuation of LH pulses as well as the estrogen-induced LH surge (Uenoyama et al., 2015), but an intravenous challenge of kisspeptin robustly enhanced LH secretion in *Kiss1* KO rats, leading to the conclusion that kisspeptin is an indispensable stimulator of GnRH/gonadotropin secretion both in the pulse and surge modes.

Kisspeptin neurons are mainly located in the hypothalamic area in two populations: the posterior population of the neurons in the arcuate nucleus (ARC); and the anterior one in the anteroventral periventricular nucleus (AVPV), periventricular nucleus (PeN), or preoptic areas (POA) in females of rodents (Gottsch et al., 2004, Adachi et al., 2007), pigs (Tomikawa et al., 2010), and ruminants (Franceschini et al., 2006, Matsuda et al., 2015, Hassaneen et al., 2016), respectively. *Kiss1* gene expression in these neurons is regulated by estrogen, negatively in the ARC and positively in the AVPV, via the estrogen receptor α in kisspeptin neurons of both nuclei in rodents (Smith et al., 2005, Adachi et al., 2007). A hypothesis has been developed over the past few decades suggesting that the GnRH pulse generator locates in the mediobasal hypothalamus including the ARC (Ohkura et al., 1991, 1992), while the GnRH surge generator locates in the AVPV/POA (Goodman, 1978, Petersen and Barraclough, 1989). Given this hypothesis, the notion that kisspeptin neurons in the two hypothalamic nuclei would be the regulators of the two modes of GnRH/gonadotropin secretion is well-accepted by many researchers. Indeed, rhythmic volleys of multiple unit activity (MUA) were found to be synchronized with LH pulses in female goats when the electrode was placed in the close proximity to the ARC kisspeptin neurons (Ohkura et al., 2009), and there was significant correlation between the LH pulse frequency and the number of *Kiss1*-expressing neurons in the ARC of maturing ewe (Redmond et al., 2011). On the other hand, when a kisspeptin antibody was infused to the POA, an adjacent brain region to the AVPV, endogenous LH surge was attenuated in female rats (Kinoshita et al., 2005). Therefore, it is plausible that ARC kisspeptin neurons are the central regulator of GnRH pulses, whereas the AVPV kisspeptin neurons are the central regulator of the GnRH surge.

As discussed above, AVPV kisspeptin neurons are considered to be a target of positive feedback of estrogen in rodents (Smith et al., 2005, Adachi et al., 2007). Similarly in pigs, kisspeptin neurons in the PeN, equivalent to rodent AVPV kisspeptin neurons, appears to be the center of the GnRH/gonadotropin surge generation because *Kiss1* expression in the region increases upon estrogen treatment in ovariectomized sows (Tomikawa et al., 2010). Likewise, in goats, monkeys and musk shrew, an increase in c-Fos, a marker

of neuronal activation, expression in the POA kisspeptin neurons is evident after the administration of preovulatory levels of estrogen (Inoue et al., 2011, Watanabe et al., 2014, Matsuda et al., 2015). Interestingly, in sheep, it is likely that both the ARC and POA kisspeptin neurons take part in GnRH surge generation, because *Kiss1* expression in both regions significantly increased during the late follicular phase compared to the luteal phase (Smith et al., 2009). Besides, administration of progesterone, but not estrogen, increases the expression of *Kiss1* in the ARC in ewes in breeding season (Smith et al., 2007). Such differences among species should be taken into account in considering the mechanism that controls GnRH/LH surge through kisspeptin neurons in livestock.

Accumulating evidence also suggests the absolute necessity of kisspeptin in the onset of puberty. For instance, the *Kiss1*-expression level is significantly higher in the post-pubertal period compared to pre-pubertal period in the hypothalamus of rats (Takase et al., 2009), ruminants (Redmond et al., 2011), and primates (Shahab et al., 2005). It is considered that *Kiss1*-expression in pre-pubertal period is suppressed by the negative feedback of estrogen, because ovariectomy causes an increase in ARC *Kiss1*-expresion as well as LH pulses in immature rats (Takase et al., 2009) and ewes (Nestor et al., 2012). Besides, kisspeptin administration induces LH secretion in prepubertal rats (Navarro et al., 2005) and primates (Shahab et al., 2005). These studies suggest that the HPG axis is already functional in terms of responsiveness of GnRH neurons to kisspeptin and that of gonadotrophs to GnRH even before puberty, and puberty occurs when the hypothalamic *Kiss1* is ready to express and secrete kisspeptin to induce GnRH and consequent gonadotropins secretion. It is considered that *Kiss1* expression is suppressed by the negative feedback of estrogen more sensitively in the pre-pubertal period than in the post-pubertal period, because a low level of circulating estrogen, which is equivalent to the diestrous level in matured animals, can robustly suppress *Kiss1* expression in immature rats (Takase et al., 2009). Therefore, investigation on the mechanism of desensitization of the kisspeptin neurons to the estrogen negative feedback could be a key to understand the precise mechanism of the onset of puberty in mammals.

GnRH pulse generation by KNDy neurons

The mechanism responsible for GnRH pulse generation could be shared by wide range of mammalian species. This common mechanism among the species may serve for development of new schemes of reproductive control in livestock. Thus, the mechanism by which ARC kisspeptin neurons control pulsatile GnRH/gonadotropin release will be discussed in this section.

The ARC kisspeptin neurons are reported to co-express two other neuropeptides, namely neurokinin B and dynorphin A, in rodents and ruminants (Goodman et al., 2007, Navarro et al., 2009, Wakabayashi et al., 2010), and therefore are often referred to as kisspeptin/neurokinin B/dynorphin A (KNDy) neurons, whereas these neuropeptides were not found in the AVPV kisspeptin neurons. As mentioned above, MUA volleys synchronizes with LH pulses when the electronic detector is placed in

close adjacent to the KNDy neurons in the ARC of female goats (Ohkura et al., 2009, Wakabayashi et al., 2010). Importantly, such MUA volleys were not observed when an electronic detector was placed in the lateral part of the median eminence, where GnRH neuronal axons project to (Ohkura et al., 2009). Therefore, it is considered that the pulsatile secretion of GnRH and gonadotropins are induced by the pulsatile activity of the ARC KNDy neurons. Interestingly, the frequency of MUA volley of KNDy neurons dramatically increases by a central administration of neurokinin B or a dynorphin A receptor antagonist in the goat whereas the volley is robustly suppressed by the dynorphin A injection (Wakabayashi et al., 2010). These results suggest that KNDy neurons regulate their own activity by autocrine and/or paracrine actions of neurokinin B and dynorphin A to determine the frequency of GnRH pulses in mammals.

Notably, a neurokinin B receptor agonist or a dynorphin A receptor antagonist induces precocious puberty in rats (Nakahara et al., 2013), and loss-of function mutations in genes encoding neurokinin B or the neurokinin B receptor cause severe hypogonadotropic hypogonadism in humans (Topaloglu et al., 2009) similar to loss-of-function mutations in *Kiss1* (Uenoyama et al., 2015) or *GPR54* encoding the kisspeptin receptor (de Roux et al., 2003, Seminara et al., 2003). These studies strongly suggest that neurokinin B positively regulates the pulsatile secretion of GnRH in mammals. On the other hand, the precise mechanism of inhibitory regulator of GnRH pulses needs further investigation. The co-expression rate of the dynorphin A in ARC kisspeptin neurons is consistent among species at 80-90% in mice, sheep, and goats (Goodman et al., 2007, Navarro et al., 2009, Wakabayashi et al., 2010), while the co-expression rate of the dynorphin A receptor (kappa-opioid receptor; KOR) in the kisspeptin neurons varies among species and has only been reported in limited number of studies (Navarro et al., 2009, Weems et al., 2016). For instance, a previous study reported that KOR is expressed only in approximately 20% of the ARC kisspeptin neurons in mice (Navarro et al., 2009), while 98% of ARC kisspeptin neurons showed KOR immunoreactivity in ewe (Weems et al., 2016). Further studies on the precise localization of each receptor of the neuropeptides are expected to contribute to drawing a clear picture of the neuronal circuit of the GnRH pulse generator.

Our previous study demonstrated a part of the mechanism of how the KNDy neurons synchronize their activity and cause pulsatile firing as a group, by using *Kiss1*-GFP transgenic mice (Ikegami et al., 2017). In the study, we demonstrated that senktide (a selective agonist for neurokinin B receptor)-induced Ca^{2+} oscillations, an indicator of neuronal activity, in cultured *Kiss1*-GFP cells were synchronized amongst themselves, as well as with those in neighboring glial cells (Ikegami et al., 2017). In addition, our study suggested that the cell-to-cell communication through gap junctions between *Kiss1*-GFP cells as well as *Kiss1*-GFP cells and neighboring glial cells is involved in synchronized activities among KNDy neurons in order to generate GnRH pulses. Considering also that among the ARC kisspeptin neurons, more than 90% express neurokinin B and the neurokinin B receptor in mice and goats (Navarro et al., 2009, Wakabayashi et al., 2010), at least neurokinin B signaling would be a stimulatory regulator of GnRH

pulse generation via autocrine manner among KNDy neurons.

The recently emerged concept that kisspeptin neurons regulate GnRH pulses using neurokinin B and dynorphin A signaling may serve for the development of new strategies and/or drugs to control reproduction in farm animals. We recently succeeded to facilitate pulsatile LH section by a single peripheral shot of a dynorphin A receptor antagonist to female goats (Sasaki et al., 2019). The regimen would be also applicable to cattle, one of the most economically important farm animals in many countries, because our study showed the presence of KNDy neurons in cows (Hassaneen et al., 2016) and a neurokinin 3 receptor-selective agonist accelerated pulsatile luteinizing hormone secretion in lactating cattle (Nakamura et al., 2017). More specific focus on the importance of controlling reproductive function in milking cows will be discussed in the following section. In addition, a mechanism mediating malfunction of reproduction caused by heat stress and malnutrition in mammals and possibility of new methods for treatment of the reproductive disorder will also be discussed.

Inhibition of GnRH/gonadotropin secretion during lactation and other stress conditions

Mammalian reproductive function is inhibited during lactation and other stress conditions such as malnutrition and heat stress, which are major causes of economic loss in both developed and developing countries. In mammals, a tremendous amount of energy is required to produce milk in lactating females. Therefore, to avoid pregnancy that requires extra energy expenditure, follicular development and ovulation are strongly suppressed during lactation (McNeilly, 2001). It has been pointed out that the conception rate and milk yields are negatively correlated in genetically improved dairy cows and that the suppressed reproductive performance may be caused by high energy expenditure required for milk production (Nebel and McGilliard, 1993). In addition, in developing countries in tropical or arid areas, extremely hot climates and a lack of food supply may cause heat-stress and malnutrition, respectively. The inhibition of reproductive function in mammals often relies on the central suppression of pulsatile GnRH and gonadotropin secretion, and the mechanisms of GnRH suppression under certain conditions such as lactation and stress, have been elucidated by recent studies. In this section, each mechanism responsible for reproductive suppression by lactation, malnutrition, or heat stress will be discussed.

The inhibition of reproductive function during lactation is at least partly caused by suckling stimulus-induced neuronal suppression on GnRH/gonadotropins pulses, because the hypothalamic deafferentation to isolate the mediobasal hypothalamus restores LH pulses in lactating mother rats (Tsukamura et al., 1990). Our group has previously demonstrated that *Kiss1* expression was suppressed by suckling stimulus in rats, while LH release was immediately restored by an administration of kisspeptin to the mother rat (Yamada et al., 2007). Interestingly, estrogen positive feedback to the hypothalamus is functional during lactation, because the LH surge can be induced by administration of estrogen at a proestrous level (Tsukamura et al., 1988)

and LH pulses were restored 12 hours after the removal of the suckling stimulus in lactating rats (Maeda et al., 1989). These findings have led us to consider that the inhibition of GnRH/LH pulses by suckling stimulus in lactating animals is mainly caused by suppression of kisspeptin production in the ARC. Further, our most recent study suggested that somatostatin signaling at least partly mediates LH pulse suppression in lactating rats (Sugimoto et al., 2019).

It is well known that malnutrition also suppresses the reproductive function in mammals. Precise analysis on plasma hormones in rodents revealed that LH pulses are strongly suppressed in fasted animals (Cagampang et al., 1990, Minabe et al., 2011). Undernourished heifers showed longer estrous cycles, poor corpus luteum formation with significantly lower plasma progesterone concentration, and reduced number or unusual development of follicles, compared to those fed with a sufficient diet (Hill et al., 1970). Leptin is a hormone mainly secreted from the adipocytes (Zhang et al., 1994) and its plasma concentration drops along with body weight loss in humans (Weigle et al., 1997), and with food restriction as in ewes (Recabarren et al., 2004). Leptin is known to affect the HPG axis via neuropeptide Y and pro-opiomelanocortin neurons in the ARC (Cunningham et al., 1999) and restores gonadotropin secretion in fasted rats (Nagatani et al., 1998) and monkeys (Finn et al., 1998). Neuropeptide Y, an orexigenic neuropeptide inhibits estrogen-induced LH surge through neuropeptide Y type 2 receptor (Clarke et al., 2005), and melanocortins, a group of anorexigenic neuropeptides produced from pro-opiomelanocortin, are mainly reported to enhance the LH surge (Backholer et al., 2009), as both studies demonstrated in ewes. Interestingly, in *ob*/*ob* mice, a mutant strain in where leptin is deficient, ARC *Kiss1* expression is partially impaired but restored by leptin administration (Smith et al., 2006). In ewes, it is also reported that leptin increased the expression level of *Kiss1* in the ARC (Backholer et al., 2010). It was demonstrated that the kisspeptin neurons interact with their adjacent neurons expressing neuropeptide Y and pro-opiomelanocortin neurons, supporting the notion that kisspeptin neurons may be involved in gating the fasting-induced suppression of reproduction (Backholer et al., 2010, Fu and van den Pol, 2010). The mRNA expression of *Ob-Rb*, a gene encoding the leptin receptor, is detected in the *Kiss1*-expressing neurons in the ARC in mice (Smith et al., 2006). It is also possible that kisspeptin neurons are under regulation by the anorexigenic neurons, because administration of melanocortin receptors agonist accelerated GnRH generator activity detected as MUA volleys in goats (Matsuyama et al., 2005).

We have previously proposed that negative energy balance of the body can be sensed by the hindbrain to inhibit GnRH/LH secretion, based on the studies demonstrating that central injections of 2-deoxy-D-glucose (a glucose metabolic inhibitor) (Murahashi et al., 1996), alloxan (an inhibitor of glucokinase, a rate-limiting enzyme for glucose metabolism) (Kinoshita et al., 2004), ketone body (by-product of enhanced fatty acid mobilization) (Iwata et al., 2011), mercaptoacetate or trimetazidine (inhibitors of fatty acid oxidization) (Sajapitak et al., 2008), or AICAR (an

inhibitor of AMP-activated protein kinase; AMPK) into the fourth ventricle in the hindbrain suppress pulsatile LH secretion in rats. It has been also shown that glucokinase, glucose transporters, and phosphorylated AMPK are evident in the ependymocytes surrounding the ventricle of the hindbrain (Maekawa et al., 2000, Minabe et al., 2015), and that the intracellular calcium concentration in the ependymocytes taken from the hindbrain increases in response to administration of a low or high level of glucose (Moriyama et al., 2004) or AICAR (Minabe et al., 2015) in the cultured medium. Our most recent study demonstrated that the ependymocytes of the fourth ventricle have neuronal connection to hypothalamic kisspeptin neurons (Deura et al., 2019). The study also implicated the possibility that the noradrenergic neurons and corticotropin-releasing hormone neurons mediate a part of the pathway from the hindbrain to the hypothalamus (Deura et al., 2019). Precise investigation on the direct neuronal inputs into the kisspeptin neurons will be the key to obtain complete understanding of the mechanism how malnutrition is sensed by the brain to suppress GnRH/gonadotropins secretion.

Cows subject to hot weather are known to display a lower frequency of LH pulses compared to those under a more controlled and cooler environment (Wise et al., 1988). Previous research demonstrated that LH pulse amplitude was decreased in animals that had lower plasma estradiol concentration which were under heat stress (Gilad et al., 1993). It should be clarified if the heat stress suppresses gonadotropin release through affecting KNDy neurons. Other types of stress such as restraint stress are reported to suppress *cfos* expression in the ARC *Kiss1* neurons while corticosterone secretion is enhanced in mice (Yang et al., 2017). This suppression could be caused by increased dynorphin A signaling in the ARC, because the promoter activity of *Pdyn*, a gene encoding dynorphin A, is enhanced by presence of the glucocorticoid receptor agonist in a cell line derived from mice hypothalamus (Ayrout et al., 2019). The link between body-temperature control and KNDy neurons has also been implicated in a previous study, which demonstrated that female rats with a functional ablation of KNDy neurons, induced by a selective neurotoxin for neurokinin B receptor-expressing cells, showed the lower and higher core body temperature compared to control animals under high (33°C) and low (11°C) ambient temperature, respectively (Mittelman-Smith et al., 2012). Collectively, considering those studies, we note the possibility that KNDy neurons may play a role in suppression of GnRH/LH pulses as well as body temperature control under heat stress.

Suppression of GnRH/LH pulses during lactation and under stress is a critical mechanism to ensure efficient use of energy and maximize the survival of an individual, and their offspring. On the other hand, in the context of livestock industry, these periods when GnRH/LH pulses, and then reproductive performance are suppressed are often a bottle neck of productivity in the field. Since kisspeptin neurons play an essential role to inhibit or enhance the reproductive function in mammals, kisspeptin and related peptides, such as neurokinin B and dynorphin A, can be the key molecules to unlock the development of innovative method to control reproductive function in livestock.

For instance, a possible novel method to treat the reproductive disorders would be the administration of neurokinin B agonists and/or dynorphin A antagonists. Development of these drugs for veterinary use would be necessary to enable peripheral administration of the drugs as well as maximizing their efficacy.

Conclusion

The discovery of kisspeptin at the beginning of this century contributed greatly to the progress in uncovering the central mechanism of the regulation of mammalian reproduction. Especially, the KNDy neurons in the ARC, where GnRH pulse generating mechanism have long been hypothesized to locate, may be responsible for the pulsatile secretion of GnRH/gonadotropin. This notion has attracted great attention among researchers in the field of mammalian reproduction. Further studies on regulatory factors on KNDy neuronal activities and the mechanisms would greatly contribute to the innovative technologies to enhance reproductive function in livestock.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgement

We appreciate Dr. Nicola Skoulding, Graduate School of Bioagricultural Sciences, Nagoya University for English proof reading.

References

Adachi S, Yamada S, Takatsu Y, Matsui H, Kinoshita M, Takase K, Sugiura H, Ohtaki T, Matsumoto H, Uenoyama Y, Tsukamura H, Inoue K, Maeda K (2007). Involvement of anteroventral periventricular metastin/kisspeptin neurons in estrogen positive feedback action on luteinizing hormone release in female rats. Journal of Reproduction and Development, 53: 367-378.

Ayrout M, Le Billan F, Grange-Messent V, Mhaouty-Kodja S, Lombes M, Chauvin S (2019). Glucocorticoids stimulate hypothalamic dynorphin expression accounting for stress-induced impairment of GnRH secretion during preovulatory period. Psychoneuroendocrinology, 99: 47-56.

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

Belchetz PE, Plant TM, Nakai Y, Keogh EJ, Knobil E (1978). Hypophysial responses to continuous and intermittent delivery of hypopthalamic gonadotropin-releasing hormone. Science, 202: 631-633.

Bronson FH (1981). The regulation of luteinizing hormone secretion by estrogen: relationships among negative feedback, surge potential, and male stimulation in juvenile,

peripubertal, and adult female mice. Endocrinology, 108: 506-516.

Burgus R, Butcher M, Amoss M, Ling N, Monahan M, Rivier J, Fellows R, Blackwell R, Vale W, Guillemin R (1972) Primary structure of the ovine hypothalamic luteinizing hormone-releasing factor (LRF) (LH-hypothalamus-LRF-gas chromatography-mass spectrometry -decapeptide-Edman degradation). Proceeding of National Academy of Science of the United States of America, 69: 278-282.

Cagampang FR, Maeda K, Yokoyama A, Ota K (1990). Effect of food deprivation on the pulsatile LH release in the cycling and ovariectomized female rat. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme, 22: 269-272.

Clarke IJ, Backholer K, Tilbrook AJ (2005). Y2 receptor-selective agonist delays the estrogen-induced luteinizing hormone surge in ovariectomized ewes, but y1-receptor-selective agonist stimulates voluntary food intake. Endocrinology, 146: 769-775.

Cunningham MJ, Clifton DK, Steiner RA (1999). Leptin's actions on the reproductive axis: perspectives and mechanisms. Biology of Reproduction, 60: 216-222.

de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E (2003) Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proceedings of the National Academy of Sciences of the United States of America, 100: 10972-10976.

Deura C, Minabe S, Ikegami K, Inoue N, Uenoyama Y, Maeda KI, Tsukamura H (2019). Morphological analysis for neuronal pathway from the hindbrain ependymocytes to the hypothalamic kisspeptin neurons. Journal of Reproduction and Development, 65: 129-137.

Evans NP, Dahl GE, Glover BH, Karsch FJ (1994). Central regulation of pulsatile gonadotropin-releasing hormone (GnRH) secretion by estradiol during the period leading up to the preovulatory GnRH surge in the ewe. Endocrinology, 134: 1806-1811.

Finn PD, Cunningham MJ, Pau KY, Spies HG, Clifton DK, Steiner RA (1998). The stimulatory effect of leptin on the neuroendocrine reproductive axis of the monkey. Endocrinology, 139: 4652-4662.

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

Fu LY, van den Pol AN (2010). Kisspeptin directly excites anorexigenic proopiomelanocortin neurons but inhibits orexigenic neuropeptide Y cells by an indirect synaptic mechanism. Journal of Neuroscience, 30: 10205-10219

Gilad E, Meidan R, Berman A, Graber Y, Wolfenson D (1993). Effect of heat stress on tonic and GnRH-induced gonadotrophin secretion in relation

to concentration of oestradiol in plasma of cyclic cows. Journal of reproduction and fertility, 99: 315-321.

Goodman RL (1978) The site of the positive feedback action of estradiol in the rat. Endocrinology, 102: 151-159.

Goodman RL, Lehman MN, Smith JT, Coolen LM, de Oliveira CV, Jafarzadehshirazi MR, Pereira A, Iqbal J, Caraty A, Ciofi P, Clarke IJ (2007). Kisspeptin neurons in the arcuate nucleus of the ewe express both dynorphin A and neurokinin B. Endocrinology, 148: 5752-5760.

Gottsch ML, Cunningham MJ, Smith JT, Popa SM, Acohido BV, Crowley WF, Seminara S, Clifton DK, Steiner RA (2004). A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. Endocrinology, 145: 4073-4077.

Hassaneen A, Naniwa Y, Suetomi Y, Matsuyama S, Kimura K, Ieda N, Inoue N, Uenoyama Y, Tsukamura H, Maeda KI, Matsuda F, Ohkura S (2016). Immunohistochemical characterization of the arcuate kisspeptin/neurokinin B/dynorphin (KNDy) and preoptic kisspeptin neuronal populations in the hypothalamus during the estrous cycle in heifers. Journal of Reproduction and Development, 62: 471-477.

Hill JR, Jr., Lamond DR, Henricks DM, Dickey JF, Niswender GD (1970). The effects of undernutrition on ovarian function and fertility in beef heifers. Biology of Reproduction, 2: 78-84.

Ikegami K, Minabe S, Ieda N, Goto T, Sugimoto A, Nakamura S, Inoue N, Oishi S, Maturana AD, Sanbo M, Hirabayashi M, Maeda KI, Tsukamura H, Uenoyama Y (2017). Evidence of involvement of neurone-glia/neurone-neurone communications via gap junctions in synchronised activity of KNDy neurones. Journal of Neuroendocrinology, 29.

Inoue N, Sasagawa K, Ikai K, Sasaki Y, Tomikawa J, Oishi S, Fujii N, Uenoyama Y, Ohmori Y, Yamamoto N, Hondo E, Maeda K, Tsukamura H (2011). Kisspeptin neurons mediate reflex ovulation in the musk shrew (Suncus murinus). Proceeding of the National Academy of Science of the United States of America, 108: 17527-17532.

Iwata K, Kinoshita M, Susaki N, Uenoyama Y, Tsukamura H, Maeda K (2011). Central injection of ketone body suppresses luteinizing hormone release via the catecholaminergic pathway in female rats. Journal of Reproduction and Development, 57: 379-384.

Kinoshita M, I'Anson H, Tsukamura H, Maeda K (2004). Fourth ventricular alloxan injection suppresses pulsatile luteinizing hormone release in female rats. Journal of Reproduction and Development, 50: 279-285.

Kinoshita M, Tsukamura H, Adachi S, Matsui H, Uenoyama Y, Iwata K, Yamada S, Inoue K, Ohtaki T, Matsumoto H, Maeda K (2005). Involvement of central metastin in the regulation of preovulatory

luteinizing hormone surge and estrous cyclicity in female rats. Endocrinology, 146: 4431-4436.

Kotani M, Detheux M, Vandenbogaerde A, Communi D, Vanderwinden JM, Le Poul E, Brezillon S, Tyldesley R, Suarez-Huerta N, Vandeput F, Blanpain C, Schiffmann SN, Vassart G, Parmentier M (2001). The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. The Journal of biological chemistry, 276: 34631-34636.

Maeda KI, Tsukamura H, Uchida E, Ohkura N, Ohkura S, Yokoyama A (1989). Changes in the pulsatile secretion of LH after the removal of and subsequent resuckling by pups in ovariectomized lactating rats. Journal of Endocrinology, 121: 277-283.

Maekawa F, Toyoda Y, Torii N, Miwa I, Thompson RC, Foster DL, Tsukahara S, Tsukamura H, Maeda K (2000). Localization of glucokinase-like immunoreactivity in the rat lower brain stem: for possible location of brain glucose-sensing mechanisms. Endocrinology, 141: 375-384.

Matsuda F, Nakatsukasa K, Suetomi Y, Naniwa Y, Ito D, Inoue N, Wakabayashi Y, Okamura H, Maeda KI, Uenoyama Y, Tsukamura H, Ohkura S (2015). The luteinising hormone surge-generating system is functional in male goats as in females: involvement of kisspeptin neurones in the medial preoptic area. Journal of Neuroendocrinology, 27: 57-65.

Matsuyama S, Ohkura S, Sakurai K, Tsukamura H, Maeda K, Okamura H (2005). Activation of melanocortin receptors accelerates the gonadotropin-releasing hormone pulse generator activity in goats. Neuroscience letters, 383: 289-294.

McNeilly AS (2001). Lactational control of reproduction. Reproduction, fertility, and development, 13: 583-590.

Minabe S, Deura C, Ikegami K, Goto T, Sanbo M, Hirabayashi M, Inoue N, Uenoyama Y, Maeda K, Tsukamura H (2015). Pharmacological and Morphological Evidence of AMPK-Mediated Energy Sensing in the Lower Brain Stem Ependymocytes to Control Reproduction in Female Rodents. Endocrinology, 156: 2278-2287.

Minabe S, Uenoyama Y, Tsukamura H, Maeda K (2011). Analysis of pulsatile and surge-like luteinizing hormone secretion with frequent blood sampling in female mice. Journal of Reproduction and Development, 57: 660-664.

Mittelman-Smith MA, Williams H, Krajewski-Hall SJ, McMullen NT, Rance NE (2012) Role for kisspeptin/neurokinin B/dynorphin (KNDy) neurons in cutaneous vasodilatation and the estrogen modulation of body temperature. Proceeding of National Academy of Science of the United States of America, 109: 19846-19851.

Moenter SM, Brand RM, Midgley AR, Karsch FJ (1992). Dynamics of gonadotropin-releasing hormone

release during a pulse. Endocrinology, 130: 503-510.

Moriyama R, Tsukamura H, Kinoshita M, Okazaki H, Kato Y, Maeda K (2004). In vitro increase in intracellular calcium concentrations induced by low or high extracellular glucose levels in ependymocytes and serotonergic neurons of the rat lower brainstem. Endocrinology, 145: 2507-2515.

Murahashi K, Bucholtz DC, Nagatani S, Tsukahara S, Tsukamura H, Foster DL, Maeda KI (1996). Suppression of luteinizing hormone pulses by restriction of glucose availability is mediated by sensors in the brain stem. Endocrinology, 137: 1171-1176.

Nagatani S, Guthikonda P, Thompson RC, Tsukamura H, Maeda KI, Foster DL (1998). Evidence for GnRH regulation by leptin: leptin administration prevents reduced pulsatile LH secretion during fasting. Neuroendocrinology, 67: 370-376.

Nakahara T, Uenoyama Y, Iwase A, Oishi S, Nakamura S, Minabe S, Watanabe Y, Deura C, Noguchi T, Fujii N, Kikkawa F, Maeda K, Tsukamura H (2013). Chronic peripheral administration of kappa-opioid receptor antagonist advances puberty onset associated with acceleration of pulsatile luteinizing hormone secretion in female rats. Journal of Reproduction and Development, 59: 479-484.

Nakamura S, Wakabayashi Y, Yamamura T, Ohkura S, Matsuyama S (2017). A neurokinin 3 receptor-selective agonist accelerates pulsatile luteinizing hormone secretion in lactating cattle. Biology of Reproduction, 97: 81-90.

Navarro VM, Castellano JM, Fernandez-Fernandez R, Tovar S, Roa J, Mayen A, Nogueiras R, Vazquez MJ, Barreiro ML, Magni P, Aguilar E, Dieguez C, Pinilla L, Tena-Sempere M (2005). Characterization of the potent luteinizing hormone-releasing activity of KiSS-1 peptide, the natural ligand of GPR54. Endocrinology, 146: 156-163.

Navarro VM, Gottsch ML, Chavkin C, Okamura H, Clifton DK, Steiner RA (2009). Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. Journal of Neuroscience, 29: 11859-11866.

Nebel RL, McGilliard M (1993). Interactions of High Milk Yeild and Reproductive Performance in Dairy Cows. Journal of dairy science, 76: 3257-3268.

Nestor CC, Briscoe AM, Davis SM, Valent M, Goodman RL, Hileman SM (2012). Evidence of a role for kisspeptin and neurokinin B in puberty of female sheep. Endocrinology, 153: 2756-2765.

Nett TM, Crowder ME, Moss GE, Duello TM (1981). GnRH-receptor interaction. V. Down-regulation of pituitary receptors for GnRH in ovariectomized ewes by infusion of homologous hormone. Biology of Reproduction, 24: 1145-1155.

Ohkura S, Takase K, Matsuyama S, Mogi K, Ichimaru T, Wakabayashi Y, Uenoyama Y, Mori Y, Steiner RA,

Tsukamura H, Maeda KI, Okamura H (2009). Gonadotrophin-releasing hormone pulse generator activity in the hypothalamus of the goat. Journal of Neuroendocrinology 21: 813-821.

Ohkura S, Tsukamura H, Maeda K (1991). Effects of various types of hypothalamic deafferentation on luteinizing hormone pulses in ovariectomized rats. Journal of Neuroendocrinology, 3: 503-508.

Ohkura S, Tsukamura H, Maeda K (1992). Effects of transplants of fetal mediobasal hypothalamus on luteinizing hormone pulses impaired by hypothalamic deafferentation in adult ovariectomized rats. Neuroendocrinology, 55: 422-426.

Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, Terao Y, Kumano S, Takatsu Y, Masuda Y, Ishibashi Y, Watanabe T, Asada M, Yamada T, Suenaga M, Kitada C, Usuki S, Kurokawa T, Onda H, Nishimura O, Fujino M (2001). Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. Nature, 411: 613-617.

Petersen SL, Barraclough CA (1989). Suppression of spontaneous LH surges in estrogen-treated ovariectomized rats by microimplants of antiestrogens into the preoptic brain. Brain research, 484: 279-289.

Recabarren SE, Lobos A, Torres V, Oyarzo R, Sir-Petermann T (2004). Secretory patterns of leptin and luteinizing hormone in food-restricted young female sheep. Biological Research, 37: 371-384.

Redmond JS, Baez-Sandoval GM, Spell KM, Spencer TE, Lents CA, Williams GL, Amstalden M (2011). Developmental changes in hypothalamic Kiss1 expression during activation of the pulsatile release of luteinising hormone in maturing ewe lambs. Journal of Neuroendocrinology, 23: 815-822.

Sajapitak S, Iwata K, Shahab M, Uenoyama Y, Yamada S, Kinoshita M, Bari FY, I'Anson H, Tsukamura H, Maeda K (2008). Central lipoprivation-induced suppression of luteinizing hormone pulses is mediated by paraventricular catecholaminergic inputs in female rats. Endocrinology, 149: 3016-3024.

Sasaki T, Ito D, Sonoda T, Morita Y, Wakabayashi Y, Yamamura T, Okamura H, Oishi S, Noguchi T, Fujii N, Uenoyama Y, Tsukamura H, Maeda KI, Matsuda F, Ohkura S (2019). Peripheral administration of kappa-opioid receptor antagonist stimulates gonadotropin-releasing hormone pulse generator activity in ovariectomized, estrogen-treated female goats. Domestic animal endocrinology, 68: 83-91.

Schally AV, Arimura A, Baba Y, Nair RM, Matsuo H, Redding TW, Debeljuk L (1971). Isolation and properties of the FSH and LH-releasing hormone. Biochemical and biophysical research communications, 43: 393-399.

Seminara S, Messager S, Chatzidaki E, Thresher R, Acierno J, Shagoury J, Bo-Abbas Y, Kuohung W, Schwinof K, Hendrick A, Zahn D, Dixon J, Kaiser U, Slaugenhaupt S, Gusella J, O'Rahilly S, Carlton M,

Crowley W, Aparicio S, Colledge W (2003). The GPR54 gene as a regulator of puberty. The New England Journal of Medicine, 349: 1614-U1618.

Shahab M, Mastronardi C, Seminara SB, Crowley WF, Ojeda SR, Plant TM (2005). Increased hypothalamic GPR54 signaling: a potential mechanism for initiation of puberty in primates. Proceeding of the National Academy Science of the United States of America, 102: 2129-2134.

Smith JT, Acohido BV, Clifton DK, Steiner RA (2006). KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. Journal of Neuroendocrinology, 18: 298-303.

Smith JT, Clay CM, Caraty A, Clarke IJ (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 JT, Cunningham MJ, Rissman EF, Clifton DK, Steiner RA (2005). Regulation of Kiss1 gene expression in the brain of the female mouse. Endocrinology, 146: 3686-3692.

Smith JT, Li Q, Pereira A, Clarke IJ (2009). Kisspeptin neurons in the ovine arcuate nucleus and preoptic area are involved in the preovulatory luteinizing hormone surge. Endocrinology, 150: 5530-5538.

Sugimoto A, Tsuchida H, Ieda N, Ikegami K, Inoue N, Uenoyama Y, Tsukamura H (2019). Somatostatin-Somatostatin Receptor 2 Signaling Mediates LH Pulse Suppression in Lactating Rats. Endocrinology, 160: 473-483.

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

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

Topaloglu AK, Reimann F, Guclu M, Yalin AS, Kotan LD, Porter KM, Serin A, Mungan NO, Cook JR, Imamoglu S, Akalin NS, Yuksel B, O'Rahilly S, Semple RK (2009). TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. Nature Genetics, 41: 354-358.

Tsukamura H, Maeda K, Ohkura S, Yokoyama A (1990). Effect of hypothalamic deafferentation on the pulsatile secretion of luteinizing hormone in ovariectomized lactating rats. Journal of Neuroendocrinology, 2: 59-63.

Tsukamura H, Maeda KI, Yokoyama A (1988). Effect of the suckling stimulus on daily LH surges induced by chronic oestrogen

treatment in ovariectomized lactating rats. Journal of Endocrinology, 118: 311-316.

Uenoyama Y, Nakamura S, Hayakawa Y, Ikegami K, Watanabe Y, Deura C, Minabe S, Tomikawa J, Goto T, Ieda N, Inoue N, Sanbo M, Tamura C, Hirabayashi M, Maeda K, Tsukamura H (2015). Lack of pulse and surge modes and glutamatergic stimulation of LH release in Kiss1 knockout rats. Journal of Neuroendocrinology, 27: 187-197.

Wakabayashi Y, Nakada T, Murata K, Ohkura S, Mogi K, Navarro VM, Clifton DK, Mori Y, Tsukamura H, Maeda K, Steiner RA, Okamura H (2010). Neurokinin B and dynorphin A in kisspeptin neurons of the arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile gonadotropin-releasing hormone secretion in the goat. Journal of Neuroscience, 30: 3124-3132.

Watanabe Y, Uenoyama Y, Suzuki J, Takase K, Suetomi Y, Ohkura S, Inoue N, Maeda KI, Tsukamura H (2014). Oestrogen-induced activation of preoptic kisspeptin neurones may be involved in the luteinising hormone surge in male and female Japanese monkeys. Journal of Neuroendocrinology, 26: 909-917.

Weems PW, Witty CF, Amstalden M, Coolen LM, Goodman RL, Lehman MN (2016). kappa-Opioid Receptor Is Colocalized in GnRH and KNDy Cells in the Female Ovine and Rat Brain. Endocrinology, 157: 2367-2379.

Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR, Kuijper JL (1997). Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. The Journal of clinical endocrinology and metabolism, 82: 561-565.

Wise ME, Armstrong DV, Huber JT, Hunter R, Wiersma F (1988). Hormonal alterations in the lactating dairy cow in response to thermal stress. Journal of dairy science, 71: 2480-2485.

Yamada S, Uenoyama Y, Kinoshita M, Iwata K, Takase K, Matsui H, Adachi S, Inoue K, Maeda KI, Tsukamura H (2007), Inhibition of metastin (kisspeptin-54)-GPR54 signaling in the arcuate nucleus-median eminence region during lactation in rats. Endocrinology, 148: 2226-2232.

Yang JA, Song CI, Hughes JK, Kreisman MJ, Parra RA, Haisenleder DJ, Kauffman AS, Breen KM (2017). Acute Psychosocial Stress Inhibits LH Pulsatility and Kiss1 Neuronal Activation in Female Mice. Endocrinology, 158: 3716-3723.

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