Review Article

The Role of Kisspeptin Signaling in Reproduction of Ruminants

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Abstract

In the present decade, kisspeptin has been a fascinating topic in the reproductive spotlight. Kisspeptin is a neuropeptide, produced mainly in the hypothalamus from Kiss1 gene. This gene was initially discovered in 1996 as a tumor suppressor gene in human malignant melanoma. Then in the year 2003, researchers found that mutations of the G protein-coupled receptor (GPR54), which is the strongly cognate receptor of kisspeptin, were associated with hypogonadotropic hypogonadism in mice and humans. There are many kisspeptin distribution and function studies (both *in vitro* and *in vivo*) in mammals. In ruminants, kisspeptin has been studied mainly in sheep and to some extent in goats and cattle. In ewes, Kiss1 is expressed in the arcuate nucleus to forward signals relevant to negative feedback regulation of gonadotropin releasing hormone (GnRH), and is also responsible for positive feedback regulation of GnRH at the time of the preovulatory GnRH/luteinizing hormone (LH) surge via the hypophyseal portal circulation. Although there are Kiss1 cells in the preoptic area, whether they play a role in the sex steroid feedback regulation of GnRH secretion has not been discovered yet. Previous research indicates, however, that kisspeptin-GPR54 signaling is a key regulator of puberty, reproductive function and fertility via the hypothalamic pituitary gonadal axis, which produces a neuroendocrine substrate to stimulate gonadotropin releasing hormone and has the potential to replay feedback effects from the sex hormones to GnRH neurons.

Keywords: kisspeptin, role, reproduction, ruminant

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บทคัดย่อ

กลไกของสัญญาณคิสเปบทีนต่อการควบคุมการสืบพันธุ์ในสัตว์เคี้ยวเอื้อง

ธัชฎาพร ไชยคุณ[1,2] พงษ์ศิวะ โสตถิพันธุ์[2] ศิริวัฒน์ ทรวดทรง[1*]

ในปัจจุบัน คิสเปบทีน จัดเป็นหัวข้อสำคัญทางด้านการสืบพันธุ์ที่น่าสนใจเป็นอย่างมาก คิสเปบทีนเป็นสารโปรตีนทางระบบประสาทที่ผลิตจากยีนคิสวันในสมองส่วนไฮโปทาลามัสเป็นหลัก ยีนนี้ได้ถูกค้นพบในปี 1996 และจัดในกลุ่มยีน tumor suppressor ที่พบการแสดงออกในมะเร็งเมลาโนมาในมนุษย์ หลังจากนั้นในปี ค.ศ. 2003 พบว่าเกิดการกลายพันธุ์ของยีนจีโปรตีน-คัพเพิล รีเซพเตอร์ ซึ่งเป็นตัวรับที่มีความสัมพันธ์กันอย่างมากกับคิสเปบทีน มีความเกี่ยวข้องกับการเกิดภาวะฮอร์โมนเพศต่ำในหนูและมนุษย์ มีการศึกษาการกระจายตัวและการทำงานของคิสเปบทีนเป็นจำนวนมากในสัตว์เลี้ยงลูกด้วยนม (ทั้งภายในและภายนอกตัวสัตว์) ส่วนในสัตว์เคี้ยวเอื้องเคยมีการศึกษาในแกะเป็นหลัก บางส่วนในแพะและโค ในแกะมีการแสดงออกของยีนคิสเปบทีนในอาร์คูเอท นิวเคลียส ซึ่งส่งสัญญาณยับยั้งต่อโกนาโดทรอปิน รีลิซซิง ฮอร์โมน (จีเอ็นอาร์เอช) และยังส่งสัญญาณกระตุ้นต่อจีเอ็นอาร์เอชเมื่อถึงเวลาก่อนการหลั่งสูงสุดของจีเอ็นอาร์เอชและลูติไนซิง ฮอร์โมน ก่อนการตกไข่ผ่านทางการไหลเวียนเลือดบริเวณต่อมใต้สมอง ถึงแม้ว่าจะพบเซลล์คิสเปบทีนในบริเวณพรีออพติค แต่ยังไม่มีการค้นพบกลไกการควบคุมย้อนกลับต่อฮอร์โมนเพศชนิดสเตียรอยด์ งานวิจัยก่อนหน้านี้บ่งบอกว่า สัญญาณจากคิสเปบทีนร่วมกับ จีโปรตีน-คัพเพิล รีเซพเตอร์ เป็นกุญแจสำคัญที่ควบคุมการเข้าสู่วัยเจริญพันธุ์ การทำงานของระบบสืบพันธุ์ และความสมบูรณ์พันธุ์ ผ่านทางแกนการทำงานร่วมกันของสมองส่วนไฮโปทาลามัส ต่อมใต้สมอง และอวัยวะสืบพันธุ์ โดยการหลั่งสารฮอร์โมนสื่อประสาทไปกระตุ้นให้เกิดการหลั่งจีเอ็นอาร์เอช และมีการตอบสนองกลับจากฮอร์โมนเพศต่อเซลล์ประสาทจีเอ็นอาร์เอชร่วมด้วย

คำสำคัญ: คิสเปบทีน กลไก การสืบพันธุ์ สัตว์เคี้ยวเอื้อง

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Introduction

Kisspeptin (syn. metastin) was discovered in 1996 by Lee et al. (1996) who identified Kiss1 gene for suppressing metastasis in human malignant melanoma. Kiss1 was named for the home of the famous Hershey chocolate Kiss (Hershey, Pennsylvania, USA) which is manufactured in the area where the gene was discovered (Gottsch et al., 2004). Kisspeptin are peptide hormones which encode a 145-amino acid peptide that produces various lengths (10-54) of biologically active peptide (Gottsch et al., 2004; Hashizume et al., 2010). The larger peptides contain some variability between species, whereas the 10 amino acid C-terminus peptide is stable and has the potency to activate its receptors (Lee et al., 1996; Muir et al., 2001; Ohtaki et al., 2001; Richard et al., 2008). G protein- coupled receptor (GPR54 or Kiss1r) is the strongly cognate receptor of kisspeptin. Kisspeptin and GPR54 have been found within the hypothalamus, brainstem, spinal cord, pituitary, ovary, prostate, liver, pancreas, intestine, aorta, coronary artery, umbilical vein and placenta (Lee et al., 1996; Muir et al., 2001; Ohtaki et al., 2001; Mead et al., 2007; Richard et al., 2008; Roseweir and Millar, 2009). In 2003, researchers found that mutations of GPR54 were associated with hypogonadotropic hypogonadism in humans (De Roux et al., 2003; Seminara et al., 2003). These studies demonstrated that kisspeptin-GPR54 signaling was necessary for pubertal activation of gonadotropin releasing hormone (GnRH) neurons and reproductive function, both of which play a pivotal role in the control of the hypothalamic pituitary gonadal (HPG) axis involving follicular development, ovulation, spermatogenesis and steroidogenesis (Roseweir and Millar, 2009; Tsukamura and Maeda, 2011). In ruminants, kisspeptin has been studied mainly in sheep and to some extent in goat and cattle, but not in buffalo.

Kisspeptin and GnRH neurons expression and distribution in the hypothalamus

The expression and distribution of kisspeptin, GPR54 and GnRH neurons are different in each species due to differences in anatomy (Colledge, 2008). *In situ* hybridization (ISH) and immunohistochemistry (IHC) are the main techniques

for researching kisspeptin, GPR54 and GnRH gene or protein expression, and localization. Many kisspeptin, GPR54 and GnRH distribution studies have been done in mammals such as mice (Gottsch et al., 2004), hamsters (Revel et al., 2006), rats (Irwig et al., 2004; Smith et al., 2006), horses (Decourt et al., 2008), pigs (Tomikawa et al., 2010) monkeys (Rometo et al., 2007), humans (Rometo et al., 2007) and sheep (Estrada et al., 2006; Smith et al., 2007).

In ewes, Kiss1 neurons were found in arcuate neucleus (ARC) and preoptic area (POA) regions with a greater cell density in ARC by ISH (Estrada et al., 2006; Smith et al., 2007). Several anti-kisspeptin antiboies have been created for localization of kisspeptin neurons by IHC. Some reports noticed that cross-reaction with other RF-amide peptides and the non-specific reactivity of anti-kisspeptin antisera in IHC might be the reason that Kiss1 expression has not been detected by ISH in some locations, thus anti-protein antibodies should be validated for specificity (Colledge, 2008). From the IHC technique, kisspeptin-immunoreactive (Kp-ir) cells have been mainly found in the ARC, POA and dorsomedial nucleus (DMN) (Rometo et al., 2007; Franceschini et al., 2006). GnRH cell bodies are presented both in the POA and ARC (Colledge, 2008).

Connections and actions of kisspeptin on GnRH neurons

The hypothesis that kisspeptin neurons stimulate GnRH neurons directly is proven by many immunofluorescence data reports. In ewes, Kp-ir fibres were detected in the POA, where the GnRH neurons reside, however co-localization studies were not reported (Franceschini et al., 2006). Additionally, Kp-ir fibres were found to extend from the ARC into the external neurosecretory zone of the median eminence (ME) (Franceschini et al., 2006; Pompolo et al, 2006). These terminals might be causative for the kisspeptin that has been identified in the ewe hypophyseal portal blood (Smith et al., 2008) Despite these data, the function of these connections is still unknown, but kisspeptins might have a non-synaptic action at the ME level to activate GnRH release (Ramaswamy et al., 2008). Moreover, in sheep the kisspeptin cell bodies are located closer to the GnRH cell bodies than in rodents (Colledge, 2008).

GnRH neurons can be directly acted on by kisspeptin to begin a sustained depolarization event. The ability of GnRH neurons to respond to kisspeptin signals is progressively regulated, with the percentage of responsive GnRH neurons rising from 25% in the pre-pubertal period to more than 90% in adults. This raise in GnRH responsiveness during the pre-pubertal period reflects an increasing in Kiss1 gene expression rather than GPR54 expression (Han et al., 2005). The study in the POA region of mice found that kisspeptin caused membrane depolarization ranging from 5 mV to 22 mV in about 90% of GnRH neurons (Han et al., 2005; Liu et al., 2008) and depolarization was sustained, lasting for up to 30 mins after kisspeptin removal. GPR54 expression by GnRH neurons does not appear to be sexually dimorphic since no sex differences were detected in the number of GnRH neurons (Liu et al., 2008).

Pharmacialogical studies and assessments of the current-voltage (I-V) relationships note that depolarization happens by the inhibition of inwardly rectifying potassium channels (Kir) and the activation of sodium-dependent non-selective cation channels (NSCC). Therefore, inhibition of Kir by extracellular barium (Ba2+) or intracellular caesium reduced the number of GnRH neurons responding to kisspeptin by 50% approximately (Liu et al., 2008) and also strongly affected the reversal potential at hyperpolarized potentials (Zhang et al., 2008). Study of NSCC activity indicated that these ion channels are might be created by members of the transient receptor potential cation (TRPC) channel family and the activity of each TRPC channel might be slightly different between individual GnRH neurons depending on subunit composition (Zhang et al., 2008). A heterologous cell expression system has shown kisspeptin binding to GPR54 couples to G protein Gq/11 to activate phosphalipase C (PLC) and increase intracellular Ins (1, 4, 5) P3 and Ca^{2+} release, arahidonic acid release, and activation of extracellular signal- related kinase 1/2 (ERK 1/2) and p38 mitogen-actvated protein kinase (MAPK) pathways (Kotani et al., 2001).

Efforts have been made to establish whether the same signaling pathway is implied in kisspeptin-stimulated GnRH secretion (Castellano et al., 2006). However, the process by which the final messengers of the Gq/11-PLC-signaling pathway interact with Kir and NSCC channels to depolarize the GnRH membrane is not completely understood (Colledge, 2008). Furthermore, kisspeptin may also have indirect effects on GnRH neurons through synaptic input from other neurons in the hypothalamus that express GPR54 (Herbison et al., 2010).

Action of kisspeptin on reproductive function

Kisspeptin and puberty: Puberty is the sexual transition from immaturity to maturity involving body growth and development (related with leptin in adipose tissue and growth hormone) (Kadokawa et al., 2008[a]; Kadokawa et al., 2008[b]; Smith et al., 2010). The onset of puberty is triggered by the activation of neurons in the forebrain which produce a neuroendocrine substrate to stimulate GnRH (Saito et al., 2012).

Many studies in mammals indicated that kisspeptin and GPR54 were key regulators of puberty due to the programmed increased in Kiss1 mRNA, GPR54 mRNA (only in females), which had been observed in the anteroventral periventicular neucleus (AVPV), the POA, and the ARC areas (through the immunoreactive method) which could in turn cause an increase in GPR54 sensitivity to kisspeptin (possibly due to increase in receptors at the cell surface). Activation of the kisspeptin system facilitates increased pulsatile and surge modes of GnRH from GnRH neurons (in the POA and the ME), then GnRH

awakens the reproductive axis bringing about pubertal maturation via hypophyseal portal circulation to stimulate the production and release of gonadotropins such as luteinizing hormones (LH) and follicle-stimulating hormones (FSH) (Kadokawa et al., 2008[a]; Roseweir and Millar, 2009). GnRH releasing is regulated by gonadal steroid feedback action (Tsukamura and Maeda, 2011).

Kisspeptin mechanism on hypothalamic pituitary gonadal axis

It is well known that steroid hormones fluctuate across the female estrous cycle and feedback from gonads regulates the HPG axis. Kisspeptin neurons express estrogen receptor α (ERα), progesterone receptor (PR) and androgen receptor (AR), and hence have the potential to relay feedback effects on the GnRH neuron (Hashizume et al., 2010). Many studies found that ovariectomy (OVX) and estrogen replacement in animals affected kisspeptin expression in different region of hypothalamus as we call "differential estrogen regulation" (Smith et al., 2005; Clarkson and Herbison, 2006; Adachi et al., 2007). The two modes of GnRH secretion are the estrogen- induced ovulatory surge of GnRH/LH, and pulsatile, basal GnRH/LH releasing modes.

The model is most well-developed in rodents. On one hand, the Kiss1 neurons in the AVPV are directly stimulated by estrogen effects via ERα (predominant in females). These neurons in turn directly stimulate GnRH neurons through GPR54 expressed on the cell bodies. This positive feedback of estrogen effect on AVPV Kiss1 neurons climaxes in the GnRH/LH surge, which generates the preovulatory LH surge, which in turn triggers ovulation. On the other hand, the pulsatile GnRH/LH release from ARC kisspeptin neurons (present in both female and male) drives tonic secretion of gonadotropin which mainly controls folliculogenesis and steroidogenesis and is negatively regulated by estrogen. Additionally, it appears that positive feedback occurs at the level of GnRH cell bodies, with estrogen responsive cells in the AVPV projecting directly to GnRH neurons, whereas negative feedback occurs primarily at the GnRH terminal level by an indirect (inter-neuronal) pathway (from estrogen-sensitive neurons in the ARC) (Wintermantel et al., 2006; Smith et al., 2010; Tsukamura and Maeda, 2011).

In ewes, kisspeptin cells in the ARC are held steady to play a role in the negative feedback control of GnRH/LH secretion by sex steriods which have been proven by OVX stimulation, estrogen and progesterone replacement (Smith et al., 2007). Practically, all kisspeptin cells in the ARC of the ewe brain express ERα and PR (Franceschini et al., 2006; Smith et al., 2007). The major site of sex steroid negative feedback action is the mediobasal hypothalamus (Caraty et al., 1998). Furthermore, the positive feedback effects of estrogen are also seen in the ARC (specifically in caudal region), which up regulate Kiss1 mRNA immediately before the preovulatory GnRH/LH surge (Estrada et al., 2006).

Therefore, kisspeptin cells in the ovine ARC appear to regulate in both negative and positive feedback control of GnRH by sex steroids and show a significant difference from the Kiss1 regulatory model presented in rodents. It is possible that subpopulations of kisspeptin cells respond differently to these stimuli (Smith, 2009). Moreover, the nature of the estrogen stimulus might induce different responses. There are three types of estrogen feedback important for GnRH releasing in ewes: short-term negative feedback, long-term negative feedback, and transient positive feedback (Smith, 2009). It is thought that kisspeptin cells may be able to discriminate between the more chronic effects of constant estrogen levels, inducing negative feedback, from the more acute increase in estrogen during the late follicular phase of the estrous cycle, inducing the switch to transient positive feedback (Smith, 2009).

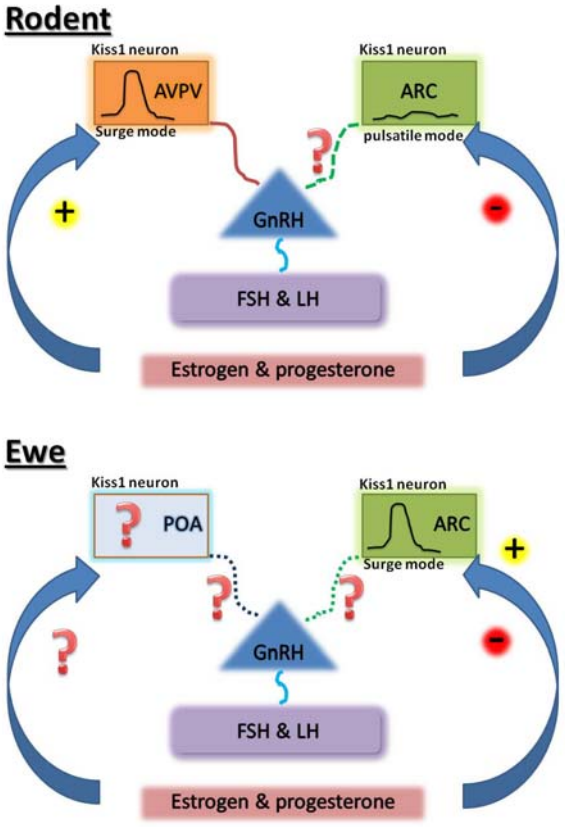

Figure 1 The positive feedback and negative feedback regulation of ovarian steroids on kisspeptin neurons and GnRH/LH stimulation. In rodents, estrogen induces the AVPV kisspeptin expression as positive feedback regulation which stimulates the GnRH/LH surge mode directly. In the ARC area, kisspeptin neurons are blocked as negative feedback by estrogen, which indirectly stimulates the GnRH neurons inducing the GnRH/LH release in basal mode. In ewes, estrogen affects the ARC kisspeptin neurons in both negative and positive feedback controls which activate the GnRH/LH sure mode. Kisspeptin neurons in the POA region have also been identified, but it is unclear what their function is in the regulation of estrogen and GnRH neurons. Though kisspeptin immunoreactive cells are detected on GnRH neurons, their exact origin is still unknown (modified from Smith, 2009).

Early research suggested no regulatory effect of sex steroids on Kiss1 mRNA expression in the POA. However, one study using twice the number of POA tissue sections reported chronic estrogen replacement after OVX increased the Kiss1 mRNA expression and kisspeptin protein in the POA, and half of the kisspeptin expressing cells co-expressed ERα. Although estrogen acts in the ARC at the mediobasal hypothalamus, not the POA, to induce the GNRH/LH surge, it is possible that POA kisspeptin cells in sheep participate in the E-induced preovulatory LH surge, similar to AVPV neurons in rodents. Thus, the sheep POA kisspeptin neurons would most likely be stimulated indirectly by the positive feedback control of estrogen (Smith, 2009). The illustrations of kisspeptin signaling on the HPG axis in rodents and ewes are shown in Fig 1.

Kisspeptin: Reproductive researches in ruminants

In ewes, kisspeptin- immunoreactive cells are located in the POA and the ARC. The mechanism of kisspeptin cells in the POA is not clearly known yet in ruminants, however kisspeptin expression in the ARC increases immediately prior to the preovulatory GnRH/LH surge in ewes. The "surge center" in ewes is located in the mediobasal hypothalamus for GnRH/LH releasing and GnRH neurons expressing GPR54. The rapid increase in LH secretion stimulated by peripheral administration of Kiss-1 peptide to ovariectomized ewes appeared to reflect a direct action on the hypothalamus (Arreguin-Arevalo et al., 2007). Also, kisspeptin cells in the ARC express ERα and PR. This evidence indicates that kisspeptin may play a role in the steroid feedback control mechanism for GnRH secretion in ruminants (Estrada et al., 2006; Hashizume et al., 2010; Smith, 2009). Since sheep are seasonal (short-day) breeders, their reproductive activity is activated by the photoperiodic hormone melatonin. During the non-breeding season (anestrus), GnRH secretion is decreased by both steroid-independent and steroid-dependent mechanisms (Robinson et al., 1985; Barrell et al., 1992; Smith et al., 2007; Smith, 2009). Interestingly, the estrogen effects on Kiss1 mRNA and kisspeptin protein expression in the ARC are greater during the non-breeding season in ewes (Smith et al., 2008). Therefore, kisspeptin cells appear to be the main candidates for facilitating the change in the feedback effect of estrogen (Smith, 2009). Furthermore, a seasonal alteration in Kiss1 expression in the ARC of OVX ewes intensely indicates that kisspeptin is basically involved in the seasonal change control in their reproductive function (Smith et al., 2007).

In cattle, kisspeptin not only stimulates lutenizing hormones (LH) but also growth hormones (GH) in OVX cows, which were injected with kisspeptin10 (Kp10) in different doses. Importantly, this study found that the dose of Kp10 used in the study (0.13 µg/kg body weight or 100 pmol/kg body weight) to provide maximal LH response was $1/20^{th}$ of the dose used in pubertal cattle, and the rapid onset and short duration of the LH response presented in this study was similar to that observed in pubertal gilts and adult ewes, which contrasted with the response in pre-pubertal cattle (Whitlock et al., 2008). *In vitro* research indicate that kisspeptin was relevant to the release of growth hormone (GH) and prolactin (PRL) as well as the release of gonadotropin in ruminants (Hashizume et al., 2010). One study indicated that kisspeptin10 treatment stimulated LH secretion from anterior pituitary cells in bovines (Ezzat et al., 2010).

In OVX goats, the peripheral infusion of Kp10 stimulates GnRH neurosecretion into hypophyseal portal circulation and the action of kisspeptin on LH releasing is mediated by GnRH (Tanaka et al., 2012). Another study found that kisspeptin stimulated LH and FSH releasing but not GH and PRL releasing during the luteal phase in female goats, and that the releasing effect of LH and FSH from intravenously kisspeptin administration was less potent than that of intravenously GnRH administration (Hashizume et al., 2010). Saito et al. (2012) reported that no studies had compared the effects of kisspeptin on the release of gonadotropin in ruminants in different stages of postnatal development. Their research found that the LH releasing response to kisspeptin-10 was greater in pre-pubertal than post-pubertal male goats and also showed that Kisspeptin-10, as well as GnRH, was able to stimulate the release of testosterone. Additionally, the negative feedback control of GnRH secretion by testicular steroids increased in post-pubertal male goats. Other studies, moreover, in the neuroendocrinology of ruminants with a multiple-unit activity used an electrophysiological technique for monitoring the neural activity of GnRH pulse generation and were able to evaluate the factors effecting the GnRH pulse (for example, male pheromones, fasting, hypoglycemia and gonadal steroids) (Okamura and Ohkura, 2007).

Several *in vivo* reports noted that the maximum LH-releasing effect of kisspeptin10 (intravenous injection) doses was observed at 0.54-0.65 µg/kg bw in OVX ewes (Caraty et al., 2007), 1 µg/kg bw in luteal phase goats (Hashizume et al., 2010), 0.13 µg/kg bw in OVX cows (Whitlock et al., 2008) and 4.76 µg/kg bw and in pre-pubertal heifers (Kadokawa et al., 2008[a]).

Potential applications of kisspeptin in farm animals

Kisspeptin research has been ongoing in many animals both *in vitro* and *in vivo*. The main point of most of these studies is to gain fundamental knowledge of, and information about, the mechanism of kisspeptin in the reproductive system. In normal condition animals it has been discovered that kisspeptin has a role involving cooperation with other neurons and hormones. Some studies have tried to focus on different status animals such as seasonal breeders (sheep and horses) and anestrous animals. Innovative uses of kisspeptin might possibly be applied in farm management in the future, for example, using kisspeptin to control the estrous cycle with or without other exogenous hormonal treatments, solving infertility problems which have causes related to kisspeptin, and controlling reproductive management in the non-breeding season

as well as in the normal season for optimal production. However, more research on a variety of animals should provide more information, which could help increase the potential applications of kisspeptin hereafter.

Conclusion

Kiss1 genes mainly appear in the hypothalamus of ruminants and other mammals to provide the negative and positive feedback regulation of GnRH secretion by gonadal steroids. In ewes, which are used as representatives of ruminants, the kisspeptin cells in the ARC are balanced to play a role in the steroid negative feedback control of GnRH. Also, the kisspeptin cells in the mediodorsal hypothalamus are involved in the surge mode, and kisspeptin expression is increased immediately before the preovulatory GnRH/LH surge. The exogenous kisspeptin administration potently stimulates gonadotropin secretion and appears to be a new tool to manipulate reproduction in farm animals. The process involved in the regulation and function of the kisspeptin cells in the POA area, however, is still not understood and further studies need to be done.

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