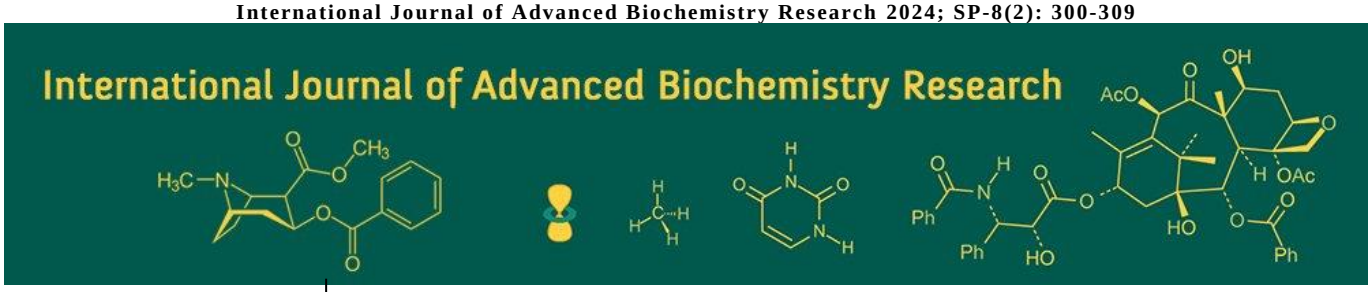

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Kisspeptin: Bridging physiology and clinical application in HPG axis regulation

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Abstract
Kisspeptin (Kp), a neuropeptide primarily produced by neuronal clusters within discrete hypothalamic nuclei from the KISS1 gene, serves as a critical initiator of puberty and regulator of ovulation in sexually mature females through central control of the hypothalamic–pituitary–gonadal (HPG) axis. Its interaction with the G protein-coupled receptor (GPR) 54 is indispensable for activating the HPG axis. Although extensively studied in various animal models including rodents, primates, and livestock such as sheep and cattle, the physiological characterization of the KISS1R/GPR54 system remains ongoing. Evidence suggests that KISS-1 neurons occupy a central role in the regulation of the gonadotropin-releasing hormone (GnRH) system. Beyond reproduction, Kp expression influences non-reproductive functions such as lactation, stress response, nutrition, cancer, and metabolism. The development of agonists and antagonists targeting KISS1R/GPR54 holds promise for therapeutic advancements. This paper provides a comprehensive review of Kp, its physiological effects, and the responses to its analogs on reproductive functions in animals, highlighting its importance for therapeutic and diagnostic interventions in animal reproductive diseases and infertility.

Keywords: Kisspeptin, KISS1R, GnRH secretagogue, HPG Axis, Animal reproduction

1. Introduction
Kisspeptins have emerged as pivotal regulators of critical aspects of reproductive axis maturation and function, encompassing sexual brain differentiation, puberty onset, adult regulation of gonadotropin secretion by gonadal hormones, as well as metabolic and environmental control of fertility. The discovery of Kp and its encoding gene KISS1, initially identified as a suppressor gene of human malignant melanoma in Hershey, Pennsylvania, USA-home to the famous Hershey's Kisses chocolates-marked a significant milestone [1, 2]. The name "KISS1" was inspired by these confections, with "SS" symbolizing the "suppressor sequence." All kisspeptins feature a C-terminal region containing an Arg–Phe–NH2 motif typical of the RF-amide peptide family, enabling full activation of KISS1R. Globally termed "Kp" due to their structural and evolutionary connection to KISS1, these peptides, originating from a 145-amino acid precursor, yield various biologically active peptide lengths (10-54 amino acids). Initially dubbed "metastin" due to its ability to inhibit tumor metastasis, Kp-54 is regarded as the primary product of the human KISS1 gene. Conversely, Kp-53 in sheep, cattle, and goats, and Kp-52 in rats and mice, represent the largest proteolytic products, with the RF-amide signature substituted by an Arg–Tyr–NH2 motif. Although variations exist, the Kp10 sequence remains relatively conserved across species, indicating a conserved physiological role [3]. Equine Kp10 displays a single amino acid difference compared to ovine Kp10, with a valine replacing an arginine at position 2 [4].
Kp is acknowledged as the primary transmitter and major regulator of GnRH synthesis and secretion. Functioning through the seven-transmembrane G-protein-coupled receptor, KISS1R (formerly known as GPR54), initially identified in the rat brain, Kp interacts strongly with this receptor to stimulate GnRH release (and subsequently FSH and LH secretion) in numerous mammals, sharing around 40% sequence similarity with the galanin receptor [5, 6]. Upon Kp binding, activation of phospholipase C ensues, leading to the recruitment of secondary intracellular messengers, inositol triphosphatase, and diacylglycerol, which in turn mediate intracellular calcium release and protein kinase C activation [7].

Numerous studies have revealed widespread expression of kisspeptins (Kps) and their putative receptor, KISS1R, in various tissues including the hypothalamus, brainstem, spinal cord, pituitary, ovary, female genital tract, prostate, testis, liver, pancreas, intestine, aorta, coronary artery, umbilical vein, and placenta [8]. This broad distribution suggests that Kps may exert direct effects on diverse tissues in an autocrine/paracrine manner, depending on specific physiological conditions.

Regarding reproductive function, emerging evidence indicates that reproductive tissues such as the ovary, placenta, and testis express functional forms of the Kp/KISSR system across various animal species, including humans [9]. Several studies have demonstrated that kisspeptin can regulate trophoblast migration and invasion, suggesting a significant production of kisspeptin by the placenta during pregnancy. Moreover, low circulating levels of kisspeptin during pregnancy have been associated with an increased risk of miscarriage, highlighting the potential of plasma kisspeptin levels as a biomarker for miscarriage risk, particularly in the first and third trimesters [10], thus revolutionizing the field of reproductive physiology.

Kps and their receptor, KISS1R, serve as critical regulators of sexual maturation during puberty onset and play pivotal roles in dynamically regulating the gonadotropic axis in adulthood. While Kp primarily acts centrally to regulate reproduction, peripheral administration of Kp has been shown to stimulate GnRH release in numerous animal studies and subsequently in human studies, with no reported adverse effects [11]. With its diverse and multifunctional nature, kisspeptin influences various physiological systems throughout the body and operates at all levels of the reproductive axis, including the brain, pituitary, gonads, and accessory organs [12].

Mutations in Kp or GPR54 are implicated in various reproductive axis disorders, such as idiopathic hypogonadotrophic hypogonadism, central precocious puberty, and normosmic idiopathic hypogonadotropic hypogonadism [13, 15]. Exogenous administration of Kp has demonstrated potent stimulation of gonadotropin secretion and holds promise as a novel tool for manipulating reproduction in farm animals. This review will primarily focus on existing literature concerning the physiological roles of Kp/KISS1R in regulating reproductive function and will summarize current understandings of the mechanisms underlying Kp's physiological actions, as well as the therapeutic implications of Kps in veterinary reproductive medicine.

2. Expression of Kisspeptin neuronal populations in the brains of various animal species.

Kisspeptin has emerged as a crucial regulator of the reproductive axis, exerting its effects hierarchically and serving as one of the most potent stimulators of GnRH-gonadotrophin secretion across various mammalian species, including humans [16, 17]. Its primary action occurs in the hypothalamus, where it activates GnRH neurons. Neuroanatomical investigations have led to the identification of discrete populations of kisspeptin neurons in different hypothalamic regions [18].

In rodents, two main populations of KISS-1-expressing neurons are found in the hypothalamus: the arcuate nucleus (ARC) and the anteroventral periventricular nucleus (AVPV). Similar populations expressing KISS-1 neurons have been observed in species such as sheep, cattle, goats, pigs, dromedary camels, and cats, with expression in the ARC and medial pre-optic area (mPOA). In pigs, Kp neurons are also found in the paraventricular nucleus (PVN). In horses, KISS-1-expressing neurons are predominantly located in the ARC, with some in the dorsomedial nucleus and ventromedial nucleus. Llamas also exhibit kisspeptin neurons in the preoptic area and arcuate nucleus, indicating their influence on gonadotropin secretion in camelids.

The majority of Kp neurons in the ARC co-express neurokinin B (NKB) and dynorphin (Dyn), forming the KNDy (Kp/neurokinin B/dynorphin) neuron group. NKB is implicated in puberty onset, as mutations in NKB or its receptor block pubertal development in humans. In sheep, an NKBR agonist stimulates LH release, while an antagonist suppresses GnRH/LH pulses. Additionally, continuous Kp10 infusion restores GnRH/LH pulses in the presence of an NKBR antagonist, suggesting that Kp action is downstream of NKB signaling. Dyn, another neuropeptide co-expressed in KNDy neurons, binds to the k-opioid receptor (KOR) and terminates each GnRH pulse, limiting GnRH release during the secretory phase. Dyn has been linked to progesterone negative feedback on pulsatile GnRH secretion in ewes and prepubertal lambs, although the specific source of Dyn and its effects require further investigation.

3. Kisspeptin, an activator of the GnRH/gonadotropin axis

The gonadotropin-releasing action of kisspeptin (Kp) may result from its direct stimulatory effect on GnRH neurons within the hypothalamus, as depicted in Figure 1. There are two primary modes of GnRH secretion: the estrogen-induced ovulatory surge of GnRH/LH and the pulsatile basal GnRH/LH releasing modes. Activation of the kisspeptin system facilitates an increase in both pulsatile and surge modes of GnRH from GnRH neurons. Subsequently, GnRH stimulates the reproductive axis via the hypophyseal portal circulation, leading to the production and release of gonadotropins such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [16, 34].

The KISS1 genes primarily appear in the hypothalamus of ruminants and other mammals, playing a crucial role in the negative and positive feedback regulation of GnRH secretion by gonadal steroids. In ewes, which are often used as representatives of ruminants, Kp cells in the arcuate nucleus (ARC) are balanced to contribute to the steroid-negative feedback control of GnRH. Kp stimulates the gonadotropic axis of ruminants *in vivo* [35]. Infusion of Kp has been shown to synchronize LH surge and ovulation in progesterone-primed cyclic ewes and anoestrus ewes by activating the hypothalamic-pituitary-gonadal axis. GnRH neurons can be directly influenced by Kp to initiate sustained depolarization events. The responsiveness of GnRH neurons to Kp signals is progressively regulated, with the percentage of responsive GnRH neurons increasing from 25% in the pre-pubertal period to over 90% in adults.

In sheep, Kp appears to act on LH via its effect on GnRH release from the hypothalamus. Kp stimulates the pulse-like release of LH within 15 minutes following intravenous injections, increasing the frequency and amplitude of LH pulses and estradiol in prepubertal ewe lambs [36, 37]. A significant increase in GnRH concentrations in the

cerebrospinal fluid, along with a parallel rise in serum LH, has been observed after intracerebroventricular (icv) administration of Kp10 [38]. In sheep, KISS1R is present in the pituitary, and LH secretion increases after the addition of Kp10 to pituitary cell cultures. However, Kp10 fails to induce LH release in ewes with hypothalamo-pituitary disconnection, while GnRH induces a significant LH release. GnRH neurons in the mediobasal hypothalamus exhibit greater Kisspeptin innervation, both in terms of the proportion of GnRH neurons displaying Kisspeptin synapses and the number of kisspeptin synapses per GnRH [39, 40].

In ovariectomized (OVX) goats, peripheral infusion of Kp10 stimulates GnRH neurosecretion into the hypophyseal portal circulation, and the action of Kp on LH release is mediated by GnRH [41]. It has been reported that no studies have compared the effects of Kp on the release of gonadotropins in ruminants at different stages of postnatal development. However, research has shown that the LH-releasing response to Kp-10 was greater in prepubertal than post-pubertal male goats, and Kp-10, as well as GnRH, were able to stimulate the release of testosterone. The negative feedback control of GnRH secretion by testicular steroids increases in post-pubertal male goats.

Moreover, peripheral administration of Kp10 can stimulate GnRH secretion [43]. Several *in vivo* studies have noted that the maximum LH-releasing effect of Kp10 (intravenous injection) doses was observed at 0.54-0.65 µg/kg BW in OVX ewes, 1 µg/kg bw in luteal phase goats, 0.13 µg/kg BW in OVX cows, and 4.76 µg/kg BW in pre-pubertal heifers. This effect is detected after intracerebral and systemic administration, across a variety of mammalian species. Kp stimulates the pulse-like release of LH within 15 minutes following intravenous injections and increases the frequency and amplitude of LH pulses and estradiol in prepubertal ewe lambs, as well as in horses [4].

4. Kisspeptin in Regulating Growth Hormone Secretion

Kisspeptin (Kp) is recognized as a key regulator of reproductive function across various species. However, recent research indicates a potential additional role for Kp in modulating Growth Hormone (GH) secretion. It appears that Kp enhances the responsiveness of reproductive steroids to the somatotropic axis, acting as a mediator of both LH and GH release, thus potentially linking metabolism, growth, lactation, and reproduction [35]. Initial studies demonstrated a stimulating effect of Kp on GH release from cultured rat and bovine pituitary cells [34]. Furthermore, peripheral administration of Kp in pigs increased serum LH concentrations without affecting FSH or GH levels. Notably, intravenous injections of a high dose of Kp into prepubertal female cattle significantly raised circulating GH concentrations, indicating a regulatory role for Kp in GH release *in vivo*. Similarly, Kp has been shown to stimulate LH and GH secretion in prepubertal heifers, suggesting potential significant connections between Kp, the reproductive axis, and the somatotropic axis. In cattle, Kp administration to ovariectomized cows resulted in increased levels of both LH and GH, indicating that reproductive steroids may enhance the sensitivity of the somatotropic axis to physiologically relevant doses of Kp, thereby integrating LH and GH release in bovines [46].

5. Kisspeptin's influence on Gonadal activity

In rodents, the expression of the KISS1 and KISS1R genes has been identified in the ovary [47], a pattern subsequently confirmed in other species such as fish [48], hamsters [49], pigs, goats [50], and primates [51]. Studies have shown that mice lacking Kp receptors or having reduced expression experienced premature ovarian failure (POF), indicating a critical role of ovarian Kp receptor expression in ovarian function. Additionally, it has been observed that ovarian Kp expression varies with the reproductive cycle.

The genes responsible for encoding Kp and its receptor are also expressed in the testes of rodents, particularly in Leydig cells, spermatid cells, seminiferous tubules, and testes [52, 53]. Recent investigations have examined the effects of Kp on the testes of adult male monkeys, as well as its presence in human and buffalo bull spermatozoa [54, 55]. Evidence suggests that Kp may modulate the fertilization capacity of spermatozoa by promoting capacitation, and studies in rats have shown that administration of a Kp antagonist reduced spermatozoa fertilization rates [52]. Moreover, Kp is implicated in steroidogenesis, spermatogenesis progression, and sperm physiology [56].

Kp may also exert direct effects on gonadal tissues and interact with metabolic pathways [57]. High concentrations of Kp have been found in porcine follicular fluid compared to serum, indicating a potential role for Kp in follicular development and suggesting either intrafollicular or systemic action [58]. These findings underscore the biological significance of Kp in potentially regulating gonadal function in both males and females.

6. Kisspeptin on Pubertal Onset

The initiation of puberty is triggered by the activation of neurons in the forebrain, which produce a neuroendocrine substrate to stimulate Gonadotropin-releasing hormone (GnRH). Puberty onset is believed to be timed by an increase in GnRH and gonadotrophin secretion, facilitated by a decrease in negative feedback inhibition of LH by estradiol. Studies in 2003 [59] first demonstrated that mice lacking the Kisspeptin receptor exhibited hypogonadotropic hypogonadism (HH), characterized by low levels of circulating gonadotropin hormones, small testes in males, and delayed vaginal opening and absence of follicular maturation in females [60]. Additionally, both Kiss1 and Kiss1R proteins have been found in the testis, cauda epididymis ligament, ovary, uterus, and trophoblast cells of dogs, suggesting a potential role in steroidogenesis, spermatogenesis progression, spermatozoa detachment from Sertoli cells, and sperm physiology [56].

In sheep, puberty onset is influenced by metabolic cues indicating sufficient growth and permissive photoperiod. During this period, the hypothalamus becomes less sensitive to negative feedback from estradiol, leading to increased pulse frequency of GnRH and luteinizing hormone (LH). This rise in GnRH/LH pulse frequency stimulates estrogen production by growing ovarian follicles, triggering an LH surge and ovulation. The timing of puberty onset significantly impacts animal productivity [63]. Repeated or continuous administration of native Kisspeptin has been shown to accelerate puberty and induce ovulation in both breeding and non-breeding seasons in sheep.

A study conducted by [64] aimed to inhibit reproduction by blocking puberty onset through targeting Kisspeptin. Male lambs (8 weeks old) were immunized against KISS1 at weeks 0, 3, and 6 of the experiment. This treatment induced a strong anti-KISS1 antibody titer and suppressed gonadal

function and sexual behavior. Thus, targeting KISS1 could be considered a novel approach for developing an immunocastration vaccine in sheep.

7. Pheromone-Induced Activation of Kisspeptin Signaling

The introduction of male sheep to seasonally anestrous female sheep has been observed to activate Kisspeptin (Kp) neurons and other cells in the hypothalamus, resulting in increased Gonadotropin-releasing hormone (GnRH)/luteinizing hormone (LH) secretion. When a ram is introduced to seasonally anestrous ewes isolated from rams for at least one month, it induces pulsatile LH secretion and can trigger ovulation outside of the breeding season, with Kp playing a role in this response. Indeed, [65] utilized a Kp antagonist (p-271) to demonstrate that Kp action is necessary for seasonally anestrous ewes to respond to ram introduction with increased LH secretion. Additionally, ram introduction increased the number of Kp-positive neurons and KISS1 mRNA expression in cells in the rostral arcuate nucleus (ARC). Interestingly, Tac2 mRNA, which encodes for neurokinin B, was readily detectable in cells expressing KISS1 mRNA, but decreased in rostral ARC cells of ewes following ram introduction. It was also reported that medial amygdala Kiss1 neurons mediate female pheromone stimulation of luteinizing hormone in male mice [66].

8. The interplay between Kisspeptin, nutrition, and fertility

It is well known that body weight affects fertility. Leptin, a peptide hormone secreted by adipocytes, playing a crucial role in this regard. Leptin deficiency can lead to delayed puberty and hypogonadotropic hypogonadism in both mice and humans, while administration of leptin can reverse infertility associated with leptin deficiency [67]. Kisspeptin (Kp) is implicated as a mediator between leptin signalling and Gonadotropin-releasing hormone (GnRH) function, as Kp neurons express the leptin receptor and exogenous leptin administration increases Kp expression [57].

Studies have shown that fasting reduces hypothalamic Kp mRNA levels and delays puberty onset in rats [68]. Research on the effects of a high-fat diet (HFD) on metabolic and reproductive parameters in adolescent and adult male rats revealed that HFD rats exhibited increased body weight, impaired glucose tolerance, reduced testosterone levels, decreased hypothalamic Kp receptor expression, and decreased LH responsiveness to Kp [68].

Additionally, a study on female mice lacking the Kp receptor found that they had increased body weight, adiposity, and leptin levels, along with reduced glucose tolerance compared to wild-type controls [69]. Another investigation on fasted monkeys revealed that they maintained testosterone release after Kp injection, suggesting that fasting-induced suppression of the reproductive axis involved attenuated responsiveness to Kp [70].

Negative energy balance or excess energy intake significantly impacts the Kp system [71]. Altering metabolic levels can change the pattern of Kp secretion, as evidenced by a study on prepubertal Tibetan ewes supplemented with concentrates or minerals. Animals receiving concentrates exhibited higher expression of KISS-1, KISS1R, and ERα mRNA in the anteroventral periventricular nucleus (AVPV) compared to those receiving mineral supplementation or consuming only oat hay. Additionally, follicular development was enhanced in supplemented prepubertal animals, suggesting that the KISS1/KISS1R system is modulated by feed intake and that reproductive performance can be improved through this treatment [72].

9. The Influence of Seasonality on the Kisspeptin System

Kisspeptin (Kp) plays a significant role in regulating seasonal reproduction in various species. For instance, in Syrian hamsters, increased Kp expression is observed during long-day conditions, correlating with heightened sexual activity. Administration of Kp 10 under photo-inhibitory conditions has been shown to restore testicular and reproductive activity in these hamsters [57]. Conversely, in sheep, which are seasonal breeders, enhanced reproductive activity is observed during short days, accompanied by increased arcuate nucleus (ARC) Kp expression [73]. During long-day periods, Kp expression in the ARC of ewes is reduced. Kp administration in seasonally acyclic ewes can induce ovulation, suggesting its crucial role in regulating reproductive activity across seasons.

Moreover, the expression of Kp receptor in Gonadotropin-releasing hormone (GnRH) neurons is greater during the breeding season compared to the non-breeding season [36]. In seasonally anestrous ewes, the GnRH and luteinizing hormone (LH) response to Kp-10 is more pronounced than in luteal ewes during the breeding season. There is an increase in Kp expression in the breeding season compared to the non-breeding season in sheep, with an elevated number of Kp contacts with GnRH neurons during the breeding season. Additionally, the sensitivity of the hypothalamic-pituitary-gonadal (HPG) axis to Kp varies not only across seasons but also during the cycle, correlated with KISS1 mRNA expression [75].

In adult ewes, KISS1 mRNA expression in the ARC is higher during the breeding season, influenced by melatonin secretion, although Kp neurons do not express melatonin receptors [78]. Furthermore, a seasonal change in estrogen sensitivity occurs at the level of Kp neurons in the ARC, facilitating the switch between breeding and non-breeding seasons. However, KISS1 mRNA expression in the preoptic area (POA) does not differ between breeding and non-breeding seasons, suggesting its role only in positive feedback inducing an LH surge, but not in controlling seasonality [39].

In dogs, Kp elicits robust gonadotrophin and oestradiol responses in anoestrous bitches, indicating the significance of canine KISS1/KISS1R in modulating reproduction [79]. Notably, Kp induces a larger increase in GnRH and LH during the non-breeding season compared to the luteal phase of the cycle. This difference is attributed to the greater KISS1R expression on GnRH neurons and the larger releasable pool of GnRH/LH during the non-breeding season, reflecting the HPG axis's responsiveness to changes in GnRH pulsatility during the transition to the breeding season.

10. Modulation of the Kisspeptin system to induce ovulation

The modulation of the Kisspeptin (Kp) system has emerged as a promising approach for inducing ovulation in various species. By targeting the Kp pathway, researchers aim to trigger the release of gonadotropins, particularly luteinizing hormone (LH), which is crucial for ovulation. Exogenous

administration of Kp has been observed to induce LH secretion, leading to ovulation in various species, including rats [80]. Additionally, the pre-ovulatory surge induced by estrogen is inhibited by anti-Kp antibodies, suggesting the involvement of Kp in follicular rupture and ovulation [51]. This local action of Kp in controlling ovulation is further supported by its stimulatory effect on ovulation in ewes and mares [44, 81].

The importance of Kp in both female puberty and the preovulatory gonadotrophin surge has been highlighted by the use of potent Kp antagonists [16, 17]. Kp has been shown to stimulate LH secretion effectively in various species, including bovines, prepubertal heifers, ewes, acyclic mares, swine, and dogs [82-85]. Furthermore, Kp has been implicated in inducing LH secretion in llamas and musk shrews [40, 86].

However, the short half-life of Kp peptides necessitates repeated injections or continuous administration for sustained gonadotropin release. Studies have shown that repeated injections or continuous infusion of Kp10 can induce LH pulsatility and ovulation in prepubertal cattle, lambs, and ewes [12, 37]. Additionally, continuous Kp10 infusion has been found to synchronize LH surges in cyclical ewes and induce ovulation in seasonally acyclic ewes [87]. Moreover, a single injection of Kp10 triggers a rapid but short-lasting increase in LH and/or follicle-stimulating hormone (FSH) in females of various species [4].

To address the limitations of Kp's short half-life, Kp10 analogs with improved pharmacological properties have been developed. These analogs have shown promising results in inducing ovulation in cyclic and anestrus animals, including buffaloes, sheep, goats, and gilts [87, 89-91]. Despite these advancements, the precise role of Kp in controlling the ovulation process requires further elucidation.

11. Kisspeptin on IVM and fertilization of oocytes

In this context, there is growing interest in utilizing Kisspeptin (Kp) as a novel tool for designing reproductive technologies in mammals. Despite limited *in vitro* studies, evidence suggests that Kp may have significant effects on *in vitro* maturation (IVM) and fertilization of oocytes in farm animals. The expression of Kp and its receptors has been detected during the IVM period in oocytes and cumulus cells of pigs and bovines, with supplementation of Kp in IVM media showing improvements in oocyte maturation efficiency in these species. Kp appears to interact locally with follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) during buffalo oocyte maturation, as suggested by previous studies in pigs and sheep. It is hypothesized that Kp exerts continuous and direct actions on oocytes and cumulus cells in an autocrine-paracrine manner.

The involvement of the Kp/KISS1R system and its downstream signaling pathways in various ovarian functions has spurred the development of potential therapeutic approaches to address ovarian pathology and infertility. Data from both animal and human studies indicate that peripheral administration of Kp-10 and Kp-54 can initiate the LH surge. This suggests the potential clinical application of Kp as a biomarker for ovarian reserve and as an indicator for ovulation induction during *in vitro* fertilization (IVF) treatment.

12. Kisspeptin in lactation and stress

The lactation status and stage of lactation have been found not to alter the sensitivity of the growth hormone (GH) system to Kisspeptin (Kp). However, there is evidence of an effect of the stage of lactation on Kp-stimulated luteinizing hormone (LH) secretion in dairy cows. During lactation, there is a suppression of Kisspeptin 1 (KISS1) expression in the arcuate nucleus (ARH) and anteroventral periventricular nucleus (AVPV). This suppression of KISS1 mRNA levels in the ARH and AVPV during lactation could be a crucial factor in inhibiting gonadotropin-releasing hormone (GnRH) secretion. The suppression of KISS1 during lactation shows similarities to the suppression observed in a fasted model, suggesting that KISS1 neurons may be particularly sensitive to metabolic signals.

In the ARH, KNDy neurons, which co-express neurokinin B (NKB) and dynorphin (DYN), show a significant inhibition of NKB mRNA during lactation, while DYN mRNA levels remain unaffected. This decrease in mRNA levels is accompanied by reductions in KISS1 and NKB neuropeptide content in the ARH. Conversely, the decrease in KISS1 mRNA in the AVPV is associated with increased neuropeptide content, suggesting the inhibition of KISS1 release. It is speculated that brainstem neurons activated by suckling, projecting to the ARH, may participate in the active suppression of KISS1 and NKB. Activation of the ventrolateral medulla (VLM) by the suckling stimulus might be involved in the inhibition of KISS1 neurons in the ARH, besides possibly contributing to lactation-induced hyperphagia.

Glucoprivation has been shown to inhibit estrous cyclicity and LH secretion, but this inhibition is blocked when neural projections from certain areas are eliminated. Stress is known to suppress reproductive function by inhibiting GnRH release, with corticotrophin-releasing factor (CRF) playing a significant role in this mechanism. In response to various stressors, including CRF injection, Kp expression and its receptor are reduced in the ARC and medial preoptic area (mPOA) of mice, suggesting that Kp may contribute to stress-induced suppression of reproductive function. The suppression of GnRH/LH pulses during lactation and stress ensures efficient energy use and maximizes individual and offspring survival. Kp neurons may also mediate the suppression of reproductive function caused by hyperprolactinemia during lactation or pituitary tumors, although further investigation is needed due to species-specific differences in experimental findings.

13. Conclusion

Kisspeptin (Kp) stands out as one of the most significant discoveries in neuropeptides related to reproductive function since the identification of GnRH. It plays a crucial role in various reproductive processes such as puberty onset, initiation of the breeding season, and the regulation of gonadotropin secretion throughout the estrous cycle. The exogenous administration of Kp has been shown to stimulate gonadotropin (LH) secretion in multiple species by stimulating GnRH secretion, ultimately leading to oocyte maturation and ovulation. Manipulating Kp signaling holds promise for novel strategies in managing livestock reproduction, including controlling ovulation in adults and modulating the timing of puberty onset.

Kp presents itself as a potential tool for designing new protocols in reproductive technologies for mammals. Traditional treatments like hCG and eCG may induce antibodies, reducing their effectiveness, and concerns over

animal welfare and ethical sourcing of eCG highlight the need for alternative treatments. Co-administration of Kp with agents modifying opioids and neurokinin B may offer precise modulation of the hypothalamic-pituitary-gonadal (HPG) axis, opening new avenues in reproductive endocrinology and infertility treatments. However, further optimization of existing analogs and experimental protocols is necessary.

Investigations into the Kp system during lactation in dairy cows may shed light on lactation-associated changes in the reproductive axis, indicating a connection between Kp and metabolic regulatory systems. As nutrition and growth hormone (GH) are essential for reproductive success, understanding the interplay between Kp and metabolic/GH systems is crucial for comprehending normal reproduction. The Kp system holds promise for developing alternative treatments for reproductive disorders characterized by low gonadotropins or anovulation, with high potency analogs under development.

Innovative applications of Kp in farm management could include controlling the estrous cycle, addressing infertility issues related to Kp, and managing reproductive activity in both breeding and non-breeding seasons for optimal production. However, further research across various animal species is needed to explore the full potential of Kp in these areas. The future developments in Kp research hold promise for advancements in reproductive technologies and farm management practices.

14. Conflict of interest

The authors report no conflicts of interest in this work.

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