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THE INFLUENCE OF VARIOUS KISSPEPTINS ON THE REPRODUCTIVE FUNCTION OF *Bos taurus*
(review)

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Abstract

Kisspeptins (KP) are a family of peptides of various lengths with a receptor (KISS1R). Kisspeptins with gonadotropin hormone-releasing hormone (GnRH), gonadotropins (luteinizing hormone and follicle-stimulating hormone) and sexual steroids are important regulators of reproductions of various animals (S. Ohkura et al., 2009; K.-L. Hu et al., 2018). Active study of KP began in 2003. However, at present, there is not enough information about the possibilities to purposefully and effectively control the sexual cycle of *Bos taurus*, especially in dairy cows, with the help of KP (B.R. Alves et al., 2015; T. Songraphasuk et al., 2021). The KP is produced mainly in neurons of various nuclei of the hypothalamus (V. Prashar et al., 2023). Considering that the location of neurons producing the KP is specific, the approaches to control with their help the reproductive function may vary (A. Gunn et al., 2020). Kisspeptin is synthesized using the gene *kiss1*. Initially, the KP is hydrolyzed to the KP-53, which later breaks up to shorter peptides (KP-14, KP-13 and KP-10) with various biological activity (A.E. Oakley et al., 2009; J. Tomikawa et al., 2010). Neurons producing KP are also coexption of peptide neurokinin B (NKB) and dinorfin, which determined the name of this population of nerve cells (KNDy-neurons, kisspeptin/neurokinin B/dinorphin) (R.L. Goodman et al., 2013; Q. Xie et al., 2022). In cattle KNDy-neurons are mainly fixed in the arcuate core of the hypothalamus, which is considered important for both positive and negative reverse regulation by sex steroids of the synthesis of GnRH (A. Hassaneen et al., 2016; Y. Uenoyama et al., 2021). Using the histochemical method, it was demonstrated that the activation of KNDy-neurons in cattle depends on the phase of the sexual cycle (A. Hassaneen et al., 2016). Kisspeptin-, neurokinin B-, and dinorfin-immunoreactive cellular bodies and fibers detecting throughout the arcuathed core of the hypothalamus in all phases. Unlike the arcuate core, numerous kisspeptin-immunoreactive cellular bodies were found in the reservoir region of the hypothalamus in the follicular phase, while only a few immunoreactive cellular bodies are recorded in the luteal phase. As for neurokinin, in the reservoir region a small amount of neurokinin of B-immunoreactive cellular bodies and fibers in both the follicular and lutein phase is naked. Dinorfin-immunoreactive cellular bodies and fibers in the follicular phase were larger than in luteal phase. In this regard, cattle are closer to sheep and primates, including human (V.M. Tanco et al., 2016). Since the initial identification of KNDy-neurons producing KP, there are a large number of unresolved issues relating to the function of various populations of these nerve cells, depending on the location, as well as the possibilities of new technologies for their study, including in relation to *Bos taurus*. There is a need to study various concentrations of kisspeptins and their influence on the ovulation of cows. This review discusses the basic information about the location and structural-functional characteristics of the *Bos taurus* KP, the distribution and functions of the KP neurons in the brain, the content of the KP in the blood and their effect on the organs of the reproductive system. Separately data

on the exogenous regulation of KP functioning of the reproductive system *Bos taurus* are discussed. The emphasis is on scientific research data, the main object of which was *Bos taurus* animals.

Keywords: *Bos taurus*, cows, hormone, estrus cycle, kisspeptin, gonadotropin-releasing hormone, neurons

Anatomical and functional development of animal reproductive system continues from early embryogenesis until puberty. Regulation of the reproductive system functions is performed mainly through the hypothalamic-pituitary-gonadal (HPG) axis. In cattle, as in other mammals, gonadotropin-releasing hormone (GnRH), the master hormone regulating reproduction is of special importance [1].

GnRH stimulates the secretion of luteinizing (LH) and follicle-stimulating (FSH) hormones in the anterior pituitary gland, which, acting on the gonads through the peripheral circulation, regulate steroidogenesis and gametogenesis [2]. The feedback action of sex steroids produced during these physiological processes regulates the GnRH secretion. This pattern of the HPG axis hormonal regulation inherent in almost all animal species has long raised several important questions. First, there was no explanation of how the sex steroid feedback regulates the GnRH release by the hypothalamic neurons which lack estrogen receptors α (ERα), the main receptors that provide both negative and positive feedback [3]. Secondly, it was not clear how tonic and pulsatile GnRH secretion is controlled.

The opportunity to answer these questions arose with the discovery of kisspeptins (KP), a family of peptides that originated from the prohormone encoded by the *kiss1* gene. In 1996, the first identified protein of this gene was named metastin due to its ability to inhibit the metastasis of cancer cells. After 3 years, the receptor for this hormone, KISS1R (previously designated GPR54, AXOR12 or Hot7T7T175), was identified. In 2003, the influence of KP on the reproductive function was discovered.

For more than 12 years, the role of the KP has been demonstrated as the main factor in the initiation of puberty and the regulation of tonic and pulsatile release of GnRH, which has a significant impact on the fertility of females, including secretion of gonadotropins, the onset of puberty, sexual differentiation of the brain, the onset of ovulation and metabolic regulation of fertility [4, 5]. There are many publications revealing the neurohumoral and physiological role of the KP in various species of wild and domestic animals [6-10].

The purpose of this review is to systematize the accumulated data on the effect of kisspeptins on reproductive function in *Bos taurus*.

Localization and structural and functional characteristics of *Bos taurus* kisspeptins. Based on data for other species, it is possible to assume a similar localization of the *kiss-1*/KISS1R system in the central nervous system (mainly in the hypothalamus) and the placenta. Expression has also been reported in the gonads, pancreas, liver, small intestine, spleen, adipose tissue, and lymph nodes [7, 9, 10].

In cattle, the *kiss1* gene on chromosome 16 encodes a prohormone, the hydrophobic protein consisting of 135 amino acid residues (aa). This prohormone is hydrolyzed into kisspeptin 53 protein (KP-53). There are data indicating further hydrolysis of KP-53 into short peptides (KP-14, KP-13 and KP-10). Similar information has not been obtained for cattle, but a similar pattern can be assumed (Fig. 1). All four forms of the peptide have affinity, bind efficiently to receptors, and are highly stable in all vertebrate species [11].

The most functionally active kisspeptin consisnts of 52-54 amino acids depending on the animal species [6]. The amino acid sequence of kisspeptin is structurally conserved among various mammalian species (Fig. 2) [11, 13]. For example, the amino acid sequence of the goat KP is 98% similar to the sheep KP, 91% similar to the cow KP, and 77% similar to the pig KP. In particular, the 10

amino acid C-terminal domain is the minimal sequence for maximum receptor activation and remains identical in most of the species mentioned (see Fig. 2). [14, 15].

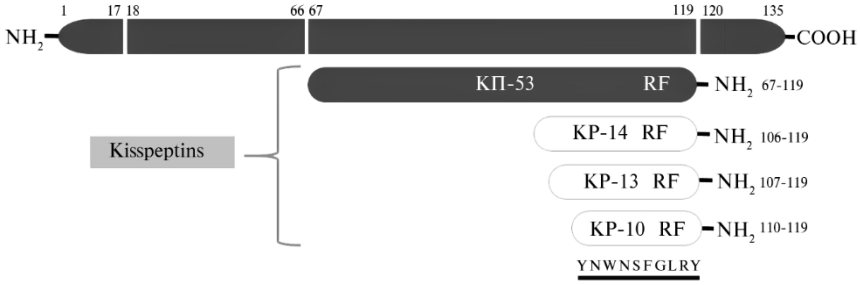

Fig. 1. Main structural features of *Bos taurus* kisspeptins resulted from post-translational modification of the prohormone. Kisspeptins presumably produced by hydrolysis of KP-53 are highlighted in white. All kisspeptins contain an RF amide that can bind and activate KISS1R (adapted from reference 12).

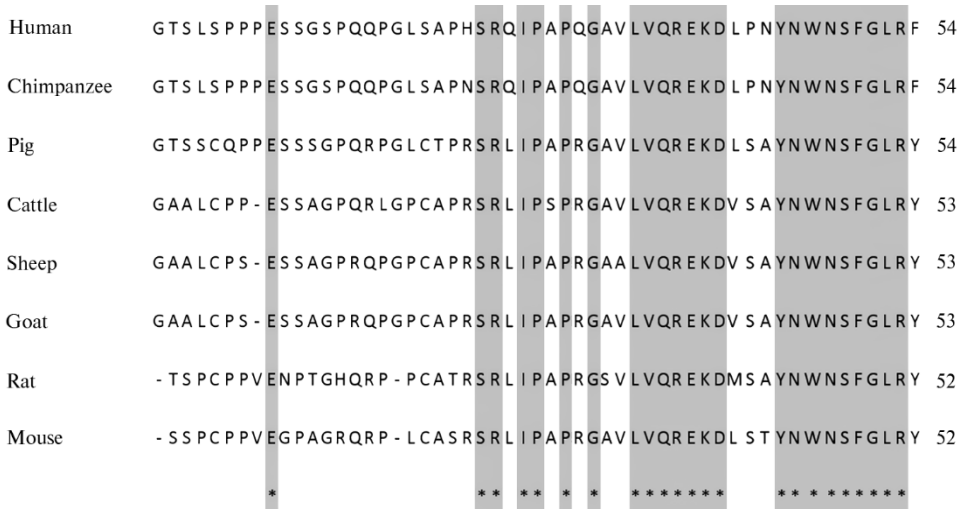

Fig. 2. Comparison of kisspeptin amino acid sequence in different mammalian species [11]. The last column indicates the number of amino acid residues. Identical amino acid residues are marked with an asterisk (*). The highlighted sequence YNWNSFGLR shows the KP-10 region which is similar in many mammalian species.

Distribution and function of kisspeptin neurons in the *Bos taurus* brain. The existence of a tonic system of Gn-RH secretion in both sexes, and a cyclic system only in females is consistent with the idea that the regulatory centers for the tonic and pulsatile systems are located in different areas of the hypothalamus. This fully applies to the areas of the hypothalamus in which neurons producing KPs have been identified. Kisspeptin neurons coexpress the peptide neurotransmitters neurokinin B (NKB) and dynorphin [16-20]. Due to co-localization of three neuropeptides, this cell population is called KNDy neurons (kisspeptin/neurokinin B/dynorphin) [21, 22].

The localization of KNDy neurons in the hypothalamus is species specific. In cattle, KNDy neurons are mainly located in the arcuate core of the hypothalamus (arcuate, ARC), which is considered important for both positive and negative feedback regulation of GnRH synthesis by sex steroids [23-25]. Most KNDy neurons express estrogen receptors α (ERα) and progesterone receptors [25-28], so the synthesis and secretion of all products of these neurons depend on the level of sex steroids. Note, there are areas of neuronal populations that produce KPs with a small amount of dynorphin and NKB [22]. However, it is important to consider

data that calls into question only the direct effect of kisspeptins on neurons producing GnRH. According to A. Gunn et al. [25], GnRH neurons in small numbers are located adjacent to kisspeptin neurons. The authors suggested that the effects of estrogen may be transmitted through kisspeptin neurons, but in cattle, unlike most other mammalian species, this is unlikely to be the main stimulatory factor.

Using a histochemical method, it was demonstrated that the activation of KNDy neurons in cattle depends on the phase of the reproductive cycle [22]. Kisspeptin-, neurokinin B-, and dynorphin-immunoreactive cell bodies and fibers are found throughout the ARC in all phases. In contrast to the ARC, numerous kisspeptin-immunoreactive cell bodies are found in the preoptic area of the hypothalamus (POA) during the follicular phase, whereas only a few immunoreactive cell bodies are recorded for the luteal phase. As for NKB, a small number of neurokinin B-immunoreactive cell bodies and fibers were found in the POA in both the follicular and luteal phases. At the same time, there were more dynorphin-immunoreactive cell bodies and fibers in the follicular phase than in the luteal phase. In this regard, as reported by V.M. Tanco et al. [29], cattle are closer to sheep and primates, including humans. For example, in rodents, cells expressing *kiss1* mRNA are located in both the ARC and POA [30]. In sheep, goats, and deer, most KNDy neurons were located in the ARC and only a small portion in the POA [26, 31, 32].

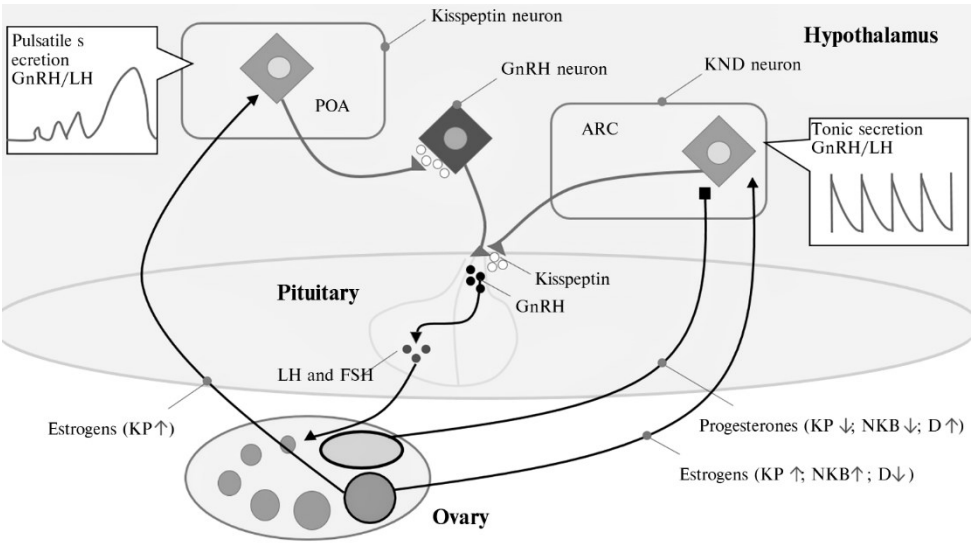

Fig. 3. Regulation of reproductive function in *Bos taurus* by kisspeptin: GnRH — gonadotropin-releasing hormone, LH — luteinizing hormone, FSH — follicle-stimulating hormone, POA — preoptic area of the hypothalamus, ARC — arcuate core of the hypothalamus, KP — kisspeptin, NKB - neurokinin B, D — dynorphin.

Secretion of the three substances by KNDy neurons in the ARC during all phases of the sexual cycle provides tonic release of GnRH (Fig. 3). This is largely possible due to the fact that KNDy neurons in the ARC, due to connections through axons and/or dendrites, form a population of neurons that mutually influence each other. Moreover, in the ARC, KNDy neurons form a dense network, which probably contributes to the synchronization of their activity [3, 22]. Synchronous activity of KNDy neurons is considered necessary for pulsatile GnRH secretion. Some authors assign the main role in such synchrony to the NKB system with its receptor NK3R [33, 34]. Kisspeptin neurons do not express KISS1R but do express receptors for dynorphin and neurokinin B. That is, KNDy neurons intercommunicate through neurokinin B and dynorphin, but not through kisspeptin [35]. NKB, secreted by a KNDy neuron, binds to its receptor NK3R

on another KNDy neuron, causing an influx of Ca^{2+} into it. An increase in intracellular Ca^{2+} concentration can promote its movement into neighboring KNDy neurons and glial cells through intercellular GAP junctions, even if these KNDy neurons do not express NK3R. This forms the synchronized activity of KNDy neurons which is induced by NKB-NK3R signaling.

Dynorphin synthesized by a KNDy neuron binds to κ-opioid receptor (KOR) on a neighboring KNDy neuron and can cause its inhibition. Each oscillation mediated by NKB/NK3R and dynorphin/KOR is thought to induce a pulsatile release of kisspeptin [33]. It is worth noting that the dynorphin/KOR system in KNDy neurons ensures negative communication through sex steroids estrogens, progesterone and testosterone [36]. These findings suggest that in *Bos taurus*, KNDy neurons in the ARC function throughout the estrous cycle and regulate follicular development and corpus luteum function through tonic release of GnRH, whereas the second large population of kisspeptin neurons found in the POA during follicular phase may be involved in the preovulatory rise in GnRH/LH concentrations [22].

Data from I.J. Clarke et al. [37] provide indirect evidence that kisspeptin-producing neurons are more active in the ARC. In cows with spontaneous ovarian cycles, *kiss1* expression in the arcuate core is almost 2 times higher compared to cows with various problems in cyclicity [37].

A. Hassaneen et al. [22] indicated kisspeptin-immunoreactive cell bodies and nerve fibers in the supraoptic ridge of the OVLT (organum vasculosum laminae terminalis). This is of interest because a dense cluster of GnRH-producing neurons and fibers has previously been found in the OVLT [29]. Ihis work also documented immunoreactive fibers in the PVN (paraventricular nucleus), DMH (dorsomedial hypo-thalamic nucleus), VMH (ventromedial nucleus), and LaH (lateral hypothalamus) [22]. Notable is the accumulation of immunoreactive fibers in the outer zone of the median eminence, indicating that kisspeptin is secreted into the portal vasculature beyond the blood-brain barrier. The inner zone contains numerous fibers that allow interaction with neuronal fibers also secreting into the portal vessels [38].

Similar data on OVLT were obtained by C.E. Leonardi et al. [39]. They studied the distribution of GnRH and KP neurons in the hypothalamus during the reproductive cycle in cows. The main clusters of KP neurons were founs in the POA, ARC, and OVLT [39]. Noteworthy is the fact that the largest number of KP neurons was recorded in the POA in the luteal phase (metestrus), and the smallest in diestrus. This is consistent with data that increased progesterone, which is characteristic of the diestrus phase, causes the synthesis and secretion of dynorphin that reduces Gn-RH production [40].

Increased levels of dynorphin in response to stress and exercise may suppress GnRH/LH secretion [36] (see Fig. 2). Stress factors (heat, transportation, veterinary manipulations) disrupt reproductive function by reducing the secretion of GnRH not only through dynorphin. This occurs in part due to cortisol acting through the type II glucocorticoid receptor. In this case, GnRH neurons do not express the receptor, but KNDy neurons are capable of the expression.

The examples with dynorphin and cortisol further prove that the hypothalamic control of fertility depends on various external factors. In cattle, one of the most important factors is the energy status of the animals. GnRH neurons lack many nutrient-dependent metabolic hormone receptors, implying the existence of neurons presynaptic to GnRH neurons. KP neurons express receptors not only for steroids, but also for metabolic hormones such as insulin, leptin, and ghrelin [41]. It can be assumed that KP neurons may be key for coordinating the energy state of animals with their reproductive function [23, 38, 42, 43].

Blood kisspeptin content in *Bos taurus*. Currently, there are very few research works on the content of kisspeptins in the body of cattle. This is explained by the complexity of experiments and the lack of kits necessary for assay. There are practically no enzyme immunoassay kits for various kisspeptins (KP-53, KP-14, KP-13, KP-10), whereas radioimmunoassay, due to its specificity, is not always available. An alternative is to quantify the kisspeptin prohormone for which commercial kits are available.

One of the few studies assessed the content of KP-10, progesterone and estradiol in dairy cows on days 10, 12, 14 and 16 after calving [44]. The KP-10 content was 116.99±35.29, 114.86±27.34, 149.5±36.67 and 124.69±42.76 pg/ml, respectively, whereas progesterone levels remained practically unchanged, approximately 0.35 ng/ml, and the estrogen concentration was 0.92±0.34, 1.33±0.4, 1.81±0.37 and 1.78±0.33 ng/ml, respectively. It can be noted that the maximum amounts of KP-10 coincided with an increase in estradiol concentrations. The authors concluded that an increase in estrogen levels may, by a positive feedback, enhance the synthesis and secretion of hypothalamic KP-10 [44]. This conclusion is confirmed by A. Rizzo et al. [45]. They recorded a significant increase in KP-10 duering post-calving period (80±15 days) in cows with follicular cysts compered to healthy cows, 125.06±34.47 vs. 97.72±21.34 pg/ml. In addition, estradiol and progesterone concentrations also increased almost 2-fold [45].

The effect of kisspeptin on the reproductive organs. A large number of publications postulate that the main function of the KP is realized in the hypothalamic regulation of the HPG axis. However, data have emerged proving their regulatory role in the functions of the ovaries, embryonic trophoblast and placenta [46], which requires more in-depth study.

H. Liu et al. [47] found expression of KP-10 in the cow's preantral follicles, i.e., in the oocytes, granulosa and theca cells, with maximum expression in the latter. This is of interest because in cattle, most follicles growing in vivo gradually become atretic during the growth phase that begins with the formation of preantral follicles. There was a dose-dependent negative effect of KP-10 on follicular growth, up to and including atresia, and it has been suggested that KP suppresses the expression of the FSH receptor [47].

During the follicular growth stage, the development and maturation of oocytes depends primarily on their connection with granulosa cells. H. Liu et al. [48] demonstrated that KP-10, by inhibiting proliferation, promotes the initiation of apoptosis in ovarian granulosa cells in cows. KP-10 may also have a slightly different effect that is not associated with cell apoptosis. Some authors note the ability of KP-10 to reduce the expression of one type of microRNA, the miR-1246 which inhibits synthesis of progesterone by granulosa cells in follicles [49].

Perhaps the inhibitory effect of KP is associated with the need to stop the appearance of new preovulatory follicles. In the early stages of embryogenesis, the influence of kisspeptin can be stimulating. M.M. Soares et al. [50] studied the effect of KP in in vitro culture of embryos and revealed that the KP-10 added to the medium increased the rate of blastocyst formation.

The expression of *kiss1*/KISS1R during placentation which has species differences is also of interest. In cows, implantation is a less invasive process. During synepithelial-chorionic placentation in ruminants, the trophoblast does not penetrate the basement membrane and stroma of the uterus, but forms villous outgrowths in the endometrial epithelium. Based on this, M.J. D'Occhio et al. [46] suggested an influence of fetal trophoblast *kiss1* expression on uterine KISS1R. The gene may influence the initial attachment of the trophoblast to the endometrial epithelium of the uterus through interaction with intercellular adhesion molecules and extracellular matrix proteins. Therefore, in livestock, the *kiss1*/KISS1R

system may play a lesser role.

The *kiss1*/KISS1R system is also involved in the regulation of placenta development in cows. N.A. Martino et al. [51] demonstrated that bovine epithelial cell cultures derived from first-trimester pregnant cows expressed KISS1R [51]. The KP-10 added to cultures both stimulated and inhibited epithelial cell proliferation in two separate cell lines. Stimulation occurred in a cell line with overregulation of KISS1R mRNA. This is especially important since the failure of ruminant embryos to attach and implant is one of the main causes of declining reproductive capacity [46, 52].

Exogenous kisspeptin regulation of reproductive functions in *Bos taurus*. Since blood KP in cattle is difficult to of measure, researchers almost immediately drew attention to testing KP by exogenous administration and analysis of its effects. However, studying these effects in cattle is still problematic due to the lack of drugs intended for the *Bos taurus* species. Most studies have used human or mouse KP-10. Considering that KP-10 is the most similar in its amino acid sequence in almost all mammalian species, this peptide is most often used.

However, note that different KP-10 may make adjustments to the data obtained. This was shown by C.E. Leonardi [53]. A single bolus or multiple intravenous injections of human KP-10 more effectively increased blood plasms level of LH compared to similar treatment with murine KP-10 [53]. Because of small number of reports on the effect of exogenous KP on *Bos taurus*, in the review we combined data from publications where various kisspeptins were used.

In this regard, the first experiments with cell cultures of the adenohypophysis of calves and adult animals are of interest in order to detect the effect of KP-10 on the content of various hormones [54-56]. A dose-dependent effect of exogenous KP-10 on increasing the amount of LH and growth hormone in the cells of the adenohypophysis, regardless of age, has been revealed [54, 55]. A.A. Ezzat et al. [56] studied the effect of KP-10 on the secretion of LH, FSH and prolactin by cells of the anterior pituitary gland of cattle, assessing the ability of sex steroids to enhance the sensitivity of gonadotropic and lactotropic cells to KP-10. KP-10 significantly stimulated LH secretion in cells treated with estradiol and testosterone, but not in cells treated with progesterone. In contrast, KP-10 did not affect FSH secretion regardless of steroid use. KP-10 significantly stimulated prolactin secretion, but no effect of sex steroids was detected. These results suggest that estradiol and testosterone increase the LH secretion by gonadotropic cells in response to KP-10. KP-10 directly stimulates the secretion of prolactin by pituitary cells, and sex steroids do not increase the sensitivity of lactotropic cells to KP-10 [56].

The results of the described experiments allow us to conclude that the stimulating effect of kisspeptin on LH secretion in cattle is expressed at least in two ways, via GnRH neurons with its secretion in the POA or ARC (cell bodies, dendrites or dendrons) and by direct stimulation of LH secretion in the pituitary gland. This may influence the generation of GnRH/LH pulses as well as the preovulatory GnRH/LH surge. In other animal species, a third way is found, that is, kisspeptin from KNDy neurons located in the ARC can act on the terminals of GnRH neurons in the median eminence, but similar experiments have not been performed in cattle [57, 58].

Stimulation activity of kisspeptin has been demonstrated in studies of injections of KP-10 and KP-53 in cows and heifers of different breeds and ages [59-61]. In most cases, kisspeptin injections resulted in a surge in LH concentrations as well as an increase in growth hormone concentrations [62-64]. However, the KP-10 was less effective compared to exogenous GnRH, whereas injections of KP-53 provided LH levels sufficient for ovulation [59, 63, 65].

A.E. Ahmed et al. [62] assessed the inhibitory effect of progesterone on

the ability of kisspeptin to increase LH concentrations. For 7 days, cycling cows were treated with a progesterone releasing intravaginal device (PRID). Animals received a single intravenous injection of KP-10 for 3 days after removal of the device. Blood progesterone concentration was higher on the date of PRID removal, followed by a decrease on days 1 and 2. KP-10 did not significantly alter plasma LH concentrations at the date of PRID removal. However, KP-10 significantly stimulated the release of LH in the following days. The authors concluded that KP-10 stimulates LH release in cycling postpartum cows, and high plasma progesterone concentrations may reduce the effect of kisspeptin on LH secretion [62].

In almost all works on the FSH level as influences by kisspeptins in cows of different breeds and ages, the concentration of FSH remained virtually unchanged. However, there are the opposite data. M.A. Rodríguez et al. [66] studied the effectiveness of KP-10 administration. In calves aged 4, 7 and 11 months intravenous administration of KP-10 increased FSH levels.

In male cattle, the positive effect of kisspeptins on FSH concentration is more often recorded. Several studies have shown that a single intravenous injection of KP-10 significantly stimulated the release of LH and FSH in calves and bulls [67, 68]. S.L. Northup et al. [69] studied the effect of intravenous administration of KP-10 to bulls during puberty on the blood LH and FSH concentrations. Acute intravenous infusion of KP-10 increased blood LH and did not change FSH. Chronic intravenous infusion had no effect on LH but decreased the blood FSH mean concentration and the amplitude of its pulsatility [69].

Summarizing the data on the exogenous administration of kisspeptins, we can note their stimulating effect on the LH synthesis and secretion. With regard to growth hormone, the effect of KP-10 is determined by the age of the animals, which may explain the positive effect of steroid hormones (progesterone and estradiol). On the effect of kisspeptins on FSH, the data are contradictory and require clarification, although in most reports the synthesis and secretion of FSH were less susceptible to stimulation. The explanations may be the greater resistance of FSH to pulsed GnRH secretion and the suppressive effect of inhibin A and B on FSH secretion [36, 70].

Interest in the exogenous (injection) administration of kisspeptins is obvious, since there is the possibility of controlling the sexual cycle through control of the secretion of GnRH and gonadotropins [71]. For example, M. Mondal et al. [72] showed that the kisspeptin-based synchronization protocol induced better follicular growth than the ovsynch (ovarian synchronization protocol). The frequency of ovulation was significantly higher, and luteolysis began earlier, even before the injection of prostaglandins [72].

However, in general, most researchers still have questions about the injection of kisspeptins to control ovulation, especially since endogenous peptides have clear limitations. For example, the half-life of KP-10 in the bloodstream is less than 1 min, and in most cases the desired effect can only be achieved by perfusion (intravenous or intracerebroventricular) or hourly injection. The use of intracerebroventricular or intravenous perfusion in agricultural settings is difficult to implement, and multiple intravenous injections require increased veterinary costs. Not surprisingly, researchers have become interested in using synthetic analogues of kisspeptins that have higher activity and longer half-lives [73].

For example, P.A. Parker et al. [74] studied the effect of a synthetic analogue of kisspeptin called Compound 6 (C6) on the concentrations of LH, FSH, and testosterone in prepubertal bulls. The in vitro activity of C6 was approximately 8 times higher than that of KP-10. It was shown that intramuscular administration of C6 increased the concentration of LH without changing the content of FSH

and testosterone in the blood. The work of C.R. Burke et al. [75] is another example of using TAK448, a synthetic analogue of kisspeptin. The authors concluded that kisspeptin treatment, if performed when there is a mature dominant follicle in the ovaries, can induce ovulation in postpartum dairy cows followed by estrous cycles of normal length [75].

S.M. Popa et al. [76] using genetically engineered mice that exhibited only 5% KISS1 expression, found that both males and females reached puberty and remained fertile. Y. Uenoyama et al. [3] confirmed this phenomenon in rats, finding that 20% of KNDy neurons are sufficient to support GnRH pulses and folliculogenesis, indicating functional redundancy of the KNDy neuron population. The authors pointed out that there is not yet enough information about the functional redundancy of KNDy neurons in mammalian species other than rodents [3].

Although much has been learned since the initial identification of KP-producing KND neurons, there are still many unresolved questions regarding the function of different populations of these nerve cells depending on location. Another aspect is new technologies for studying KND neurons, including KND neurons of *Bos taurus*. It is necessary to analyze the effect of different concentrations of kisspeptins on ovulation in cattle. Importantly, there is a problem that has not yet been solved even in human medicine. M.A. Hussain et al. [77] noted that commercially available methods for quantifying blood kisspeptin in various mammals are not reliable enough due to large differences in assay methods, detection ranges, and uncertainty about which forms of kisspeptin (e.g. KP-10, KP54) are detected [77]. Thereof, methods for measuring kisspeptin concentrations in biological fluids should be improved.

Thus, the effect of kisspeptin on the reproductive function of *Bos taurus* is of interest. Despite accumulated information on the issue, the data are mostly obtained upon exogenous administration (injection) of kisspeptin-containing drugs and measurements of the resulting hormonal levels in animals. There is an obvious lack of information about the natural levels of kisspeptin in young animals, the concentration of kisspeptin in the blood of cycling and pregnant cows, and the relationship between kisspeptin concentrations, reproduction and productivity. The available information is often obtained for other animal species. Kisspeptins used in experiments on cows are in most cases non-species specific or synthetic. In general, despite the fact that the role of kisspeptin in reproductive function has been established long ago, this hormone is interesting for further study, provided that the necessary tools for accurate detection and measurement of its concentrations become available.

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