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Abstract

The discovery of the kisspeptin (Kp) system stirred a burst of research in the field of reproductive neuroendocrinology. In the last 15 years the organization and activity of the system including its neuroanatomical structure, its major physiological functions, and its main pharmacological properties were outlined. To this endeavor, the use of genetic tools to delete and to restore Kp system functionality in a specific tissue was essential. At present, there is no question as to the key role of the Kp system in mammalian reproduction. However, easily applicable genetic manipulations are unavailable for domestic animals. Hence, many essential details on the physiological mechanisms underlying its action on domestic animals require further investigation. The potentially different effects of the various Kp isoforms, the precise anatomical localization of the Kp receptor, and the respective role played by the two main populations of Kp cells in different species are only few of the questions that remain unanswered and that will be illustrated in this review. Furthermore, the application of synthetic pharmacological tools to manipulate the Kp system is still in its infancy but has produced some interesting results suggesting the possibility to develop new methods to manage reproduction in domestic animals. In spite of a decade and a half of intense research effort, much work is still required to achieve a comprehensive understanding of the influence of the Kp system on reproduction. Furthermore, Kp system ramifications in other physiological functions are emerging and open new research perspectives.

Key words: Kisspeptin, reproduction, ovulation, GnRH, LH, pharmacology, agonist.

1. Introduction

The discovery that reproduction in mammals is mastered by a brain neuropeptide, kisspeptin (Kp), is the most recent breakthrough in reproductive neuroendocrinology. Our knowledge of the Kp system has grown exponentially and several excellent reviews on the subject are available [1-4]. To elucidate Kp system functions, most studies used rodents or primates, but investigations in domestic animals are less copious. Nevertheless, these studies are extremely interesting from a comparative point of view and capable of opening new opportunities to manage livestock reproduction. In the following sections we will briefly delineate the biological action of Kp and point to a few outstanding questions that we deem particularly interesting. Namely, we will discuss the anatomical distribution of the Kp system, its main physiological effects in reproduction and some pharmacological aspects crucial for the development of applications to manage domestic animal reproduction. Many other important and exciting features of the Kp system still require a substantial research effort. To gain a broader perspective on these aspects the reader could refer to other reviews on domestic animals [5-8].

2. The Kp system

A role for Kp in reproduction was first discovered in human patients showing loss of function mutations of the Kp receptor, *KISS1R* (also known as GPR54). These mutations lead to hypogonadotropic hypogonadism, lack of puberty and infertility [9, 10]. Further studies showing that GnRH neurons express *Kiss1r* [11], and the fact that Kp administration triggers GnRH release [12, 13] demonstrated that Kp stimulates the reproductive axis by a direct action on GnRH neurons. Kp also has a role in modulating the timing of puberty onset as shown in rodents, where repeated administration of Kp to prepubertal female rats advanced vaginal opening [14]. Two major populations of Kp neurons in the hypothalamus have been identified: one in the preoptic area (POA)

region and the other in the arcuate nucleus (ARC). Neurons of both populations bear estrogen receptor alpha, progesterone receptors and/or androgen receptors [15-18]. These finding and others [19] imply that Kp neurons are the target of sexual steroid feedback. Kp neurons in the ARC colocalize with neurokinin B and dynorphin and are referred to as KNDy neurons from the initials of the neurotransmitters they produce [20]. Localization and pharmacological studies have shown that KNDy neurons express the neurokinin B receptor NK3R [21] and the K-opioid receptor that is the cognate dynorphin receptor [22]. These results were instrumental in formulating the hypothesis that by an autocrine mechanism neurokinin B would initiate Kp release from KNDy neurons and dynorphin would terminate it [23]. The concept that KNDy neurons are the GnRH pulse generator is now widely accepted [24].

3. *KISS1* and *KISS1R* genes

In vertebrates, three different genes encoding for Kp and four genes encoding for its receptor have been identified. In mammals, with the exception of the monotremata (e.g. platypus), only one gene for the Kp (*KISS1*) and one for its receptor (*KISS1R*) are present [25].

KISS1R is a G protein-coupled receptor encoded by a highly conserved sequence. In humans *KISS1R* loss or gain of function mutations result in infertility [9, 10] and precocious puberty [26] respectively. Study in transgenic mice further support the importance of *Kiss1R* in puberty onset and fertility [27]. The existence of a loss of function mutation in a species or breed of domestic animals would be unexpected due to the importance of reproductive capacities in these animals and the resulting loss with such mutation. However, the identification of polymorphisms in the *KISS1* or *KISS1R* and their correlation with reproductive traits is an interesting topic for investigation. In goat breeds the presence of specific *KISS1* and/or *KISS1R* single nucleotide polymorphisms (SNPs) was correlated with variations in litter size and age at puberty [28, 29] as well as with an increase in circulating levels of estradiol and progesterone [30]. In *Kiss1R* haplo-insufficient mice, the litter size is reduced compared

to wild type mice [31]. This would concur with a role of the Kp system to define the litter size. Nevertheless, a more thorough characterization is required to establish firmly if the reported SNPs would be responsible or not for the modifications observed in these reproductive traits. A better understanding of the functional correlate of *KISS1R* SNPs may reveal new criteria for the genetic selection of domestic animals.

Processing of the Kp precursor leads to several endogenous Kp isoforms. The shortest active sequence, 10 amino acids (Kp10), corresponds to the C-terminal part of the precursor. This sequence is extremely well conserved in mammals with only few variants identified. The remaining of the sequence is significantly less conserved. Three other Kp isoforms have been described, two short ones (Kp13 and Kp14, the number indicates the length of the amino acid sequence), and a longer one that varies among species studied (i.e. Kp54 in humans, Kp53 in cattle, in the sheep, and in the goat, and Kp52 in the mouse). Preliminary data obtained in the rat and in the sheep showed the existence of an additional isoform, Kp16, and suggested that Kp16 and Kp13 are actually the most abundant isoforms [32]. Unfortunately, a complete account of these results was never published. The presence of different isoforms raises a major question: do the different isoforms serve different physiological functions? This question remains at present unanswered. The vast majority of the knowledge gained on the Kp system was obtained by applying the Kp10 and to a much lesser extent the Kp54. The other isoforms have received very little attention. To the best of our knowledge, *in vitro* data are available only for the hKp13, showing binding affinity and potency in a calcium mobilization assay similar to that of hKp10 [33] .

The small variations observed in the Kp10 sequence may have physiological consequences, but these are not easily predictable based on the sequence's structure. Interestingly the human (h) and ovine Kp10 (hKp10 and oKp10) differ in the C-terminal: such that the last amino acid is a phenylalanine in the human sequence and a tyrosine in the sheep (figure 1A). This amino acid is part of the pharmacophore region, the region bearing the biological activity [34]. A modification of this region

could influence molecule's activity. However, when tested *in vitro* in a calcium mobilization assay (for detail on the assay see [35]) hKp10 and oKp10 have superimposable profiles (figure 1B). Furthermore, when injected into the ewe both peptides produced an increase of LH and FSH plasma levels that were identical in amplitude and duration [36]. Studies in goats using intravenous administration of hKp10 produced similar results [37, 38]. Surprisingly, in Hereford cross-bred heifers, a comparison of the effect of hKp10 and cattle Kp10, that is structurally identical to oKp10 (figure 1A), on LH plasma concentration showed that hKp10 elicited a more pronounced effect than cattle Kp10. In addition, repeated injections of hKp10 over a 2-hour period triggered ovulation, but this was not the case when cattle Kp10 was used [39].

The equine Kp10 (eKp10) also has a single amino acid difference compared to the oKp10, in position 2 an arginine is substituted by a valine. This position is outside the Kp10 pharmacophore [34] and, therefore, this modification should bear no consequence on the activity. At odds with this prediction, when tested in a calcium mobilization assay on HEK293 cells transfected with the human *KISS1R*, the eKp10 was actually less potent that oKp10 and hKp10 (figure 1B). Consistent with this result when injected iv in the ewe (15 nmol/ewe, N=5 per group) the eKp10 is less potent *in vivo* than the oKp10 (figure 1C and D). The effect of eKp10 was extensively studied in the horse, where it was able to stimulate the release of gonadotropins. However, in contrast to what found with oKp10 in the sheep [36], it was unable to trigger or advance ovulation [40] and significantly disrupted normal sexual receptivity in the estrous mare [41]. At present, it is difficult to draw any conclusion from this scattered data. Nonetheless, they indicate that a single amino acid modification could result in a significant change of Kps' capacity to activate the receptor of a different species. Considering that often the hKp10 was used to study the physiology of the Kp system in other mammals, these data point to the importance of using the appropriate endogenous ligand for the species under evaluation in order to obtain meaningful physiological data.

4. Anatomical localization of the Kp system

148 Initial studies by RT-PCR reported the expression of *KISS1R* gene in several tissues including the brain,
149 the pituitary gland, the placenta and the pancreas [42-44]. Subsequent research focusing on the
150 brain showed its presence in the hypothalamus [45]. However, only a handful of studies describing a
151 more precise brain localization of *Kiss1r* are available. This information was obtained by applying *in*
152 *situ* hybridization in the rat [46, 47] and in the rhesus monkey [48] or by using a β-gal reporter gene
153 in the mouse [11]. Receptor expression was detected in several brain regions including the olfactory
154 bulb, the hypothalamus, the septum, the hippocampus, the thalamus, and the brainstem. The
155 distribution pattern appears significantly discordant between species suggesting either a different
156 sensitivity of the methods or a species-specific dependent expression of the receptor. This last
157 possibility deserves further investigation because it might have important implications to understand
158 the physiological role of the Kp system in different species.

159 On the other hand, double labelling studies in rats, monkeys and ewes consistently reported the
160 expression of *KISS1R* gene in GnRH neurons [47-49]. These data confirm that a direct effect of Kp on
161 GnRH neurons is a common feature.

162 Detection of the KISS1R protein has proven a difficult task. Most of the available antibodies are
163 insufficiently characterized lacking for example specificity validation performed in Kiss1r knockout
164 animals. Evaluation of the antibody using western blotting often results in multiple bands and
165 inconsistent molecular weights. Furthermore, use of antibodies raised against the rodent or human
166 receptor to detected KISS1R in other species without performing stringent controls in the target
167 species is common, leading to arguable results. This represents an obstacle to our understanding of
168 Kp action. The development of new tools, either dependable antibodies against the receptor or
169 tagged molecules allowing its detection and localization, would be an important advance in the field.
170 An attempt in this direction has been done by the creation of synthetic Kp-based probes labelled
171 with a fluorescent tag [50]. These probes were characterized successfully in *in vitro* systems, but
172 were not assessed for receptor localization on tissue. We have recently described the synthesis of a

tagged Kp-derived molecule capable of covalently binding KISS1R *in vitro* [51]. Further development of this molecule to improve its stability is ongoing and might become a valuable tool for receptor localization studies.

The expression of the *KISS1* gene and that of the related protein has been investigated in several species. In mammals, two main neuronal populations sited in the diencephalon have been described. One, located in the ARC, has been recognized in rodents [52-54], in primates [48, 55-57], in the sheep [15, 58], in the goat [59, 60], in the horse [61], in the pig [62], in cattle [63], in the dromedary camel [64], and in the cat [65]. The presence of the second one, located in the POA, has been reported in rodents [52-54], in the sheep [15, 58], in the goat [59], in cattle [63], in the dromedary camel [64], and in the cat [65], but data are contradictory in the horse [61, 66] and in primates [56, 57]. The precise localization of this second population also varies with species: in rodents it is adjacent to the ventricle whereas it is more lateral in other mammals [53].

Discrepancy on the localization of Kp in the horse could arise from the use of different antibodies. In one study, Kp-like immunoreactivity was found not only in the POA but also in the dorso-medial nucleus [66]. In this study an antibody from Phoenix Pharmaceutical was used, which have shown to have cross reactivity with other members of the RFamide family [67] and *NPVF* (the precursor mRNA for RFamide-related peptide 3) has been detected in the dorsomedial paraventricular hypothalamic nuclei of the mare [68]. This antibody gave similar results in the sheep [69], but there is no evidence of the presence of mRNA encoding for Kp in the dorso-medial region of the sheep hypothalamus [58]. There is a single report of this antibody labelling GnRH neurons in the sheep [69], but these results could not be reproduced using an antisera specific for Kp [53]. It is likely that this antibody cross reacts with other neuropeptides of the RFamide family, such as NPVF, showing a sequence similar to that of Kp. Nevertheless, the existence of a Kp POA population cannot be ruled out based on antibody cross-reactivity alone because NPVF was not detected in the POA [68].

In addition, the Kp POA population is sexually dimorphic with females having a larger number of cells. Indeed, several reports indicate an important role of sex steroids, in particular estrogens (Es), in regulating the size of this population [1]. High levels of E, similar to that observed during the pre-ovulatory period, would increase the number of neurons expressing Kp. In contrast, ovariectomy, ablating ovarian E production, would decrease it to an almost undetectable level. The study reporting the absence of Kp in the POA of the mare was performed on brain dissected between 2 and 4 hours after ovulation [61]. At this stage of the ovulatory cycle E concentration is rapidly declining and is lowered by 2/3 compared to its maximal level [70], leading to an E negative feedback. It is possible that localization studies performed at a moment of the cycle when circulating E is high (i.e. the pre-ovulatory phase) may confirm the existence of a neuronal population expressing Kp in the POA of the mare.

The question about the existence of this neuronal population is not trivial. In the current view, the Kp ARC population would be mainly involved in controlling the pulsatile release of GnRH and submitted to the E negative feedback. Optogenetic studies in rodents [71-73] and combined pharmacological and electrophysiological analyses in goats [74] converge in favor of this hypothesis. On the other hand, the POA population would relay the E positive feedback triggering the increase of GnRH secretion that leads to the pre-ovulatory surge [75]. However, in the sheep data are less clear-cut. Some data suggest a rodent-like mechanism showing an activation, concomitant with the LH surge, of POA neurons, but not of ARC neurons [76]. Others support a combined contribution of the two populations in generating the ovine pre-ovulatory surge [77, 78]. Finally, an E implant in the mediobasal hypothalamus induces a surge [79], and cells expressing *KISS1R* mRNA in the caudal ARC were more abundant in the pre-ovulatory period than during the other phase of the cycle. These data suggest a prominent role for the ARC Kp population in pre-ovulatory surge induction [80].

In the ewe the caudal portion of the Kp neurons of the ARC are less sensitive to E negative feedback during the breeding season [81]. The abundance of the estrogen receptor α in the Kp neurons of the ARC of pre-pubertal ewe was compared in ovariectomized (OVX) and OVX and E (OVX+E) replaced

223 animals. The OVX+E showed a decrease of estrogen receptor α in the rostral part on the nucleus and
224 a tendency to a decrease in the medial part and no effect on the caudal portion. Similar results were
225 obtained for the number of neurons expressing Kp [82]. The possibility that the ARC Kp neurons of
226 the ewe (and perhaps other species) would convey both positive and negative estrogen feedback to
227 GnRH neurons is an interesting hypothesis that remains to be further substantiated. If the absence of
228 a POA Kp population in the mare is confirmed, this species would be an interesting research model to
229 test this hypothesis.
230 In the ewe, a combined regulatory effect of sexual steroids and photoperiod has also been observed.
231 In OVX+E ewes, a lower level of Kp was observed in the mediobasal hypothalamus and in POA during
232 the non-breeding season compared to the breeding season [19] and switching from long to short
233 photoperiod induced an increase of Kp expressing neurons in POA and ARC [83].
234
235 A third population of Kp neurons has been detected in the medial amygdala of rodents [52, 84], a
236 region shaping social, emotional as well as sexual behaviors. Kp expression in these neurons is
237 sexually dimorphic, with males showing a larger population. Gonadectomy decreased *Kiss1*
238 expression in both sexes whereas treatment with either testosterone or E increased it [84].
239 Application of anterograde and retrograde tracers showed the existence of reciprocal connections
240 between Kp neurons located in the amygdala and the accessory olfactory bulb. A direct connection of
241 amygdala Kp neurons with GnRH neurons of the POA was also observed. Therefore, Kp neurons in
242 the medial amygdala could be part of the pathways relaying information controlling sexual behaviors
243 related to odor cues [85]. In small ruminants the so-called male effect, applied to resume the
244 ovulatory cycle during the seasonal anoestrus, is mediated mainly by odor cues. In the goat,input
245 from the medial nucleus of the amygdala to Kp neurons of the ARC has been proposed to play a role
246 in the male effect [86]. However, in domestic animals the role of Kp neurons located in the central
247 amygdala in processing and relaying odor cues remains unexplored. Actually, even the existence of

248 such a population in domestic species has not been demonstrated yet. This interesting research area
249 warrants further investigation.
250
251 The presence of the Kp system in the pituitary gland and an *in vitro* direct effect of Kp administration
252 on LH and FSH secretion and gene expression was reported in several species [42-44, 87-91] (see
253 [92, 93] for review on the subject). Even though available results suggest a potential direct
254 stimulation of gonadotrophs by Kp, nevertheless effects were mainly of small amplitude. The most
255 compelling evidence has suggested a minor, if any, role of Kp in stimulating the pituitary gland. In
256 particular, rescue of Kp signaling in GnRH neurons in *Kiss1r* KO mice completely reinstates a
257 reproductive phenotype [94, 95]. In hypothalamo-pituitary disconnected ewes there was no
258 temporal correlation between Kp levels recorded in the portal blood and LH pulsatile pattern
259 measured in the jugular vein [88]. Conversely, Kp peripheral administration is highly correlated with
260 GnRH release and its effect on gonadotropin secretion is blocked by a GnRH receptor antagonist [48,
261 96-98].
262
263 Expression and localization data indicate the presence of Kp and its receptor also in the reproductive
264 organs. In the female of various species, both Kp and its receptor have been localized in the ovary, in
265 the uterus and in the placenta [93]. Immunoreactivity was observed in granulosa cells, cumulus cells,
266 theca cells, stromal cells, trophoblast giant cells, etc. Localization differs between species and
267 sometime contrasting results in the same species were reported [99]. Contradictory findings could be
268 explained partially by the use of tissue obtained at different times of the estrus cycle under the
269 influence of a different hormonal milieu. Nevertheless, this variability is puzzling and methodological
270 biases are a concern. Some immunohistochemistry results suffer from poor characterization of the
271 antibodies that make them unreliable. Caution should be taken also when considering expression
272 data obtained by RT-PCR. They are sometime undependable due to an excessively high number of
273 amplification cycles leading to the generation of false positives.

Despite these notes of caution, some convincing results for a local role of Kp on the female reproductive apparatus of rodents are available. In *Kiss1r* null mice ovary and uterus have a reduced weight and no ovulation occurs [9]. In these mice, a treatment combining GnRH and gonadotrophins triggers ovulation. However, null mice release a significantly reduced number of oocytes than WT suggesting that *Kiss1r* in the ovary is required for a full recovery of ovarian activity [31]. In line with this result *Kiss1r* haplo-insufficient mice display a syndrome resembling premature ovarian failure with decline in ovulatory rate and reduction of pre-antral follicles [31]. The potential implication of Kp in this phenomenon was discovered by studying the neurotrophin BDNF (brain-derived neurotrophic factor) and its receptor NTRK2 (neurotrophic tyrosine kinase receptor type 2). In mice a specific deletion of NTRK2 in oocyte results in oocyte death and early adulthood infertility similar to that observed in *Kiss1r* haplo-insufficient mice. Experimental data suggest that Kp would act in concert with BDNF to mediate the effect of gonadotropins on NTRK2 and on ovarian function [100]. Clarifying the physiological action of Kp in the ovary of domestic animals would be particularly interesting and relevant to optimize ovulation rate. Expression of the Kp system in the uterus has been associated with embryo implantation [101]. In *Kiss1*$^{-}$/$^{-}$ mice application of E and gonadotropins coupled to a superovulation protocol induces normal ovulation, egg fertilization and initial embryo development; however implantation fails. When *Kiss1*$^{-}$/$^{-}$ embryos are transferred to a WT female they implant successfully, indicating a maternal defect in the *Kiss*$^{-}$/$^{-}$ females. *Kiss1r* expression in the uterus is greater in Kiss-/- females and the distribution within the subluminal epithelium is different from the WT females. Treatment with Kp10 failed to rescue embryos' implantation in *Kiss1*$^{-}$/$^{-}$ mice [101]. In the uterus of *Kiss1*$^{-}$/$^{-}$ mice endometrial glands are poorly developed [101, 102] evoking the possibility that this defect is the consequence of the lack of a Kp local action during uterus development. Results showing that E treatment of *Kiss1*$^{-}$/$^{-}$ mice or selective restoration of Kp signaling in GnRH neurons of *Kiss1r*$^{-}$/$^{-}$ mice only partially restored endometrial gland formation support this hypothesis [95]. Furthermore, the expression of the cytokine leukemia inhibitory factor,

299 essential for embryo implantation, is reduced in *Kiss1*$^{-/-}$ uterine gland. Administration of leukemia
300 inhibitory factor to hormone-primed *Kiss1*$^{-/-}$ partially rescued implantation [101].

301 Similarly to rodents, endometrial gland formation in sheep and pigs takes place during the postnatal
302 period, pointing to the relevance of these results for the physiology of domestic animals.

303 The presence and physiological significance of the Kp system in the male reproductive apparatus has
304 received less attention. In the mouse, the most convincing data indicate that the expression of the
305 genes for Kp and *Kiss1r* in the gonad is restricted to haploid spermatids. However, no protein for Kp
306 was detected, suggesting a translational repression, and Kp administration had no effect on
307 testosterone release from an immortalized Leydig cell line [103]. Conversely, in Leydig cells from goat
308 testes *Kiss1* and *Kiss1r* genes expression was increased by gonadotropins treatment. Incubation of
309 Leydig cells with the Kp antagonist Kp234 reduced testosterone secretion implying a potential role of
310 Kp in androgen production [104]. The conflicting results obtained in these studies could be due to a
311 physiological difference between species or due to the use of a cell line versus cells from primary
312 culture. A drawback of the goat study is the lack of evidence about a positive effect (i.e. stimulation
313 of testosterone production by Kp administration). Of note, Kp234 has been applied to counteract the
314 effect on Kp in various experimental systems [105]. However, in the dog it was unable to block the
315 effect of Kp both *in vitro* and *in vivo* [106]. Furthermore, it has been reported that p234 and p356,
316 the next generation analog of p234, exhibit weak agonist activity at *Kiss1r* in mice [107] and *in vitro*
317 [106]. These findings raise doubts that the observed effect would be mediated by the Kp system. The
318 fact that GnRH-specific rescue of *Kiss1r* null mice was sufficient to restore a normal fertile phenotype
319 and testes were not different from that of WT [94] support a minor role, if any, of Kp in modulating
320 testicular functions. Nevertheless, further studies on the subject would be welcome to clarify this
321 issue.

322

323

5. Pharmacological modulation of the kisspeptin system

A thorough understanding of Kp system functions requires the capacity to pharmacologically modulate its action. Modulation of the Kp system was initially explored by injecting the endogenous Kps. Of the various isoforms, Kp10 has been the most broadly used because it is easy to synthesize and highly efficacious. Except in humans, where the effects of hKp54 have been quite well studied (see [108] for a review on the subject), few studies have evaluated the effect of the longer isoforms. The effect of mKp52 was tested by icv injection in the mouse, showing that at femtomolar concentration it was able to increase the plasma concentration of LH and FSH [52]. In the cow, intravenous administration of cattle Kp53 triggered an increase of LH, but not FSH, plasma concentration and ovulation in 1 out of 4 cows [109]. In females of several species (rodents, ewe, goat, cow, pig, horse, and dog) a single injection of Kp10 triggers a rapid, albeit short-lasting, increase of LH and/or FSH [36, 37, 40, 45, 52, 54, 97, 110-113]. In the ewe during the non-breeding season a perfusion of Kp10 lasting 24 hours or longer induced the ovulation in about 75% of the treated animals [36, 114]. In prepubertal ewes, hourly administration of Kp10 for 24 hours induced an increase of LH and ovulation [115]. These results corroborated the idea that the Kp system would be an interesting target to modulate the female reproductive cycle. A major drawback in using Kp10 is its short half-life due to rapid degradation and excretion. This is a relevant obstacle when prolonged activation of the system is required, as in the case of ovulation induction or for the treatment of chronic reproductive pathologies in humans (e.g. hypothalamic amenorrhea).

A possible way to overcome this problem is the use of the longer isoforms eliciting a protracted activation of the system. In the male rat rKp52 produced an increase of LH of larger amplitude and longer duration never matched, even at high doses, by rKp10 or hKp10 [116]. Consistently, experiments performed in the male mouse indicated an increase of LH of longer duration and larger amplitude after hKp54 compared to hKp10 injection [117]. At odds with these results, however, a study on human males reported no significant difference in the effect of hKp10 and hKp54 [118]. The hKp54 isoform was also used in women but a prolonged action was only obtained after perfusion for

several hours [119]. Interestingly, in women administration of hKp54 increased plasma LH and FSH level regardless to the phase of the ovulatory cycle. Conversely, hKp10 is effective on the pre-ovulatory phase but not in the follicular phase [120]. In ruminants Kp10 injection during the luteal phase triggered an increase of LH [37, 121, 122] even though, at least in the ewe, it was lower than that induced during the mid and late follicular phase [122, 123].

Regardless to the different effects observed between sexes, isoforms, or different phases of the ovulatory cycle the overall duration of the stimulation after a single injection remains relatively short. To tackle this problem synthetic analogs based on Kp10 structure were designed to reduce degradation and increase their circulating half-life. Their potential use to manage livestock reproduction has been recently reviewed [7, 124]. The effects of these analogs (TAK683 and C6) have been extensively characterized in small ruminants. TAK683 was tested under different conditions in the goat. It consistently increased LH plasma level, with an efficacy depending on the phase of the ovulatory cycle [125-128], and induced ovulations. However, no data are available on the fertility of these ovulations [126]. The other analog, C6, induced fertile ovulations in small ruminants (sheep and goats) when injected as a substitute of PMSG (pregnant mare serum gonadotropin) after a progestogen priming [129, 130]. The treatment was efficacious both in the breeding as well as in the non-breeding season. These results are proof of the concept that the Kp system is a suitable target for ovulation synchronization and induction in small ruminants.

C6 was recently evaluated in steroid-primed pre-pubertal gilts where initial results showed that it evokes an LH surge and ovulation [131]. While non data are available on the effect of Kp analogs in the cow, data obtained with Kp10 in pre-pubertal and adult cows demonstrate an increase of plasma LH levels [112, 121, 132-135]. Furthermore, in Hereford cross-bred heifers under low plasma progesterone concentrations repeated administration of hKp10 induced ovulation [39]. Interestingly, a difference in the capacity of cattle Kp10 to trigger LH increase was observed in different cattle species with Holstein cows (*Bos taurus*) being more responsive than Gyr cows (*Bos indicus*) [112]. The potential impact of this difference on the capacity to trigger ovulation remains to be explored.

There is only a handful of studies on the effect of Kp and its analogs on male domestic animals. In adult and pre-pubertal male goats an intravenous injection of hKp10 stimulated the release of LH and testosterone [38]. Similar results were also obtained in adult rams by intramuscular injection of the Kp analog C6 [124]. In pre-pubertal Japanese bull calves a bolus injection of hKp10 increased both LH and FSH with a temporal profile similar to that observed in other species. Interestingly the effect was more pronounced in males compared to females of the same age [133]. This result is reminiscent of data obtained with the C6 in the mouse showing a longer lasting effect on LH plasma concentration in males compared to females [129].

hKp10 and C6 were recently tested in pre-pubertal Holstein bull calves using a treatment consisting of a daily intramuscular injection for 4 days. hKp10 had no effect on LH and FSH concentration on both the first and last day of treatment [136]. This is not completely surprising because of the low dose used (20 nmol/calf) and the route of administration. Experiments comparing intravenous and intramuscular injection of hKp10 in pre-pubertal bull calves applying a higher dose (about 550 nmol/calf) showed a significantly reduced effect of intramuscular compared to intravenous injection [133]. On the other hand, the first injection of C6 in bull calves increased LH plasma concentration for several hours but the increase after the fourth injection was limited if any. Interestingly, C6 had no immediate effect on circulating FSH, but at the fourth injection FSH level were lower compared to control [136].

Further investigations will be required to evaluate if repeated administration of Kp or its analogs may be a valuable approach to stimulate male sexual behaviors during the non-breeding season in small ruminants and to advance the onset of puberty in bulls.

6. Conclusions

In mammals an overall similar scenario has emerged showing that the Kp system plays a key role in stimulating the hypothalamo-pituitary-gonad axis. Yet significant species-specific differences exist and their potential implications in shaping the peculiar reproductive features of various domestic species is still unclear. To fully appreciate the many facets of these species-specific differences and their physiological reverberations in domestic animal reproduction, several aspects need to be further investigated. In particular differences in receptor and peptide localization, the physiological relevance of the different Kp isoforms, the creation of new tools for receptor localization, the implication of the Kp system in mediating sexual behavior, etc. are all exciting under-investigated areas of research.

Outside reproduction, the involvement of the Kp system in other physiological functions ranging from control of metabolism to motivation to stress responses has been proposed. These functions are indirectly involved in reproduction and may complement the more direct effect of the Kp system on the reproductive axis. However, they have been only partially characterized in primates and rodents and remain almost unexplored in domestic animals. To conclude we could say that while much has been learned, more remains to be discovered on the physiological ramifications of the Kp system in reproduction control.

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Figure Caption

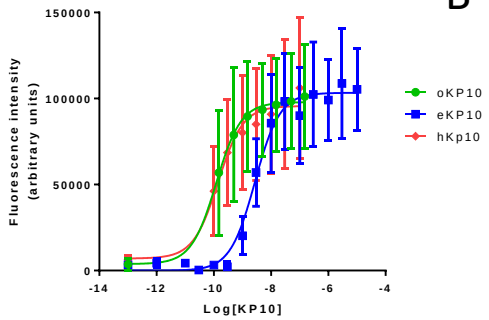
Figure 1. (A) Kp10 sequences of representative species. In red are indicated the amino acid variants in the sequence. (B) Concentration activity response of human, ovine, and equine Kp10 on a calcium mobilization assay on HEK293 transfected with the human *KISS1R*. Human and ovine Kp10 have very similar potency and efficacy, whereas equine Kp10 is about 10 times less potent. (C & D) Effect of the injection of ovine or equine Kp10 (15 nmol/animal) in the ewe. Blood samples (2 mL) were collected from the jugular vein every 15 minutes from 30 minutes before the injection of the drug to 45 minutes after, and then every 30 minutes until experiment end. LH was measured with an RIA methods as previously described [129]. Both molecules increase the LH plasma concentration. However, the maximal amplitude (C) and the total amount (D) of LH released are significantly less after the injection of the equine Kp10. The black arrow in C indicates the time of molecule injection.

Figure 1

A

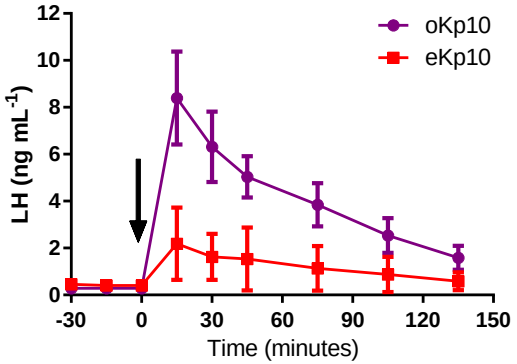
Homo sapiens	YNWNSFGLRF
Pan troglodytes	YNWNSFGLRF
Macaca mulatta	YNWNSFGLRF
Capra hircus	YNWNSFGLRY
Ovis aries	YNWNSFGLRY
Bos taurus	YNWNSFGLRY
Sus scrofa	YNWNSFGLRY
Equus caballus	YRWNSFGLRY
Mus musculus	YNWNSFGLRY
Felis catus	YNWNSFGLRY
Canis lupus familiaris	YNWNVFGLRY

B



	oKP10	hKp10	eKP10
EC50	1.209e-010	1.406e-010	2.746e-009

C



D