The emerging therapeutic potential of kisspeptin and neurokinin B

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Abbreviations

KP, Kisspeptin; NKB, neurokinin B; GnRH, Gonadotropin-releasing hormone; CHH, congenital hypogonadotropic hypogonadism; PCOS, polycystic ovary syndrome; *TAC3R*, gene encoding NKB3 receptor; FSH, follicle stimulating hormone; LH, luteinizing hormone; POA, pre-optic area; AVPV, anteroventral periventricular area; NPFF, neuropeptide FF; GPCR, G-protein-coupled receptor; *KISS1R*, gene encoding for kisspeptin receptor; PLC, phospholipase C; IP3, inositol triphosphate; DAG, diacylglycerol; PKC, protein kinase C; ERK, extracellular signal-related kinase; GPER, G protein estrogen receptor; GRK, GPCR serine/threonine kinases; RP3V, rostral periventricular area of the third ventricle; NK3R, neurokinin 3 receptor; HA, hypothalamic amenorrhea; PCOM, polycystic ovarian morphology on ultrasound; HPG, hypothalamic-pituitary-gonadal; E2, estradiol; PRL, prolactin; KNDy, kisspeptin-neurokinin B-dynorphin; CDGP, constitutional delay of growth and puberty; CPP, central precocious puberty; IUGR, intra-uterine growth restriction; MAFLD / NASH, metabolic fatty liver disease / non-alcoholic steatohepatitis; IVF, *in-vitro* fertilization; OHSS, ovarian hyperstimulation syndrome.

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ABSTRACT

Kisspeptin (KP) and neurokinin B (NKB) are neuropeptides that govern the reproductive endocrine axis through regulating hypothalamic gonadotropin-releasing hormone (GnRH) neuronal activity and pulsatile GnRH secretion. Their critical role in reproductive health was first identified after inactivating variants in genes encoding for KP or NKB signaling were shown to result in congenital hypogonadotropic hypogonadism (CHH) and a failure of pubertal development. Over the past two decades since their discovery, a wealth of evidence from both basic and translational research has laid the foundation for potential therapeutic applications. Beyond KP's function in the hypothalamus, it is also expressed in the placenta, liver, pancreas, adipose tissue, bone, and limbic regions, giving rise to several avenues of research for use in the diagnosis and treatment of pregnancy, metabolic, liver, bone, and behavioral disorders.

The role played by NKB in stimulating the hypothalamic thermoregulatory center to mediate menopausal hot flashes has led to the development of medications that antagonize its action as a novel non-steroidal therapeutic agent for this indication. Furthermore, the ability of NKB antagonism to partially suppress (but not abolish) the reproductive endocrine axis has supported its potential use for the treatment of various reproductive disorders including polycystic ovary syndrome (PCOS), uterine fibroids, and endometriosis. This review will provide a comprehensive up-to-date overview of the preclinical and clinical data that have paved the way for the development of diagnostic and therapeutic applications of KP and NKB.

I. INTRODUCTION

Kisspeptin (KP) and neurokinin B (NKB) are hypothalamic neuropeptides that play a pivotal role in the regulation of reproductive physiology. In 2003, inactivating variants in the gene encoding for the kisspeptin receptor (*KISS1R*) was shown to result in congenital hypogonadotropic hypogonadism (CHH) and a failure of pubertal development [1,2]. Following this, inactivating variants of the *KISS1* gene were also found to result in normosomic CHH [3]. Conversely, in 2008, activating variants in genes encoding for *KISS1R* resulted in premature activation of the hypothalamic-pituitary-gonadal (HPG) axis and central precocious puberty [4]. Thus, KP was shown to play a key role in regulating reproductive hormonal secretion and puberty, and it is now established that KP acts to stimulate gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus and the downstream reproductive axis [5–7] **(Figure 1).**

NKB was also discovered through the study of patients with CHH who were found to have inactivating variants affecting NKB signaling [8]. KP co-localizes with NKB and dynorphin (Dyn) in neurons known as 'KNDy' neurons in the arcuate nucleus of the hypothalamus (equivalent to

the infundibular nucleus in humans) [9,10]. These KNDy neurons are now recognized to function as the 'GnRH pulse generator', regulating the pulsatile secretion of GnRH [9,10]. NKB stimulates, whereas Dyn inhibits, the activity of these KNDy neurons, in an auto / paracrine manner to result in the pulsatile release of KP and, in turn, GnRH [11,12]. Pulsatile GnRH secretion subsequently induces the synthesis and secretion of pituitary gonadotropins (i.e. luteinizing hormone; LH, and follicle stimulating hormone; FSH) [13,14], which in turn stimulate sex-steroid production (estrogen and testosterone), and gametogenesis within the gonads (oocytes in ovaries and sperm in testes) [15] **(Figure 1).**

KP neurons integrate sex-steroid and metabolic signals from the periphery, either directly or via inter-neurons, to impact on GnRH secretion and the HPG axis. Several functional reproductive disorders are due to a disturbance in hypothalamic KP neuronal activity, which has sparked interest in the clinical application of KP for both the treatment and diagnosis of pubertal and reproductive disorders. Furthermore, KP is expressed in multiple organs beyond the hypothalamus including the placenta, liver, pancreas, adipose tissue, bone, and limbic regions, which predicate its use in the diagnosis and treatment of conditions related to pregnancy, metabolism, liver, bone, and behavior [16,17] **(Figure 2).** Discovery of the critical role of NKB in stimulating the hypothalamic thermoregulatory center has resulted in the use of compounds that block NKB action as treatment for menopausal hot flashes [18–20]. As these antagonists of NKB action partially suppress (but not abolish) reproductive hormone secretion, they have also emerged to have utility in the treatment of uterine disorders such as endometriosis and uterine fibroids [21].

In this review, we provide a comprehensive up-to-date overview of the relevant preclinical and clinical data that have paved the way for the development of novel diagnostic and therapeutic applications of KP and NKB.

II. DISCOVERY OF KISSPEPTIN, NEUROKININ B AND THEIR RECEPTORS

IIA) Kisspeptin and its gene

Kisspeptin (KP) was first discovered in 1996 as a tumor-suppressor and initially termed 'metastin' due to its anti-metastatic action in malignant melanoma cell lines [22]. It later acquired the name 'kisspeptin' in homage to its discovery in Hershey (Pennsylvania; USA), which is the hometown of the famous chocolate 'Hershey's kisses' [22]. The gene for KP in humans is called *'KISS1'* with the suppressor sequence denoted by 'SS'. Whilst *KISS1* is used to indicate the gene in humans, *Kiss1* is used for non-human KP genes [23]. In 2003, kisspeptin's obligatory role in regulating hypothalamic GnRH neuronal function was first described in two landmark reports by de Roux *et al.* and Seminara *et al.* [1,2].

In humans, KP is predominantly expressed in two distinct hypothalamic nuclei: the infundibular nucleus [24,25] (analogous to the arcuate nucleus in rodents [26]) and the rostral pre-optic area [24,25] (analogous to the pre-optic area, POA, including the anteroventral periventricular area, AVPV, and periventricular nucleus, PeVN in rodents [26]). KP is also expressed within the limbic system (in the amygdala, caudate nucleus, cingulate gyrus, globus pallidus, hippocampus, medial and superior frontal gyrus, nucleus accumbens, parahippocampal gyrus, putamen, striatum, substantia nigra, and thalamus) [16,17] and has been recognized to play a role in mood and sexual behaviors. Beyond the brain, *KISS1* mRNA is also highly expressed in the placenta (particularly by syncytiotrophoblasts [27,28]), gonads [16,29], adipose tissue [16], pancreas [16,29], liver [29], small intestine [29] and bone (particularly osteoblasts) [30] **(Figure 2).**

The *KISS1* gene is mapped to the long arm of chromosome 1 (1q32-q41) and comprises four exons of which only two are translated [31]. The resultant 145 amino acid prepropeptide is then post-translationally cleaved into biologically active KP peptides of different amino acid lengths

indicated by their suffix: e.g. KP -54, -14, -13, and -10 [17,29,31]. All native KP peptides share a common C-terminal decapeptide sequence, equivalent to KP-10, which includes a terminal RF-amide sequence (Arg-Phe-NH2) [17]. This C-terminal amide sequence is important for the binding and activation of the KP receptor. In particular, amidation of the C-terminal is essential for receptor activation, with higher binding affinities observed with KP-10 (Ki = 0.042 nM) and KP-54 (Ki = 0.34 nM) than a C-terminally unamidated form (Ki = 640 nM) [29]. KP-10 has a shorter terminal half-life than KP-54 ($t_{1/2}$ 3 vs 28mins) [7,13,32]. Other RF-amide family members such as neuropeptide FF (NPFF), prolactin-releasing peptide, and neuropeptide Y do not activate the KP receptor [33].

IIB) Kisspeptin receptor

The KP receptor (encoded by *KISS1R*) was described in 1999 [23], and was previously known as hOT7T175 [29], AXOR12 [16], or GPR54 [22]. The KP receptor is a 398-amino-acid peptide encoded by a gene on chromosome 19 (19p13.3) with five coding exons interrupted by four introns [16]. The KP receptor is part of the rhodopsin-like family of G-protein-coupled receptors (GPCRs), which is the largest group of GPCRs, and binds its ligand in the binding site within the transmembrane domain [16]. KP has a single high-affinity binding site at the human KP receptor (dissociation constant, Kd, 1.9 ± 0.4 nM using 500 nM of 125I-KP10) [17] and induces a biphasic response in downstream signaling, with an acute response (lasting ~5 min) and a prolonged phase (lasting >30 minutes) [34]. Whilst *KISS1R* is expressed in similar areas of the body as *KISS1*, it is also expressed at low levels in tissues including the stomach, thymus, spleen, lung, gonads, heart, kidney, adrenal gland, bone, and fetal liver [16,29,35] **(Figure 2).**

During the basal state (in the absence of KP), the KP receptor couples to $G\alpha_{q/11}$ at the cell surface which triggers KP-independent signaling and downstream activation of phospholipase C (PLC),

the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) to inositol triphosphate (IP3) and diacylglycerol (DAG), and intracellular calcium mobilization [36]. In the presence of KP, the KP receptor displays increased $G\alpha_{q/11}$ signaling through recruitment of GPCR serine/threonine kinases (GRK2) and β-arrestin from the cytosol to the plasma membrane [36]. GRK2 phosphorylates the KP receptor (at the intracellular loop and carboxyl terminus) and subsequently facilitates the binding of β-arrestin whilst preventing further coupling to G proteins [37,38]. β-arrestin subsequently induces receptor desensitization by uncoupling the KP receptor from $G\alpha_{q/11}$ and simultaneously triggers receptor sequestration by trafficking the desensitized KP receptor to the cell surface clathrin-coated pit [36]. The sequestered KP receptor (linked to β-arrestin) undergoes β-arrestin-dependent signaling resulting in receptor internalization and the formation of clathrin-coated vesicles [36]. Following this, the KP receptor dissociates from β-arrestin and is either resensitized and recycled back to the cell surface (ready to signal) or targeted for degradation [36] **(Figure 3).** Prolonged KP receptor signaling is also dependent on the continuous influx of calcium into the cell as well as maintaining a dynamic pool of receptors at the cell surface including both recycled and nonrecycled receptors [36]. Whilst KP receptor mainly signals via $G\alpha_{q/11}$, it can also activate the extracellular signal-regulated kinase 1/2 (ERK1/2) β-arrestin dependent pathway that also contributes to GnRH secretion [39]. Additionally, the KP receptor can form homodimers, heterodimers, or even oligomers with modified actions [40]. For instance, the KP receptor heterodimerizes with the G protein estrogen receptor (GPER), which reduces its expression at the cell-surface and decreases KP receptor signaling [40].

The KP receptor is vulnerable to tachyphylaxis, whereby the receptor response is reduced following repeated doses or continuous high doses of KP administration [37]. For instance, in agonadal juvenile and adult male monkeys, a 98-hr intravenous (IV) infusion of KP10 induced a

maximal LH response at 3-hrs, however, a rapid decline then followed by 12-hrs [41,42]. Moreover, an additional bolus of GnRH but not KP-10 resulted in an LH rise, thus indicating that tachyphylaxis is occurring at the level of the KP receptor [41,42]. Likewise, in women with hypothalamic amenorrhea, twice-daily administration of KP-54 resulted in a reduced LH response within a few days [43]. Interestingly, KP's responsiveness was maintained with a twice-weekly dosing interval suggesting that chronic stimulation with KP is possible using an appropriate dosing protocol [43]. Furthermore, although tachyphylaxis occurs after persistent high-dose exogenous KP, this may not be the case with physiological endogenous KP. Indeed, optogenetic activation of KP neurons in the rostral periventricular area of the third ventricle (RP3V) of female mice can persistently stimulate GnRH neuronal firing [44].

IIC) Neurokinin B and its gene

Neurokinin B (NKB) was first discovered as a central regulator of reproduction in 2009, whereby loss-of-function variants in either NKB or its receptor (NK3R) were identified in four of nine multiplex families affected by hypogonadotropic hypogonadism using genome-wide single nucleotide polymorphism (SNP) analysis [8]. In humans, NKB is predominantly expressed in the infundibular nucleus, anterior hypothalamic area septal region, diagonal band of Broca, bed nucleus of the stria terminalis, amygdala, and neocortex [45]. The gene encoding NKB (*TAC3* in higher primates and *Tac2* in rodents) is located on chromosome 12 and is divided into seven exons, five of which are translated to form the preprotachykinin B peptide [46–48]. Following proteolytic cleavage, this precursor peptide leads to, first, proneurokinin B, and then NKB (initially contained in exon 5) [46]. NKB belongs to the tachykinin family of peptides which is characterized by a

common C-terminal amino-acid sequence (Phe-X-Gly-Leu-Met-NH2) and includes substance P, neurokinin A and NKB, as well as neuropeptide K, neuropeptide γ, and hemokinin-1 [46,48].

IID) Neurokinin B receptor

Three tachykinin receptors have been identified, NK1R, NK2R, and NK3R, with the latter having a longer amino acid sequence [46]. The genes encoding the three tachykinin receptors are all divided into five exons with identical distribution of intronic sequences [46]. NKB is an agonist for all three receptors, however, it exhibits strong preferential binding for NK3R (encoded by *TACR3*) [49,50]. Following NKB binding, NK3Rs are activated and result in increased intracellular Ca^{2+} (through inositol phospholipid hydrolysis) and increased intracellular cAMP levels (through adenylate cyclase activation), and are then internalized [51].

Like NKB, NK3R is also expressed within the central nervous system and spinal cord, although it has also been reported in the uterus, mesenteric vein, gut neurons, and placenta [45]. NK3Rs also display species-differences and exert differing actions. For instance, whilst NK3R antagonists have similar potency on NK3Rs in the gerbil, guinea pig, dog, and human, they have lower activity on NK3R in the rat and mouse [52].

III. HYPOTHALAMIC KISSPEPTIN-NEUROKININ B-DYNORPHIN (KNDY) NEURONS AND DISCRETE KISSPEPTIN NEURONAL POPULATIONS

Kisspeptin (KP) neuronal bodies are located in two discrete hypothalamic nuclei in rodents; the arcuate nucleus (ARC), and the rostral periventricular area of the third ventricle (RP3V) which includes the anteroventral periventricular (AVPV) and periventricular (PeN) nuclei [26]. The

analogous regions in humans are the infundibular nucleus and the rostral preoptic area (POA), respectively [24,25]. Both ARC and RP3V KP neuronal populations innervate gonadotropin-releasing hormone (GnRH) neurons and are responsible for regulating GnRH pulsatility and the mid-cycle luteinizing hormone (LH) surge, respectively [53–57] **(Figure 1)**. The number and distribution of KP neurons differs between sexes. For instance, whilst female mice require high hypothalamic *Kiss1* expression levels to preserve fertility, male mice only need 5% of *Kiss1* expression [58]. In rodents and sheep, the proportion of KP neurons in both the AVPV [59] and ARC [26,60] is greater in females than males. Consistent with this, the number of KP immune-positive cell bodies found in the infundibulum of human brain autopsies is sevenfold higher in women compared to men [24,25].

IIIA) Arcuate kisspeptin neurons

KP neurons in the ARC nucleus co-express NKB and dynorphin (Dyn) and are hence known as **K**isspeptin-**N**eurokinin-**Dy**norphin (**KNDy**) neurons [61]. KNDy neurons are regulated in an autocrine / paracrine manner, with NKB stimulating (via NKB receptor – mainly TAC3R) [61] and Dyn inhibiting (via kappa opioid receptor) [25] neuronal activity. This synchronized episodic action results in KP release which in turn activates distal dendrons of GnRH neurons and leads to the secretion of GnRH pulses [62]. Considering KP receptors are highly expressed within GnRH neurons and absent in KNDy neurons, KP's action predominantly occurs via GnRH neurons [62].

ARC-KP neurons are key regulators of GnRH pulsatile secretion and are referred to as the 'GnRH pulse generator' [9,60]. Indeed, optogenetic activation of the channel rhodopsin expressing ARC KP-neurons in *Kiss1-Cre* mice induced pulsatile LH secretion, whereas inhibition suppressed it [63,64]. Likewise, knock out of greater than 90% of ARC *Kiss1*-neurons resulted in marked suppression of LH pulses in ovariectomized female rats [65].

However, a recent report has challenged the KNDy hypothesis suggesting that synchronization within the ARC is dependent on a 'glutamate two-transition' mechanism in male mice [66]. In this model, the first transition is dependent on glutamate but gated by Dyn tone to initiate neuron synchronization, and the second transition is dependent on NKB which potentiates that synchronization [66].

ARC-KP neurons are tightly regulated by intricate feedback mechanisms in response to several modulators including sex-steroids such as estradiol (E2). In the presence of low circulating E2 levels, negative feedback effect is exerted on ARC-KP neurons. Indeed, a recent RNA sequencing study in mice identified 1583 estrogen responsive genes in the ARC with majority of the genes being suppressed in response to a low E2 environment [67]. Whilst negative feedback is present continuously in males, in females it occurs during most of the follicular and luteal phases of the menstrual cycle [68]. Negative feedback in response to E2 is mediated by the 'non-classical pathway', whereby the interaction between E2 and its receptor (ERα) results in the recruitment of estrogen response element (ERE) independent transcriptions factors [69,70]. E2-ERα signaling leads to *Kiss1* promoter histone deacetylation, which inhibits chromatin loop formation between the *Kiss1* promoter and the *Kiss1* gene enhancer, resulting in reduced ARC-specific *Kiss1* gene expression.

IIIB) Rostral periventricular area of the 3rd ventricle kisspeptin neurons

KP neurons in the RP3V, which includes the AVPV and PeN, innervate the soma and proximal dendrites of GnRH neurons to stimulate GnRH secretion [67]. This KP neuronal network is mainly regulated by positive feedback from higher levels of E2. In the presence of high E2, RP3V-KP neurons in rodents (rostral POA neurons in humans) continuously produce GnRH leading to an

LH surge [71,72] which occurs during the proestrus phase in rodents and during the late follicular phase (mid-cycle) in women [73]. Of note, 222 genes within RP3V-KP neurons are upregulated in response to high E2 levels demonstrating their importance to facilitating positive feedback [67]. The mechanism responsible for positive feedback predominantly involves E2-ERα signaling and recruitment of cofactors to ERE in the 'classical pathway' [69,70]. In contrast to the ARC, *Kiss1* promoters within the AVPV undergo histone acetylation and subsequent increased AVPV-specific *Kiss1* gene expression. The role of these neurons remains uncertain in male mammals who have lower KP expression than female mammals in RP3V-KP neurons [74].

IV. KISSPEPTIN AND NEUROKININ B IN HEALTHY MEN AND WOMEN

IVA) Kisspeptin in healthy men

In healthy adult men, acute administration of kisspeptin-54 (KP-54) induced dose-dependent rises in circulating luteinizing hormone (LH) and to a lesser degree, follicle stimulating hormone (FSH) [13] **(Table 1A).** In particular, KP-54 (intravenous; IV infusion 0.24 nmol/kg/hr over 90 minutes) increased mean LH levels 2.6-fold higher than placebo [13]. Similarly, an IV bolus of KP-10 (0.77 nmol/kg) potently evoked LH secretion from 4.1 to 12.4 ± IU/L and a continuous IV infusion (3.07 nmol/kg/hr) of KP-10 led to persistent LH secretion over 22.5hrs [75]. The shorter isoform, KP-10, has a briefer half-life and duration of gonadotropin release, with LH levels rising within 30-40 minutes after an IV bolus administration (0.3 to 1.0 nmol/kg) [76]. In a direct equimolar comparison between KP-54 and KP-10 (hypothalamic stimulation) against gonadotropin-releasing hormone (GnRH, pituitary stimulation), LH and FSH responses were greater following GnRH, then KP-54 and then KP-10 [32]. Although GnRH is more potent than KP, KP is hypothesized to induce the release of GnRH from a limited endogenous pool [77], which could be preferable when stimulating

reproductive hormone secretion in a clinical context where there is an unwanted risk of over-stimulation.

The pulsatile secretion of GnRH is critical for reproductive function. Indeed, KP-10 (IV infusion 3.07 nmol/kg/hr over 22.5 hrs) increased LH pulse frequency from 0.7 to 1.0 pulses per 1hr in men [75]. KP has also been shown to reset the 'GnRH pulse generator' in healthy men but not women [78]. KP-10 (IV bolus 0.24 nmol/kg) resulted in sustained GnRH neuronal activation lasting ~17 minutes and immediately induced an LH pulse (irrespective of the timing of the preceding endogenous pulse) and increased the LH pulse amplitude by 2.4-fold [78]. Furthermore, the following native pulse was delayed by an interval approximating the usual inter-pulse interval, indicating that KP-10 had reset the schedule of pulses [78].

IVB) Kisspeptin in healthy women

In healthy premenopausal women, acute administration of KP-54 (subcutaneous; SC bolus 0.4 nmol/kg) increased circulating LH during all phases of the menstrual cycle, with the highest LH levels being observed during the pre-ovulatory (20.64 ±2.91 IU/L) compared to the follicular (0.12 ± 0.17 IU/L) or luteal (2.17 ± 0.79 IU/L) phases of the cycle [14] **(Table 1B).** Similarly, whilst KP-10 (IV bolus 10 nmol/kg) increased gonadotropins during the preovulatory phase (mean area under the curve; AUC: LH = 30.3 ± 7.7 IU/L, FSH = 6.9 ± 0.9 IU/L), it was least sensitive during the follicular phase [76]. However, KP-54 (SC bolus 0.30-0.60nmol/kg) can still increase LH pulsatility (by 2.33 pulses per 4hrs) during the follicular phase in premenopausal women [79].

The effects of chronic KP administration have also been evaluated in healthy women. For instance, twice daily KP54 (SC bolus 6.4 nmol/kg) injections for 1-week increased maximal change in LH from baseline on day 7 (8.6 ± 3.4 IU/L), day 11 (8.3 ± 2.4 IU/L) and day 14 (12.7 ± 8.1 IU/L) of

the menstrual cycle [80]. Furthermore, an infusion of KP54 (SC 0.3-1.0 nmol/kg/hr over 8 hrs) induced a mean LH rise (>8 IU/L) during the early follicular phase [81]. KP receptor analogs have been shown to stimulate longer LH responses and are similarly cost-effective to manufacture [82]. For example, MVT-602 (formerly known as TAK-448) generated similar LH amplitude responses as KP-54 during the follicular phase, but the peak LH level was later at ~21-hrs compared to KP-54 (~5-hrs) resulting in a four-fold increase in AUC of LH secretion [82].

IVC) Neurokinin B in healthy men and women

Although neurokinin B (NKB) administration increased LH concentration in male juvenile monkeys [83], no significant changes in circulating LH, FSH, or testosterone concentrations were observed in healthy men during either a 90-minute (doses 0.04 to 5.12 nmol/kg/hr), a 4-hr (doses 2.56 and 5.12 nmol/kg/hr), or 8-hr (dose 5.12 nmol/kg/hr) IV infusion of NKB [84] **(Table 2A).** Similarly, no significant differences in either LH pulsatility or mean LH, FSH and extradiol (E2) levels have been observed in healthy premenopausal women [84]. Interestingly, NKB induced vasoactive effects in healthy men (IV infusion 10.24 nmol/kg/hr) [84] and in 80% of premenopausal healthy women (IV infusion 5.12 nmol/kg/hr) [85] **(Table 2B).** These data highlighted the potential of NKB-signaling blockade for the management of vasomotor symptoms in postmenopausal women and / or following cancer therapy (e.g., breast or prostate cancer). Thereafter, several safe and efficacious NKB receptor (mainly NK3R) antagonists have been investigated for this indication, which are discussed in later sections of this review. Recent *in-vitro* data has also suggested that the NKB receptor, NK1R, may have a role in promoting breast [84] and non-small cell lung cancer [86], hence it is possible that antagonists against NK1R could have a therapeutic role in addition to the relief of vasomotor symptoms.

V. CLINICAL APPLICATIONS OF KISSPEPTIN

VA) IN DISORDERS OF PUBERTY

Puberty is characterized by the acquisition of secondary sexual characteristics and reproductive capacity, and the development of important psychosocial behaviors [87]. Pubertal onset is dependent on the reawakening of the pulsatile secretion of gonadotropin-releasing hormone (GnRH) and activation of the downstream reproductive endocrine axis [87]. During fetal life and infancy, there are two periods of transient activations of the hypothalamic-pituitary-gonadal (HPG) axis termed 'mini puberty', followed by a period of relative quiescence until the onset of puberty [87] **(Figure 4).**

VA1) Diagnosing Delayed Puberty

Delayed puberty is defined as the absence of testicular enlargement (testicular volume < 4ml) in boys, or breast development in girls, at an age that is > 2 standard deviations (SD) later than the population mean, typically 14 years in boys and 13 years in girls [88]. The commonest cause of delayed puberty is constitutional delay of growth and puberty (CDGP), affecting 60-80% of boys and 30-55% of girls [89]. In CDGP, although puberty is delayed, it is initiated spontaneously without treatment [88,103]. Another important but less common cause of delayed puberty is congenital hypogonadotropic hypogonadism (CHH). CHH affects 10-20% of adolescents with delayed puberty and is characterized by failure of GnRH action resulting in absent or incomplete puberty [89]. It is caused by genetic variants that either impair developmental GnRH neuronal migration or alter GnRH secretion and / or action [89]. The cause of CDGP remains unknown; however, 50-75% of patients have a family history of delayed puberty and there is some overlap with genes causing

CHH, as well as with nutritional status [89]. Accurately diagnosing these conditions is crucial, as although CDGP can be managed conservatively or symptomatically with sex-steroids, timely pubertal induction in CHH could safeguard future reproductive, sexual, bone, metabolic and psychological health [90]. Currently, differentiating CDGP and CHH is challenging due to their overlapping clinical presentations, biochemical profiles, and the lack of a 'gold standard' diagnostic test [91].

Animal data

Kisspeptin (KP) is a central regulator of the HPG axis and has a critical role in pubertal initiation and maintenance. Numerous animal studies have investigated KP signaling in the context of delayed or absent puberty. Indeed, *Kiss1r*-deficient male mice have small testes and female mice have delayed vaginal opening and absent follicular maturation [2]. Likewise, targeted disruption of the KP receptor in male and female mice resulted in reduced internal and external reproductive organ size (e.g. testicular volume: 0.2±0.04ml in controls, 0.02±0.01ml in *Kiss1r* knockout mice), altered organ weight/body weight ratios and infertility [92]. Furthermore, specific knockout of *Kiss1r* only in GnRH neurons led to infertile mice with reduced serum LH and FSH levels [93]. External abnormalities, including microphallus and reduced anogenital distance in male mice, and acyclicity in female mice, were also observed [93]. Furthermore, intracerebral administration of a KP antagonist (p234) in female rats suppressed markers of puberty including vaginal opening and an increase in uterine weight [94].

Disruption of the *Kiss1* gene also results in pubertal failure [95], however, it appears that knockout of *Kiss1* results in a less severe phenotype (higher gonadal weight and larger vaginal opening) than

knockout of *Kiss1r* [96]. Interestingly, the degree of disruption of pubertal progression caused by aberrant KP signaling can vary. For instance, female *Kiss1* and *Kiss1r* knockout mice can still progress through estrus, suggesting there is some level of retained GnRH activity [97]. Likewise, another study showed that female mice with *Kiss1* ablation had normal timing of puberty and remained fertile [98]. Collectively, this data indicates pubertal maturation can occur despite impaired KP signaling, however, its development is not entirely normal.

Human data

KP's role in puberty was first identified in humans when loss of function variants in *KISS1R* resulted in failure of pubertal progression and CHH [1,2]. Following this, researchers identified that CHH patients with impaired *KISS1R* signaling were homozygous for a single variant causing substitution of leucine with proline [99]. These patients were still able to respond to exogenous GnRH, suggesting that pituitary function was still intact [99]. Likewise, functional *KISS1* is also required for normal pubertal development. In a large consanguineous family, members with homozygous (but not heterozygous *KISS1* variants) had CHH, thus indicating that one copy of *KISS1* is sufficient for functioning of the HPG axis [3].

KP's ability to directly stimulate hypothalamic GnRH release could enable its use as a novel diagnostic tool in identifying patients with CHH. As CHH is predominantly caused by hypothalamic defects, it is expected that the majority of patients with CHH will fail to respond to KP but not to GnRH [100]. However, many also fail to respond to an initial dose of exogenous GnRH as they typically have "sleepy" pituitary glands, which have not been primed resulting in false negative interpretations [100]. To avoid this, researchers used intermittent exogenous GnRH exposure

to "prime" pituitary gonadotrophs [100]. The first study to evaluate KP as a diagnostic test in adult CHH patients was conducted in 2014 [100] **(Table 1C).** Here, whilst an IV bolus of GnRH induced mild and robust LH responses during the 'pre-priming' and 'post-priming' stages, respectively; no response was observed with KP-10 (IV bolus 0.24 nmol/kg) [100]. Some CHH patients can undergo spontaneous activation of their HPG axis and restoration of reproductive function, termed 'reversal' [101]. KP-10 (IV bolus 0.24-2.4 nmol/kg) induced LH pulses (within 30 minutes) in patients with sustained reversal but not in those who suffered a relapse of CHH, thus confirming KP's ability to assess current GnRH neuronal functional capacity [101].

Another study using KP-54 (IV bolus 6.4 nmol/kg) found that patients with CHH had lower LH responses after KP-54 (0.4 IU/L) than in healthy controls (12.5 IU/L) [102]. KP-54 had higher discriminatory power than GnRH to accurately differentiate CHH from healthy men with an area under receiver operating characteristic curve (AUCROC) of 1.0 (95% CI 1.0–1.0) versus 0.88 (95% CI 0.76–0.99), respectively [102]. Additionally, CHH patients with anosmia or those with an identified pathogenic variant in causative genes e.g. *ANOS1*, *FGFR1*, *PROKR2*, or *SEMA3A*, had even lower LH rises following KP-54 than other men with CHH [102].

In patients with delayed puberty, KP-10 has been shown to predict subsequent progression through puberty, which could be used to differentiate CHH from CDGP [103]. For instance, "KP responders (LH ≥ 0.8 mIU/mL)" proceeded through puberty spontaneously (i.e. CDGP) whereas "KP non-responders (LH ≤ 0.4 mIU/mL)" did not (i.e. CHH) [103]. This test had 100% sensitivity and specificity and predicted outcomes more accurately than previously described basal / stimulated hormonal markers and genetic testing [103]. These data demonstrate the potential of KP in the context of delayed puberty to differentiate CDGP and CHH.

VA2) Diagnosing Precocious Puberty

Precocious puberty is pubertal development occurring earlier than that which is expected for gender, ethnicity, and race, typically occurring at <9 years in boys and <8 years in girls [104]. Precocious puberty can be classified as either a GnRH-dependent or a GnRH-independent process. GnRH-dependent or central precocious puberty (CPP) results from the premature activation of the HPG axis, whereas GnRH-independent or peripheral precocious puberty results from the unregulated gonadal production of sex-steroids [104]. CPP affects around 1 in 5,000-10,000 Caucasian children and is ten-fold more prevalent in girls than boys [105]. As early exposure of high sex-steroid concentrations causes premature epiphyseal fusion and reduced final height, as well as psychosocial issues, early diagnosis and treatment of CPP is critical [105]. Differentiating CPP from premature thelarche (PT), a condition characterized by isolated breast development with no growth or bone problems, is challenging [105]. Although a GnRH stimulation test is often used as a biochemical parameter for diagnosis, it has low sensitivity, thus new markers are required [105].

Animal data

KP has been shown to precociously activate the HPG axis. Indeed, male, and female rats persistently express hypothalamic *Kiss1* and *Kiss1r* during postnatal life, with maximum levels expressed at puberty [106]. Furthermore, KP induced complete vaginal opening (in 74%) and increased uterine weight (by 3-fold), serum LH (by 10-fold) and serum E2 (by 2-fold) levels in immature female rats compared to controls [107]. Likewise, female monkeys with intact ovaries demonstrate increased *Kiss1* and *Kiss1r* (by 3-fold) mRNA levels in the arcuate (ARC) nucleus of

the hypothalamus during puberty [108]. Furthermore, administration of KP-10 to juvenile monkeys has been shown to elicit robust and precocious LH surges [108].

Human data

Activating variants of the KP gene and receptor have been identified in patients with CPP. For instance, an autosomal dominant mutation involving substitution of proline for arginine at codon 386 (Arg386Pro) of *KISS1R* was discovered in a girl with CPP [4]. *In-vitro* studies revealed that this KP receptor variant induced a prolonged response to KP through a reduced rate of degradation [4,109]. Furthermore, two *KISS1* missense mutations, p.P74S (heterozygous) and p.H90D (homozygous) have also been identified in CPP, with the p.P74S variant displaying higher KP resistance to degradation [110].

Considering gain of function variants in KP gene or receptor result in CPP, KP has been investigated as a potential marker of early pubertal activation and CPP. Indeed, serum KP levels have been shown to be higher in CPP (14.62 ± 10.2 pmol/L) than in age-matched prepubertal controls (8.35 ± 2.98 pmol/L) [111], however, there was some overlap between the groups **(Table 1D).** Similarly, a systematic review and meta-analysis (11 studies, CPP n=316, controls n=251) demonstrated higher KP levels in CPP versus controls with a bias-corrected standardized mean difference (SMD) of 1.53 (95% CI 0.56-2.51) [112]. Subgroup analyses revealed a positive correlation between serum KP and age in the CPP cohort, and an association between serum KP levels and precocious thelarche [112]. A more recent study demonstrated higher KP levels in age and BMI-matched CPP (0.43 ± 0.16 ng/ml) versus PT (0.26 ± 0.10 ng/ml) and controls (0.18 ± 0.07 ng/ml) [113]. Whilst a KP cutoff of ≥0.41 ng/mL was indicative of CPP, a KP level <0.21 ng/mL excluded CPP (AUC = 0.830) [113]. KP also positively correlated with increasing bone age, a

cardinal feature of CPP [113]. Taken together, circulating KP levels may provide a useful adjunct in the diagnosis of CPP especially when the levels are at one end of the spectrum.

VB) IN ADULT DISORDERS OF REPRODUCTIVE FUNCTION

Kisspeptin's (KP) ability to directly stimulate hypothalamic gonadotropin-releasing hormone (GnRH) release and regulate reproductive hormone secretion can be utilized to assess hypothalamic function and treat common ovulatory disorders **(Figure 5).**

VB1a) Diagnosing Hypothalamic Amenorrhea

Hypothalamic amenorrhea (HA) affects 1-4% of women and is characterized by an acquired functional deficiency of hypothalamic function and reduction in GnRH secretion [114]. HA is diagnosed by the presence of menstrual disturbance (menstrual cycle length persistently >45 days or amenorrhea >3 months), low body-weight, excessive exercise, psychological stress and the hypogonadotropic hypo-estrogenism (typically <184 pmol/L) [115]. Diagnosing HA can be challenging as it requires the exclusion of other causes of amenorrhea before a diagnosis can be made and there can be overlap in features with other common causes of menstrual disturbance [116].

Animal data

Reproductive suppression through food deprivation and/ or stress is mediated by hypothalamic KP. For instance, in calorie-restricted sheep models, *Kiss1* mRNA expression is reduced in the arcuate nucleus (ARC) and the preoptic area (POA) of the hypothalamus [117–119]. In male castrated sheep with reduced food-intake, mean serum luteinizing hormone (LH) and hypothalamic ARC

Kiss1 mRNA expression were decreased [120]. Cows with non-ovulatory cycles have a 2-fold reduction in ARC *Kiss1* expression compared to controls [121]. Stress induced by lipopolysaccharide (LPS) administration also decreased hypothalamic *Kiss1* mRNA expression and serum LH levels in female rats [122]. Similarly, central and peripheral activation of the hypothalamic-pituitary-adrenal (HPA) axis by corticotropin and corticosterone respectively, reduced ARC KP expression in female mice [122].

Human data

Circulating KP levels are reduced by 13% in HA and are particularly low in women with reduced LH (KP = 1.7 ± 0.1 ng/ml) compared to those with normal LH (KP = 2.6 ± 0.3 ng/ml) levels [123] **(Table 1E).** Women with HA with lower KP levels had higher levels of stress hormones such as corticotropin releasing hormone (CRH) compared to controls [124]. Furthermore, KP levels have been shown to negatively correlate with physical activity [125]. Whilst circulating KP levels could be used to diagnose HA, it is important to note that they are challenging to detect accurately at low levels using current methods of measurements, thereby limiting their potential clinical use.

VB1b) Treating Hypothalamic Amenorrhea

HA is a chronic endocrine disorder associated with serious negative health consequences including infertility, osteoporosis, and cardiovascular disease [123]. Although pulsatile GnRH pump therapy is recommended as the first-line treatment, it has limited availability [114]. Estrogen supplementation offers symptom control and only some protection against osteoporosis [114]. Furthermore, some women with HA seeking fertility can respond poorly to clomiphene citrate during ovulation induction protocols as estradiol (E2) is already low [114]. Considering kisspeptin's (KP) direct potent

stimulatory effects on the hypothalamic-pituitary-gonadal (HPG) axis, it has potential for use to restore reproductive function in women with HA.

Animal data

The potential of KP for reinstating reproductive function has been explored in calorie-restricted animal models. For instance, food-deprived prepubertal rats with low hypothalamic *Kiss1* and high *Kiss1R* expression, have enhanced LH responses (~62.5-fold increase) following exogenous KP [126]. Although KP did not alter food intake, chronic KP administration induced vaginal opening (in ~60%) and elicited rises in FSH and E2 [126].

Human data

Women with HA had an earlier rise in LH (6.2hrs) than healthy women (15hrs), and also had increased follicle stimulating hormone (FSH) and E2 levels following administration of the kisspeptin (KP) receptor agonist (MVT-602) [82] **(Table 1E).** In women with HA, KP54 (SC bolus 6.4 nmol/kg twice daily) induced robust LH rises on the first day of treatment (max LH increase = 24.0 ±3.5 IU/L above baseline at 4hrs post injection) [43]. However, LH responses were markedly reduced by 2 weeks of treatment (max LH increase = 2.5±2.2 IU/L above baseline), consistent with tachyphylaxis at the KP receptor [43]. To prevent receptor desensitization and maintain stimulation, the dosing interval can be extended to twice-weekly [43]. This dosing protocol maintained stimulation with maximal LH increases of: 21.5 ±10.7 IU/l (at baseline), 10.0 ± 4.3 IU/l (at 2 weeks), 9.0±4.1 IU/l (at 4 weeks), 8.9 ± 3.5 IU/l (at 6 weeks), and 7.9 ± 4.5 IU/l (at 8 weeks) [43]. Furthermore, unlike GnRH-based therapies, KP can induce pulsatile secretion of GnRH / LH even when administered in a non-pulsatile manner. For example, women with HA receiving

an intravenous infusion of KP-54 had a 3-fold rise in the number of LH pulses and a 6-fold increase in mean peak LH pulse secretory mass [114]. Thus, chronic KP administration could offer a novel approach to restoring physiological LH pulsatility in women with HA.

VB2a) Diagnosing Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is a multifactorial condition influenced by genetic and environmental factors, and results in heterogenous clinical phenotypes including neuroendocrine and metabolic abnormalities [127]. PCOS affects 2-13%[112] of women of reproductive age and is currently diagnosed by the presence of two of the following three features: (i) menstrual irregularity, (ii) hyperandrogenism, or (iii) polycystic ovarian morphology on ultrasound (PCOM) [128]. A key pathological feature responsible for PCOS is androgen excess [129]. Androgens induce PCOS features through a central mechanism via the HPG axis and increase GnRH pulsatility [130]. Considering GnRH neurons lack androgen receptors, other intermediate pathways providing afferent inputs to GnRH neurons, such as KP neurons, are crucial to mediating the altered sex-steroid feedback found in PCOS [131]. Indeed, testosterone exposure upregulates the androgen receptor but downregulates progesterone receptor expression in ARC KP neurons, thus indicating that androgen exposure in PCOS disrupts progesterone-induced negative feedback through a direct action on ARC KP neurons [132]. The consequent unrestrained LH secretion stimulates ovarian theca cell androgen production, which in turn reduces sex-steroid mediated negative feedback, thus establishing a vicious cycle [130].

Animal data

Hypothalamic *Kiss1* expression differs in various PCOS animal models. For instance, in testosterone and dihydrotestosterone (DHT) induced PCOS rat models, *Kiss1* gene expression is reduced [133]. Conversely, ARC KP expression is increased in pre-natal androgen (PNA) models featuring irregular cycles, increased LH and testosterone levels [133]. Likewise, prenatal exposure of androgens to sheep and other non-human primate models recapitulates many of the cardinal features of PCOS [129]. In rodent models of PCOS induced by letrozole, *Kiss1* expression is upregulated in the ARC compared to the AVPV thus suggesting ARC KP neurons mediate the impaired sex-steroid feedback in PCOS [134]. Overall, it appears that *Kiss1* expression is increased in PCOS phenotypes with higher LH levels and normal body weight.

Human data

A recent meta-analysis (of 23 studies) reported that circulating KP levels were raised in PCOS (standard mean difference = 0.47 and [95% CI] = [0.17 to 0.77]) and had a diagnostic odds ratio of 13.71 and an AUC of 0.835 to differentiate PCOS from controls in BMI-matched women[135] **(Table 1F).** Additionally, two further studies have also observed higher KP levels in PCOS than controls: 1.79 ng/ml vs 1.05 ng/ml [136] and 0.131 ng/ml vs 0.076 ng/ml [137]. As KP is a potent stimulator of GnRH and LH release, one would expect a positive correlation between KP and the high serum LH levels observed in PCOS. However, whilst oligomenorrheic PCOS women have loss of temporal coupling of KP and LH pulses, coupling is preserved in PCOS women with eumenorrhea [138].

VB2a) Treating Polycystic Ovary Syndrome

PCOS treatments are currently directed towards a specific symptom of PCOS e.g. ovulation induction for infertility, rather than aiming to treat the underlying pathophysiological process. Approximately 40% of women with PCOS have increased LH pulse frequency (22–24 vs. 16 pulses per 24hrs), with PCOS often being described as a state of relative FSH deficiency [139]. Considering kisspeptin (KP) administration induces a greater LH than FSH response, KP could exacerbate the relative FSH deficiency potentially limiting its use as an agent to restore folliculogenesis in PCOS [130]. Furthermore, KP can evoke differential gonadotropin and ovulation responses in different PCOS phenotypes thus indicating the need for individualized management of women with PCOS.

Animal data

KP-54 (SC bolus 100µg/kg) increased both LH and FSH levels in prenatal, neonatal, and post-weaning androgenized PCOS-like rat models [140]. In anovulatory rats with neonatal androgen exposure, KP induced marked LH and FSH responses, increased follicle growth and rescued ovulation (increased number of corpora lutea) [140]. However, in post-weaning androgenized rats with persistently raised androgen levels, KP had blunted LH responses and failed to induce ovulation[140]. These data indicate that KP responses are more robust in PCOS phenotypes linked to early androgenization, without marked elevation of circulating androgens.

Human data

Like animal data, women with PCOS also have increased LH and FSH responses following administration of a kisspeptin (KP) receptor agonist, MVT-602 (SC bolus 0.01 - 0.03 nmol/kg) [82] **(Table 1F).** However, in women with PCOS receiving KP-54 (SC bolus 3.2 and 12.8 nmol/kg

twice daily for 21 days), LH (from 10.8 to 13.4 IU/L) but not FSH (from 3.9 to 3.5 IU/L) levels were raised [140]. Similarly, KP-10 (IV infusion 4 μg/kg/h for 7 hrs) increased LH (from 5.2 to 7.8 IU/L) and E2 concentrations but did not increase FSH secretion in women with PCOS [141]. However, pretreatment with a neurokinin B receptor 3 (NK3R) antagonist increased the FSH-rise following KP [141]. Thus, the relative FSH deficiency observed in women with PCOS could be exacerbated by KP and limit its use as a sole agent to restore healthy folliculogenesis. In two women with PCOS and amenorrhea but no biochemical hyperandrogenism, KP (SC bolus 9.6 nmol/kg twice daily over 3 weeks) stimulated follicle growth and ovulation, and these effects continued even after KP administration ceased [140]. Consistent with animal data, KP is more effective in PCOS phenotypes linked to anovulation without marked elevation of circulating androgen levels.

VB3) Treating Hyperprolactinemia

Hyperprolactinemia has an annual incidence of 23.9 per 100,000 person years and is a major cause of anovulatory infertility in women of reproductive age [142]. Elevated prolactin (PRL) levels suppress GnRH release and result in reduced LH pulse frequency and amplitude and hypogonadotropic hypogonadism [143]. Dopamine agonists (e.g. cabergoline, bromocriptine) are the first-line treatment for hyperprolactinemia as they effectively normalize PRL levels and restore gonadal function. However, up to 30% of patients have drug resistance and others cease therapy due to intolerable side-effects such as impulse-control disorders [144].

Animal data

The mechanism of PRL action on GnRH neurons has remained elusive. However, as most GnRH neurons do not express PRL receptors, PRL inhibitory action is thought to be mediated indirectly through PRL sensitive afferent pathways such as KP neurons [145]. Indeed, PRL induced anovulatory female mice have reduced *Kiss1* and *GnRH* expression levels [146]. KP neurons within the ARC of the hypothalamus regulate PRL-mediated LH suppression. For instance, lactating female rats with elevated PRL levels have a 58% reduction in ARC KP neuron immunoreactivity versus non-lactating rats [147]. Furthermore, PRL induces greater inhibitory signal transduction responses in ARC KP neurons (70.6% ± 5.9%) versus KP neurons of the rostral periventricular area of the third ventricle (RP3V) (38.5% ± 6.7%) [148]. Additionally, KP administration restored cyclicity and ovulation rate (number of corpora lutea following KP: 7.8 ± 0.6, controls: 7.5 ± 0.6) in female mice with hyperprolactinemia [146]. Consistent with this, specific knockout of the PRL receptor within ARC KP neurons prevents prolactin-induced suppression of LH secretion [147].

Tubero-infundibular dopamine (TIDA) neurons within the ARC, which are essential for maintaining PRL homeostasis, can be modulated by dynorphin action [145,149]. As ARC KP neurons co-express dynorphin (and NKB) and dynorphin cells project onto TIDA neurons, KP neurons may be directly involved in regulating PRL secretion [145,149]. Furthermore, KP has been shown to regulate PRL release through suppression of TIDA neuronal activity [150].

Human data

Considering PRL exerts its effects on fertility through suppression of KP inputs to GnRH neurons, KP could have the potential to be used for treatment of hyperprolactinemia. In women with PRL-induced chronic amenorrhea, KP-10 (IV infusion 1.5 mg/kg/h over 12 hrs) increased LH pulse frequency, serum LH, FSH, and ovarian hormones (E2, inhibin B, and testosterone) levels [151]

(Table 1G). Similarly, an IV bolus of KP-10 (0.24 nmol/kg) given every hour for 10 hrs increased LH pulse frequency from 4.5 ±0.9 to 7.5 ±0.5 per 10hrs and elevated mean LH levels from 3.32 ± 0.60 IU/L to 5.91 ± 0.65 IU/L in women with hyperprolactinemia [152].

VB4) *In-vitro* fertilization

Infertility is the inability to conceive after 12 months or more of regular unprotected sexual intercourse and affects one in six couples [153]. *In-vitro* fertilization (IVF) is the main treatment offered and has resulted in over 8 million live births worldwide over the past 40 years [154,155]. In brief, IVF involves the use of supra-physiological doses of FSH to induce follicle development, followed by human chorionic gonadotropin (hCG) or a GnRH agonist to provide LH-like exposure and induce oocyte maturation [156]. The half-life of exogenous hCG is double that of the endogenous physiological LH surge and hence exogenous hCG may persist in the circulation for up to 7 days [157]. A serious life-threatening complication of hCG treatment is severe ovarian hyperstimulation syndrome (OHSS) affecting 2-6% of women [158], and women with PCOS are at higher risk [130]. In this condition, excessive ovarian stimulation causes aberrant release of vascular endothelial growth factor (VEGF) [159], which results in increased vascular permeability and third-spacing of fluids, ultimately leading to the development of ascites, pleural effusions and hemoconcentration [160]. Thus, treatments that effectively trigger an LH-surge to induce oocyte maturation, whilst avoiding over-stimulation and OHSS are of clinical value.

Animal data

KP induces the LH surge necessary for ovulation and oocyte maturation. Indeed, approximately 30% of KP neurons in the RP3V are activated during the LH surge [161]. Transgenic mice null of

Kiss1 or its receptor lack the LH surge and GnRH neuronal activity [161], and treatment of the hypothalamic POA with a neutralizing monoclonal antibody inhibits ovulation in female rodents [72]. Notably, KP administration generated an LH-surge inducing ovulation to a similar degree as hCG in gonadotropin pretreated rats [162]. KP also stimulated ovulation in other mammals including ewes [163] and musk shrews [162] thus suggesting that hypothalamic KP signaling is requisite for physiological ovulation.

Human data

In humans, KP induces a more similar LH rise to that observed after the physiological mid-cycle LH surge than either GnRH agonist or hCG, and therefore could be a promising ovulation induction agent in IVF **(Table 1H).** For instance, the physiological midcycle LH surge has a mean amplitude of 56.5 IU/L (SD 23.4, range 25-144 IU/L)[164], which is similar to the LH rise at 4-6 hrs following KP (LH ~45 IU/L) [165] whereas that induced by GnRH agonists is supraphysiological (LH 140.4 IU/L) [166]. In 2014, KP-54 (SC bolus 1.6, 12.8 nmol/kg) was administered during a GnRH antagonist co-treated IVF cycle to 53 women with subfertility [165]. KP-54 resulted in the retrieval of at least one mature oocyte in 51 of 53 women, one embryo for implantation in 49 of 53 women, and the birth of 12 healthy babies (8 singleton, 2 twin pregnancies) [165].

KP's has also been proposed to suppress VEGF levels through a direct action at the ovary and potentially reduce the risk of OHSS, making it a safe and attractive therapeutic agent for IVF [167]. In women with high risk of OHSS, 95% had oocyte maturation (highest oocyte yield = 121%) and 90% formed embryos following KP-54 (SC bolus 3.2-12.8 nmol/kg) [167]. The rates of biochemical pregnancy, clinical pregnancy and live births per transfer were 85, 77, and 62%, respectively following a dose of 9.6 nmol/kg of KP-54 [167]. Importantly, none of the women developed

moderate, severe, or critical OHSS [167]. To determine whether the duration of the physiological LH surge (24-28 hrs) is crucial for IVF treatment, KP-54 (SC bolus 9.6 nmol/kg) was administered as either a single dose or two doses (10 hrs apart), to women at high risk of OHSS [160]. Women receiving two doses of KP-54 had higher oocyte yields (71% vs 45%), implantation rates (37% vs 23%) and live birth rates (39% vs 19%) compared to those receiving a single dose [160]. Critically, two doses of KP-54 still did not result in OHSS despite extending the duration of LH-exposure [160]. The KP analog, MVT-602, induced a similar amplitude of LH-surge as KP-54 [82] but a longer duration of LH rise, and therefore also has potential as a trigger for oocyte maturation.

In a retrospective single-center comparison, the risk of OHSS was greater following hCG (OR 33.6, CI 12.6-89.5) and GnRH agonist treatment (OR 3.6, CI 1.8-7.1) than KP-54 [155]. Ovarian volumes were larger by 20-fold with hCG, 8-fold with GnRH agonist, and 5-fold with KP-54, compared to baseline pre-stimulation ovarian volumes [155]. Similarly, mean ascitic volumes were greatest following hCG (62 ± 84ml) than GnRH agonist (9 ± 44ml) or KP-54 (5 ± 8ml) [155]. Collectively, this data highlights KP's use as a safe and efficacious agent for oocyte maturation in IVF protocols.

VC) IN DISORDERS OF PREGNANCY

Kisspeptin (KP) is a putative regulator of trophoblast invasion [168] and placentation [169] in pregnancy. The *Kiss1* gene is abundantly expressed in syncytiotrophoblasts, whereas its receptor is expressed in both cytotrophoblasts and syncytiotrophoblasts [27,28]. KP levels increase linearly during healthy pregnancy from <8 pmol/L (non-pregnant levels) to 1230 pmol/L in the first trimester, and 9590 pmol/L in the third trimester [170] **(Table 1I).** Whilst high circulating KP levels are associated with advanced maternal age, lower KP levels are associated with Afro-Caribbean

ethnicity, smoking, and high body mass index (BMI) [170]. Importantly, KP has emerged as a promising biomarker to predict several adverse pregnancy complications **(Figure 6).**

VC1) Miscarriage

Miscarriage is the spontaneous loss of an intrauterine pregnancy before 24 weeks of gestation and affects 20% of pregnancies [171]. Miscarriage can be difficult to diagnose as a pregnancy can be failing for a period before miscarriage is conclusively confirmed. Therefore, biomarkers that could aid in the evaluation of miscarriage, such as KP, are valuable.

Human data

KP levels adjusted for gestation are reduced (by 79%) in women with miscarriage compared to healthy pregnancy [170,172–175] and are particularly low in complete versus incomplete (retained products of conception) or missed (empty gestational sac with absent heartbeat) miscarriage **(Table 1J).** Unlike beta human chorionic gonadotropin (β-hCG), KP has been shown to maintain a high diagnostic performance throughout the first trimester [170,172]. Indeed, a combined KP and β-hCG measurement had the highest diagnostic accuracy to predict miscarriage at all gestations with an area under receiver operating characteristic curve (AUCROC) of 0.92 (0.89-0.95) [170].

VC2) Hypertensive disorders of pregnancy

Pregnancy-induced hypertension and pre-eclampsia are defined as new onset hypertension (blood pressure ≥140/90 mmHg) following 20 weeks' gestation. Pre-eclampsia also includes the presence of proteinuria (>3g per 24 hrs), neurological complications and a high risk of significant end-organ dysfunction [176].

Human data

KP levels vary according to pre-eclampsia subtype, severity, and time of disease onset. KP concentrations are generally reduced in pre-eclampsia, especially during the first and second trimesters [177–183] and levels decline further with increasing disease severity [182,183] **(Table 1K).** In contrast, KP levels were found to be increased during the third trimester of pregnancy in keeping with placental KP expression data [184].

VC3) Ectopic Pregnancy

Ectopic pregnancy (EP) occurs when a fertilized ovum implants outside of the uterine cavity and affects 2% of pregnancies [185]. Its current diagnostic methods (serial β-hCG measurements and laparoscopy) have low sensitivity and specificity and are associated with high morbidity [185].

Human data

Whilst some studies have reported low levels of KP in EP [175,186] others did not find any significant differences after adjusting for confounding variables [184] **(Table 1L).** These differing results are likely due to the early gestational age at presentation of EP.

VC4) Fetal growth restriction and pre-term birth

Fetal growth restriction (FGR) encompasses intrauterine growth restriction (IUGR; fetal weight <10th centile for GA with abnormal doppler artery results [187]) and small for gestational age (SGA; weight at delivery <10th percentile for gestational age) [180,184,188,189].

Human data

KP levels are consistently reduced in IUGR [180,189] and pregnancies with SGA [184,188] and therefore KP could aid in the assessment of these conditions **(Table 1L).** In contrast, in pre-term birth (PTB; delivery prior to 37 weeks' gestation [190]), circulating KP levels were increased during the first trimester but were unaltered in the third trimester [184].

VC5) Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) affects up to 20% of pregnancies worldwide [191] and develops when pancreatic β-cells fail to respond to the physiological increase in insulin resistance that occurs during pregnancy [192,193]. *In-vitro* and *in-vivo* studies suggest that KP could potentiate glucose-stimulated insulin secretion (GSIS) [194–197] and thus, improve glucose tolerance [198] (further discussed in the later section on metabolism in this review).

Human data

In studies involving women with GDM, KP concentrations were either decreased [177,198] or not significantly different [184,199] **(Table 1L).**

Although evidence to date is convincing regarding KP's utility for diagnosing miscarriage, further larger studies with sufficiently sized control cohorts, and adjustments for gestation, BMI, comorbidities, and disease severity, are required to assess KP's potential as a biomarker in other pregnancy complications.

VD) IN DISORDERS OF METABOLISM

VD1) Glucose Homeostasis

Glucose regulation is dependent on the meticulous control of blood glucose concentrations by several hormones released from central and peripheral tissues [200]. Kisspeptin (KP) and its receptor are expressed in murine and human pancreatic β-cells, liver, and adipose tissue [16,29,194,201], suggesting that it could have a putative role in glucose regulation.

In vitro data

The effect of KP on glucose stimulated insulin secretion (GSIS) is conflicted within the literature. Using isolated islets and/or perfused pancreata from mice [202,203] and rats [204], KP induced an inhibitory effect on insulin secretion. In contrast, studies employing static incubation and/or peri-perfusion experiments using islets from mice [194–197], rats [197] and pigs [197] found that KP potentiated insulin secretion. These differing results may be due to the differences in experimental protocols used. Indeed, human islets incubated with glucose (3- and 17-mM) and KP (0, 2.7 and 1000 nM), demonstrated that KP stimulates GSIS in a dose-dependent manner in the presence of high (but not low) glucose levels [205]. Consistent with this, KP stimulates insulin secretion at higher ambient glucose concentrations (20 mM) versus lower concentrations (2 mM) in human islet cells [194,196]. Taken together, these findings suggest that KP stimulates insulin release at high ambient glucose concentrations.

Animal data

Female but not male mice null of *Kiss1r* have higher fasted basal glucose levels, impaired glucose tolerance, and increased body weight [206,207] which suggests that KP-signaling may influence glucose homeostasis in a sexually dimorphic manner. As global *Kiss1r* KO animals are also profoundly hypogonadal and lack gonadal sex steroids, this could influence the impact on glucose tolerance [208]. To account for this, *Kiss1r* KO mice with selective re-introduction of *Kiss1r* only in GnRH cells were generated, thus preserving gonadal function [207]. Using this approach, females with preserved gonadal function still displayed perturbed glucose tolerance, albeit with a milder phenotype [207]. In pregnant mice, specific knockout of *Kiss1r* in pancreatic β-cells caused glucose intolerance[198] . These changes were not observed in the non-pregnant state which suggests that KP has an adaptive role in compensating for gestational insulin resistance through regulation of β-cell function [198].

In adult male rats, peripheral (IV) rather than central (by intracerebroventricular injection) KP administration induced rapid rises in plasma insulin levels (4-fold), suggesting that KP's effects are peripherally mediated [196]. Likewise, peripheral injections (intraperitoneal) of KP-10 resulted in a 3-fold increase in plasma insulin concentrations [209]. Furthermore, KP administration significantly heightened GSIS in both fed and fasted monkeys [210]. However, despite rises in insulin secretion, no changes in glucose tolerance have been observed following short-term administration of KP [209].

Human data

The first study evaluating the effects of KP on GSIS in humans (n=15) was conducted in 2018 [205] **(Table 1M).** Here, an IV infusion of KP-54 (1 nmol/kg/hr) increased both insulin secretion and

the deposition index (an assessment of β-cell function) by 35%, compared to placebo [205]. This effect was only observed in response to an intravenous glucose tolerance test (IVGTT) and not a mixed meal tolerance test [205]; thus suggesting that KP increases insulin only in the presence of high glucose levels. On the contrary, an IV infusion of KP-54 (1 nmol/kg/hr) did not influence pre-prandial and postprandial glucose and insulin levels in women with overweight or obesity [211]. These data indicate that KP could have a potential role in glucose metabolism, especially during pregnancy, a state of insulin resistance.

VD2) Appetite regulation and Obesity

Appetite is intricately regulated by hypothalamic arcuate (ARC) neurons including proopiomelanocortin (POMC), agouti-related peptide (AgRP) and neuropeptide Y (NPY) neurons [212]. Whilst POMC neurons are anorexigenic (appetite-suppressing) [213], NPY and AgRP neurons are orexigenic (appetite-stimulating) [214,215]. Considering KP has a critical role in reproduction, and that adequate reproductive function is dependent on sufficient energy stores, studies have investigated the anatomical and functional reciprocal connections between KP, POMC and NPY/ AgRP neurons [212].

VD2a) Appetite regulation

Animal data

Evidence of the interactions between KP, POMC and NPY neurons is controversial. Whilst KP has been shown to stimulate POMC and AgRP [216], and inhibit NPY neurons [217] (overall reduced food intake) other studies have reported the opposite [119,218]. Notably, toxin-induced silencing of

ARC *Kiss1* neurons altered circadian food intake (less food eaten during the dark phase) but not total food intake[219]. Likewise, global knockout of *Kiss1r* in mice resulted in reduced food intake in both dark and light phases [206,220], suggesting that KP has appetite suppressive effects. Interestingly, like glucose homeostasis, appetite regulation also displays sexual dimorphism. For instance, whilst female *Kiss1r* null mice have reduced food intake, the male counterparts have either similar or only mildly reduced food intake than controls[206]. However, this effect is lost in *Kiss1r* knockout male mice with preserved gonadal function [207], thus indicating that the effects of KP on food intake is mediated by changes in gonadal sex steroids in males.

KP's effect on appetite regulation varies within the literature and differences can occur according to the species type involved. For instance, central (ICV, intracerebroventricular) administration of KP-10 reduced food intake in fasted adult male mice [221] and female jerboas [222] but had no effect in fasted prepubertal [126] or adult male rats [5]. However, higher doses (4.6 nmol) of ICV KP-10 markedly reduced food intake in rats [223]. Similarly, peripheral (intraperitoneal) injections of KP-10 have been shown to decrease food intake in mice in some [209] but not all studies [221,224]. In contrast, chicks had increased food intake following administration of ICV KP-10 [205]. These species differences are likely due to alterations in experimental methodology (e.g. food intake being measured in light vs dark phases, KP administration to fed vs fasted animals) between studies, but could be due to the presence of different appetite circuits between species.

Human data

In fasted healthy men, KP-54 (IV infusion 1 nmol/kg/h over 2hrs) had no effect on self-reported hunger or objective food intake [205]. Furthermore, in healthy men, an IV bolus of KP-54 did not alter brain signal responses (limbic and hypothalamic) to visual food stimuli [225] **(Table 1N).**

VD2b) Obesity

Animal data

Knockout of the KP receptor in adult female mice display increased adiposity and leptin levels from as early as 6 weeks of age followed by a dramatic rise in bodyweight (BW) of 30% [206]. Although increased BW did not correlate with increased food intake, it was associated with lower respiratory rates, energy expenditure and locomotor activity [206]. The estradiol (E2) deficient state following *Kiss1r* knockout could have also contributed to the changes in BW observed. However, a higher BW was observed in ovariectomized (OVX) versus gonadal intact in *Kiss1r* knockout mice [206], thus suggesting KP's effect on energy homeostasis is likely to be mediated in both a direct (via energy expenditure) and indirect (via sex steroid hormones) manner [206]. Once again, sexual dimorphism was exhibited as male *Kiss1r* null mice had normal BW [206].

Brown adipose tissue (BAT), a marker of energy expenditure, regulates thermogenesis and metabolic rate. Interestingly, selective *Kiss1r* knockout from BAT (BAT- *Kiss1r* KO) reduced BW and increased energy expenditure, locomotor activity, body temperature, and BAT gene expression (specifically Cox8b) in female mice [226]. Collectively, these data indicate that the obesity and decreased metabolism in global *Kiss1r* KOs reflect impaired KP signaling in non-BAT tissues and that BAT specific KP induction could be a potential target for obesity treatment [226]. More research elucidating the specific tissues and cell types where KP signaling influences metabolic and thermogenic parameters is required.

Human data

The first human study to evaluate the acute effects of KP in obesity was conducted in 2023. Here, an IV infusion of KP-54 (1 nmol/kg/h over 2hrs) administered to women with overweight or obesity, had no effect on self-reported appetite or objective food intake [211]. Thus, the appetite regulatory effects of KP appear to be species specific, with no changes being observed in humans.

VD3) Metabolic Fatty Liver Disease

Metabolic fatty liver disease (MAFLD) is highly prevalent with global rates reaching 25% and is a leading cause of liver transplantation in the UK [227]. It encompasses a spectrum of disease from excessive liver fat / steatosis ('non-alcoholic fatty liver' [NAFL]), necroinflammation and fibrosis ('non-alcoholic steatohepatitis' [NASH]), to NASH-cirrhosis and ultimately hepatocellular carcinoma [228,229]. MAFLD is associated with significant comorbidities including central obesity, type 2 diabetes mellitus, dyslipidemia, and the metabolic syndrome [230]. From a therapeutic perspective, there are currently no approved pharmacotherapeutic options for the treatment of MAFLD.

VD3a) Diagnosing Metabolic Fatty Liver Disease

Animal data

To study the effects of KP signaling in MAFLD, mouse models have been generated in which wild-type mice are administered high-fat diets over several weeks **(Figure 7)**. In MAFLD mice, hepatic *Kiss1* and *Kiss1r* mRNA expression is enhanced and circulating KP levels are 50% higher than controls [231]. This could indicate that KP increases as a compensatory response to liver damage from MAFLD/ NASH.

Human data

Liver biopsies from men with MAFLD and NASH have increased expression of both *KISS1* and *KISS1R* (mRNA and protein levels) and have 3-fold higher plasma KP levels, compared with healthy controls [231] and thus could have potential as a marker for grading MAFLD severity **(Table 1O).**

VD3b) Treating Metabolic Fatty Liver Disease

Animal data

In MAFLD mice, specific deletion of *Kiss1r* has been shown to worsen hepatic steatosis, impair glucose tolerance and upregulate markers of inflammation (such as macrophage inflammatory protein-2 and chemokines IFN-γ–induced protein 10) and fibrosis (such as collagen, smooth muscle actin and matrix metalloproteinases) [231] **(Figure 7).** Conversely, enhanced stimulation of *Kiss1r*, through administration of a KP receptor agonist (MVT-602), alleviated hepatic steatosis and metabolic deterioration in MAFLD mice and prevented liver fibrosis in NASH mice [231]. The mechanism by which KP exerts these protective effects is via activation of hepatic AMPK with resultant inhibition of triglyceride accumulation. However, KP failed to protect against NAFLD livers deplete of AMPK or *Kiss1r* [231]. Thus, KP receptor signaling plays an important role in the suppression of MAFLD / NASH disease progression by reducing hepatic lipogenesis, and therefore could have potential as future treatment targets for these conditions.

VE) IN DISORDERS OF BONE

VE1) Direct effects of Kisspeptin in Bone with potential to treat osteoporosis

From an evolutionary perspective, during the physiological response to starvation, energy demanding processes such as skeletal integrity and reproduction may be relinquished [232]. Therefore, it is unsurprising that an established relationship between bone and reproductive hormones exists, with hormones from all levels of the HPG axis implicated in the growth and maintenance of the mammalian skeleton [reviewed recently and extensively in [233]].

The importance of the interaction between reproductive hormones and bone is clearly illustrated by reproductive disorders, which contribute to the clinical burden of low bone mineral density, such as Primary Ovarian Insufficiency, Hypothalamic Amenorrhea, Congenital Hypogonadotropic Hypogonadism and Hyperprolactinemia. In addition, post-menopausal bone loss is a central risk factor for developing osteoporosis [234,235], with higher risk and prevalence of fractures resulting in disability, poor quality of life and increased mortality [236]. Taken together, this stresses the need to better understand bone physiology and the pathogenesis of bone loss, to identify new safe and effective therapeutic targets.

In Vitro Studies

Bone mass is maintained by a tight balance between osteoclastic bone resorption and osteoblastic bone formation [233]. Kisspeptin receptor expression has been detected on osteoclast cell lines differentiated *in vitro* from CD14-selected monocytes [237]. Moreover, both *KISS1* mRNA and protein are strongly expressed in the normal human osteoblast cell line hFOB1.19 [30]. This compares with *KISS1* mRNA and protein expression, which are moderate, weak, and almost lost in the human osteosarcoma cell lines U-2 OS, Saos-2 and MG-63, respectively [30]. Interestingly, the cell invasion ability of these cell lines reveals a gradually increasing aggressive phenomenon in U-2 OS, Saos-2 and MG-63, suggesting that lower *KISS1* expression might be associated with

a stronger invasive capability [30]. Regarding the kisspeptin receptor, *Kiss1r* mRNA and protein have been observed in normal canine osteoblasts [238], as well as high expression of KISS1R protein on MG-63 osteoblast-like osteosarcoma cells [239]. KISS1R expression has also been reported on osteoblast precursors, including primary human mesenchymal stem cells and osteoprogenitor cells [240].

Rodent data reveals that kisspeptin enhances osteoblast differentiation (osteoblastogenesis). In C3H10T/2 mouse mesenchymal stem cells, incubation with kisspeptin increases the expression of osteogenic marker genes, including distal-less homeobox 5 (Dlx5), runt-related transcription factor 2 (Runx2) and alkaline phosphatase (ALP) [241]. Of note, the growth factor bone morphogenetic protein 2 (BMP2) stimulates bone formation by activating these osteogenic genes [242,243]. It is therefore pertinent that kisspeptin has been documented to stimulate osteoblast differentiation by increasing the expression and activation of BMP2 in C3H10T/2 cells (via the transcriptional factor NFATc4), whereas in Kiss1r null cells, osteoblast differentiation was suppressed [241]. Collectively, this reveals that in C3H10T/2 cells, kisspeptin (acting via Kiss1r) stimulates osteoblastogenesis through NFATc4-mediated BMP-2 expression and activation [241].

Moving from rodents, recent work provides the first evidence for direct effects of kisspeptin on human bone metabolism. Using the human cell line hMSCs, exposure to kisspeptin for 7-days induced a 41.1% increase in ALP activity, signifying enhanced osteoblastogenesis [244]. It is notable that kisspeptin administration had no effect on ALP activity in either osteoblast monoculture or cocultures, indicating that kisspeptin does not modulate mature osteoblast activity but instead has a predominant effect on osteoblastogenesis at least *in vitro*. In terms of human osteoclasts, *KISS1R* mRNA was identified throughout the 10-day process of osteoclastogenesis (i.e., from $CD14^+$ to mature human osteoclast). Indeed, kisspeptin administration exerted a potent and dose-dependent

antiresorptive effect on osteoclast activity in both monocultures and osteoclast/osteoblast cocultures. In cocultures, this inhibitory effect ranged from 26.2% (0.01 nM kisspeptin) to 53.4% (10 nM kisspeptin) [244]. Taken together, these *in vitro* data reveal that in humans kisspeptin enhances osteoblastogenesis and potently inhibits osteoclast activity.

In Vivo Non-Human Studies

Using a combination of genetic models and stereotaxic surgery, recent pivotal work has identified a neuroskeletal axis, whereby deleting estrogen receptor alpha (ERα)-signalling in the ARC promotes significant increases in bone mass without affecting food intake [245]. This skeletal phenotype was sex-specific (occurring in female but not male mice) with a remarkable increase in trabecular bone mass of ~700%, an average 80% increase in bone volume over total volume, as well as increases in trabecular number and thickness and overall mechanical strength of long bones [245]. These changes were accompanied by a significant increase in bone formation rate and mineralized surface (indicating enhanced osteoblastic functions) and upregulation of BMP signaling and osteoblast differentiation on transcriptional profiling [245]. Notably, acute ablation of ARC ERα after ovariectomy resulted in a 50% increase in bone density, demonstrating that even in the absence of gonadal hormones, the brain circuit remains intact [245]. Finally, loss of ERα specifically in kisspeptin-expressing ARC recapitulated this bone phenotype, defining *central* kisspeptin-signaling as a key node in the ER-neuroskeletal circuit regulating sex-dependent bone remodeling in females [245].

In Vivo Human Studies

Translating the preclinical evidence into humans, a recent clinical study investigated the acute effects of kisspeptin administration on bone turnover markers in humans for the first time [244] **(Table 1P).** Involving 26 healthy eugonadal young men, an acute 90-minute infusion of kisspeptin elicited a 20.3% maximal increase in total osteocalcin (an established marker of bone formation) and 24.3% maximal increase in carboxylated osteocalcin (which predominates in bone remodeling) but had no acute effects on circulating P1NP levels (a further bone formation marker) in this short time course. Interestingly, a comparable magnitude of increase in osteocalcin along with bone-forming effects has been observed with short-term teriparatide administration (a recombinant parathyroid hormone used for the treatment of osteoporosis) [246]. Moreover, during the acute experimental time-course, kisspeptin administration had no significant effects on the bone resorption marker CTx (which may require a longer experimental duration to detect changes) or on downstream testosterone levels [244]. Collectively, these data highlight that kisspeptin administration acutely increases the bone formation marker osteocalcin in healthy men, independently of downstream sex-steroid levels.

Taken together, across a series of experimental models, an emerging and favorable link between kisspeptin and bone metabolism has been identified. Importantly, human evidence demonstrates that kisspeptin enhances osteoblastogenesis and potently inhibits osteoclast activity *in vitro*, whilst also acutely increasing the bone formation marker osteocalcin in healthy men. Therefore, these findings suggest that kisspeptin administration may beneficially uncouple bone turnover in humans, which warrants further investigation in chronic kisspeptin administration studies and in patients with disorders of bone metabolism to examine kisspeptin's clinical therapeutic potential.

VF) IN DISORDERS OF SEXUAL BEHAVIOUR

Reproductive behaviors are complex strategies related to the ultimate production of offspring. They include the identification of suitable mating partners (principally using olfactory and auditory signals), as well as copulatory and sexual behaviors [247]. Furthermore, advanced species (including humans) have evolved to gain reward and satisfaction from sex itself and its precursors (sexual desire and arousal) [248]. A persistent disturbance with any stage of normal sexual activity can result in sexual dysfunction (i.e., sexual desire, arousal, and orgasmic disorders).

Along these lines, it is therefore pertinent that beyond the hypothalamus, KP and its receptor have been localized to numerous limbic brain structures in rodents [22] and humans [18] which are areas implicated in the neurocircuitry regulating sexual and emotional behaviors [249]. Consistent with this, a wealth of literature implicates KP-signaling in the neuroendocrine control of all aspects of reproductive behavior across a range of species as discussed below **(Figure 8).**

VF1) Male Reproductive Behavior

In Vivo Non-Human Studies

Olfactory processing: In adult male rats, reciprocal connectivity between the accessory olfactory bulb (AOB) and amygdala KP neurons has been visualized [250]. Given the established role for the AOB in relaying pheromonal signals [251], this suggests that amygdala KP neurons are targeted directly by pheromonal pathways. Moreover, amygdala KP neurons project to GnRH neurons in the hypothalamic POA, with approximately 15% receiving inputs from this amygdala KP population [250]. Collectively, these neuroanatomical data define a physiological framework for how KP-signaling serves as a relay between olfactory signals and the HPG axis.

To provide biological significance for the neuroanatomical connections, rodent models have investigated whether sex-related olfactory signals can modulate central KP expression. In male mice, exposure to female urine (as a pheromone stimulus) for 30-minutes has been observed to increase the number of KP-neurons co-expressing c-Fos in the medial amygdala (MeA) by two-fold, with a concomitant rise in LH release within 15-minutes [252]. Notably, no changes in AVPV or ARC KP activity was observed [252]. Building on these findings, the acute effects of olfactory signals in male rats has been recently examined [253]. In this study, within 5-minutes of exposure to a female rat, KP expression was significantly enhanced in the AVPV and periventricular nucleus (PeN), resulting in significant increases in LH and testosterone levels, followed by increased male sexual behavior [253]. In contrast, exposure to solely female-soiled bedding failed to increase KP expression in the AVPV/PeN or testosterone levels, suggesting that a physical stimulus animal is required to induce AVPV/PeN KP expression in male rats [253]. In contrast to the earlier discussed mouse study [252], neither exposure to a female rat or female-soiled bedding affected KP expression in the MeA (or ARC), which may be accounted for by species differences, or the experimental model.

Sexual partner preference: Gonad intact *Kiss1r* knockout (KO) male mice display no partner preference for either male or female stimulus animals [254]. Specifically, despite normosmia (determined using a 'hidden cookie test'), they spend an equal investigatory duration with male and female stimulus animals (48% versus 52%, respectively), whereas wildtype male mice spend >70% with females [254]. Notably, this behavioral deficit is not rescued by testosterone replacement [254], suggesting that the KP receptor is indispensable for regulating sexual partner preference in male mice. Along similar lines, using a chemogenetic approach, DREADDs-stimulation of KP

neurons in the posterodorsal MeA (MePD) has been reported to double s the time male mice spend investigating an estrous female over another gonadally-intact male [255]. Furthermore, to define direct KP effects, a recent study investigated sexual motivation in male rats following three interventions: intranasal administration of a GnRH analogue, intraperitoneal KP or intranasal KP [256]. Using this experimental paradigm, intranasal GnRH augmented circulating testosterone levels but did not affect sexual motivation, whereas intraperitoneal KP increased both testosterone and sexual motivation [256]. Importantly, despite not affecting testosterone levels, intranasal KP increased sexual motivation [256], highlighting KP is a GnRH/testosterone-independent regulator of sexual motivation in male rats.

Sexual and copulatory behaviors: Direct infusion of KP into the MePD of male rats ~dose-dependently results in multiple ex-copula erections, an effect which is blocked by pre-treatment with a KP receptor antagonist (peptide-234) [257]. Comparatively, when KP is infused into the lateral cerebroventricle, despite a similar rise in circulating LH, no erections are observed [257], indicating GnRH/LH-independence and site-specificity of the MePD for KP's erectile response in rodents. Given the previous data highlighting that testosterone replacement fails to restore sexual partner preference in *Kiss1r* KO male mice [254], it is interesting to consider whether this happens with other reproductive behaviors. When paired with a hormone-primed receptive female for 45-minutes, *Kiss1r* KO male mice display an absence of all normal male-like sexual parameters (mounts, thrusts, intromissions, and ejaculation) [254]. In contrast, castration followed by testosterone-replacement elicits a robust increase in mounts and thrusts at a ratio with that of testosterone-treated wildtype males [254]. This is highly congruent with evidence in *Kiss1* KO male rats [258] and mice [259], whereby testosterone-supplemented males show mounting behavior, but not ejaculation

(which may be attributable to incomplete penile development) in mating trials. Taken together, these findings indicate that restoration of testosterone levels partly rescues some but not all sexual behaviors (especially mounting) in both *Kiss1r* and *Kiss1* KO male rodents.

Moving from rodents into male domestic animals, recent data provide s evidence for the relationship between circulating KP levels and sexual behavior in buffalo bulls [260]. In this study, it was observed that KP levels were significantly lower in bulls with longer reaction times (i.e., time from exposure to mounting the female). Moreover, on approach to the female, males displaying characteristic aggressive behaviors (i.e., uncontrollable, and extremely eager to mount and approach with full vigor) had significantly higher KP levels, compared to dull males (i.e., proceeding with a dull expression and longer time to mount). In keeping with earlier rodent evidence [254,257], males with lower KP levels also exhibited incomplete penile erection and protrusion [260]. Hence, these findings suggest that circulating KP levels may offer a novel biomarker for sexual behavior in male domestic animals.

In Vivo Human Studies

The application of functional neuroimaging (including functional MRI [fMRI] and proton magnetic resonance spectroscopy) has been indispensable to facilitate the non-invasive study of sexual brain processing in humans by mapping activated areas of brain [261]. To date, clinical studies have been undertaken in both healthy men and patients with low sexual desire to investigate the effects of KP across a range of behavioral domains as detailed below **(Table 1Q)**.

Resting brain activity: KP's effects on resting brain activity has been explored using two established neuroimaging techniques. Firstly, using fMRI in healthy heterosexual men, peripheral

KP administration has been shown to modulate resting brain connectivity [262], which is an important element of human behavior, frequently disrupted in psychosexual and emotional disorders [263]. Specifically, KP modulated the default mode network (the most defined resting state [264]), which correlated with enhanced limbic brain activity later in response to visual sexual images [262]. Additionally, KP's modulation of this network was greater in men with less reward drive and correlated with reduced sexual aversion [262]. In a further study, proton magnetic resonance spectroscopy was employed to examine the *in vivo* effects of KP administration on central levels of the key inhibitory neurotransmitter GABA in the human brain [265]. Using this approach, peripheral KP administration significantly decreased endogenous GABA by 15% in the anterior cingulate cortex of healthy men [265]. Of note, a similar magnitude of GABA change has previously been reported in psychological studies with functional impact [266,267].

Olfactory processing: In healthy heterosexual men, peripheral KP administration has been observed to enhance limbic brain activity when men are exposed to an established feminine olfactory stimulus, 'Chanel No.5' [268]. Specifically, brain activation was demonstrated in limbic regions implicated in olfactory processing, hedonic valuation of olfactory stimuli and sexual arousal, including the amygdala, hippocampus, and insula [269]. Comparatively, KP did not affect brain activity in the motor cortex (which was employed as a control region), highlighting the specificity of KP's effects in olfactory and limbic circuits regulating sexual behavior on exposure to a feminine olfactory stimulus [270].

Sexual partner preference: Attraction is an important initiating step in human sexual behavior, involving numerous aesthetic brain regions, including the medial pre-frontal cortex [271–273] and

superior frontal gyrus [274]. In healthy heterosexual men, peripheral KP administration increases brain activity in both regions in response to viewing female faces [270]. From a functional perspective, significant correlations were observed between KP-enhanced brain activity and important psychometric parameters. For example, the effects of KP in the anterior cingulate cortex and insula were more pronounced in men with lower baseline reward and sexual quality of life, which is relevant given these areas are implicated in sexual arousal [275], facial attraction [274] and motivation towards reward [276,277]. It is interesting to speculate about the biological significance of this differential effect. From an evolutionary perspective, KP's enhancement of these brain regions may serve to strengthen feelings of reward, attraction, and motivation in individuals with lower sexual quality of life, in order to promote sexual attraction and ultimately encourage reproduction at a population level.

Sexual behavior: In healthy heterosexual men, peripheral KP administration enhances limbic brain activity when men are exposed to visual sexual stimuli (but not other stimuli, such as negative, neutral, happy, or fearful-themed images), including in the anterior and posterior cingulate and amygdala [278]. Additionally, the more KP was observed to enhance brain activity in key limbic structures involved in sexual arousal (such as the putamen, anterior cingulate and globus pallidus), the less aversion to sex healthy men displayed [278]. Thus, given that desire for sexual stimulation is a fundamental component of the human sexual response [279], these findings laid the foundation for potential clinical application of KP for the treatment of patients with psychosexual dysfunction. Along these lines, in recently published work, the clinical and mechanistic effects of KP administration were investigated in men with distressing low sexual desire due to Hypoactive Sexual Desire Disorder (HSDD) [280]. This condition is characterized by increased activity of higher

cortical and cognitive brain regions, which inhibits lower limbic and emotional regions, thus interfering with sexual desire [281]. It is therefore significant that in response to watching erotic videos in the fMRI scanner, KP administration was observed to significantly deactivate brain regions involved in self-monitoring and introspection (such as the parahippocampus, frontal pole and precuneus), whilst increasing brain activity in sexual arousal centers (such as the anterior cingulate) in this cohort of men with psychosexual dysfunction [280]. Indeed, in response to KP's restoration of sexual brain processing, significant increases in penile tumescence (by 56% more than placebo) and behavioral measures of sexual desire (including increased 'happiness about sex') were observed, providing functional and behavioral relevance [280].

In this collection of neuroimaging studies [280], an [262,265,270,278] identical administration protocol with peripheral KP-54 was employed. Although different KP isoforms display different degrees of blood-brain barrier penetrance, it is well-established that peripheral KP-54 can activate GnRH neuron dendritic terminals before the blood-brain barrier [282], as well as cross the blood-brain barrier to directly access deeper brain structures expressing KP receptors [278]. In all the highlighted clinical studies, KP largely modulated brain regions matching KP receptor expression in humans [16,17,29] which could suggest direct actions of KP on its receptor. In addition, the administration protocol was selected to ensure steady-state levels of KP during the data collection period (brain imaging and behavioral testing), while avoiding downstream testosterone increases which occurs later [76]. Finally, across this series of studies, KP modulated brain activity in relation to sexual and emotional tasks. Indeed, of note, recent data reveals that using the same administration protocol, KP does not affect brain responses to visual food stimuli in healthy young men [225], highlighting that KP's effects on limbic brain regions are specific to sexual and emotional stimuli.

VF2) Female Reproductive Behavior

In Vivo Non-Human Studies

Olfactory processing: In seasonally anestrous ewes, the introduction of a novel male sheep has been observed to result in 9-fold and 3-fold increases in KP c-Fos activity in the rostral and mid ARC, respectively [283]. This was associated with increases in LH pulse amplitude and pulse frequency, an effect which was abolished by central infusion of a KP antagonist (peptide-271) [283]. Turning to rodents, in female mice exposure to opposite-sex (but not same-sex) urinary pheromones induces KP c-Fos activity by almost 40% in the AVPV [284]. This is in close agreement with data from ovariectomized female rats (implanted with preovulatory levels of estradiol), whereby exposure to male-soiled bedding (but not clean or female-soiled bedding) significantly activated AVPV (but not ARC) KP neurons, as well as inducing cell activation in key limbic regions (including the MeA, BnST and cortical amygdala) [285]. Importantly, concomitant LH surges were also evident in those female rats exposed to male-soiled bedding, with maximal LH stimulation within 1-2 hours of the onset of bedding exposure [285].

Auditory processing: Certain male species, such as rodents, emit song-like ultrasonic vocalizations (USV) in order to communicate their motivational state, facilitate female approach behavior and ultimately promote reproduction [286]. Evidence reveals that these USVs promote fertility in female mice by activating hypothalamic KP neurons [287]. As part of these experiments, females were housed in a soundproof chamber and exposed to a sound file consisting of either male mice USVs or background noise (as a control sound) repeatedly for 20 minutes. This significantly increased the number of KP neurons expressing pCREB (an indicator of neural activation) in the ARC (but not the AVPV) after exposure to male USVs, compared with background noise. To provide

functional relevance for the enhanced neuronal activity, it was observed that a positive correlation existed between ARC KP neuronal activity and the duration of female searching behavior, suggesting that the female's approaching behavior towards USVs of male mice relates to the activation of KP neurons [287]. Collectively, these data suggest KP's key involvement in the mechanism by which USVs of male mice promote copulation in female mice by activating their approaching behavior.

Sexual partner preference: Ovariectomized and hormone-primed *Kiss1* KO female mice do not display male-directed preference [288]. In fact, an equivalent perturbation is observed following selective viral ablation of AVPV KP neurons [288]. Notably, in both experimental paradigms, normal male-directed sexual preference is rescued following a single peripheral injection of KP [288], highlighting site-specificity of AVPV KP neurons in the control of mate preference in female mice. Regarding the downstream pathways, exploiting a transgenic GnRH deficient mouse model (which progressively loses GnRH expression during adulthood) results in female mice displaying female rather than male-directed preference. Functionally, this behavioral deficit normalizes following a single peripheral injection of GnRH (but not KP as downstream GnRH lacking) [288], indicating that KP signals through GnRH to regulate sexual partner preference.

Copulatory and sexual behavior: In a sexually receptive female rodent, fertile copulation involves the adoption of a posture which facilitates intravaginal ejaculation to occur, termed lordosis [289]. Regarding this key reproductive behavior, both peripheral and central KP administration to female mice robustly stimulates lordosis [288]. Interestingly, when ovariectomized *Kiss1r* KO female mice are hormone-primed, they display normal lordosis [254], suggesting that the KP receptor may not be

essential for lordosis (given it is rescued by gonadal sex hormone replacement). In contrast, even when hormone-primed, ovariectomized *Kiss1* KO female mice fail to display lordosis behavior, whereas this deficit normalizes following a single peripheral injection of KP [288]. In terms of the neurocircuitry controlling lordosis, acute ablation of AVPV KP neurons results in a profound deficit in lordosis behavior in ovariectomized and hormone-primed female mice, whereas optogenetic stimulation enhances lordosis [288]. Using mutant female mice that lack GnRH secretion in adulthood, reveals that unlike male-directed preference (which is abolished), lordosis behavior is not affected [288], indicating that lordosis is independent of GnRH-signaling.

Viral tracing studies reveal that AVPV KP neurons communicate with two populations of neurons that express nitric oxide synthase (nNOS) in the ventrolateral part of the ventromedial hypothalamus (VMHvL) [288] and the paraventricular nucleus (PVN) [290]. This is pertinent given that female mice deficient in nNOS display a strong decrease in lordosis and whereas an injection of KP or GnRH fails to stimulate lordosis, a nitric oxide donor (SNAP+BAY) restores lordosis [288]. Moreover, administration of SNAP+BAY to *Kiss1* KO female mice also restores lordosis, confirming that nitric oxide acts downstream of KP neurons to mediate lordosis [288]. Recent experiments have sought to elucidate which neuronal population expressing nNOS are the target of AVPV KP-signaling. In these studies, central administration of KP or a nitric oxide donor (SNAP+BAY) into the VMHvL significantly increased lordosis, whereas administration of a nNOS inhibitor (I-NAME) decreased lordosis [291]. Moreover, central administration of KP into the PVN had no effect on lordosis, indicating that KP modulates lordosis behavior through nNOS neurons in the VMHvL [291].

<u>*In Vivo* Human Studies</u>

Unlike the aforementioned functional neuroimaging studies in healthy men investigating the effects of KP on sexual and emotional brain activity, there are currently no published studies in healthy women. However, a recent study examined KP's effects on sexual and attraction brain processing in premenopausal women with low sexual desire due to Hypoactive Sexual Desire Disorder (HSDD) [292] **(Table 1Q).** In response to erotic videos, KP administration was observed to deactivate the inferior frontal and middle frontal gyri (regions involved in inhibitory control [293,294]) and activate the postcentral and supramarginal gyri (areas known to be activated in the context of sexual arousal [295–297]). It is well-established that women with HSDD are characterized by specific alterations in the motivational component of men's perception [298]. It is therefore pertinent that in this patient cohort of women with HSDD, KP administration deactivated the temporoparietal junction (an area whose deactivation is linked with reducing negative perception of others and reducing self-consciousness [299]) in response to viewing male faces [292]. Of note, KP's enhancement of posterior cingulate activity in response to male faces was observed to correlate with reduced sexual aversion, providing behavioral and functional significance [292]. To what extent KP influences sexual brain processing and associated physiological and behavioral measures of sexual desire and arousal in postmenopausal women with HSDD is currently unknown but would be a fruitful area for study given its high prevalence [300].

Taken together, an explosion of experimental evidence reveals important neuromodulatory roles for KP-signaling in all aspects of reproductive behavior from regulating sexual partner preference and sexual motivation through to copulatory and sexual behaviors. In addition, clinical studies in men and patients with low sexual desire illustrate the emerging influence of KP in human sexual and emotional brain processing. Given these exciting data, future studies in broader patient cohorts

(such as different sexual identities and orientations) and other forms of sexual dysfunction (such as erectile dysfunction) are much warranted to provide further evidence for clinical applications of KP-based therapies in patients with common reproductive and psychosexual disorders.

VI. CLINICAL APPLICATIONS OF NEUROKININ B ANTAGONISM

VIA) Treating Polycystic Ovary Syndrome

Polycystic ovary syndorme (PCOS) is a heterogenous condition affecting 2-13%[112] of women of reproductive age and is currently diagnosed by the Rotterdam criteria [128]. PCOS is associated with adverse endocrine, reproductive, metabolic (insulin resistance, dyslipidemia) and psychological features [128]. Despite its high prevalence and significant clinical burden, current treatment strategies for PCOS are suboptimal as they rely on treatment of symptoms rather than the underlying pathophysiological process. The lack of mechanism-based treatments is attributable to the complex and unclear etiology of PCOS, and hence defining the causative factors driving PCOS pathogenesis has been of interest.

A cardinal feature of PCOS is androgen excess driven by increased gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) pulsatility [301]. As hypothalamic arcuate (ARC) kisspeptin-neurokininB-dynorphin (KNDy) neurons regulate GnRH pulse generation and express androgen receptors, KNDy neurons have been implicated in mediating the androgenic effects of PCOS **(Figure 5).** Indeed, neurokinin B (NKB) and kisspeptin (KP) gene expression are increased in some PCOS-like animal models, thus suggesting that overactivity of KNDy neurons is

responsible for the increased GnRH pulsatility observed in PCOS [35,131]. Additionally, patients with PCOS with inactivating variants in the NKB gene (*TAC3*) or NKB receptor (TACR3) have low baseline LH secretion and low LH pulse frequency [8]. However, women with functionally null *TAC3* can still conceive and mice lacking NKB (gene or receptor) can generate LH pulses, thus indicating that GnRH impairment is reduced rather than abolished [8,302]. This diminished action that NKB inhibition has on GnRH pulsatility is of therapeutic benefit as it enables GnRH pulsatile secretion to be normalized rather than terminated. Thus, there has been great interest in the use of NKB signaling blockade as a therapeutic agent in targeting the central pathophysiology of LH hypersecretion and hyperandrogenism in PCOS. Considering neurokinin B-3 receptors (NK3R) have a high binding affinity for NKB and are highly expressed in humans, antagonists of NK3R have been the preferential developmental agents for PCOS treatment [8].

Animal data

In peripubertal dihydrotestosterone (DHT)-induced PCOS mice, NK3R antagonism (MLE4901) improved several metabolic parameters (eg. adiposity, adipocyte hypertrophy, glucose tolerance), but failed to ameliorate reproductive phenotypes (eg. ovarian acyclicity) [303]. NK3R antagonist treatment reduced adipocyte area without affecting food intake, energy expenditure or locomotor activity, but altered metabolic status by utilizing carbohydrate as the predominant fuel source [303]. In parallel, NK3R antagonism also reduced circulating leptin levels [303]. Although NK3R blockade did not alter fasting glucose levels, NK3R antagonism reduced the effects of DHT induced hyperglycemia [303]. The lack of a reproductive phenotype may be due to KNDy neurons not being hyperactive in this model of PCOS (chronic DHT), as other models of androgenization (eg. pre-natal) do recapitulate KNDy neuronal overactivity. Alternatively, the dose of the NK3R antagonist

may have been inadequate and was unable to overcome the elevated androgens observed in this chronic DHT model.

Human data

In a randomized multicenter clinical trial, women with PCOS received the NK3R antagonist MLE4901 (also known as AZD4901) at doses of either 20mg/day, 40mg/day or 80mg/day; or placebo for 28 days [304] **(Table 2C).** Women receiving 80mg/day of MLE4901 demonstrated a 52% baseline-adjusted reduction in area under the curve of LH, a 79% reduction in basal LH secretion and an LH pulse decrease of 3.6 pulses/8hr, compared to placebo [304]. Similarly, total testosterone and free testosterone levels were reduced by 29% and 19%, respectively [304]. These effects were marked following 7 days of treatment and continued to be effective until the end of treatment (28 days) in women who did not ovulate during the study [304]. A more recent study using a similar dose of MLE4901 (40 mg orally twice a day for 7 days) demonstrated a reduction in LH secretion (from 6.5 to 4.0 IU/l), LH pulse frequency (from 0.8 to 0.5 pulses/h) and FSH levels (2.5 to 2 IU/l) compared to placebo in women with PCOS [141].

Another NK3R antagonist, fezolinetant (60mg QD or 180mg QD for 12 weeks), reduced the LH:FSH ratio and suppressed hyperandrogenism in women with PCOS [305]. Whilst both doses reduced LH and FSH throughout the study, only fezolinetant 180mg QD reduced testosterone levels at all timepoints, thus indicating a dose-dependent response [305]. Overall, fezolinetant 180mg/day reduced testosterone by 33%, LH by -10.17 IU/L and FSH by -1.46 IU/L, whilst fezolinetant 60mg/day reduced testosterone by 17% nmol/L, LH by -8.21 IU/L and FSH by -0.92 IU/L [305]. No changes were observed in estradiol (E2) and progesterone levels, endometrial thickness, follicle development or menstrual cycle irregularity over the 12-week study [305]. The

lack of ovulation may have been due to the increased suppressive effects of fezolinetant on NK3R signaling. To avoid this, a different dose or shorter duration of therapy of fezolinetant may be more successful in restoring ovulation. Overall, manipulation of neuroendocrine signaling with NK3R antagonism may provide novel therapeutic approaches to treat specific phenotypic features of PCOS.

V1B) Treating Uterine Disorders

Uterine fibroids and endometriosis are common disorders of the reproductive system affecting up to 80% and 15% of women of reproductive age, respectively [306,307]. Uterine fibroids are benign smooth muscle tumors of the uterus, whereas endometriosis is the presence of endometrial glands or stroma-like lesions outside of the uterine cavity [306,307]. Both conditions cause severe symptoms including abnormal uterine bleeding, chronic pelvic pain, and infertility [306,307]. Women with early age menarche and short menstrual cycle length are at high risk of developing these conditions, which suggests that continuous exposure of the endometrium and myometrium to estrogen is a key pathological driver of the disease [306,307]. Thus, suppressing E2 levels through downregulation of the hypothalamic-pituitary-gonadal (HPG) axis using GnRH modulators (agonists and antagonists), is a clinically validated therapeutic approach for the treatment of these disorders [308,309]. However, the approved duration of GnRH therapy is restricted due to its castrating effects and consequent menopausal-like symptoms, including bone loss and vasomotor hot flashes [310,311]. An ideal therapy would be one that offers a more refined modulation of the HPG axis and lowers estrogenic drive to endometriosis and fibroid cell growth without causing the adverse events that are associated with current treatments. Indeed, lowering E2 levels to a range between 110-184 pmol/L has been recommended to be effective in reducing the symptoms of uterine fibroids and

endometriosis [312,313]. One such novel therapeutic approach is to use NKB receptor antagonists to reduce LH whilst preserving FSH secretion **(Figure 5).**

Animal data

In ovariectomized ewes, NK3R antagonism (MRK-08) decreased LH pulse frequency whilst maintaining FSH concentrations [314]. Likewise, in castrated non-human primates *(Macaca fascicularis)*, repeated daily dosing of the NK3R antagonist (ESN364) decreased plasma LH levels, inhibited the LH surge, but did not change FSH concentrations [315]. NK3R blockade also lowered E2 levels in a dose-dependent manner, although nadir levels of E2 were maintained well above menopausal levels [315].

Human data

Several NK3R antagonists have also shown similar patterns of gonadotropin secretion (reduced LH with preserved FSH) in healthy women **(Table 2).** For instance, AZD4901 (also known as MLE4901, formerly AZD2624) reduced E2 levels, endometrial thickness and folliculogenesis [316] during the follicular phase. In the early mid-follicular phase, AZD4901 resulted in reduced basal LH levels and a delayed LH-surge (by 7 days), without altering LH pulse frequency [317]. Another NKB antagonist, Fezolinetant (ESN364), led to a dose-dependent (doses 40-120mg once daily for 21 days) reduction in LH but not FSH, and reduced endometrial thickness. The dual NK1,3R antagonist elinzanetant (40, 80, and 120 mg once daily) administered orally over a full menstrual cycle safely reduced serum LH in a dose-dependent manner, although in a non-significant trend [21]. Progesterone levels consistent with ovulation were reduced, especially during the luteal phase of the cycle [21]. Moreover, the highest dose of 120mg of elinzanetant once a day lowered E2 to a

level ideal for treating uterine fibroids and endometriosis, and lengthened menstrual cycles from 27 to 34 days [21]. Thus, NKB antagonism is a promising treatment option and studies are now required to evaluate their use in women with uterine disorders.

VIC) Treating Menopausal hot flashes

Menopause is the complete cessation of menstruation due to ovarian insufficiency and occurs between the ages of 45-55 years [318]. Hot flashes and sweats, collectively known as vasomotor symptoms (VMS), are the most debilitating symptom described by over 80% of women during the menopausal transition [20]. On average, symptoms last for seven years, but they can persist, with 1 in 10 women experiencing symptoms for up to 12 years [318]. Although hormone replacement therapy (HRT) or menopausal hormone therapy (MHT) is an effective treatment for VMS, it is contraindicated in women at high risk of breast and endometrial cancer as well as thromboembolic disease [20]. Therefore, alternative treatments that can safely and effectively alleviate VMS are desired.

The median preoptic nucleus (MnPO) of the hypothalamus is the control center for body temperature regulation and downstream thermoregulatory pathways [19]. This thermoregulatory center is dysregulated during the menopause and results in the activation of inappropriate heat dissipation responses including VMS [19]. As ARC KNDy neurons project onto both NK3R expressing neurons in the MnPO and GnRH neurons in the median eminence, they have been implicated in the pathogenesis of menopausal VMS **(Figure 5)** [19].

Animal data

E2 deficiency increases LH pulsatility and hot flashes and this close temporal relationship between temperature and reproduction is mediated by KNDy neuronal activity [19]. Indeed, whilst ovariectomy (E2 deficient state) increased ARC KNDy gene expression and neuronal hypertrophy, E2 supplementation reversed it [24,319,320], suggesting that E2 withdrawal leads to increased KNDy expression in rodents. Furthermore, tract tracing studies revealed that KNDy neurons project to the MnPO (thermoregulatory center) and GnRH axons in the median eminence of the hypothalamus [321]. The MnPO, which is altered by E2 and temperature, also express NK3R mRNA and protein [322], thus indicating KNDy neurons influence heat dissipation responses through projections to NK3R-expressing neurons in the MnPO. Notably, direct activation of NK3R in the MnPO by a NKB agonist (senktide) reduced core body temperature and activated heat dissipation effectors (tail skin vasodilatation) [323]. Likewise, NKB agonist administration increased tail skin vasodilatation in ovariectomized (OVX) mice, however, this effect was lost following E2 replacement, suggesting that E2 lowers the sensitivity of the thermoregulatory center to NKB/ NK3R signaling [324]. Furthermore, selective toxin ablation of ARC KNDy neurons reduced both cutaneous vasodilatation and LH secretion in female mice [325], thus supporting the role of KNDy neurons in mediating temperature and reproduction regulation. Additionally, whilst E2 replacement restored body temperature regulation in OVX rats with intact KNDy neurons, this was not observed in KNDy ablated OVX rats [325]. These studies strongly support NKB- and NK3R signaling as important mediators of postmenopausal flushing and therefore this pathway could be targeted for future therapies.

Human data

KNDy neurons in the infundibular nucleus of the hypothalamus are hypertrophied and overexpressed during E2 deficient states such as the menopause [326]. Furthermore, genome-wide association studies revealed that menopausal women with VMS had single nucleotide polymorphisms in the TACR3 locus, the gene that encodes NK3R [327]. Additionally, NKB has been shown to induce hot flushing in healthy women to a similar degree as those experienced by women in the menopause [328]. These data indicate that antagonism of NKB/ NK3R signaling could provide a novel, non-hormone-based approach for the management of menopausal hot flashes.

The NK3R antagonist, MLE4901 (oral pavinetant), was the first drug to demonstrate a reduction in the number (by 45%) and severity of weekly hot flashes experienced by menopausal women [329] **(Table 2D).** Another NK3R antagonist, fezolinetant (ESN364, oral 90mg twice daily for 12 weeks), reduced VMS scores (fezolinetant: -26.5 vs placebo: -12.2) and improved VMS severity and quality-of-life measures [330]. Furthermore, all doses of fezolinetant (30mg once daily to 90mg twice daily) except the lowest one, reduced moderate / severe VMS (>2 per day) by 4 and 12 weeks [331]. A more recent phase 3 trial involving fezolinetant 30mg or 45mg once daily, reduced the severity of VMS at week 4 (-0.15, -0.19) and week 12 (-0.24, -0.2). Furthermore, the improvements in VMS frequency and severity were sustained over 52 weeks [332]. The dual NK1R/NK3R antagonist NT-814 (elinzanetant, dose 150mg once daily for 2 weeks) also reduced hot flashes (-84%) versus placebo (37%) in menopausal women [333]. Whilst NK1R antagonism alone is ineffectual in attenuating VMS, its anti-emetic and anxiolytic effects may benefit the poor sleep quality that women experience during the menopause [334]. Indeed, nocturnal awakening due to night sweats in menopause was reduced following NT-814 (-81%) compared to placebo (32%) [333].

NK3R antagonists display distinct side-effect profiles. For instance, MLE4901 was discontinued following its association with transient rises in liver enzymes. Although ESN364 and NT-814 have

been associated with headaches, gastrointestinal disturbance and fatigue, no clinically significant impact on liver enzymes have been reported. Furthermore, E2 levels [330] and endometrial thickness or hyperplasia [331] remain unaffected, indicating that NK3R action is independent of effects on ovarian hormones [330]. This data demonstrates that NK3R antagonists provide a safe and efficacious treatment option for managing menopausal women with VMS.

CONCLUSION

Kisspeptin (KP) and upstream neurokinin B (NKB) govern the reproductive endocrine axis through their critical role in regulating gonadotropin-releasing hormone (GnRH) neuronal activity and stimulating GnRH pulsatile secretion. Their fundamental role in reproductive hormone secretion has opened several avenues for their use in diagnosing and treating several pubertal, reproductive, metabolic, bone, and behavioral disorders.

For instance, KP induces lower luteinizing hormone (LH) rises in patients with congenital hypogonadotropic hypogonadism (CHH) than in those with constitutional delay of growth and puberty (CDGP) or in healthy controls. Additionally, higher circulating KP levels are observed in central precocious puberty (CPP), thus highlighting KP's utility in diagnosing puberty-related disorders.

KP levels rise linearly with advancing pregnancy and therefore it could be developed as a promising marker for predicting pregnancy complications. In particular, the reduced KP levels associated with miscarriage and intra-uterine growth restriction (IUGR) could enable its use in risk-stratifying women presenting with possible complications during pregnancy.

Metabolic fatty liver disease/ non-alcoholic steatohepatitis (MAFLD / NASH) is associated with upregulated hepatic-KP signaling and raised circulating KP concentrations; therefore, KP

measurements could potentially be used to discriminate patients with MAFLD/ NASH from healthy controls. Thus, assessing gonadotropin responses to KP or measuring circulating KP levels directly, could aid in the diagnosis of common disorders. However, further studies to validate KP's diagnostic accuracy are necessary.

KP-based therapies have been extensively explored over the past decade. In hypogonadal disorders such as hypothalamic amenorrhea (HA), hyperprolactinemia, and diabetes-induced hypogonadism, KP induces gonadotropin rises that could restore reproductive function. KP and KP receptor agonists also mirror the physiological ovulatory mid-cycle LH surge and thus could be used therapeutically to induce oocyte maturation during *in vitro* fertilization (IVF) protocols in women seeking fertility. Further studies evaluating KP's safety and efficacy in comparison to current agents, especially in women at high risk of ovarian hyperstimulation syndrome (OHSS), are warranted. The intricate connections between KP neurons and hypothalamic neurons involved in appetite regulation has implicated a potential role for KP in obesity-related disorders. Although absence of KP has been associated with increased bodyweight, KP's effects on appetite in animals and humans remain unclear.

KP receptor agonism has also been shown to alleviate hepatic steatosis and fibrosis and thus could play an important role in suppressing the progression of hepatic lipogenesis in patients with MAFLD. With regards to bone metabolism, KP enhances osteoblastogenesis and inhibits osteoclast activity *in vitro*, and therefore could be used as a complementary treatment for osteoporosis. KP also has potential as a therapy for men and women with psychosexual dysfunction, as it has been shown to enhance sexual brain processing and associated physiological and behavioral measures of sexual function in patients with distressing low sexual desire.

NKB antagonism, in particular potent NK3 receptor antagonists, have emerged as an advantageous therapeutic tool for treating PCOS, uterine fibroids and endometriosis through their unique ability to partially suppress (and not abolish) the reproductive endocrine axis. Additionally, the critical interaction between NKB and the hypothalamic thermoregulatory center has resulted in the development of NKB antagonists as efficacious non-hormonal treatment options for women with menopausal vasomotor symptoms.

Since the pivotal discoveries of KP and NKB's role in reproduction in 2003 and 2009 respectively, there has been an abundance of basic science and translational studies demonstrating their function in the pathophysiology of several disorders including reproduction, metabolism, bone, and behavior. The wealth of evidence accumulated over the past two decades, alongside the development of potent KP and NKB antagonist-based therapies, has provided the opportunity for these peptide hormones to be investigated as promising diagnostic and management tools in the coming years.

FIGURE LEGENDS

FIGURE 1: Kisspeptin and neurokinin B in the regulation of the hypothalamic-pituitary-gonadal (HPG) axis

Kisspeptin (KP) is released from the Preoptic Area (POA) (equivalent to rostral periventricular area of the third ventricle, RP3V, in non-humans) and infundibular nucleus (arcuate, ARC, nucleus in non-humans) of the hypothalamus. The KP neurons in the infundibular nucleus co-express neurokinin B (NKB) and dynorphin (known as KNDy neurons) and are involved in the autosynaptic regulation of pulsatile KP secretion via the NKB receptor (NK3R) and kappa opioid peptide receptor (KOR) respectively. Dynorphin inhibits, whereas NKB stimulates KP release.

Following KP's release from the hypothalamus, KP stimulates the hypothalamic gonadotropin releasing hormone (GnRH) neurons to release GnRH in a pulsatile manner, which stimulates anterior pituitary production of gonadotropins (luteinizing hormone (LH), follicle stimulating hormone (FSH)) and subsequent production of gonadal (testicular/ovarian) sex-steroids (Estrogen; E2, Testosterone; T). The gonadotropins' effect on the ovary stimulates follicular development, oocyte maturation and ovulation. The KNDy neurons in the infundibular nucleus mainly receive negative feedback (red) (E2, T) from sex-steroids, whereas KP neurons in the pre-optic area receive positive feedback from estrogen in females (green) (high E2), which is involved in the pre-ovulatory LH surge. Sex-steroid communication with the pre-optic area has not yet been fully established in males.

Abbreviations: Kisspeptin' KP, Neurokinin B; NKB, Follicle Stimulating Hormone; FSH, Gonadotropin Releasing Hormone; GnRH, Kisspeptin receptor; KISS1R, kappa opioid peptide receptor; KOR, Luteinizing Hormone; LH, Neurokinin 3 receptor; NK3R, Estrogen; E, Progesterone; P, Rostral periventricular area of the third ventricle; RP3V, Testosterone; T, Preoptic Area; POA. Figure created with BioRender.com

FIGURE 2: *KISS1* and *KISS1R* human gene expression in areas where kisspeptin signaling has well-identified roles

Expression is abundant in other areas of the human body, not illustrated in Figure 2, in which the full role of kisspeptin (KP)-signaling has yet to be elucidated. This widespread distribution of *KISS1* and *KISS1R*, reflects the pleiotropic action of KP, beyond reproduction. In humans, the tissue distribution of *KISS1* and *KISS1R* has been identified using RT-PCR methods. *KISS1*

mRNA is predominantly expressed in the placenta, with the next highest level in the testis, and moderate levels in the pancreas, liver, uterus, gonads and small intestine). *KISS1* mRNA is also strongly expressed in bone, in particular the osteoblasts. *KISS1R* expression is particularly abundant in the placenta, pituitary, spinal cord, liver, pancreas and bone (osteoblasts and osteoclasts), but expressed at lower levels in other tissues, such as the stomach, uterus, small intestine, thymus, spleen, lung, gonads, heart, kidney, adrenal gland, bone and fetal liver. Both *KISS1* and *KISS1R* are also expressed in the brain, and in particular the human hypothalamus, as well as extra-hypothalamic regions, such as the amygdala, caudate nucleus, cerebellum, cingulate gyrus, globus pallidus, hippocampus, medial frontal gyrus, nucleus accumbens, para-hippocampal gyrus, putamen, spinal cord, striatum, substantia nigra, superior frontal gyrus and thalamus, as localized by RT-PCR.

Abbreviations: KISS1; kisspeptin gene, KISS1R; kisspeptin receptor gene, reverse transcription polymerase chain reaction; RT-PCR. Figure created with BioRender.com

FIGURE 3: Kisspeptin receptor induces differential responses in downstream signaling

Kisspeptin (KP) has a high-affinity binding site for the human KP receptor and induces a biphasic response in downstream signaling, with an acute (lasting ~5 min) and prolonged response (lasting >30 minutes). *KISS1R* (coupled to Gαq/11) triggers the activation of phospholipase C (PLC) and subsequent recruitment of secondary intracellular messengers, inositol triphosphate (IP3) and diacylglycerol (DAG), which in turn mediate intracellular calcium release. DAG additionally activates protein kinase C (PKC) and induces downstream phosphorylation of extracellular signal-related kinase (ERK) 1 and 2. Kisspeptin binding results in the recruitment of β-arrestin and GPCR

serine/threonine kinases (GRK2) which leads to desensitization and internalization of the kisspeptin receptor (through uncoupling of Gαq/11). β-arrestin traffics the desensitized *KISS1R* to the clathrin-coated pit resulting in sequestration which results in β-arrestin-dependent signaling. Internalized *KISS1R* eventually dissociates from β-arrestin and the majority of kisspeptin receptors become resensitized and traffic back to the cell surface, thus maintaining a continuous pool of receptors at the cell surface which are ready to signal while a lesser population of *KISS1R* are targeted for degradation.

Abbreviations: KISS1R; kisspeptin receptor gene, PLC; phospholipase C, IP3; inositol triphosphate, DAG; diacylglycerol, PKC; protein kinase C, ERK; extracellular signal-related kinase, GRK2; GPCR serine/threonine kinases. Figure created with BioRender.com

FIGURE 4: Role of kisspeptin in disorders of puberty

Puberty is triggered by the pulsatile secretion of gonadotropin releasing hormone (GnRH) and subsequent downstream activation of the hypothalamic-pituitary-gonadal (HPG) reproductive axis. The pulsatile secretion of GnRH requires adequate development and migration of GnRH neurons from the olfactory bulb to the hypothalamus. The HPG axis is transiently activated at two distinct phases; during early developmental life, termed 'mini puberty', and at the onset of puberty. Kisspeptin (KP) stimulates an LH response during the later stages of puberty (Tanner stage 5) thus suggesting *KISS1R* sensitivity on GnRH neurons develops during the later part of puberty. *KISS1* gain in function variants can lead to premature activation of the HPG axis resulting in early central precocious puberty (CPP). Kisspeptin (KP) levels are increased in CPP versus age-matched healthy controls and thus KP has potential in aiding in the diagnosis of early puberty. *KISS1* loss

in function variants cause aberrations in GnRH neuronal development or migration and impair GnRH secretion resulting in congenital hypogonadotropic hypogonadism (CHH) and delayed puberty. Constitutional delay of growth and puberty (CDGP) is another common cause of delayed puberty and can be challenging to accurately differentiate from CHH. Kisspeptin (KP), a potent stimulator of GnRH and luteinizing hormone (LH) release, induces differential responses in CDGP (increased LH) and CHH (absent/ reduced LH) and thus can aid in the diagnosis of delayed puberty.

Abbreviations: Gonadotropin releasing hormone (GnRH); central precocious puberty (CPP); Kisspeptin (KP); congenital hypogonadotropic hypogonadism (CHH); Constitutional delay of growth and puberty (CDGP); luteinizing hormone (LH). Figure created with BioRender.com

FIGURE 5: Therapeutic potential of kisspeptin and neurokinin B in female reproductive disorders

Activation of hypothalamic kisspeptin (KP) neurons directly stimulates gonadotropin releasing hormone (GnRH) release and regulates reproductive hormone secretion. Absent or reduced GnRH and luteinizing hormone (LH) pulses observed in hypothalamic amenorrhea (HA) and hyperprolactinemia can be restored using exogenous KP. Whilst GnRH/ LH pulsatility is retained in patients with endometriosis/ uterine fibroids, patients with polycystic ovary syndrome (PCOS) have high pulsatility. During the menopause, increased kisspeptin-neurokinin b-dynorphin (KNDy) neuronal activity results in very high GnRH/ LH pulses and induction of vasomotor symptoms through dysregulation of the thermoregulatory center. Considering NKB antagonism partially suppresses (but does not abolish) the reproductive endocrine axis, NK3R antagonists have

been developed for the therapeutic potential of these disorders. NK3R antagonism can be used to treat endometriosis/ uterine fibroids (by reducing estradiol; E2), PCOS (by reducing androgens) and menopausal hot flashes (by reducing vasomotor symptoms).

Abbreviations: Kisspeptin (KP); Gonadotropin releasing hormone (GnRH); luteinizing hormone (LH); hypothalamic amenorrhea (HA); Polycystic ovary syndrome (PCOS); kisspeptin-neurokinin b-dynorphin (KNDy); estradiol (E2). Figure created with BioRender.com

FIGURE 6: The utility of kisspeptin in the prediction of pregnancy complications

Kisspeptin (KP) regulates trophoblast invasion and placentation during pregnancy and has emerged as a promising biomarker to predict several adverse pregnancy complications. The *KISS1* gene is abundantly expressed in syncytiotrophoblasts, whereas its receptor (*KISS1R*) is expressed in both cytotrophoblasts and syncytiotrophoblasts. Circulating KP levels increase linearly in healthy pregnancy but are reduced in miscarriage during early pregnancy. KP can accurately predict the risk of miscarriage with average/ above average levels of KP being associated with a <1% risk of miscarriage. KP levels are reduced in fetal growth restriction (FGR) and gestational diabetes mellitus (GDM) and raised in pre-eclampsia (PET) during the later stages of pregnancy. Abbreviations: Kisspeptin (KP); kisspeptin gene (*KISS1*); kisspeptin receptor (*KISS1R*); fetal growth restriction (FGR); gestational diabetes mellitus (GDM), pre-eclampsia (PET). Figure created with BioRender.com

FIGURE 7: Effects of liver-specific Kiss1r knockout and enhanced kisspeptin signaling

Liver-specific *Kiss1r* knockout mice model placed on high fat diet exhibited increased lipogenesis, triglyceride synthesis and reduced mitochondrial β oxidation compared to controls. This resulted in increased triglyceride levels, serum alanine transaminase levels (indicating hepatocellular injury) and hepatic steatosis. Increased body weight and reduced energy expenditure were observed. Higher fasting glucose and basal insulin levels, indicating glucose intolerance and insulin resistance was also observed. Moreover, markers of inflammation and early stages of fibrosis were upregulated.

Effects of enhanced kisspeptin signaling: Wildtype mice were placed on high fat diet for 6 weeks prior to administration of MVT-602, a kisspeptin receptor agonist, for 5 weeks on high fat diet. MVT-602 alleviated hepatic steatosis and metabolic deterioration through improvements in insulin sensitivity, lower basal insulin levels, reduced triglyceride and ALT levels. MVT-602 treated mice had slightly lower body weight compared to controls with increased energy expenditure in the light phase. Mechanistically MVT-602 treatment under high fat diet conditions significantly reduced triglyceride synthesis, increased lipolysis and mitochondrial β oxidation compared to controls. Markers of inflammation and early stages of fibrosis were downregulated.

Abbreviations: ALT, Alanine transaminase; Kiss1r, Kisspeptin receptor; TG: triglyceride. Figure created with BioRender.com

FIGURE 8: The effects of kisspeptin signaling on key reproductive behaviors in rodents, sheep and humans including olfactory processing, sexual partner preference, copulatory behavior and arousal and bonding

Kisspeptin is widely expressed in limbic and paralimbic regions of the brain, that are involved in reproductive behaviors. Olfaction: KP expression and activity increased in several brain regions in response to opposite sex olfactory cues in male mice and female mice and ewes. In humans, KP administration enhanced limbic brain activity when men were exposed to a pleasant feminine scent. Sexual partner preference and bonding: Studies showed that the KP MePD neurons regulate partner preference in male mice, whereas intraperitoneal and intranasal administration of KP to male rats increases sexual motivation. When peripheral KP was administered to Kiss KO female mice, normal male-directed sexual preference was restored. In healthy heterosexual men, peripheral KP administration increased brain activity in aesthetic brain regions in response to viewing female faces. Copulatory behavior and arousal: KP stimulation in the MePD resulted in erections in male rats, whereas both peripheral and central KP administration to female mice robustly stimulated lordosis. In healthy heterosexual men, peripheral KP administration enhanced limbic brain activity when exposed to visual sexual stimuli. KP administration to males with HSDD deactivated brain regions involved in self- monitoring and introspection, and increased brain activity in sexual arousal centers, in response to watching erotic videos in the fMRI scanner. KP administration also led to increases in penile tumescence. KP administration to pre-menopausal women with HSDD, deactivated brain regions involved in inhibitory control and activated areas known to be activated in the context of sexual arousal in response to erotic visual cues.

Abbreviations: AVPV; anteroventral periventricular nucleus, fMRI; functional magnetic resonance imaging, gonadotropin releasing hormone, KP; kisspeptin, KO; knock out, MePD; posterodorsal subnucleus of the medial amygdala, HSDD; hypoactive sexual desire disorder. Figure created with BioRender.com

1 **TABLE 1: Clinical trials involving kisspeptin**

Author	Study design	Cohort	Intervention	Results
A: KISSPEPTIN IN HEALTHY MEN				
Author	**Study design**	**Cohort**	**Intervention**	**Results**
Dhillo et al. (2005) [13]	Double-blind placebo-controlled crossover	6 men	KP54 (IV infusion 4pmol/kg/min for 90 min) versus vehicle	KP54 increased LH (by 2.6-fold), FSH (by 1.2-fold) and testosterone.
Chan et al. (2011) [78]	Prospective study	13 men	Baseline sampling (10min for 6hrs) followed by KP10 (IV bolus 0.24 nmol/kg)	KP10 induced immediate LH pulses, regardless of the timing of the previous endogenous pulse. KP10 induced larger amplitude pulses than endogenous pulses (amplitude 5.0 ± 1.0 vs. 2.1 ± 0.3 mIU/ml).
George et al. (2011) [75]	Placebo-controlled	6 men (acute studies) 4 men (chronic studies)	KP10 (IV bolus 0.01, 0.03, 0.1, 0.3, 1.0, and 3.0 µg/kg) versus vehicle Baseline sampling (10min for 9hrs) followed by bolus KP10 (IV bolus 3.0 µg/kg) then (IV infusion 1.5 µg/kg/hr for 22.5hrs)	KP10 (IV bolus 1 µg/kg) induced max LH response (4.1 ± 0.4 to 12.4 ± 1.7 IU/L) KP10 (IV infusion 1.5 µg/kg/hr) increased - LH (5.2 ± 0.8 to 14.1 ± 1.7 IU/L) - LH pulse frequency (0.7 ± 0.1 to 1.0 ± 0.2 pulses/hr)
Jayasena et al. (2011) [76]	Single-blind placebo-controlled	4-5 per group	KP10 (IV bolus at 0.3, 1.0, 3.0, or 10 nmol/kg) versus vehicle	KP10 elevated LH, FSH and testosterone levels at doses as low as 0.3 and 1.0 nmol/kg, respectively

Jayasena et al. (2015) [32]	Single-blind placebo-controlled	5 men	KP10, KP54, GnRH or vehicle (IV infusion 0.1, 0.3 and 1.0 nmol/kg/hr for 3hrs)	Serum LH and FSH ~ 3-fold higher during GnRH versus KP10. Serum LH and FSH ~ 2-fold higher during GnRH versus KP54.
B: KISSPEPTIN IN HEALTHY PRE-MENOPAUSAL WOMEN				
Author	**Study design**	**Cohort**	**Intervention**	**Results**
Dhillo et al. (2007) [14]	Double-blind placebo-controlled	8 women	KP54 (SC bolus 0.4 nmol/kg)	KP54 increased mean LH ± SEM (IU/L) during the follicular (0.12 ± 0.17), pre-ovulatory (20.64 ±2.91) and luteal (2.17 ± 0.79) phases of the menstrual cycle
Jayasena et al. (2011) [76]	Single blind placebo-controlled	4-5 per group	KP10 (IV bolus 1-10 nmol/kg) (SC bolus 2-32 nmol/kg) (IV infusion 20-720 pmol/kg/min) KP54 (IV bolus 1 nmol/kg)	KP10 (all doses and routes) did not alter LH and FSH in the follicular phase of the menstrual cycle KP10 (IV bolus 10nmol/kg) increased mean AUC LH (30.3 ± 7.7 h·IU/L) and FSH (6.9 ± 0.9 h·IU/L) in the preovulatory phase
Chan et al. (2012) [97]	Prospective study	3-14 per group	KP 112-121 (IV bolus 0.24, 0.72 nmol/kg)	KP112-121 induced higher LH responses and LH pulses in the luteal and preovulatory phases, but not the early-mid follicular phase of the menstrual cycle
George et al. (2012) [335 335]	Prospective study	10 women	KP10 (IV bolus 0.3 µg/kg)	KP10 increased LH but not FSH during early follicular phase of the menstrual cycle

Jayasena et al. (2013) [79]	Randomised single-blinded placebo-controlled trial	6 women	KP54 (SC bolus 0.30, 0.60 nmol/kg) versus vehicle	KP54 increased mean LH pulses (KP54; −0·17 ± 0·54, saline; +2·33 ± 0·56) during the follicular phase
Jayasena et al. (2013) [80]	Prospective single-blinded, placebo-controlled 1-way crossover trial	5 women	KP54 (SC bolus 6.4 nmol/kg, twice daily, during days 7 to 14 of menstrual cycle) versus vehicle.	KP54 does not cause tachyphylaxis KP54 induced a shorter menstrual cycle length (d26.8 vs d28.6) an earlier LH peak (d13 vs d15.2) and an earlier luteal phase vs saline (d15.8 vs d18) versus vehicle
Narayanaswamy et al. (2016) [81]	Prospective single-blinded placebo-controlled trial	4 women	KP54 (SC infusion 0.3-1.0 nmol/kg/hr for 8hrs) during early follicular phase of 4 menstrual cycles	KP54 induced a mean rise in LH (>8 IU/l) KP54 positively correlated with baseline E2 levels (KP54 dose of 1.0 nmol/kg/hr → 100pmol/l rise in baseline E2 associated with a 1·0 IU/L increase in LH)
Abbara et al. (2020) [82]	Single-blinded randomised controlled trial	9 women	MVT-602 (SC bolus 0.01, 0.03 nmol/kg) KP-54 (SC bolus 9.6 nmol/kg) during early follicular phase	MVT-602 and KP54 had similar LH amplitude rises LH peak delayed with MVT-602 vs KP54 (21.4 vs 4.7 hrs) AUC of LH exposure increased with MVT-602 vs KP54 (169 vs. 38.5 IU·h/L) MVT-602 induced a longer duration of GnRH neuronal firing than KP54 (115 vs 55 min)

C: KISSPEPTIN IN DELAYED PUBERTY				
Author	**Study design**	**Cohort**	**Intervention**	**Results**
Chan et al. (2014) [100]	Longitudinal cohort study, proof of concept	11 CHH (adult) 1 with reversal of CHH	KP10 (IV bolus 0.24 nmol/kg) GnRH (IV bolus 75 ng/kg)	KP10 (unlike GnRH) failed to induce an LH response in CHH, but produced an LH response in reversal of CHH
Lippincott et al. (2016) [101]	Single-blinded randomised controlled trial	4 with reversal of CHH 2 with relapsed CHH	KP10 (IV bolus 0.24-2.4 nmol/kg) GnRH (IV bolus 75ng/kg)	KP10 stimulated LH pulses in reversal of CHH (within 30min) but not in relapsed CHH
Chan et al. (2020) [103]	Longitudinal cohort study	16 with delayed puberty	KP10 (IV bolus 0.313 µg/kg) GnRH (IV bolus 75 ng/kg)	KP10 increased LH in CDGP (≥ 0.8 mIU/mL) but not in CHH (≤ 0.4 mIU/mL)
Abbara et al. (2021) [102]	Single-blinded randomised controlled trial	21 CHH 21 Controls	KP54 (IV bolus 6.4 nmol/kg) GnRH (IV 100mcg)	KP54 had reduced LH responses in CHH (0.4 iU/L) than controls (12.5 iU/L), and had an AUCROC of 100% (95% CI 100-100%) to differentiate CHH from healthy
D: KISSPEPTIN IN PRECOCIOUS PUBERTY				

Author	Study design	Cohort	Intervention	Results
Cintra et al. (2021) [112]	Systematic review	Systematic review and meta-analysis 316 CPP 251 controls	KP measurement	KP increased in CPP vs controls (Std MD and [95% CI] = 1.53 [0.56-2.51]) KP positively correlated with age and was associated with precocious thelarche
Vuralli et al. (2023) [113]	Cross-sectional study	51 CPP 48 PT 42 controls	KP measurement (ng/ml)	KP increased in CPP (0.43 ± 0.16) vs PT (0.26 ± 0.10) vs controls (0.18 ± 0.07)
E: KISSPEPTIN IN HYPOTHALAMIC AMENORRHOEA				
Author	**Study design**	**Cohort**	**Intervention**	**Results**
Podfigurna et al. (2020) [123]	Prospective cohort	HA: 58 low-LH 13 normal-LH	KP measurement (ng/ml)	KP reduced in HA women with low-LH (1.7 ± 0.1) versus normal-LH (2.6 ± 0.3)
Podfigurna et al. (2020) [123]	Prospective cohort	41 HA 40 Controls	KP measurement (ng/ml)	KP reduced in HA (0.17 ± 0.11) versus controls (0.3 ± 0.36)
Hofmann et al. (2017) [125]	Prospective cohort	38 HA (anorexia)	KP measurement	KP negatively correlated with physical activity ($r = -0.41$)
Jayasena et al. (2009) [43]	Prospective, randomized, double-blinded	10 HA	KP54 (SC bolus 6.4 nmol/kg, twice daily for 2 weeks) versus vehicle	Acute KP54 (after 4hrs) increased LH (to 24 IU/L) and FSH (to 9.1 IU/L) Chronic KP54 (after 2wks) lowered LH (to 1.5 U/L) and FSH (to 0.5 IU/L) due to tachyphylaxis

Jayasena et al. (2010) [43]	Randomized, double-blinded, placebo-controlled	20 HA	KP54 (SC bolus 6.4 nmol/kg, twice weekly for 8 weeks)	KP54 (after 1d) increased LH (to 21.5 IU/L) and FSH (to 6.4 IU/L) KP54 (after 2wks) reduced LH (to 10 IU/L) and FSH (to 2.7 IU/L) KP54 (after 4wks) maintained LH (9 IU/L) and FSH (2.6 IU/L) KP54 (after 6wks) maintained LH (8.9 IU/L) and FSH (2.4 IU/L) KP54 (after 8wks) maintained LH (7.9 IU/L) and FSH (2.7 IU/L)
Jayasena et al. (2014) [114]	Randomised single-blinded placebo-controlled	5 HA	KP54 (IV infusion 0.01-0.3 nmol/kg/h, for 8hrs; 1.0 nmol/kg/h for 10hrs)	Highest dose of KP54 increased LH greater than 10-fold vs placebo (Placebo 1.26 ± 0.56, KP54 15.42 ± 3.57 IU/L) Highest dose of KP-54 increased LH pulses by 3-fold (no. of LH pulses over 8hrs: Placebo 1.6 ± 0.4, KP54 5.0 ± 0.5)
Abbara et al. (2020) [82]	Single-blinded RCT	6 HA 9 Controls	MVT-602 (SC bolus 0.03 nmol/kg)	MVT-602 increased LH sooner in HA (6.2 hrs) vs controls (15.1hrs) MVT-602 increased FSH and E2 levels in HA
F: KISSPEPTIN IN POLYCYSTIC OVARY SYNDROME				
Author	**Study design**	**Cohort**	**Intervention**	**Results**
Tang et al. (2019) [133]	Systematic literature review	12 studies	KP measurement	KP increased in PCOS than controls in 9 studies
Varikasuvu et al. (2019) [135]	Meta-analysis	23 studies	KP measurement	KP increased in PCOS than controls (Std MD and [95% CI] = 0.47 [0.17 to 0.77]) Diagnostic OR 13.71, AUC 0.835 to differentiate PCOS from controls

Ibrahim et al. (2020) [336]	Prospective	60 PCOS 40 Controls	KP measurement (ng/ml)	KP increased in PCOS (1.79 ± 0.98) than controls (1.05 ± 0.86)
Akad et al. (2022) [137]	Prospective case-control	37 PCOS 24 Controls	KP measurement (pg/ml)	KP increased in PCOS (130.5) than controls (76.2), 95% CI 7.55 – 11.50
Romero-Ruiz et al. (2019) [140]	Pilot exploratory cohort	12 PCOS	KP54 (SC bolus 3.2-12.8 nmol/kg for 21 days)	KP54 increased LH (from 10.8 to 13.4 IU/L) and E2 levels, but did not change FSH
Skorupskaite et al. (2020) [141]	Single-blinded placebo-controlled trial	15 PCOS	KP10 (IV infusion 4 µg/kg/h for 7 hrs)	KP10 increased LH (from 5.2 to 7.8 IU/L) and E2 levels, but did not change FSH
Abbara et al. (2020) [82]	Single-blinded RCT	6 PCOS 9 Controls	MVT-602 (SC bolus 0.01- 0.03 nmol/kg)	MVT-602 did change LH, FSH or E2 concentrations in PCOS
G: KISSPEPTIN IN HYPERPROLACTINAEMIA				
Author	**Study design**	**Cohort**	**Intervention**	**Results**
Millar et al. (2017) [151]	Prospective exploratory study	2 women with high PRL	KP10 (IV infusion 1.5 mg/kg/h for 12hrs) versus vehicle	KP10 increased LH from 5.3 to 25.4 IU/L and from 1.22 to 5.2 IU/L in each patient
Hoskova et al. (2022) [152]	Prospective study	11 high PRL (F)	KP112–121 (IV bolus 0.24 nmol/kg, every hr for 11hrs)	KP112-121 increased LH pulses from 4.5 ± 0.9 to 7.5 ± 0.5 pulses KP112-121 decreased LH inter-pulse interval from 2.7 ± 0.5 hrs to 1.3 ± 0.1 hrs

				KP112-121 did not change LH pulse amplitude, FSH, E2 or PRL levels
H: KISSPEPTIN IN IVF				
Author	**Study design**	**Cohort**	**Intervention**	**Results**
Jayasena et al. (2014) [165]	Phase 2 randomized	53 undergoing IVF	KP54 (SC bolus 1.6-12.8 nmol/kg)	≥1 mature oocyte: 51/53 (96.2%) ≥1 fertilised egg: 49/53 (92.5%) Embryo transfer: 49/53 (92.5%) Clinical pregnancy rate per transfer: 12/49 (24.5%) Live birth rate per transfer: 10/49 (20.4%) Moderate to severe OHSS:0
Abbara et al. (2015) [167]	Phase 2, open label randomized	60 with high risk of OHSS undergoing IVF	KP54 (SC bolus 3.2-12.8 nmol/kg)	≥1 mature oocyte: 57/60 (95.0%) ≥1 fertilised egg: 54/60 (90.0%) Embryo transfer: 51/60 (85.0%) Clinical pregnancy rate per transfer: 27/51 (52.9%) Live birth rate per transfer: 23/51 (45.1%) Moderate to severe OHSS: 0
Abbara et al. (2017) [160]	Phase 2, placebo-controlled, randomized	62 with high risk of OHSS undergoing IVF	KP54 (SC bolus 9.6 nmol/kg, 1 dose vs 2 doses)	≥1 mature oocyte: 61/62 (98.4%) ≥1 fertilised egg: 61/62 (98.4%) Embryo transfer: 60/62 (96.8%) Clinical pregnancy rate per transfer: 19/60 (31.7%) Live birth rate per transfer: 18/60 (30.0%) Moderate to severe OHSS: 1/62 (1.6%)
I: KISSPEPTIN IN HEALTHY PREGNANCY				

Author	Study design	Cohort	Intervention	Results
Abbara et al (2021) [170]	Case-control trial	39 pregnant 10 non-pregnant	KP measurement (pmol/l)	KP increased linearly with advancing pregnancy
J: KISSPEPTIN IN MISCARRIAGE				
Author	**Study design**	**Cohort**	**Intervention**	**Results**
Silva et al. (2023) [337]	Systematic review	7 case–control studies	KP measurement	KP is reduced in miscarriage KP had a better discriminatory score than b-hCG to differentiate miscarriage from healthy pregnancy (in 3 out of 7 studies)
K: KISSPEPTIN IN HYPERTENSIVE DISORDERS OF PREGNANCY				
Author	**Study design**	**Cohort**	**Intervention**	**Results**
Perez-Lopez et al. (2021) [338]	Meta-analysis	7 studies 214 Pre-eclampsia/ gestational hypertension 263 normotensive	KP measurement	KP is reduced in pre-eclampsia or gestational hypertension than in normotensive pregnancies (SMD -0.68); $I^2 = 77\%$
Abbara et al. (2022) [184]	Case-Control	265 Controls 20 Pre-eclampsia	KP measurement	KP reduced in all hypertensive disorders (at 28-40 weeks of gestation)

		12 Gestational hypertension		KP increased in late-onset pre-eclampsia and reduced in early-onset pre-eclampsia (at 9-13weeks gestation)
L: KISSPEPTIN IN OTHER PREGNANCY COMPLICATIONS				
Author	**Study design**	**Cohort**	**Intervention**	**Results**
i. GESTATIONAL DIABETES MELLITUS (GDM)				
Cetcovic (2012) [177]	Prospective Case-Control	25 Controls 20 GDM	KP measurement (nmol/l)	KP is reduced in GDM (21-25wks; 4.51, 32-36wks; 11.64) than controls (21-25wks; 10.33, 32-36wks; 20.48)
Bowe et al. (2019) [198]	Case-Control	62 Controls 26 GDM	KP measurement (pmol/l)	KP is reduced in GDM (889) than controls (1270) at 26-34 weeks of gestation
Arslan et al. (2020)[199]	Cross sectional	82 Controls 76 GDM	KP measurement (pmol/l)	KP remained unchanged in GDM versus controls at 24-26 weeks of gestation
Abbara et al. (2022) [184]	Case-Control	265 Controls 35 GDM	KP measurement	KP remained unchanged in GDM versus controls in all trimesters
ii. PRE-TERM BIRTH				
Torricelli et al. (2008) [339]	Observational	30 Controls 10 Preterm	KP measurement (ng/ml)	KP remained unchanged in pre-term birth
Abbara (2022) [184]	Case-Control	265 Controls 11 Preterm	KP measurement	KP increased in pre-term birth than controls in all trimesters
iii. FOETAL GROWTH RESTRCITION				
Smets et al. (2008) [188]	Case-Control	31 Controls 31 SGA	KP measurement (pmol/L)	KP is reduced in SGA (1376) than controls (2035)
Armstrong et al. (2009) [180]	Retrospective Case Control	317 Controls 118 IUGR	KP measurement (pg/ml)	KP is reduced in IUGR (1164) than controls (1188)

Khaled et al. (2018) [189]	Case-Control	10 Controls 10 10 PE & IUGR 10 IUGR	KP measurement (ng/ml)	KP is reduced in IUGR (with PE;1640 and without PE; 1630) than controls (2900)
Abbara et al. (2022) [184]	Case-Control	265 Controls 17 FGR	KP measurement	KP is reduced in FGR in all trimesters
M: KISSPEPTIN IN GLUCOSE CONTROL				
Author	**Study design**	**Cohort**	**Intervention**	**Results**
Izzi-Engbeaya et al. (2018) [205]	Randomised blinded two-way crossover	15 healthy men	KP54 (IV infusion 1nmol/kg/h for 2hrs) versus vehicle	KP induced: -higher mean post-glucose load insulin secretion 4.1µU/ml -higher disposition index (IVGTT-DI) 2768±484 units
Izzi-Engbeaya et al. (2023) [211]	Single-blinded, crossover study	17 women with overweight or obesity	KP54 (IV infusion 1nmol/kg/h for 2hrs)	KP had no effect on pre and post prandial insulin and glucose levels
N: KISSPEPTIN IN APPETITE REGULATION AND OBESITY				
Author	**Study design**	**Cohort**	**Intervention**	**Results**
Izzi-Engbeaya et al. (2018) [205]	Randomised blinded two-way crossover	15 healthy men	KP54 (IV infusion 1nmol/kg/h for 2hrs) versus vehicle	KP had no effect on self-reported hunger (assessed by visual analogue scores) or objective food intake

Yang et al. (2021) [225]	Double-blinded, randomized, placebo-controlled, crossover study	27 healthy men	KP54 (IV infusion 1nmol/kg/h for 75 min) versus vehicle	KP did not elicit brain responses to visual food stimuli or psychometric parameters
Izzi-Engbeaya et al. (2023) [211]	Single-blinded, crossover study	17 women with overweight or obesity	KP54 (IV infusion 1nmol/kg/h for 2hrs)	KP had no effect on self-reported hunger (assessed by visual analogue scores) or objective food intake
O: KISSPEPTIN IN MAFLD				
Author	**Study design**	**Cohort**	**Intervention**	**Results**
Guzman et al. (2022) [231]	Observational	31 T2DM 34 NAFL 25 NASH 31 healthy men	KP measurement (pmol/L)	KP increased in NAFL (19.2±2.6) and NASH (18.9±2.4) compared with controls (6.6±0.8) or patients with type 2 diabetes (7.1±0.7)
P: KISSPEPTIN IN BONE DISORDERS				
Author	**Study design**	**Cohort**	**Intervention**	**Results**
Comninos et al. (2022) [244]	Randomized, placebo-controlled,	26 healthy men	KP54 (IV infusion 1nmol/kg/h for 90 min)	KP54 increased osteoblast activity (20.3% increase in osteocalcin, 24.3% increase in carboxylated osteocalcin)

	double-blind, 2-way crossover			
Q: KISSPEPTIN IN PSYCHOSEXUAL DYSFUNCTION				
Author	**Study design**	**Cohort**	**Intervention**	**Results**
Comninos et al. (2017) [278]	Randomized, double-blind, 2-way crossover, placebo-controlled, fMRI study	29 healthy heterosexual men	KP54 (IV infusion 1nmol/kg/h, for 75 min) versus vehicle	In response to sexual stimuli, KP54 enhanced brain activity in the amygdala, globus pallidus, posterior cingulate, putamen and thalamus, compared to placebo. Correlation between baseline reward scores and KP hippocampal enhancement, and change in sexual aversion and KP putamen enhancement.
Comninos et al. (2018) [262]	Randomized, double-blind, 2-way crossover, placebo-controlled, fMRI study	29 healthy heterosexual men	KP54 (IV infusion 1nmol/kg/h, for 75 min) versus vehicle	KP's modulation of the default mode network correlated with increased limbic activity in response to sexual stimuli. KP's DMN modulation was greater in men with less reward drive and predicted reduced sexual aversion.
Yang et al. (2020) [268]	Randomized, double-blind, 2-way crossover, placebo-	33 healthy heterosexual men	KP54 (IV infusion 1nmol/kg/h, for 75 min) versus vehicle	In response to a feminine olfactory stimulus, KP54 enhanced brain activity in the amygdala, caudate, globus pallidus, putamen and thalamus, compared to placebo. In response to female faces, KP54 enhanced brain activity in the medial prefrontal cortex and superior frontal gyrus, compared to placebo.

	controlled, fMRI study			
Comninos et al. (2021) [265]	Randomized, double-blind, 2-way crossover, placebo-controlled, MR spectroscopy study	19 healthy heterosexual men	KP54 (IV infusion 1nmol/kg/h, for 75 min) versus vehicle	Significant decrease (14.1–15.7%) in total endogenous GABA levels in the anterior cingulate cortex during KP, compared to vehicle.
Thurston et al. (2022) [292]	Randomized, double-blind, 2-way crossover, placebo-controlled, fMRI study	32 eugonadal women with Hypoactive Sexual Desire Disorder	KP54 (IV infusion 1nmol/kg/h, for 75 min) versus vehicle	In response to erotic videos, KP54 deactivated the inferior frontal and middle frontal gyri and activated the postcentral and supramarginal gyri, compared to placebo. In response to male faces, KP54 deactivated the temporoparietal junction, compared to placebo.
Mills et al. (2023) [280]	Randomized, double-blind, 2-way crossover, placebo-controlled, fMRI study	32 eugonadal men with Hypoactive Sexual Desire Disorder	KP54 (IV infusion 1nmol/kg/h, for 75 min) versus vehicle	In response to erotic videos, KP54 deactivated the parahippocampus, precuneus, frontal pole, and posterior cingulate, whilst activating the anterior cingulate, middle frontal gyrus, fusiform gyrus, visual cortex. Associated with significant increases in penile tumescence (by 56% more than placebo) and behavioral measures of sexual desire, most notably increased 'happiness about sex'.

Abbreviations: AUC; area under the curve, CDGP; constitutional delay of growth and puberty, CHH; congenital hypogonadotropic hypogonadism, CPP; central precocious puberty, d; day, E2; estradiol, EP; ectopic pregnancy, F; female, FSH; follicle stimulating hormone, GA; gestational age, GDM; gestational diabetes mellitus, fMRI; functional magnetic resonance imaging, GnRH; gonadotropin releasing hormone, HA; hypothalamic amenorrhea, HCG; human chorionic gonadotropin, IV; intravenous, IVF; in-vitro fertilisation, IVGTT-DI; intravenous glucose tolerance test - disposition index, IUGR; intrauterine growth retardation, KP; kisspeptin, LH; luteinizing hormone, M; male, N; number, NAFL ; metabolic fatty liver, MAFLD; non-alcoholic fatty liver disease, NASH; non-alcoholic steatohepatitis, OHSS; ovarian hyperstimulation syndrome, PRL; prolactin, RCT; randomised controlled trial, SC; subcutaneous, SGA; small for gestational age.

TABLE 2: Clinical Trials involving Neurokinin B and NKB antagonism

Author	Study design	Cohort	Intervention	Results
A: NKB IN HEALTHY MALES				
Author	**Study design**	**Cohort**	Intervention	**Results**

Jayasena et al. (2014) [84]	Randomized single-blinded placebo-controlled trial	23 healthy men	NKB (IV infusion 0.4-5.12 nmol/kg/h over 90 min, 5.12 nmol/kg/h over 4hrs)	NKB did not alter LH, FSH or testosterone levels at all doses.
Narayanaswamy et al. (2016) [340]	Randomized single-blinded placebo-controlled trial	5 healthy men per group	Naltrexone (oral 50mg) NKB (IV infusion 2.56 nmol/kg/h over 8hrs) KP54 (IV infusion 0.1 nmol/kg/h over 8hrs)	Whilst naltrexone and KP54 increased LH levels, NKB did not alter LH or FSH
B: NKB IN HEALTHY FEMALES				
Author	**Study design**	**Cohort**	**Intervention**	**Results**
Jayasena et al. (2014) [84]	Randomized single-blinded placebo-controlled trial	5-8 pre-menopausal women per group	NKB (IV infusion 0.32, 0.64, 1.28, 2.56, or 5.12 nmol/kg/h for	No change in LH, FSH and estradiol at all doses throughout the menstrual cycle

			3hrs) versus vehicle	
Jayasena et al. (2015) [85]	Randomized, double-blinded, placebo-controlled, 2-way cross-over trial	10 pre-menopausal women	NKB (IV infusion 5.12 nmol/kg/h over 30 min) versus vehicle during follicular phase	NKB induced hot flashes in 8/10 women, and elevated heart rate, skin temperature and thermal imaging
C: NK3R ANTAGONISM IN POLYCYSTIC OVARY SYNDROME				
Author	**Study design**	**Cohort**	**Intervention**	**Results**
George et al. (2016) [304]	Double-blind, placebo-controlled, phase 2 trial	65 PCOS	AZD4901 (oral 20mg, 40mg, 80mg, once daily for 28 days)	Highest dose of AZD4901 reduced: -LH AUC by 52.0% (95% confidence interval [CI], 29.6–67.3%) -LH pulses by 3.55 LH pulses/8 hrs (95% CI, 2.0–5.1) -Total testosterone by 28.7% (95% CI, 13.9–40.9%)
Skorupskaite et al. (2020) [141]	Prospective study	15 PCOS	MLE4901 (oral 40mg twice daily for 7 days) versus vehicle	MLE4901 vs vehicle reduced: -LH (4.0 ± 0.4 vs 6.5 ± 0.8 IU/l) -LH pulse frequency (0.5 ± 0.1 vs 0.8 ± 0.1 pulses/h) -FSH secretion (2.0 ± 0.3 vs 2.5 ± 0.4 IU/l)
Fraser et al. (2021) [305]	Phase 2a, randomized,	73 PCOS	Fezolinetant (oral 60mg,	Highest dose of Fezolinetant reduced testosterone by 33%, LH by -10.17 IU/L and FSH by −1.46 IU/L

	double-blind, placebo-controlled		180mg, four times a day)	
D: NK3R ANTAGONISM IN MENOPAUSAL HOT FLASHES				
Author	**Study design**	**Cohort**	**Intervention**	**Results**
Prague et al. (2017) [329]	Phase 2, randomized, double-blind, placebo-controlled, crossover	28 menopausal women	MLE4901(oral 40mg twice daily for 4 weeks) versus vehicle	MLE4901 reduced hot flash frequency versus vehicle (19·35 vs 49·01 per week) and decreased hot flash severity versus vehicle (3·27 vs 5·70 per week).
Depypere et al. (2019) [330]	Double-blind, randomized, placebo-controlled	87 menopausal women	Fezolinetant (oral 90mg twice daily for 12 weeks) versus vehicle	Fezolinetant reduced VMS score versus vehicle (-26.5 vs -12.2) and decreased frequency of moderate/severe VMS by five episodes per day.
Fraser et al. (2020) [331]	Phase 2b, double-blind, randomized,	287 menopausal women	Fezolinetant (oral 15, 30, 60, 90 mg twice daily or 30, 60, 120	All doses of fezolinetant, except the lowest one, reduced moderate/severe VMS (>2 per day) by 4 and 12 weeks

	placebo-controlled		mg once daily for 12 weeks) versus vehicle	
Trower et al. (2020) [333]	Double-blind, randomized, placebo-controlled	76 menopausal women	NT-814 (oral 50, 100, 150, 300 mg once daily for 14 days) versus vehicle	NT-814 reduced hot flash frequency by 24% (50mg), 59% (100mg), 84% (150mg), and 66% (300mg).
Lederman et al. (2023) [332]	Double-blind, randomized, placebo-controlled	522 menopausal women	Fezolinetant (oral 30mg or 45mg once daily for 12 weeks) versus vehicle followed by a 40-week active treatment extension	Fezolinetant reduced VMS frequency at week 4 (difference in change in least squares mean –1·87; 30mg, –2·07; 45mg) and week 12 ((–2·39; 30mg, –2·55; 45mg) Fezolinetant 30mg or 45mg once daily, reduced the severity of VMS at week 4 (-0.15, -0.19) and week 12 (-0.24, -0.2).

Abbreviations; FSH; follicle stimulating hormone; h; hrs, IV; intravenous, KP; kisspeptin, LH; luteinizing hormone, N; number, NK3R; neurokinin 3 receptor, NKB; neurokinin B, PCOS; polycystic ovary syndrome, VMS; vasomotor symptoms

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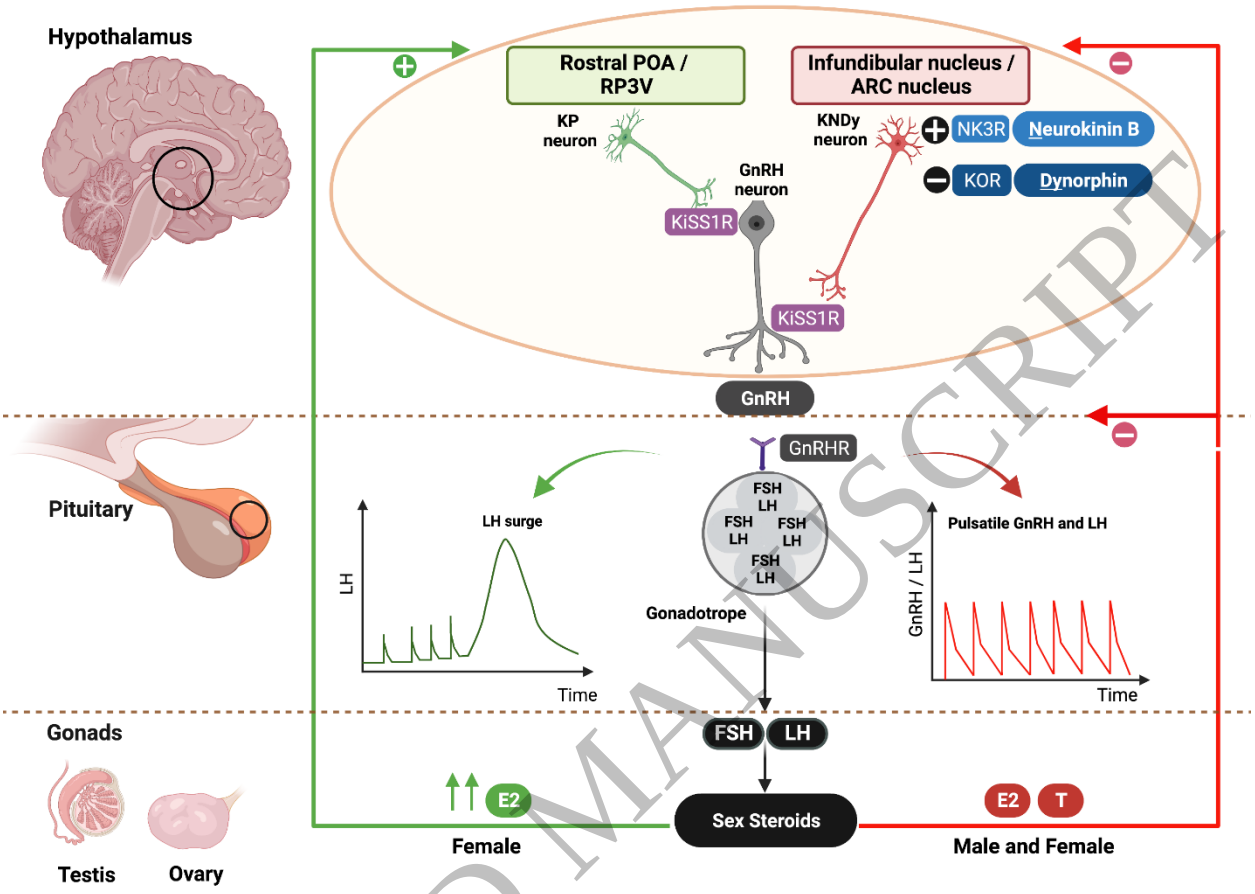

Figure 1

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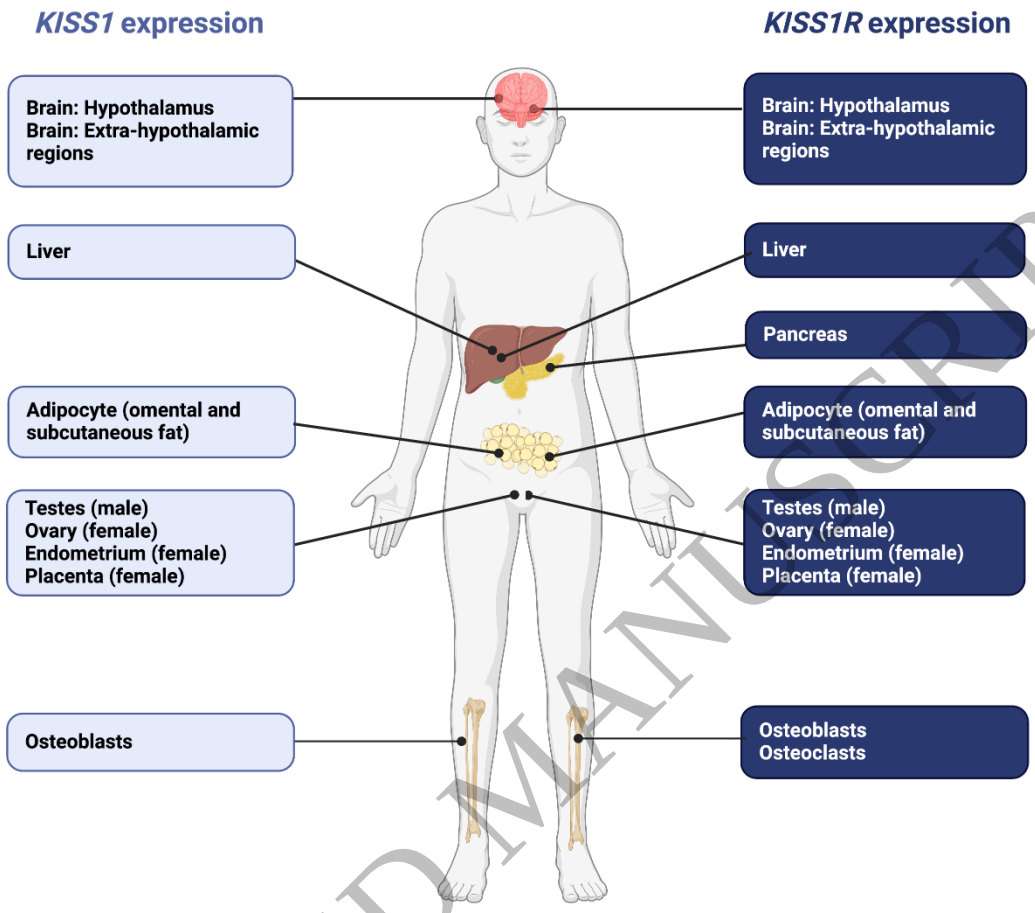

Figure 2

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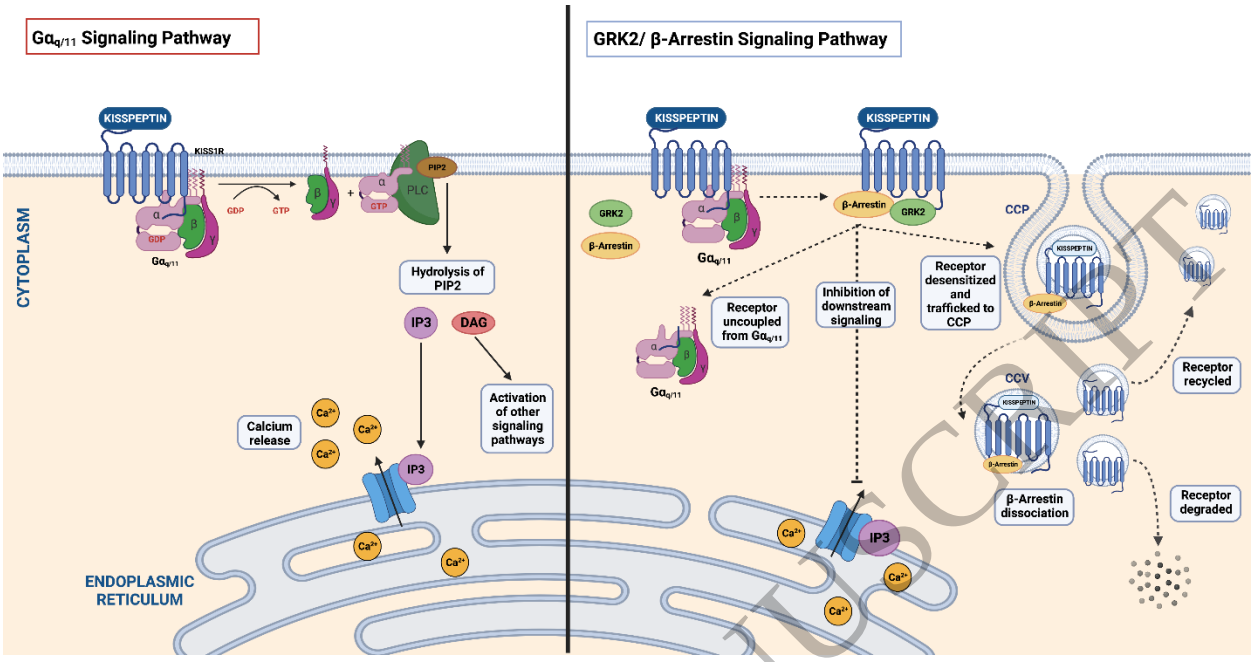

Figure 3

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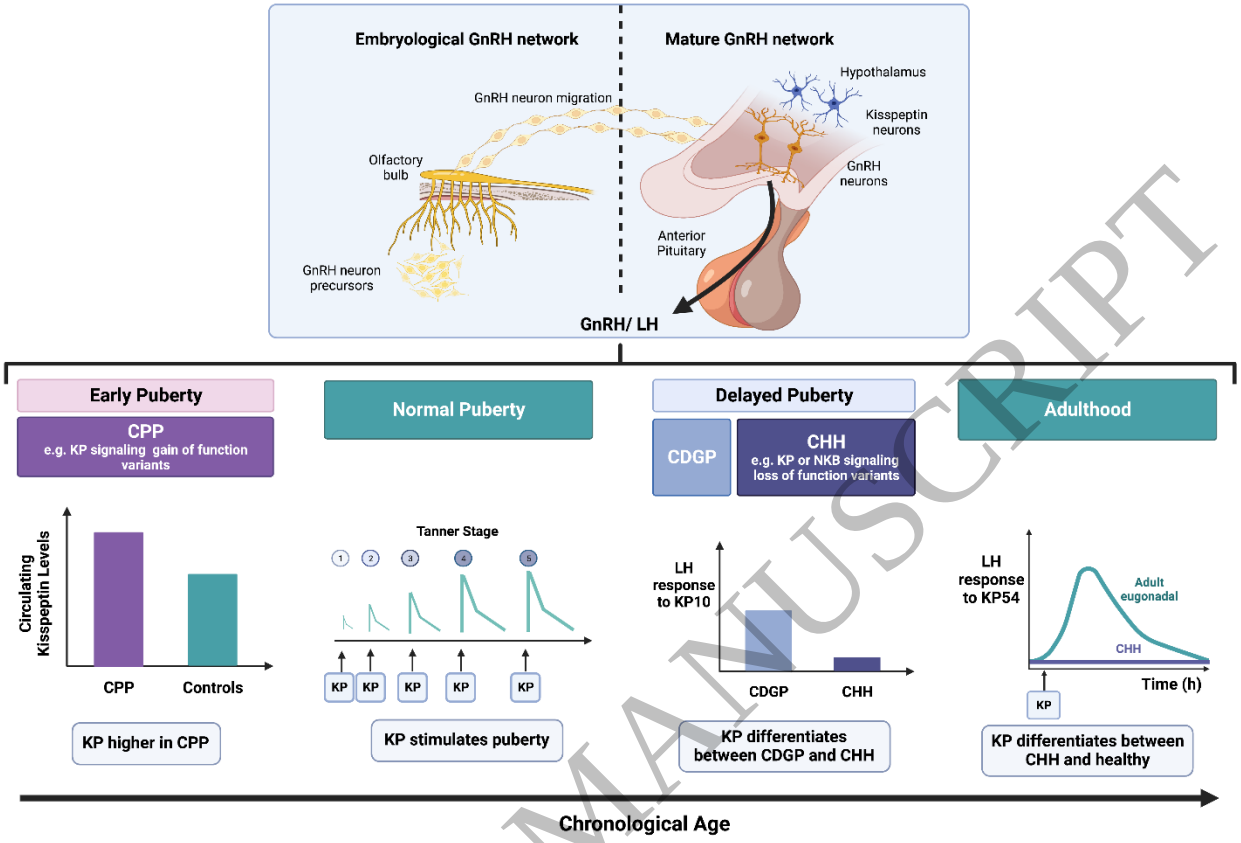

Figure 4

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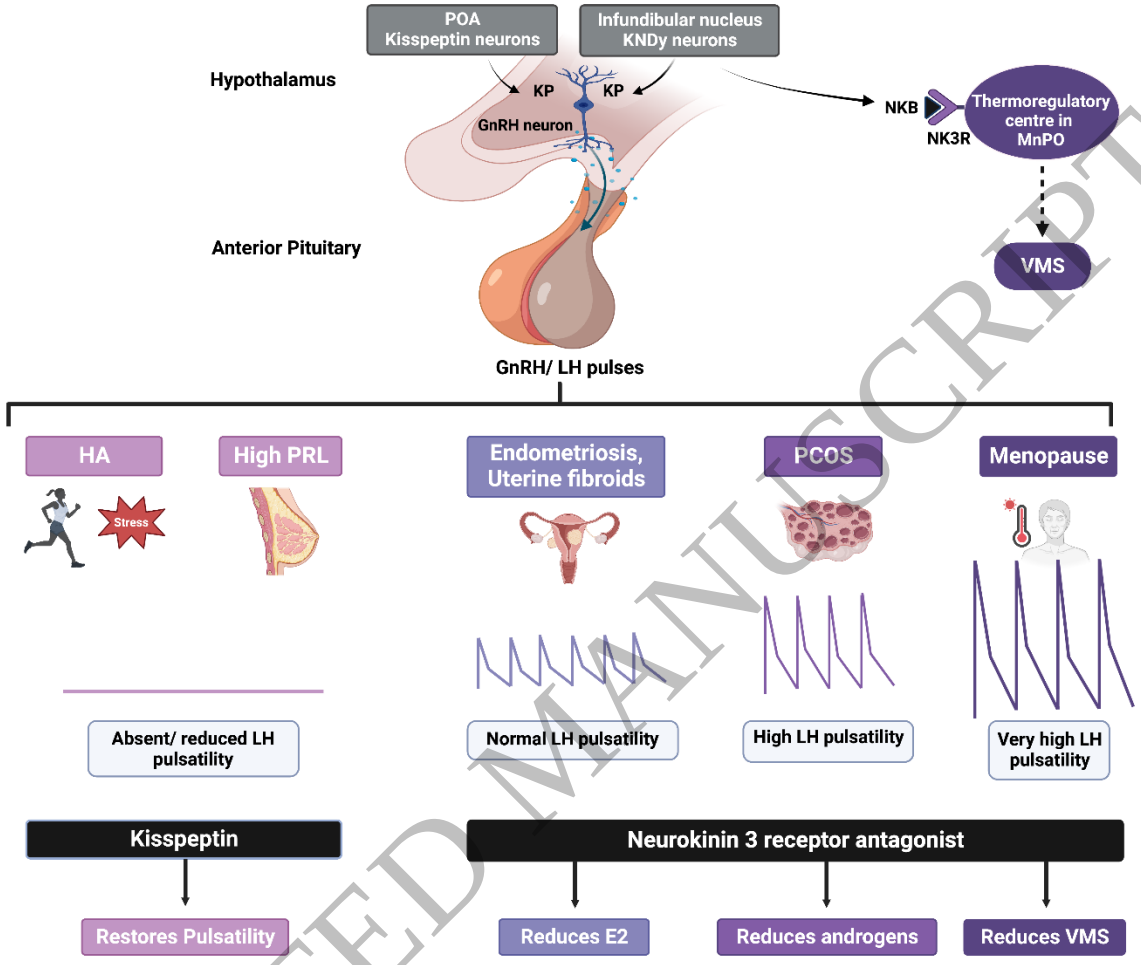

Figure 5

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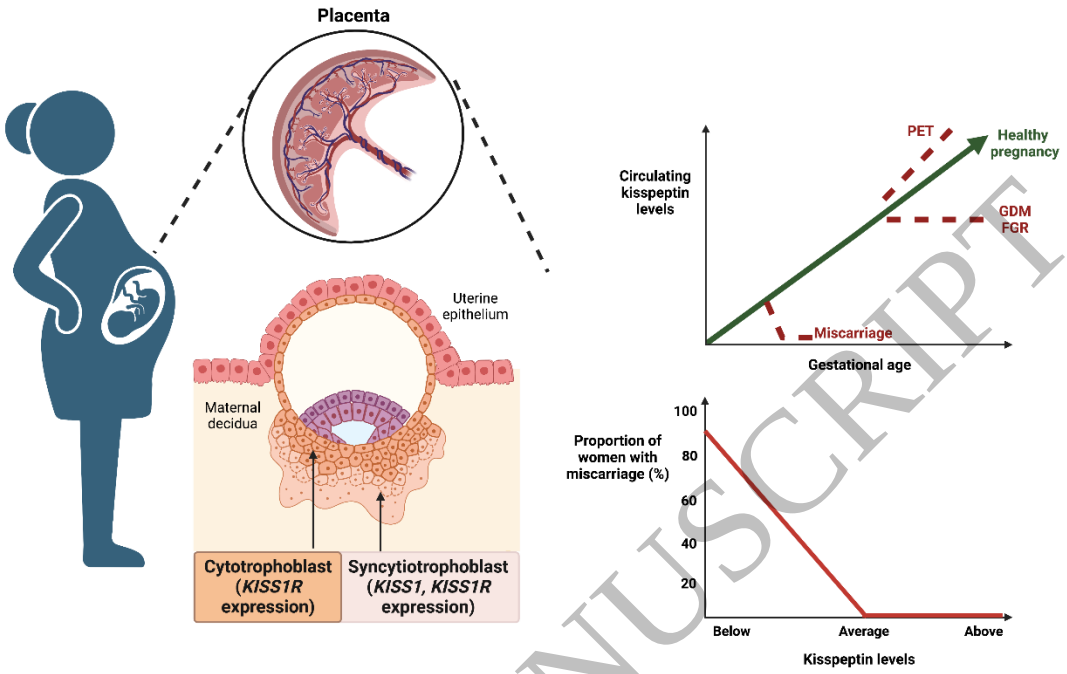

Figure 6

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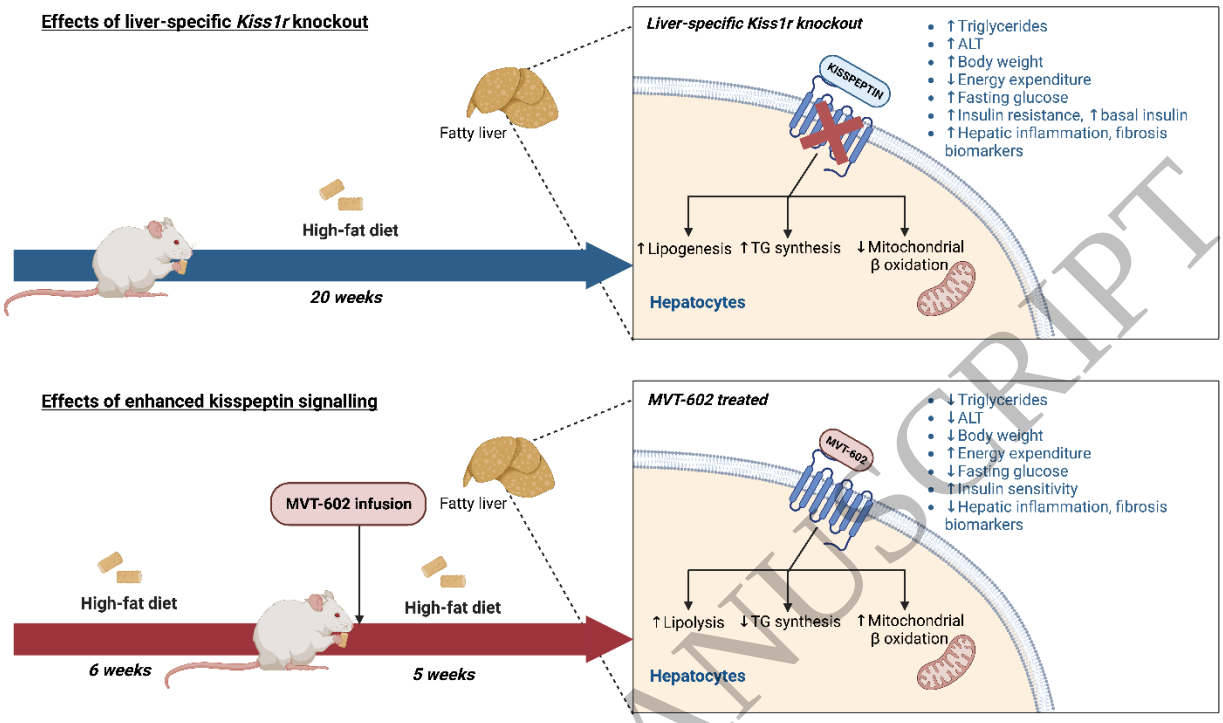

Figure 7

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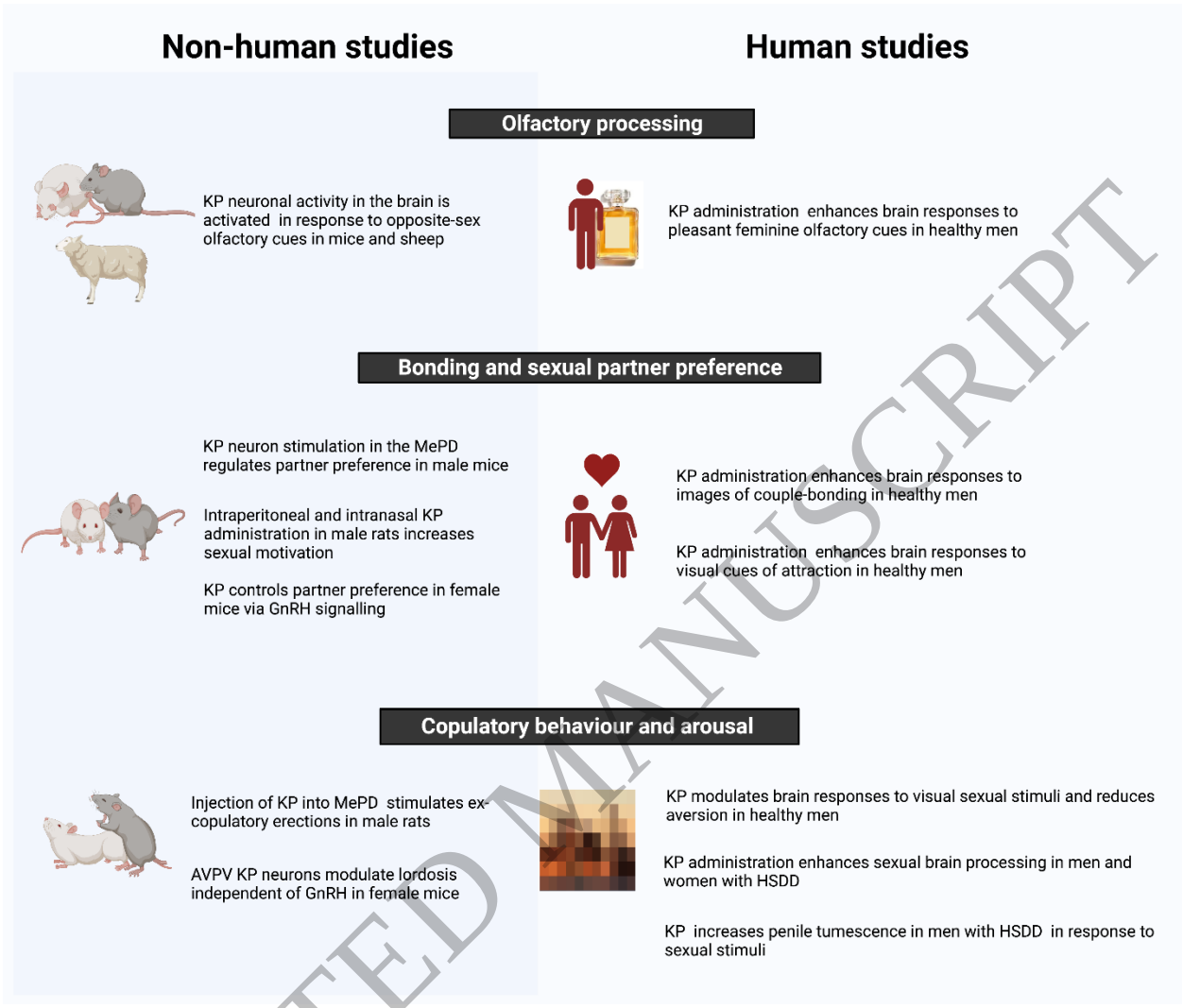

Figure 8

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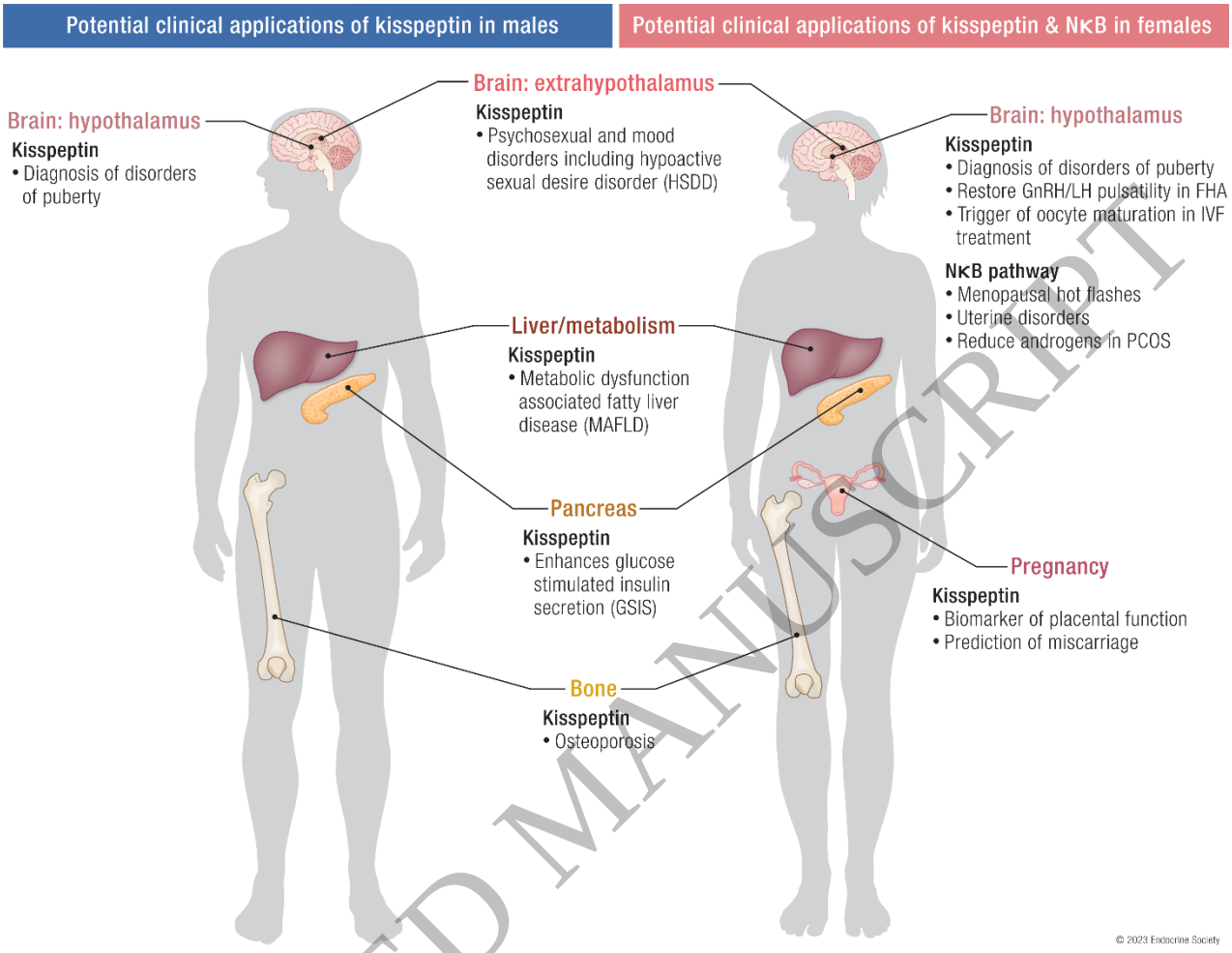

Graphical Abstract
168x129 mm (x DPI)

ESSENTIAL POINTS

- Kisspeptin (KP) and neurokinin B (NKB) stimulate the pulsatile secretion of gonadotropin-releasing hormone (GnRH) and thus are considered key regulators of the reproductive endocrine axis.
- KP has emerged as a promising diagnostic and therapeutic tool for disorders of puberty, reproduction, pregnancy, metabolism, liver, bone, and behavior.

Therapies acting through antagonism of NKB action provide potential therapeutic options for women with menopausal hot flashes, polycystic ovary syndrome (PCOS), uterine fibroids, and endometriosis.