Clinical Translational Studies of Kisspeptin and Neurokinin B

Tia Hunjan, MBChB, BSc, MRCOG[1] Ali Abbara, MBBS, PhD, MRCP[1]

[1] Division of Diabetes, Endocrinology and Metabolism, Imperial College London, London, United Kingdom

Semin Reprod Med 2019;37:119–124

Address for correspondence Ali Abbara, MBBS, PhD, MRCP, Division of Diabetes, Endocrinology and Metabolism, Imperial College London at Hammersmith Campus, Commonwealth Building, Du Cane Road, London, W12 ONN, United Kingdom (e-mail: ali.abbara@imperial.ac.uk).

Abstract

Kisspeptin and neurokinin B (NKB) are hypothalamic neuropeptides that are vital for reproductive health. An absence of either kisspeptin or NKB signaling results in hypogonadotrophic hypogonadism and a failure to proceed through puberty. In recent years, several studies have demonstrated potential avenues for the clinical utility of medications that act through these pathways in the assessment and treatment of reproductive disorders. Kisspeptin acts to stimulate hypothalamic gonadotrophic-releasing hormone (GnRH) secretion from the hypothalamus. Kisspeptin induces gonadotrophin secretion in both healthy men and women, and in women with reproductive disorders such as hypothalamic amenorrhea (HA). Kisspeptin-based treatments hold promise for use during in vitro fertilization (IVF) treatment; a bolus of kisspeptin-54 induces an LH surge of 12 to 14 hours of duration sufficient to induce oocyte maturation, but with markedly reduced rates of the most significant complication of IVF treatment, ovarian hyperstimulation syndrome (OHSS). Kisspeptin could also be used chronically to restore reproductive health in patients with functional hypogonadism, such as those with HA. Furthermore, kisspeptin has potential as a diagnostic test of hypothalamic function; a "kisspeptin test" could be used in children with delayed puberty to identify the subset with genetically determined deficits in hypothalamic pathways (congenital hypogonadotrophic hypogonadism [CHH]). In addition to its role in hypothalamic GnRH pulse generation, NKB plays a critical role in the occurrence of one of the most troubling symptoms of the menopause, the "hot flush." Neurokinin-3 receptor (NK3R) antagonists are highly effective as treatments for hot flushes in postmenopausal women, with several compounds now in late-phase development. Furthermore, NK3R antagonism leads to a reduction in LH secretion by reducing GnRH pulsatility in the hypothalamus and has been shown to reduce androgen levels in women with polycystic ovary syndrome (PCOS) (in whom GnRH pulsatility is often increased). In summary, although further detailed evaluation in several clinical settings is ongoing, medications based on kisspeptin and NKB pathways have prodigious potential in the assessment and treatment of reproductive disorders.

Keywords

- Kisspeptin
- neurokinin B
- reproductive disorders

Kisspeptin and neurokinin B (NKB) are hypothalamic neuropeptides that are obligate for reproductive health. In 2003, two seminal publications reported that inactivating mutations in the gene encoding for the kisspeptin receptor (previously known as GPR54) cause hypogonadotrophic hypogonadism and a failure of pubertal development.[1,2] Subsequently, it was found that an inactivating mutation of the *KISS1* gene also results in normosmic hypogonadotrophic hypogonadism.[3] Conversely, activating mutations of the kisspeptin receptor result in central precocious puberty.[4] Thus, kisspeptin was

Issue Theme Kisspeptin, Neurokinin B and New Players in Reproduction: Part 2; Guest Editor, Waljit S. Dhillo, MD

DOI https://doi.org/10.1055/s-0039-3400240.
ISSN 1526-8004.

shown to play a key role in orchestrating reproductive hormonal secretion and in the regulation of normal puberty.

Similarly, mutations in genes encoding for NKB or its receptor (TAC3R) also result in congenital hypogonadotrophic hypogonadism (CHH). The decapeptide NKB is encoded for by the TAC3 gene and activates the neurokinin 3 receptor (NK3R) encoded by the TAC3R gene.[5–9] Patients with NK3R mutations have reduced gonadotrophin-releasing hormone (GnRH) pulsatility with an increase in follicle-stimulating hormone (FSH) to luteinizing hormone (LH) ratio.[10] Patients with either TAC3 or TAC3R mutations were shown to remain responsive to exogenous kisspeptin administration, consistent with NKB exerting its action upstream of kisspeptin.[10,11]

Since then, it has been established that kisspeptin acts to stimulate hypothalamic GnRH neurones and thus the remainder of the reproductive axis. In rodent models, kisspeptin was found to co-localize with NKB and dynorphin to form "KNDy" neurones. These neuropeptides are thought to act in a paracrine manner to result in the pulsatile secretion of GnRH. Both central and peripheral administration of kisspeptin in animal models results in robust stimulation of gonadotrophin secretion.[12,13]

Kisspeptin in Healthy Men and Women

In 2005, Dhillo and colleagues conducted the first administration of kisspeptin to healthy men and observed a dose-dependent increase in gonadotrophin secretion.[14] Thereafter, the same group administered kisspeptin to healthy women; while significant increases in gonadotrophins were observed in the follicular and luteal phases, LH-rise was most prominent during the preovulatory phase of the menstrual cycle.[15] Similarly, Chan et al observed that women were least sensitive to an intravenous bolus of kisspeptin-10 during the follicular phase of the menstrual cycle.[16]

A key feature of a healthy reproductive axis is the pulsatile secretion of GnRH [*by proxy* serum LH]. A single subcutaneous bolus of kisspeptin was shown to increase LH pulsatility in healthy women during the follicular phase.[17] Furthermore, a single bolus of kisspeptin-10 could reset the GnRH pulse generator in men,[18] but this did not appear to be the case in women.[16]

Acute LH responses to intravenous bolus administration of kisspeptin-10 were investigated in healthy men (dose range: 0.01–3 μg/kg) and the highest LH rises were observed after 1 μg (0.77 nmol/kg).[19] Moreover, a continuous intravenous infusion of 4 μg/kg per hour (3.07 nmol/kg per hour) of kisspeptin-10 persistently stimulated LH secretion over 22.5 hours.[19] Intriguingly, the LH response to an acute intravenous bolus of kisspeptin-10 (0.3 μg/kg) was similar in obese hypogonadal diabetic men to that in healthy control subjects.[20] Furthermore, robust LH stimulation was observed to an intravenous infusion of kisspeptin-10 (4 μg/kg per hour) in obese hypogonadal diabetic men for 11 hours.[20] Additionally, responses to kisspeptin in healthy older men were also maintained.[21] Thus, in future, kisspeptin-based therapeutics could be developed for the treatment of functional hypogonadism such as diabetes, obesity, or age-related hypogonadism.

Kisspeptin in Hypothalamic Amenorrhea

Hypothalamic amenorrhea (HA) is a condition characterized by a reduction in the physiological pulsatile secretion of GnRH. HA frequently occurs in the context of low body weight, excessive exercise, reduced energy availability, psychological stress, or genetic predisposition.[22]

Notably, the acute LH response to kisspeptin was increased by four-fold in women with HA compared with the same dose in healthy women during the follicular phase.[23] Data from a rodent model of HA could explain this observation; *Kiss1* expression in the hypothalamus is reduced in undernourished rodents, whereas kisspeptin receptor expression is increased.[24] Thus, HA could be considered as a state of hypothalamic kisspeptin deficiency, and thus there has been great interest in investigating the use of kisspeptin to restore physiological hormonal secretion in these women. Twice daily subcutaneous administration of kisspeptin-54 (6.4 nmol/kg) for 2 weeks was investigated in five women with HA.[23] While robust increases in serum gonadotrophin levels were observed on day 1 of administration, these were reduced within a few days of administration,[25] and markedly attenuated by day 14.[23] The majority of the tachyphylaxis to kisspeptin is likely to have occurred at the level of the kisspeptin receptor as GnRH responsiveness was maintained.[23] However, twice weekly administration of subcutaneous kisspeptin was able to persistently stimulate gonadotrophin secretion.[25] Thus, whilst bolus administration of kisspeptin has potential to restore normal reproductive function in women with HA, there is a risk of tachyphylaxis with excessive doses of kisspeptin. An alternative approach is to use a continuous intravenous infusion of kisspeptin, which when administered at lower doses can restore physiological LH pulsatility without tachyphylaxis.[26] Ongoing studies are assessing whether continuous subcutaneous administration of kisspeptin could be used to restore ovulatory function in women with HA.

Kisspeptin During *In Vitro* Fertilization

Kisspeptin signaling is requisite for physiological ovulation; infusion of a kisspeptin neutralizing antibody directly into the preoptic area of the hypothalamus results in abolition of the midcycle LH surge.[27] Furthermore, kisspeptin administration to superovulated prepubertal rats was able to induce ovulation to a similar extent as human chorionic gonadotrophin (hCG).[12] Additionally, the LH response to kisspeptin is dramatically increased in the preovulatory phase of the menstrual cycle, suggesting that it could induce an ovulatory LH surge.[15]

Indeed in 2014, Jayasena and colleagues conducted a "proof of concept" study demonstrating that a single subcutaneous bolus of kisspeptin-54 (1.6–12.8 nmol/kg) was able to induce an LH surge with a peak of approximately 40 IU/L that lasted for 12 to 14 hours.[28] This was sufficient to induce oocyte maturation in 51 of 53 healthy subfertile women undergoing *in vitro* fertilization (IVF) treatment.[28] Furthermore, kisspeptin safely induced high rates of oocyte maturation in 60 women at increased risk of ovarian hyperstimulation syndrome (OHSS)

based on elevated ovarian reserve markers without causing this most serious of complications of current IVF treatment.[29] OHSS is predominantly caused by hCG stimulating the release of vascular endothelial growth factor (VEGF) from the ovary, which increases vascular permeability and leakage of fluid into the third spaces of the body, resulting in ascites, pleural effusions, renal impairment, and rarely even death.[30] Kisspeptin reduced the odds of severe OHSS by 33.6-fold (95% CI: 12.6–89.5) compared with hCG.[31] More recently, kisspeptin has been hypothesized to play a direct role in the pathogenesis of OHSS, by directly reducing estradiol-induced VEGF production.[32] This is consistent with clinical data, suggesting that extending the duration of the LH surge with a second dose of kisspeptin further enhances oocyte maturation, but without causing OHSS.[33]

Kisspeptin is known to be present in the ovary and has been suggested to play a direct ovarian role in addition to its predominant mode of action through hypothalamic GnRH secretion. Ovarian kisspeptin expression is undetectable in immature oocytes, but is increased at ovulation.[34] Kisspeptin has been shown to increase in vitro maturation of ovine[35] and porcine immature oocytes,[36] and smaller follicles contributed more to the oocyte yield than following other triggers in humans.[37] Furthermore, intrafollicular kisspeptin levels correlate with the number of mature oocytes retrieved.[5]

Owens and colleagues[38] examined the *in vivo* and *in vitro* actions of using kisspeptin-54 trigger on gene expression relating to ovarian reproductive function, steroidogenesis, and OHSS in granulosa lutein cells, compared with current triggers.[38] They observed that triggering oocyte maturation with kisspeptin-54 increased the expression of genes involved in ovarian steroidogenesis such as LH/hCG and FSH receptor, steroid acute regulatory (STAR) protein, aromatase, and estrogen receptors.[38]

In summary, kisspeptin stimulates release of an LH surge sufficient to induce effective oocyte maturation and achieve at least comparable pregnancy rates in subfertile women undergoing IVF treatment. Crucially, rates of OHSS are dramatically reduced by kisspeptin triggering, and this could in part be achieved by a further direct ovarian action of kisspeptin on VEGF production. Randomized controlled trials directly comparing kisspeptin to current triggers of oocyte maturation with accurate determination of clinical outcomes, such as OHSS rates, are now indicated.

Kisspeptin as a Diagnostic Test of Hypothalamic Function

Kisspeptin acts to stimulate the hypothalamus to secrete GnRH and thus has potential as a diagnostic test of hypothalamic reproductive function, where currently no direct test exists. The hypothalamus is known to play a key role in controlling the onset of puberty; mutations in several genes involved in the regulation of hypothalamic GnRH function are known to result in CHH and absent puberty. Responses to kisspeptin in CHH have been shown to be attenuated[39]; however, patients with reversal of CHH (which can occur in up to one-fifth of such patients) regain responsiveness to kisspeptin.[40] The majority of patients with delayed puberty have constitutional delay of growth and puberty (CDGP) and will proceed through puberty with time; however, a subset of patients will have CHH and are unlikely to proceed through puberty without treatment. Thus, kisspeptin has been evaluated as a diagnostic test in children with delayed puberty and a wide variety of responses to a kisspeptin challenge test were observed.[41] Follow-up of puberty onset in these children will reveal whether those who responded to kisspeptin were more likely to have CDGP than CHH.

In summary, mutations affecting structural components of hypothalamic GnRH signaling (e.g., anosmin1) are unlikely to respond to kisspeptin (unless reversal has occurred)[40]; however, functional hypogonadotrophic hypogonadism (e.g., HA[23] or hyperprolactinemia[42]) does respond to kisspeptin. Thus, a "kisspeptin test" can be used to more accurately evaluate the hypothalamic contribution to hypogonadotrophic hypogonadism.

NKB Administration in Humans

NKB administration in male agonadal juvenile monkeys increases LH levels.[43] However, infusions of NKB were unable to stimulate gonadotrophin secretion or pulsatility in either healthy men or women.[44] Nevertheless, an interesting phenomenon was reported by some participants who had received higher doses, in that they felt hot and appeared flushed.[44] This was investigated in more detail in 10 healthy women who received a 30-minute infusion of either NKB or vehicle in random crossover design.[45] Sweating, heart rate, and skin temperature were increased during NKB administration, which are characteristic features of menopausal flushing.[45] Following this, NK3R antagonists have been developed as a novel therapy for menopausal flushing, reducing the total number and severity of flushes by 41 to 45%.[46] Thus, these promising agents could revolutionize the treatment of women with hot flushes, especially those at risk of side effects from sex-steroid–based therapies, and several compounds are now in late-phase development.

NK3 Receptor Antagonism in Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is a condition characterized by anovulation, hyperandrogenism, and polycystic ovarian morphology.[47] Increased GnRH pulsatility is implicated in the pathogenesis of PCOS and is often reflected by an increase in serum LH level, which is estimated to be present in half of women with PCOS. Thus, kisspeptin and NKB pathways could be targeted to treat women with PCOS. George and colleagues investigated the use of pharmacological blockade of NKB action to reduce GnRH pulsatility, and assessed the impact of this on clinical features of PCOS such as hyperandrogenism.[48] Women were randomized to receive an oral NK3R antagonist (AZD4901 at a dose of 20, 40, or 80 mg/day) or placebo for 28 days.[48] After 7 days of 80 mg/day, a 52% reduction in area under the curve (AUC) of LH levels and a reduction in LH pulsatility by 3.6 LH pulses

per 8 hours ($p < 0.05$) was observed.[48] This was associated with a reduction in total testosterone levels by 28.7%.[48] A secondary analysis revealed that this reduction in androgen levels predominantly occurred in anovulatory women.[48] Further longer-term studies are indicated to evaluate the efficacy of this novel approach in the treatment of ovulatory dysfunction in women with PCOS.

Conclusion

Kisspeptin is a critical regulator of hypothalamic GnRH function, and thus offers a significant opportunity to better evaluate and treat conditions caused by hypothalamic dysfunction. Several kisspeptin analogs are in development,[49–54] which can aid in the translation of kisspeptin-based therapeutics to the bedside. Medications targeting NKB signaling are likely to represent a huge advance in the management of women with postmenopausal hot flushes and could have benefit in other conditions such as PCOS.

Funding
The Section of Endocrinology and Investigative Medicine is funded by grants from the MRC, BBSRC, and NIHR and is supported by the NIHR Biomedical Research Centre Funding Scheme. T.H. is supported by an Imperial Health Charity Research Fellowship and NIHR Research Professorship awarded to Professor Waljit S. Dhillo. A.A. is supported by National Institute of Health Research (NIHR) Clinician Scientist Award.

Conflict of Interest
There are no conflicts of interest to declare.

References

1 de Roux N, Genin E, Carel J-C, Matsuda F, Chaussain J-L, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc Natl Acad Sci U S A 2003;100(19):10972–10976
2 Seminara SB, Messager S, Chatzidaki EE, et al. The GPR54 gene as a regulator of puberty. Obstet Gynecol Surv 2004;59(05):351–353
3 Topalglu AK, Tello JA, Kotan LD, et al. Inactivating KISS1 mutation and hypogonadotropic hypogonadism. Obstet Gynecol Surv 2012; 67(06):352–353
4 Teles MG, Bianco SDC, Brito VN, et al. A GPR54-activating mutation in a patient with central precocious puberty. N Engl J Med 2008;358(07):709–715
5 Taniguchi Y, Kuwahara A, Tachibana A, et al. Intra-follicular kisspeptin levels are related to oocyte maturation and gonadal hormones in patients who are undergoing assisted reproductive technology. Reprod Med Biol 2017;16(04):380–385
6 Guran T, Tolhurst G, Bereket A, et al. Hypogonadotropic hypogonadism due to a novel missense mutation in the first extracellular loop of the neurokinin B receptor. J Clin Endocrinol Metab 2009;94(10):3633–3639
7 Gianetti E, Tusset C, Noel SD, et al. TAC3/TACR3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood. J Clin Endocrinol Metab 2010;95(06):2857–2867
8 Young J, Bouligand J, Francou B, et al. TAC3 and TACR3 defects cause hypothalamic congenital hypogonadotropic hypogonadism in humans. J Clin Endocrinol Metab 2010;95(05):2287–2295
9 Francou B, Bouligand J, Voican A, et al. Normosmic congenital hypogonadotropic hypogonadism due to TAC3/TACR3 mutations: characterization of neuroendocrine phenotypes and novel mutations. PLoS One 2011;6(10):e25614
10 Young J, George JT, Tello JA, et al. Kisspeptin restores pulsatile LH secretion in patients with neurokinin B signaling deficiencies: physiological, pathophysiological and therapeutic implications. Neuroendocrinology 2013;97(02):193–202
11 Lippincott MF, León S, Chan Y-M, et al. Hypothalamic reproductive endocrine pulse generator activity independent of neurokinin B and dynorphin signaling. J Clin Endocrinol Metab 2019;104(10): 4304–4318
12 Matsui H, Takatsu Y, Kumano S, Matsumoto H, Ohtaki T. Peripheral administration of metastin induces marked gonadotropin release and ovulation in the rat. Biochem Biophys Res Commun 2004;320(02):383–388
13 Messager S, Chatzidaki EE, Ma D, et al. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. Proc Natl Acad Sci U S A 2005;102(05):1761–1766
14 Dhillo WS, Chaudhri OB, Patterson M, et al. Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. J Clin Endocrinol Metab 2005;90(12):6609–6615
15 Dhillo WS, Chaudhri OB, Thompson EL, et al. Kisspeptin-54 stimulates gonadotropin release most potently during the preovulatory phase of the menstrual cycle in women. J Clin Endocrinol Metab 2007;92(10):3958–3966
16 Chan YM, Butler JP, Sidhoum VF, Pinnell NE, Seminara SB. Kisspeptin administration to women: a window into endogenous kisspeptin secretion and GnRH responsiveness across the menstrual cycle. J Clin Endocrinol Metab 2012;97(08):E1458–E1467
17 Jayasena CN, Comninos AN, Veldhuis JD, et al. A single injection of kisspeptin-54 temporarily increases luteinizing hormone pulsatility in healthy women. Clin Endocrinol (Oxf) 2013;79(04):558–563
18 Chan YM, Butler JP, Pinnell NE, et al. Kisspeptin resets the hypothalamic GnRH clock in men. J Clin Endocrinol Metab 2011;96(06):E908–E915
19 George JT, Veldhuis JD, Roseweir AK, et al. Kisspeptin-10 is a potent stimulator of LH and increases pulse frequency in men. J Clin Endocrinol Metab 2011;96(08):E1228–E1236
20 George JT, Veldhuis JD, Tena-Sempere M, Millar RP, Anderson RA. Exploring the pathophysiology of hypogonadism in men with type 2 diabetes: kisspeptin-10 stimulates serum testosterone and LH secretion in men with type 2 diabetes and mild biochemical hypogonadism. Clin Endocrinol (Oxf) 2013;79(01):100–104
21 Abbara A, Narayanaswamy S, Izzi-Engbeaya C, et al. Hypothalamic response to kisspeptin-54 and pituitary response to gonadotropin-releasing hormone are preserved in healthy older men. Neuroendocrinology 2018;106(04):401–410
22 Gordon CM, Ackerman KE, Berga SL, et al. Functional hypothalamic amenorrhea: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 2017;102(05):1413–1439
23 Jayasena CN, Nijher GMK, Chaudhri OB, et al. Subcutaneous injection of kisspeptin-54 acutely stimulates gonadotropin secretion in women with hypothalamic amenorrhea, but chronic administration causes tachyphylaxis. J Clin Endocrinol Metab 2009;94(11):4315–4323
24 Castellano JM, Navarro VM, Fernández-Fernández R, et al. Changes in hypothalamic KiSS-1 system and restoration of pubertal activation of the reproductive axis by kisspeptin in undernutrition. Endocrinology 2005;146(09):3917–3925
25 Jayasena CN, Nijher GMK, Abbara A, et al. Twice-weekly administration of kisspeptin-54 for 8 weeks stimulates release of reproductive hormones in women with hypothalamic amenorrhea. Clin Pharmacol Ther 2010;88(06):840–847
26 Jayasena CN, Abbara A, Veldhuis JD, et al. Increasing LH pulsatility in women with hypothalamic amenorrhoea using intravenous infusion of Kisspeptin-54. J Clin Endocrinol Metab 2014;99(06): E953–E961

27 Kinoshita M, Tsukamura H, Adachi S, et al. Involvement of central metastin in the regulation of preovulatory luteinizing hormone surge and estrous cyclicity in female rats. Endocrinology 2005; 146(10):4431–4436

28 Jayasena CN, Abbara A, Comninos AN, et al. Kisspeptin-54 triggers egg maturation in women undergoing in vitro fertilization. J Clin Invest 2014;124(08):3667–3677

29 Abbara A, Jayasena CN, Christopoulos G, et al. Efficacy of kisspeptin-54 to trigger oocyte maturation in women at high risk of ovarian hyperstimulation syndrome (OHSS) during in vitro fertilization (IVF) therapy. J Clin Endocrinol Metab 2015;100(09):3322–3331

30 Abbara A, Clarke SA, Dhillo WS. Novel concepts for inducing final oocyte maturation in in vitro fertilization treatment. Endocr Rev 2018;39(05):593–628

31 Abbara A, Islam R, Clarke SA, et al. Clinical parameters of ovarian hyperstimulation syndrome following different hormonal triggers of oocyte maturation in IVF treatment. Clin Endocrinol (Oxf) 2018;88(06):920–927

32 Zhai AJ, Liu J, Zhao H. Kisspeptin-10 (Kp-10) inhibits ovarian hyperstimulation syndrome (OHSS) by suppressing vascular endothelial growth factor (VEGF) secretion. Reproduction 2017;154 (04):355–362

33 Abbara A, Clarke S, Islam R, et al. A second dose of kisspeptin-54 improves oocyte maturation in women at high risk of ovarian hyperstimulation syndrome: a Phase 2 randomized controlled trial. Hum Reprod 2017;32(09):1915–1924

34 Castellano JM, Gaytan M, Roa J, et al. Expression of KiSS-1 in rat ovary: putative local regulator of ovulation? Endocrinology 2006; 147(10):4852–4862

35 Byri P, Gangineni A, Reddy KR, Raghavender KBP. Effect of kisspeptin on *in vitro* maturation of sheep oocytes. Vet World 2017;10 (03):276–280

36 Saadeldin IM, Koo OJ, Kang JT, et al. Paradoxical effects of kisspeptin: it enhances oocyte in vitro maturation but has an adverse impact on hatched blastocysts during in vitro culture. Reprod Fertil Dev 2012;24(05):656–668

37 Abbara A, Vuong LN, Ho VNA, et al. Follicle size on day of trigger most likely to yield a mature oocyte. Front Endocrinol (Lausanne) 2018;9:193

38 Owens LA, Abbara A, Lerner A, et al. The direct and indirect effects of kisspeptin-54 on granulosa lutein cell function. Hum Reprod 2018;33(02):292–302

39 Chan YM, Lippincott MF, Butler JP, et al. Exogenous kisspeptin administration as a probe of GnRH neuronal function in patients with idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab 2014;99(12):E2762–E2771

40 Lippincott MF, Chan YM, Delaney A, Rivera-Morales D, Butler JP, Seminara SB. Kisspeptin responsiveness signals emergence of reproductive endocrine activity: implications for human puberty. J Clin Endocrinol Metab 2016;101(08):3061–3069

41 Chan YM, Lippincott MF, Kusa TO, Seminara SB. Divergent responses to kisspeptin in children with delayed puberty. JCI Insight 2018;3(08):99109

42 Millar RP, Sonigo C, Anderson RA, et al. Hypothalamic-pituitary-ovarian axis reactivation by kisspeptin-10 in hyperprolactinemic women with chronic amenorrhea. J Endocr Soc 2017;1(11): 1362–1371

43 Ramaswamy S, Seminara SB, Ali B, Ciofi P, Amin NA, Plant TM. Neurokinin B stimulates GnRH release in the male monkey (Macaca mulatta) and is colocalized with kisspeptin in the arcuate nucleus. Endocrinology 2010;151(09):4494–4503

44 Jayasena CN, Comninos AN, De Silva A, et al. Effects of neurokinin B administration on reproductive hormone secretion in healthy men and women. J Clin Endocrinol Metab 2014;99(01):E19–E27

45 Jayasena CN, Comninos AN, Stefanopoulou E, et al. Neurokinin B administration induces hot flushes in women. Sci Rep 2015; 5:8466

46 Prague JK, Roberts RE, Comninos AN, et al. Neurokinin 3 receptor antagonism as a novel treatment for menopausal hot flushes: a phase 2, randomised, double-blind, placebo-controlled trial. Lancet 2017;389(10081):1809–1820

47 Teede HJ, Misso ML, Costello MF, et al; International PCOS Network. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Fertil Steril 2018;110(03):364–379

48 George JT, Kakkar R, Marshall J, et al. Neurokinin B receptor antagonism in women with polycystic ovary syndrome: a randomized, placebo-controlled trial. J Clin Endocrinol Metab 2016; 101(11):4313–4321

49 Clarke S, Abbara A, Eng PC, et al. A single subcutaneous injection of the kisspeptin analogue, MVT-602, induces a more prolonged LH surge compared with kisspeptin-54 in healthy women. J Endocr Soc 2019;3(Suppl 1):OR33-4

50 MacLean DB, Matsui H, Suri A, Neuwirth R, Colombel M. Sustained exposure to the investigational Kisspeptin analog, TAK-448, down-regulates testosterone into the castration range in healthy males and in patients with prostate cancer: results from two phase 1 studies. J Clin Endocrinol Metab 2014;99(08): E1445–E1453

51 Nishizawa N, Takatsu Y, Kumano S, et al. Design and synthesis of an investigational nonapeptide KISS1 receptor (KISS1R) agonist, Ac-d-Tyr-hydroxyproline (Hyp)-Asn-Thr-Phe-azaGly-Leu-Arg (Me)-Trp-NH_2 (TAK-448), with highly potent testosterone-suppressive activity and excellent water solubility. J Med Chem 2016; 59(19):8804–8811

52 Nishizawa N, Asami T, Nishibori K, et al. A new class of pentapeptide KISS1 receptor agonists with hypothalamic-pituitary-gonadal axis activation. Bioorg Med Chem Lett 2019;29 (04):654–658

53 Decourt C, Robert V, Anger K, et al. A synthetic kisspeptin analog that triggers ovulation and advances puberty. Sci Rep 2016;6(01): 26908

54 Decourt C, Robert V, Lomet D, et al. The kisspeptin analog C6 is a possible alternative to PMSG (pregnant mare serum gonadotropin) for triggering synchronized and fertile ovulations in the Alpine goat. PLoS One 2019;14(03):e0214424