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Current Opinion in Pharmacology

Novel therapeutic avenues for kisspeptin

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Abstract

Kisspeptin is a hypothalamic neuropeptide that acts via the hypothalamus to stimulate hypothalamic gonadotrophin-releasing hormone secretion and downstream gonadotrophin release. In health, kisspeptin induces normal puberty and modulates ovulation in healthy women. Hypothalamic kisspeptin expression is reduced in several functional reproductive disorders; thus, treating such conditions with kisspeptin is conceptually attractive. Recent studies have demonstrated that kisspeptin can induce a more physiological degree of oocyte maturation during *in vitro* fertilisation treatment that can reduce the risk of potentially life-threatening complications such as ovarian hyperstimulation syndrome seen with human chorionic gonadotrophin. Furthermore, chronic use of kisspeptin could potentially restore reproductive health in females with hypothalamic amenorrhoea, treat hyposexual drive disorder in otherwise healthy males and has potential indications in polycystic ovary syndrome, osteoporosis and metabolic dysfunction-associated fatty liver disease. Finally, kisspeptin analogues could potentially overcome some of the pharmacological challenges associated with the natural forms of kisspeptin such as short duration of action and development of tachyphylaxis.

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Current Opinion in Pharmacology 2022, **67:**102319

This review comes from a themed issue on **Endocrine and metabolic diseases**

Edited by **Stephanie Constantin** and **Ivana Bjelobaba**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.coph.2022.102319

Background

The kisspeptins are a family of peptides encoded by the *KISS1* gene in humans (*KISS1* in non-human primates and *Kiss1* in other mammals) [1]. The prepropeptide consists of 145 amino acids that is subsequently proteolysed into shorter peptides of lengths denoted by their suffix, such as kisspeptin-54 (KP-54), −14, −13 and −10 (KP-10) [2]. All forms share a common C-terminal decapeptide sequence, equivalent to KP-10, which is important for their binding to the G-protein-coupled kisspeptin receptor, KISS1R (formerly known as the orphan receptor GRP54) [2]. Kisspeptin primarily stimulates the hypothalamus to regulate the hypothalamic−pituitary−gonadal axis [3]. Indeed, the decreased KISS1R signalling in humans results in absent puberty and hypogonadotrophic hypogonadism [4,5], whereas increased KISS1R signalling results in precocious puberty [6].

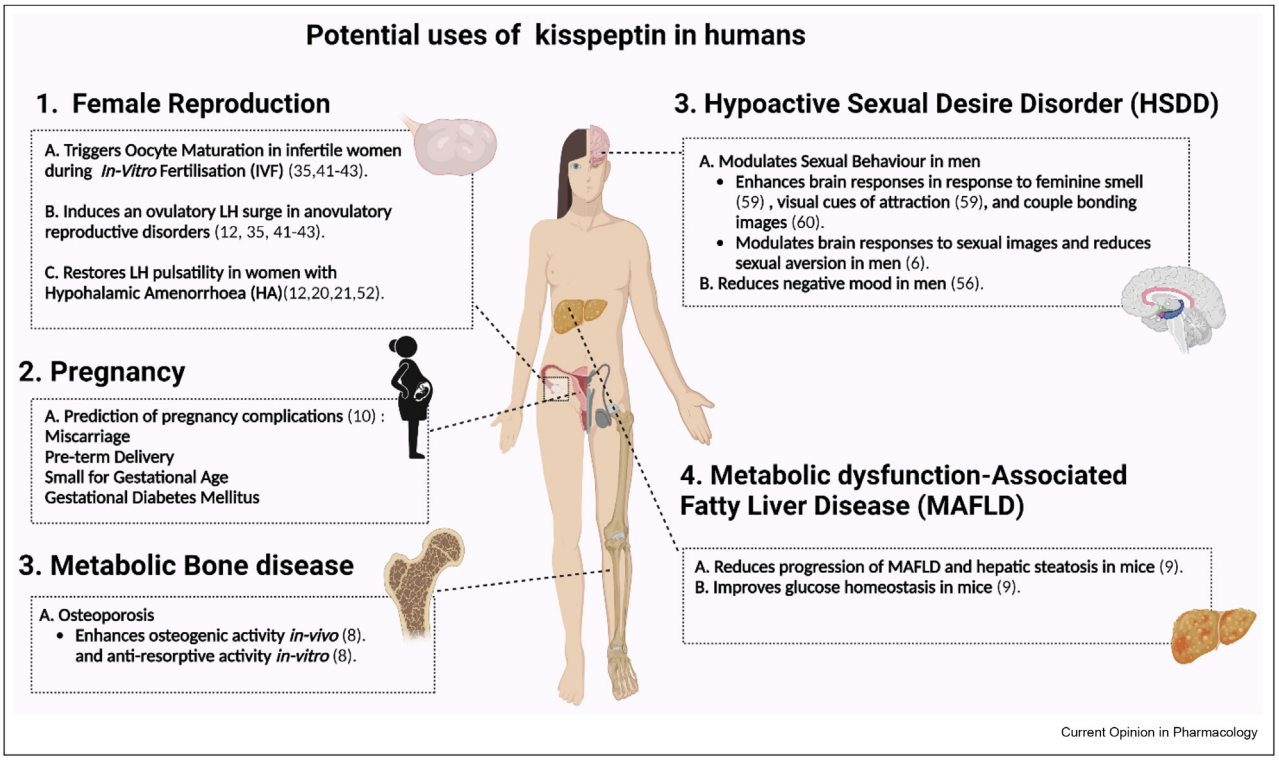
Outside the human hypothalamus [7], kisspeptin and its receptor are expressed in the brain in key limbic and paralimbic regions [7], and in peripheral tissues such as the gonads, placenta, liver, adipose tissue and bone [7]. Consequently, beyond its central role in stimulating hypothalamic gonadotrophin-releasing hormone (GnRH) secretion, kisspeptin has been studied in sexual and emotional brain processing [7], bone turnover [8], metabolism [9], and as a biomarker of pregnancy complications [10]. Herein, we summarise data on the pharmacological use of kisspeptin in reproductive disorders and fertility treatment, as well as its putative utility in hypoactive sexual desire disorder (HSDD), osteoporosis and non-alcoholic fatty liver disease, now known as metabolic dysfunction-associated fatty liver disease (MAFLD) (Figure 1).

Kisspeptin trials in healthy men and women

KP-10, KP-54 and kisspeptin receptor agonists such as TAK-683 [11] and MVT-602 (formerly known as TAK-448) [12] are the kisspeptin peptides that have been studied in humans to date. KP-10 is potent against KISS1R *in vitro* but is not believed to cross the blood−brain barrier and has a shorter half-life than KP-54 ($t_{1/2}$ 3 vs. 28 min) due to significant enzymatic degradation, making it less suitable for bolus administration [2]. Native KP-54 has a longer half-life and induces greater LH rises *in vivo* after bolus administration but is more expensive to manufacture than KP-10 due to its longer peptide length [13]. KISS1R-analogues, such as TAK-683 and MVT-602, which have been recently developed by modification of KP-10, possess increased stability and potency, hence enable more cost-effective peptide manufacture [11,12].

Exogenous kisspeptin has been reported to potently stimulate GnRH, and in turn luteinising hormone (LH),

Figure 1



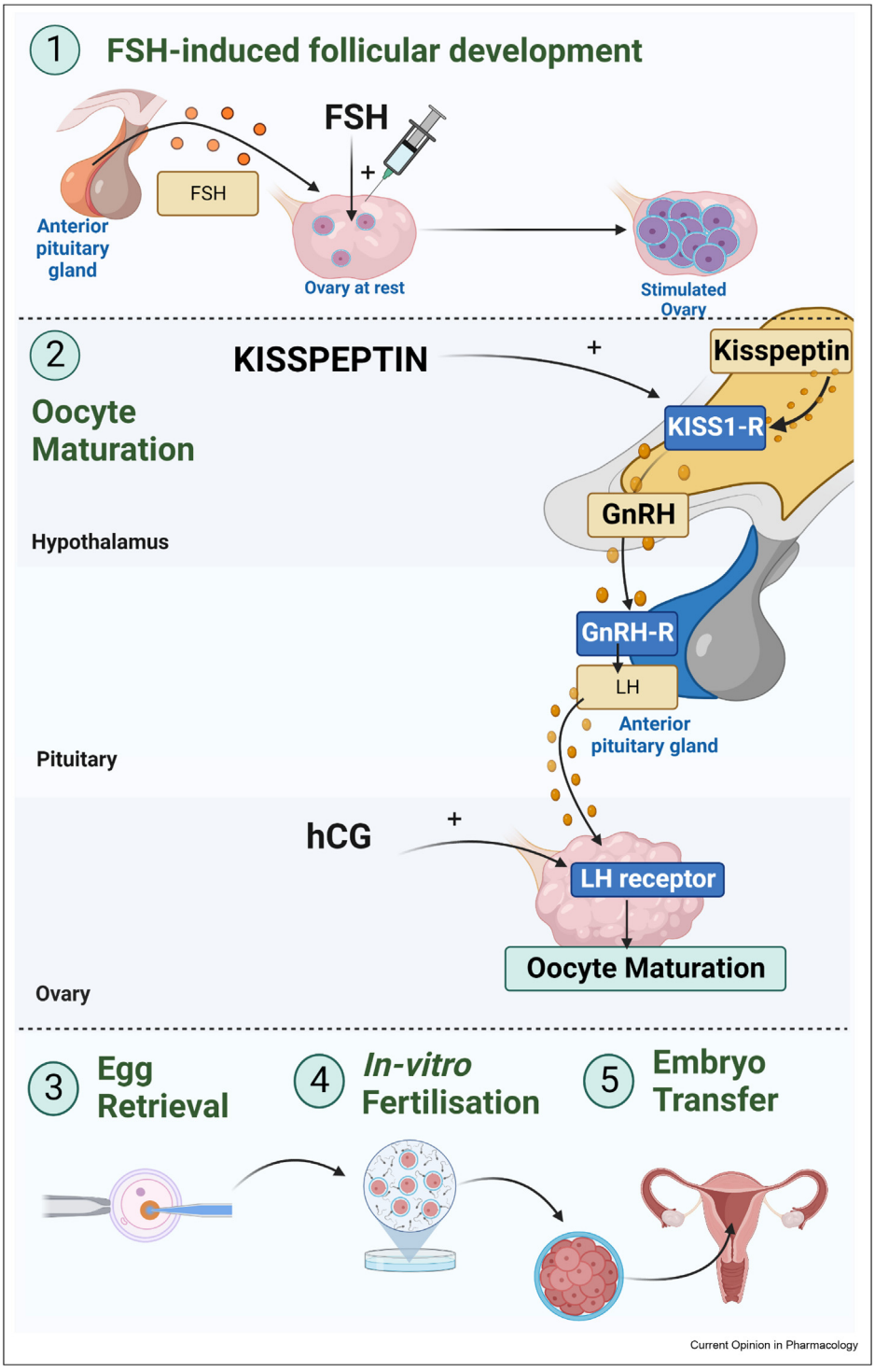
Novel therapeutic avenues using kisspeptin. Kisspeptin has a putative therapeutic benefit in humans in reproductive anovulatory conditions (e.g. hypothalamic amenorrhoea (HA), polycystic ovary syndrome (PCOS), during *in vitro* fertilisation (IVF) treatment, in the treatment of metabolic bone disease, in metabolic associated fatty liver disease (MAFLD), in hyposexual drive disorder (HSDD) and as a biomarker to predict pregnancy complications such as miscarriage. ***Created with*** BioRender.com.

in healthy men and women, and in patients with reproductive disease, using different kisspeptin peptide forms (KP-54, KP-10, KP-analogue), durations, frequencies (bolus or continuous infusion) and administration routes (central, subcutaneous, intranasal or intravenous) [2,14–19]. Subcutaneous KP-54 stimulated gonadotrophin secretion in healthy females throughout all phases of their menstrual cycle [18,20,21], but with greatest LH rises during the preovulatory phase [18,22–24]. Intravenous KP-10 was least effective during the follicular phase of the menstrual cycle and evoked no gonadotrophin response when administered subcutaneously [25,26].

In healthy men, both an intravenous bolus and a continuous infusion of KP-10 produced significant LH responses with the latter maintaining LH secretion for at least 22.5 h [17,25]. Acute and chronic administration of intravenous KP-10 induced LH increases in obese hypogonadal diabetic men [27] and healthy older men [28], thereby highlighting promising therapeutic avenues for the use of kisspeptin in male functional hypogonadism related to diabetes, obesity or age.

Recently, kisspeptin receptor agonists, including MVT-602 and TAK-683, were shown to potently increase LH secretion in men and women [12,29–31]. MVT-602 administered during the follicular phase of the menstrual cycle of healthy women triggered a similar LH amplitude to KP-54 yet produced a more sustained LH rise, with correspondingly increased area under the curve of LH rise [12]. However, pharmacokinetic properties were similar between MVT-602 and KP-54, suggesting that the longer duration of effect was centred on differential activation of the kisspeptin receptor [12]. When studied *in vitro* on mouse GnRH neurons, MVT-602 was more potent and induced a more sustained duration of GnRH-neuronal firing than KP-54 (115 vs. 55 min) [12]. Importantly, kisspeptins have been administered to a few hundred patients by different research groups and to different populations but have not been associated with any adverse effects [11,15–17,32–34]. Indeed, kisspeptin levels increase dramatically during pregnancy from non-pregnant levels (8 pmol/L) to 1230 pmol/L during the first trimester and 9590 pmol/L during the third trimester [35–37], consistent with the reported wide therapeutic safety window [10].

Figure 2



IVF treatment sequence and illustration of the pharmacological stimulation of oocyte maturation. IVF treatment is a supraphysiological process that stimulates the physiological processes of the human menstrual cycle. It involves (1) follicular development and oocyte stimulation, (2) oocyte maturation, (3) egg retrieval, (4) *in-vitro* fertilisation, and (5) embryo transfer/implantation into the endometrium. FSH is initially administrated to induce oocyte stimulation. Exogenous kisspeptin activates the kisspeptin receptor on GnRH neurons in the hypothalamus and induces the release of an endogenous pool of GnRH, with subsequent secretion of LH which induces oocyte maturation. Exogenous hCG acts directly on the ovarian LH receptors to stimulate oocyte maturation and is not under endocrine feedback control and carries a higher risk of OHSS. Abbreviations: FSH, follicle stimulating hormone; GnRH, gonadotrophin-releasing hormone; GnRH-R, gonadotrophin-releasing hormone receptor; KISS1-R; kisspeptin receptor; LH, luteinising hormone; OHSS, ovarian hyperstimulation syndrome; hCG, human chorionic gonadotrophin. *Created with* BioRender.com.

Kisspeptin trials in reproductive disorders

Kisspeptin and oocyte maturation in *in vitro* fertilisation treatment

According to the World Health Organisation, infertility affects 15% of reproductive-aged couples worldwide, which has led to a rise in the number of couples undergoing *in vitro* fertilisation (IVF) treatment [38]. In order to prepare the oocytes for surgical retrieval during IVF, LH-like exposure is needed to 'mature' them in order that they attain competence for fertilisation by sperm [39]. Approximately, three quarters of IVF cycles use human chorionic gonadotrophin (hCG) to trigger oocyte maturation [40] (Figure 2), which can result in excessive ovarian stimulation due to its longer duration of action (7–10 days) and an increase in ovarian release of vascular endothelial growth factor (VEGF) [41]. VEGF increases vascular permeability causing fluid extravasation into the third spaces of the body, contributing to the development of the 'ovarian hyperstimulation syndrome' (OHSS) [42,43], which can manifest as ascites, renal impairment and rarely even death in its most severe form [43].

Emerging data propose kisspeptin as a promising agent for inducing oocyte maturation as part of IVF protocols, particularly in women at high risk of OHSS (Figure 2). A single subcutaneous bolus of KP-54 generates an LH surge sufficient to mature oocytes in women undergoing IVF treatment [44]. In women at high risk of OHSS, KP-54 achieved live birth rates per transfer of 45% without causing any clinically significant OHSS [45]. Administration of a second dose of KP-54 10-h following the first extended LH exposure and led to increased reliability in the induction of oocyte maturation but importantly without increasing the incidence of clinically significant OHSS [46]. The longer duration of action of MVT-602 could enable a closer replication of the physiological mid-cycle LH surge after a minimal stimulation protocol and represents a desirable LH profile for the induction of oocyte maturation during IVF treatment but has yet to be tested in clinical trials during IVF treatment [47]. Recently, it has been suggested that kisspeptin may have an additional direct action at the ovary in reducing VEGF secretion [48]. Thus, kisspeptin can also reduce the risk of OHSS through a second mechanism, aside from the more physiological degree of increase in LH levels [48]. In a retrospective comparison, KP-54 reduced the likelihood of OHSS by 33-fold compared to hCG [42]. Finally, kisspeptin is a potentially superior choice to hCG during IVF as it also stimulates a follicle-stimulating hormone (FSH) surge, whereas hCG possesses only LH-like activity [39]. FSH is known to increase LH receptor expression in granulosa cells [39] and might have an added effect in enhancing oocyte maturation, hence making kisspeptin a more attractive choice than bHCG [39]. Prospective studies directly comparing KP-54's efficacy against that of established triggers of oocyte maturation during IVF treatment are awaited.

Kisspeptin and polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is the leading cause of anovulatory subfertility and affects 8–13% of reproductive-aged women [49]. PCOS is driven by abnormally increased GnRH pulsatility and a preferential increase in LH over FSH secretion [50]. Increased LH levels promote ovarian hyperandrogenism and reduce the sex–steroid negative feedback on hypothalamic kisspeptin neurons, further exacerbating the unrestrained LH secretion [50]. The relative FSH deficiency promotes follicular arrest and polycystic ovary morphology [50]. Women with PCOS seeking fertility are at increased risk of OHSS when using hCG to induce oocyte maturation (Figure 2) [50]. Recently, MVT-602 was shown to evoke a similar gonadotrophin response in women with PCOS to that of healthy women [12]. In pre-clinical PCOS-like female rodent models, chronic KP-54 administration led to variable FSH responses, contingent on their baseline gonadotrophin profile, whereas in anovulatory women with PCOS, it had no significant effect on the FSH levels [51]. Baseline LH levels are already high in PCOS, and kisspeptin could raise them further; therefore, it is possible that some form of pre-treatment (e.g. with an NK3R antagonist) might be required to predicate desirable increases in gonadotrophin levels in women with PCOS [52]. In the absence of this, chronic KP-54 produced an LH surge sufficient to provoke ovulation in only two of the seven women with PCOS [51]. Indeed, accounting for baseline hormonal profiles in women with PCOS and close monitoring after treatment are needed to identify specific subgroups who might benefit from kisspeptin treatment. Kisspeptin antagonists inhibit GnRH/LH release in animal models, hence could hypothetically reduce LH levels and improve the relative FSH deficiency observed in PCOS but have yet to be trialled in humans [53,54].

Kisspeptin and hypothalamic amenorrhoea

Hypothalamic Amenorrhoea (HA) describes loss of menstrual cycles in women due to a combination of low body weight, weight loss, excessive exercise and stress, on a background of genetic predisposition [33]. HA is characterised by the loss of the physiological pulsatile pattern of GnRH/LH secretion, which leads to reduced ovarian follicular development, low oestradiol and anovulation [55].

KP-54 administration to women with HA increased LH pulsatility [12,20,21,56] and led to a fourfold higher LH rise than in healthy women [21,23]. This could be attributed to increased hypothalamic *KISS1R* expression as seen in an undernourished rodent model [57]. However, on chronic administration of twice daily

subcutaneous kisspeptin, the LH response was markedly attenuated by day 14 signifying tachyphylaxis, most likely due to desensitisation of the kisspeptin receptor [23]. Extending the dose interval to twice weekly, persistent LH stimulation has been achieved over 8 weeks [20]. Thus, tachyphylaxis appears to be related to the frequency, dose of KP-54 used and sensitivity to that dose (e.g. increased in HA). A continuous intravenous KP-54 infusion at lower doses could be used as an alternative approach to increase pulsatile LH secretion in women with HA, without causing tachyphylaxis [56].

Moreover, in HA, the peak rise in LH after MVT-602 was advanced (6.2 vs. 15.1 h) compared to healthy women, whereas the FSH and oestradiol rise were markedly augmented despite similar pharmacokinetic profiles [12]. Because of the long duration of gonadotropin secretion after MVT-602, less frequent administration could be used during chronic stimulation protocols, which could minimise the risk of tachyphylaxis; however, chronic studies with MVT-602 are still pending.

Finally, kisspeptin restored GnRH/gonadotrophin release and ovulation in other analogous functional hypogonadal disorders, for example, hyperprolactinaemia. Kisspeptin overcame GnRH suppression in hyperprolactinaemia mice [58] and increased LH levels in amenorrhoeic women with cabergoline-resistant microprolactinomas [59,60].

Kisspeptin as a therapeutic in other conditions

Kisspeptin and metabolic dysfunction-associated fatty liver disease

MAFLD is the commonest liver disease, affecting 25% of the population [61]. It is associated with obesity, hyperlipidaemia and type 2 diabetes and can progress to non-alcoholic steatohepatitis, fibrosis, and even cirrhosis [61]. MAFLD is characterised by dysregulated hepatic fat metabolism and accumulation (steatosis).

Kiss1r-knockout mice on high-fat diet developed liver steatosis, glucose intolerance and insulin resistance compared with high-fat diet-fed controls [9]. MVT-602 administration to high-fat diet-fed mice and MAFLD mouse models improved glucose homoeostasis and reduced hepatic steatosis and non-alcoholic steatohepatitis progression [9]. Therefore, in rodents, the activation of the kisspeptin receptor is protective against steatosis.

In humans, there was a compensatory increase in KISS1/KISS1R expression in MAFLD liver biopsies, and circulating kisspeptin levels were increased in MAFLD patients compared to type 2 diabetes and healthy participants [9]. Therefore, kisspeptins could potentially be used to treat or delay the progression of MAFLD; however, further translational human studies are awaited.

Kisspeptin and hypoactive sexual desire disorder

Psychosexual disorders affect up to one in three people worldwide [62], with detrimental effects on quality of life, interpersonal relationships and fertility [7]. Recent human studies utilised functional magnetic resonance imaging to demonstrate that kisspeptin enhanced signalling in limbic and paralimbic regions of the human brain (amygdala, cingulate gyrus, basal ganglia) as well as the pre-frontal cortex in response to sex-related olfactory and visual cues, respectively [63,64]. The KP-54-driven brain enhancement occurred to a greater extent in participants with lower baseline reward scores and sexual quality of life [63,64]. Thus, kisspeptin might serve to amplify feelings of attraction and sexual drive, and it could prove to be a valuable therapeutic for patients with related reproductive and psychosexual disorders.

Kisspeptin and bone metabolism

Osteoporosis is characterised by low bone mass and structural bone deterioration, which predisposes to bone fragility and fractures, and is associated with increased morbidity and mortality [65]. In humans, both kisspeptin and its receptor are expressed in foetal osteoblast cells [66] and osteosarcoma bone specimens [66], whereas the kisspeptin receptor is also found in osteoprogenitor [67] and skeletal stem cells [67]. *In vitro* kisspeptin treatment increased alkaline phosphatase levels in human bone marrow mesenchymal stem cells by 41% and inhibited osteoclastic resorptive activity by up to 53% [8]. Kisspeptin's osteogenic effect *in vitro* was of a similar magnitude to that seen with teriparatide [68], whereas its anti-resorptive effect was similar to that of zoledronate on osteoclast activity [69]. Acute KP-54 administration to healthy men increased osteocalcin (a marker of osteoblast activity) by up to 24% [8], at a magnitude comparable to teriparatide's effects [70]. Large-scale clinical trials in disease groups with disrupted bone turnover are required to establish the effects of chronic kisspeptin administration and assess its potential as a putative treatment for osteoporosis.

Conclusion

Kisspeptin acts via the hypothalamus to stimulate endogenous GnRH secretion and downstream gonadotrophin release. Hypothalamic kisspeptin expression is reduced in several functional reproductive disorders, for example, hyperprolactinaemia [58], obesity [71] and undernutrition-related hypogonadism [57]. Thus, treating such conditions with kisspeptin to replace the deficient kisspeptin is conceptually attractive.

Furthermore, kisspeptin is part of the physiological mechanism of ovulation [72] and thus can be used to

restore ovulation in patients where this is lost. In this setting, it can induce a more physiological degree of stimulation that can reduce the risk of complications, such as OHSS seen with hCG. There is also increasing interest in extra-hypothalamic actions of kisspeptin, such as in the ovary, where it may directly reduce VEGF secretion and further relieve the risk of OHSS.

In summary, kisspeptin could have therapeutic benefit in several clinical settings including the restoration of reproductive health in women with HA, as a trigger of oocyte maturation during IVF treatment, as a treatment for HSDD, as well as possible therapeutic indications in osteoporosis and MAFLD pending further clinical studies. The development of kisspeptin analogues has the potential to overcome some of the pharmacological challenges encountered using the natural forms of kisspeptin although further clinical trials are needed to realise this potential.

Author contributions

JTwrote the manuscript and designed the figures. AA and WSD reviewed and edited the manuscript and are the corresponding authors. All authors have made a substantial, direct and intellectual contribution to the work and approved the manuscript before its submission.

Funding

This work was supported by grants from the National Institute of Health Research (NIHR), the NIHR/Wellcome Trust Imperial Clinical Research Facility and the NIHR Imperial Biomedical Research Centre. The Section of Endocrinology and Investigative Medicine was funded by grants from the Medical Research Council (MRC), Biotechnology and Biological Sciences Research Council (BBSRC), NIHR and was supported by the NIHR Biomedical Research Centre Funding Scheme. The views expressed are those of the authors and not necessarily those of the MRC, BBSRC, the NHS, the NIHR or the Department of Health. JT is supported by the NIHR Biomedical Research Centre Funding Scheme. AA is supported by an NIHR Clinician Scientist Award (No. CS-2018-18-ST2-002). WSD is supported by an NIHR Senior Investigator Award (NIHR RP-2014-05-001).

Declaration of competing interest

AA and WSD have consulted for Myovant Sciences who are developing a kisspeptin receptor agonist.

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