Kisspeptin across the human lifespan: evidence from animal studies and beyond

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Abstract

Since its first description in 1996, the *KISS1* gene and its peptide products, kisspeptins, have increasingly become recognised as key regulators of reproductive health. With kisspeptins acting as ligands for the kisspeptin receptor KISS1R (previously known as GPR54 or KPR54), recent work has consistently shown that administration of kisspeptin across a variety of species stimulates gonadotrophin release through influencing gonadotrophin-releasing hormone secretion. Evidence from both animal and human studies supports the finding that kisspeptins are crucial for ensuring healthy development, with knockout animal models, as well as proband genetic testing in human patients affected by abnormal pubertal development, corroborating the notion that a functional kisspeptin receptor is required for appropriate gonadotrophin secretion. Given the large body of evidence that exists surrounding the influence of kisspeptin in a variety of settings, this review summarises our physiological understanding of the role of these important peptides and their receptors, before proceeding to describe the varying role they play across the reproductive lifespan.

Key Words
- reproduction
- kisspeptin
- human
- fertility

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Introduction

Kisspeptins are a group of endogenous peptides, encoded for by the *KISS1* gene, which act as ligands for the G-protein-coupled receptor 54. Formed from the proteolytic breakdown of the 145-amino acid product of the *KISS1* gene, they possess a common C-terminal decapeptide, critical for ensuring bioactivity at the kisspeptin receptor (Clements *et al.* 2001, Kotani *et al.* 2001, Ohtaki *et al.* 2001).

Lee and coworkers first described the *KISS1* gene, who observed it to be absent in metastatic melanoma cell lines, leading them to hypothesise that its expression might be useful in distinguishing metastatic from non-metastatic melanoma (Lee *et al.* 1996). Although early research focussed on the role of the kisspeptin gene in oncological disease (Lee *et al.* 1996, Ohtaki *et al.* 2001), the abundance of *KISS1* gene expression in placental tissue, hypothalamus and gonads led researchers to speculate that kisspeptin might play a role in reproductive health (Lee *et al.* 1996, Muir *et al.* 2001).

Throughout life, the reproductive axis is active from the significant changes that occur at puberty, to the reproductive health necessary for fecundity, to the symptoms that occur following the withdrawal of sex steroids with increasing age. Each stage depends on the finely balanced hypothalamic–pituitary–gonadal system (Boehm *et al.* 2015) and secretion of hormones, including gonadotrophin-releasing hormone (GnRH), gonadotrophins (luteinising hormone (LH) and follicle-stimulating hormone (FSH)) and sex steroids

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(which include testosterone, oestrogen, and progesterone) (Boehm *et al.* 2015).

Pathology may affect any of the above structures and processes and can lead to a variety of clinical presentations. Infertility is estimated to affect one in seven couples in the UK (Hull *et al.* 1985, Templeton *et al.* 1990), resulting in significant psychological and physical burden. Increasing age and the subsequent withdrawal of sex steroids may also be associated with a psychosocial cost. Approximately 80% of women experience menopausal symptoms, the most common of which are vasomotor symptoms, often associated with significant effects on quality of life (Sarri *et al.* 2015). Reproductive health is, therefore, an important clinical issue.

Current treatments used in a variety of reproductive settings may be associated with side effects. For example, ovarian hyperstimulation syndrome (OHSS) is a rare but potentially life-threatening complication of *in vitro* fertilisation treatment (Mathur *et al.* 2006). Standard hormone replacement therapy for persistent menopausal symptoms also poses risks with evidence linking it to an increased risk of some malignancies and strokes (Panay *et al.* 2013).

Given the health burden associated with reproductive pathology, novel treatments are continually being sought. Studies in animals (Thompson *et al.* 2004) and humans have consistently shown that kisspeptin stimulates gonadotrophin secretion (Dhillo *et al.* 2005, Dhillo *et al.* 2007), and this has led to several exciting advances looking at the therapeutic potential of this group of peptides in treating reproductive pathology.

Although there have been several chronological reviews of research surrounding kisspeptin, there exists a large body of evidence regarding the role of kisspeptin at each stage of the reproductive life cycle. Much of this research has resulted from animal studies (in particular rodent studies; Colledge *et al.* 2010), partly due to the relative ease with which their genetic composition can be manipulated and the ability to study the reproductive axis in relatively close detail. Furthermore, there are recognised similarities across species with the periovulatory LH surge being a common physiological event (Plant 2015). However, the reproductive axis in humans does differ from that of rodents in several critical ways, with the control of the hypothalamic–pituitary axis being arguably more complex in rodents than in humans (Colledge *et al.* 2010) and with rodent sexual maturation being evident soon after birth, compared with the quiescent period observed in humans postnatally (Plant 2015).

Despite this, much of our understanding of the reproductive axis, and the role that kisspeptin plays, has been obtained by investigating rodent models, as well as primate studies and human clinical research. Therefore, we review the current literature investigating the role of kisspeptin across the reproductive lifespan, using evidence from both animal and human studies, with clarification provided where the application is species specific.

It should be noted that the literature contains inconsistencies regarding the terminology applied to the kisspeptin gene and its products. However, an agreed nomenclature has been proposed (Gottsch *et al.* 2009). In the remainder of this review, the terminology suggested will be utilised, with *KISS1* and *KISS1R* denoting the kisspeptin and kisspeptin receptor gene in humans, and *Kiss1* and *Kiss1r* denoting the kisspeptin and kisspeptin receptor gene in non-primate species, respectively. Although it was recommended that the kisspeptin gene products be termed *Kiss1r* mRNA in non-human species and *KISS1R* mRNA for the product of the human gene, the term 'kisspeptins' is also widely used for the product of both human and non-human genes (Pinilla *et al.* 2012) and will be used throughout the remainder of this review.

Kisspeptin: its structure, receptor and actions

Kisspeptins are peptides with a common arginine–phenylalanine residue at the amino terminal (Clements *et al.* 2001, Kotani *et al.* 2001), conferring their effects at their receptors (Clements *et al.* 2001, Stafford *et al.* 2002). They result from proteolytic cleavage of *KISS1* gene amino acid product. The product is first cleaved to a 54-amino acid protein, previously named 'metastin' due to its inhibitory effects on metastatic disease (Ohtaki *et al.* 2001). Further proteolysis may result in peptides of 10, 13 or 14 amino acids in length (Kotani *et al.* 2001).

The kisspeptin receptor has been shown to be present in the human placenta and brain (Ohtaki *et al.* 2001), with quantitative reverse transcriptase-polymerase chain reaction demonstrating its abundance in the cerebellum, cerebral cortex and brain stem (Muir *et al.* 2001). *KISS1* and *KISS1R* genes are widely expressed throughout the body, also being present in the brain, pituitary gland, placenta, gonads, gastrointestinal tract and liver, as well as the vascular system (Lee *et al.* 1996, Ohtaki *et al.* 2001, Mead *et al.* 2007).

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Kisspeptin neurons were initially localised in the human infundibular nucleus of females in samples taken at autopsy (Rometo *et al.* 2007). This finding is replicated across species with the infundibular/arcuate nucleus consistently demonstrating kisspeptin neuron possession (Smith *et al.* 2005*a*, Clarke *et al.* 2009, Xu *et al.* 2012). Evidence suggests that a second population of kisspeptin neurons exists in humans in the rostral preoptic area (Hrabovszky *et al.* 2010). This area appears to be species specific, being ill-defined in humans (Oakley *et al.* 2009, Hrabovszky *et al.* 2010), but with the anteroventral periventricular nucleus (AVPV) and the periventricular nucleus being densely populated by kisspeptin neurons in rodents (Clarkson *et al.* 2009).

Given the predominance of kisspeptin receptors in structures involved in the regulation of the reproductive system, and the anatomical distribution of kisspeptin neurons, research has focussed on the effects of kisspeptin on the hypothalamus–pituitary–gonadal axis.

Kisspeptin and its physiological role as a GnRH modulator

GnRH secretion is crucial for the hypothalamic–pituitary–gonadal axis (Colledge *et al.* 2010) with increased pulsatility stimulating pubertal development (Plant 2015). Researchers have long attempted to establish factors that drive this (the 'GnRH pulse generator') (Pinilla *et al.* 2012), and with kisspeptin being a potent stimulator of GnRH release *in vitro* and *in vivo*, its role as a regulator proved an exciting step in establishing the control of reproductive health.

Administration of kisspeptin to rodents increases circulating gonadotrophins, with this effect being notably absent in *Kiss1r*$^{-/-}$ mice (Messager *et al.* 2005), suggesting that kisspeptin stimulates the hypothalamic–pituitary axis. Administration of kisspeptin antagonist prevents GnRH secretion (Irwig *et al.* 2004, Roseweir *et al.* 2009), suggesting it acts upstream of GnRH. These findings are replicated across species and in humans (Seminara *et al.* 2003, Gottsch *et al.* 2004, Dhillo *et al.* 2005, Messager *et al.* 2005). Recent evidence, however, has found that other coregulatory peptides influence the relationship between kisspeptin and GnRH.

A subpopulation of kisspeptin neurons (subsequently termed 'KNDy neurons') in the infundibular/arcuate nucleus have been described as co-localising neurokinin B (NKB) and dynorphin (Dyn) (Goodman *et al.* 2007, Skorupskaite *et al.* 2014). These neurons, in close contact with GnRH-secreting neurons, project to the median eminence in monkeys, rodents and sheep (Ciofi *et al.* 2006, Ramaswamy *et al.* 2008, Dahl *et al.* 2009, Goodman *et al.* 2014), and are in direct contact with GnRH-secreting neurons in humans. Dyn (acting via the κ-opioid receptor (KOR)) and NKB (acting via the NK3 receptor) both influence LH secretion (Navarro *et al.* 2009). Garcia-Galiano (2003) reported that although the NK3 agonist senktide stimulated LH secretion in wild-type mice, this effect was lost in *Kiss1r* knockout mice suggesting effects upstream of *Kiss1r*.

Although evidence from human studies has at times appeared conflicting with Hrabovszky and coworkers reporting a low prevalence of Dyn fibres in the infundibular nucleus of human males (Hrabovszky *et al.* 2012), studies have consistently found Dyn neurons to be present in females in both animal and human studies (Rometo and Rance 2008, Goodman *et al.* 2014). Furthermore, naloxone, an opioid receptor antagonist, has been shown to increase LH secretion in women in the prefollicular and luteal phases with naltrexone, an opioid agonist, increasing LH release in hypothalamic amenorrhoea (HA), a condition characterised by low-circulating gonadotrophins and sex steroids (Genazzani *et al.* 1995).

Additional supportive evidence regarding the role of the KNDy neuron in humans includes the finding that inactivating mutations affecting TAC3 and TACR3 (the term given to the NK3 receptor in humans) have been reported in patients presenting with congenital hypogonadotrophic hypogonadism, suggesting that certainly NKB neurons are crucial to signalling within the reproductive axis (Topaloglu *et al.* 2009).

Evidence suggests that oestrogen acts via the oestrogen receptor ERα on kisspeptin neurons (Dubois *et al.* 2015) to exert negative feedback on GnRH release (Smith *et al.* 2005*a*,*b*). Expression of Dyn and NKB is also influenced by oestrogen, with expression of Dyn, NKB, the KOR gene and the NKB receptor gene all inhibited by oestrogen in animal models created (Navarro *et al.* 2009). Following ovariectomy, Dyn/KOR signalling has also been reported as being disrupted (Navarro *et al.* 2009). It appears that the kisspeptin neurons mediating this negative feedback are located in the arcuate nucleus of rodents or the infundibular nucleus of humans (Rometo *et al.* 2007, Pinilla *et al.* 2012).

By contrast, a second population of kisspeptin neurons, located in the AVPV in animals, appear to be stimulated by oestrogen in a response mediated by ERα (Roa *et al.* 2008), potentially explaining the LH surge in the menstrual cycle. Supporting this, administration of

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oestrogen into the AVPV has been shown to cause an LH surge, with blockage of ERα preventing this in animals (Smith *et al.* 2005*a*,*b*).

Having briefly reviewed current evidence from animal and human studies, it is clear that the peptide products of the kisspeptin gene act as potent stimulators of GnRH release through a complex mechanism, involving other neuroregulatory peptides.

Kisspeptin across the lifespan

Research has found that from the very beginning of life, kisspeptin appears to have a role in reproductive function (Fig. 1).

Implantation and placental function

When initially described, the *KISS1* gene was reported as being abundant in placental tissue, as well as the hypothalamus and gonads, and it was research using placental proteins that first elicited the ligand for the kisspeptin G-protein receptor (Kotani *et al.* 2001). Further evidence supporting the role of kisspeptin during pregnancy was provided by the finding that circulating kisspeptin rises throughout gestation, peaking in the third trimester at levels up to 7000 times that of non-pregnant controls (Horikoshi *et al.* 2003). Interestingly, at 5 days post-delivery, levels were shown to have reduced dramatically, indicative of placental production accounting for the changes observed. Recent evidence suggests that kisspeptin may play a role in several vital processes necessary for ensuring a successful pregnancy.

Implantation has been demonstrated as requiring *Kiss1* function to ensure adequate adhesion in rodents. Calder and coworkers described that in *Kiss1*$^{-/-}$ knockout mice, a failure of embryo implantation was observed due to a lack of adhesion and penetration (Calder *et al.* 2014). This was found to be a function of uterine dysfunction, as evidenced by the fact that the *Kiss1*$^{+/-}$ embryos created were able to be successfully implanted in wild-type female mice.

Following embryo implantation, decidualisation occurs, characterised by stromal cell proliferation and differentiation. Zhang and coworkers reported uterine expression of *Kiss1* and *Kiss1r* mRNA was significantly greater with decidualisation ($P<0.01$) (Zhang *et al.* 2014). Interestingly, this was also observed in pseudopregnancy, suggesting a process independent of the developing embryo. Downregulation of *Kiss1* expression using an siRNA against *Kiss1* was found to prevent the increase of *Kiss1* and *Kiss1r* demonstrated *in vitro* in a stromal cell culture model.

Although the process of decidualisation is different in humans, occurring before implantation in contrast to the post-implantation process occurring in rodents (Zhang *et al.* 2014), the findings suggested a possible mechanism underlying the relationship between kisspeptin and placental function. Evidence has also demonstrated that not only does *Kiss1* and *Kiss1r* mRNA expression increase, but that in rodents during implantation, a functional kisspeptin signalling system exists.

Having previously demonstrated phosphorylation of mitogen-activated protein kinases p38 and ERK1/2 to be a marker of activation of *Kiss1r*, Fayazi and coworkers reported that exogenous administration of kisspeptin-54

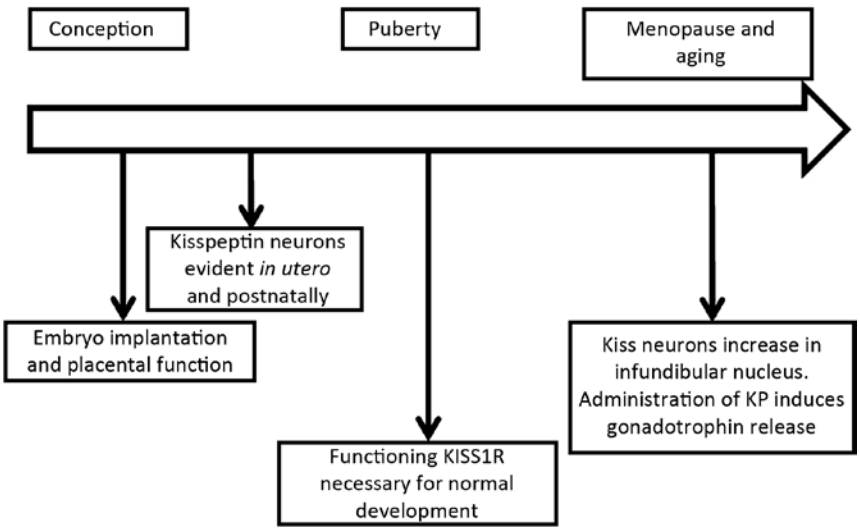

Figure 1
Kisspeptin across the lifespan.

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on day 4 of pregnancy in mice led to significant activation of kisspeptin receptors with only a weak response seen in the uteri of non-pregnant mice (Fayazi *et al.* 2015). The authors suggested that these observations supported the notion that kisspeptin signalling is important in regulating the pregnant uterine endothelium. Although it is difficult to comment on the ability to extrapolate these findings from a small study in rodents to humans, studies in man also suggest that kisspeptin is influential in pregnancy.

Using placental tissue from the first trimester and full-term delivery pregnancies, Bilban and coworkers found placental expression of *KISS1* gene to be 29-fold higher in the first trimester human placentas compared with full-term placentas (Bilban *et al.* 2004), with the highest levels of expression being found in the syncytiotrophoblast cells, replicating that of earlier work (Horikoshi *et al.* 2003). This study interestingly showed that kisspeptin-10 appeared to block trophoblast migration, acting as an inhibitor of implantation. Although this perhaps appears counter-intuitive, the authors purported that expression of kisspeptin and its receptor is greatest during the first trimester when cytotrophoblast invasion is maximal, thereby supporting the notion that kisspeptin plays a regulatory role in placentation. *In vitro* studies support this finding, demonstrating kisspeptin to inhibit trophoblast migration (Francis *et al.* 2014).

Given that placentation is crucial to pregnancy outcomes, it might be expected that kisspeptin expression alters with placental pathology. Smets and coworkers demonstrated circulating kisspeptin to be lower in the first trimester in small for gestational age pregnancies at delivery (Smets *et al.* 2008). Participant numbers were small ($n=31$ in each group), and these findings do contrast with findings of *in vitro* studies, where, given that kisspeptin appears to inhibit trophoblast expression (Bilban *et al.* 2004), it might be expected that higher levels of placental kisspeptin would correlate with poor outcomes. However, the authors suggested that both series of results reflect the determining role that kisspeptin plays in moderating implantation in the first trimester. Other studies have replicated these findings, with Armstrong and coworkers finding lower circulating kisspeptin values at 16–20 weeks' gestation in patients who subsequently developed pre-eclampsia and intra-uterine growth restriction (Armstrong *et al.* 2009) and Cetovic and coworkers demonstrating significantly lower circulating kisspeptin in pregnancies affected by type 1 diabetes, pre-eclampsia, gestational hypertension and gestational diabetes (Cetovic *et al.* 2012).

Not only does circulating kisspeptin appear to correlate with placental function of ongoing pregnancies, but evidence has been provided that kisspeptin levels are lower in pregnancies that subsequently miscarry. Jayasena and coworkers showed that circulating kisspeptin at antenatal booking correlated with risk of miscarriage (Jayasena *et al.* 2014*a*), with gestation-corrected kisspeptin being 60% lower in women with a singleton pregnancy compared with unaffected singleton pregnancies (gestation-corrected multiple of median kisspeptin 1.06 ± 0.42 singleton no miscarriage vs 0.42 ± 0.39 singleton miscarriage, $P<0.001$ with values representing mean$\pm$S.D.). The relationship of kisspeptin with miscarriage was also demonstrated in twin pregnancies, where death of one foetus was associated with a lower kisspeptin level than those without complications. Kisspeptin was also found to have a higher diagnostic performance with respect to miscarriage compared with human chorionic gonadotrophin (hCG), using receiver operator characteristic.

Although much remains to be discovered regarding the intricacies of the role of kisspeptin in embryo implantation, it is clear that it has an important role in successful and safe pregnancies, and evidence suggests that its importance continues through into the early stages of life.

Kisspeptin *in utero* and the neonate

Research has provided evidence that kisspeptin-expressing neurons exist in human foetuses (Morelli *et al.* 2008). Several animal studies have sought to delineate kisspeptin's influence throughout embryonic development and have revealed interesting insights into its role in early life.

Kiss1r has been identified in the mediobasal hypothalamus of mice from embryonic day 13.5 onwards as well as the pre-optic area (Fiorini & Jasoni 2010, Kumar *et al.* 2014, 2015), with studies also providing evidence of kisspeptin expression itself (Kumar *et al.* 2014). The presence of both the kisspeptin receptor and its ligand suggests that even early in life, this system is active (Kumar *et al.* 2014). Interestingly, this time period correlates with GnRH neuron migration into the preoptic area. Although conflicting evidence has been presented regarding whether kisspeptin stimulates GnRH neuron number (Fiorini & Jasoni 2010, Kumar *et al.* 2015), *in vitro* and *in vivo* studies have consistently shown that kisspeptin influences GnRH neuron growth with Fiorini and Jasoni (2010), demonstrating that GnRH neurite length significantly increased in the presence of KISS1 compared to controls without KISS1.

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It has long been known that sex differentiation is initiated during embryonic development, and although female gonadogenesis occurs autonomously, male development requires foetal testicular function and androgen synthesis (Pakarinen *et al.* 2002). Much remains unknown regarding the process by which foetal testosterone increases; however, this surge is thought to be crucial to external genitalia masculinisation (Clarkson & Herbison 2016). Recently, Clarkson and coworkers showed that kisspeptin neurons are implicated in activating GnRH neurons in the male mouse during the perinatal period, and that the subsequent GnRH surge generates testosterone necessary for ensuring sexual differentiation of the male mouse brain (Clarkson *et al.* 2014). These findings were in contrast to those of Poling and Kaufmann (2012), who found no difference in testosterone levels between wild-type mice and global *Kiss1r* knockout mice post birth. The narrower sampling window in the Clarkson and workers' study meaning that they are likely to have been able to detect more subtle differences in testosterone values (Clarkson *et al.* 2014). Moreover, the presence of kisspeptin neurons in close opposition to GnRH neurones does suggest they likely have a role in the testosterone surge (Skorupskaite *et al.* 2014).

Puberty

Some of the earliest work surrounding kisspeptin focussed on its role in puberty. An intact hypothalamic–pituitary–gonadal axis is required for normal sexual differentiation, pubertal development and fertility, with GnRH acting as a key messenger stimulating pituitary release of gonadotrophins and the reactivation in GnRH neuron secretion being the crucial process that instigates pubertal changes (Ebling 2005). Two seminal papers, released in quick succession, provided evidence that kisspeptin influenced pubertal development.

de Roux and coworkers looked at a series of patients with isolated hypogonadotrophic hypogonadism (IHH) (de Roux *et al.* 2003), a condition characterised by inadequate gonadotrophin secretion in isolation and without anosmia. Although research had previously described a loss-of-function mutation affecting the GnRH receptor (de Roux *et al.* 1997), the authors were able to identify a mutation affecting the *KISS1R* using a genome-mapping strategy as the responsible genetic basis for their presentation.

This paper was followed by that of Seminara and coworkers, who used linkage analysis and genetic screening in a large consanguineous family with IHH, and an unrelated patient with IHH, to identify mutations in *KISS1R* (Seminara *et al.* 2003). They then went on to develop a mouse model with *KISS1r*-deficient mice. These mice possessed a phenotype with an absence of pubertal development. Not only were they physically immature, but biochemical differences were noted, with the *KISS1r* knockout mice having low-circulating gonadotrophins.

Since these early papers, other loss-of-function mutations affecting *KISS1R* have been identified. Tenenbaum-Rakover and coworkers performed a detailed evaluation of five patients presenting with IHH with all being homozygous for a single mutation leading to a leucine substitution with proline, which was found to completely inhibit *KISS1R* signalling (Tenenbaum-Rakover *et al.* 2007). Patients were found to have LH pulses at normal frequency, but lower amplitude than expected. They all responded to exogenous GnRH stimulation, although there was reduced pituitary response in *KISS1R*-mutated patients. The authors concluded that *KISS1R*-mutated patients have delayed pubertal maturation of the gonadotrophic axis, rather than a total absence of pubertal maturation, with pituitary and gonadal function remaining intact.

Not only have mutations affecting the kisspeptin receptor being shown to influence pubertal development in humans, but Topaloglu and coworkers reported the finding of a loss-of-function mutation affecting *KISS1* being responsible for the presentation of idiopathic hypogonadotrophic hypogonadism in a family with four affected members (Topaloglu *et al.* 2012). These findings suggest that not only is the kisspeptin receptor necessary, but also a functioning kisspeptin peptide, and further emphasises the requirement for normal signalling within the kisspeptin system for pubertal development.

These findings from clinical research have been supported by several animal studies. Funes and coworkers generated a mutant mouse with disruption of the *Kiss1r* receptor (Funes *et al.* 2003). Phenotypic analysis was performed showing that in both male and female knockout mice, abnormalities in the development of external and internal genitalia were seen, along with altered organ weight-and-body weight ratios (in male wild-type mice, testis volume was 0.2 ± 0.04, compared with 0.02 ± 0.01 in homozygous mutant mice).

Later work by Novaira and coworkers looked at kisspeptin signalling specifically at the level of the GnRH neuron (Novaira *et al.* 2014). Developing a mouse model with deletion of the *Kiss1r* gene only in the GnRH neuron, they found that male knockout mice had microphallus and decreased anogenital distance compared with wild-type

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controls. Both processes are androgen specific, suggesting that disruption to the kisspeptin receptors on the GnRH neuron specifically is capable of altering the production of sex steroids. Delay in pubertal onset was also seen in both male and female knockout mice, and circulating gonadotrophins were lower than in wild-type mice. Influences on fertility were observed, with no pregnancies observed when knockout males were mated with female mice previously shown capable of becoming pregnant. Although several papers have reported *Kiss1r* knockout mice to lack pubertal features and have low-circulating gonadotrophins (d'Anglemont de Tassigny *et al.* 2007), these findings were particularly pertinent, suggesting that the role of kisspeptin at the level of the GnRH neuron influences pubertal onset and reproductive health.

Despite these findings, rodent studies have at times suggested a more nuanced role of kisspeptin. Lapatto and coworkers used targeted deletion to establish whether the phenotype of *Kiss1* and *Kiss1r* knockout mice would differ (Lapatto *et al.* 2007), revealing that although all *Kiss1* and *Kiss1r* knockout mice have abnormal sexual maturation, a subpopulation of *Kiss1* knockout females appeared to have a phenotype more similar to wild-type controls, having larger gonadal weights, larger vaginal openings and persistent evidence of vaginal cornification. The authors postulated that although additional factors could be at play, including genetic polymorphisms, these findings indicated that mutations affecting the *Kiss1* gene could still allow a near-normal progression in rodents towards sexual maturity.

Other studies have similarly described a variation in the degree of disruption to sexual maturation caused by mutations affecting the kisspeptin system.

Chan and coworkers reported that in their population of female *Kiss1*$^{-/-}$ and *Kiss1r*$^{-/-}$ knockout mice (Chan *et al.* 2009), despite puberty being delayed, the majority experienced oestrus. Interestingly, administration of a competitive GnRH antagonist impacted on oestrus in both mice, suggesting that GnRH activity was responsible for their progression through oestrus. They subsequently demonstrated that in both male and female *Kiss1*$^{-/-}$ knockout mice, and *Kiss1r*$^{-/-}$ knockout mice, gonadectomy followed by GnRH antagonist resulted in reduction in circulating gonadotrophins, again suggesting that these populations of knockout mice possessed some degree of GnRH activity.

In contrast to much of current literature, Mayer and Boehm (2011) reported that in mice in whom *Kiss1*-expressing cells had been ablated, although gonadal development appeared to have been affected, the timing of puberty was not affected in these female mice who were also found to be fertile, with the authors suggesting that another unmeasured regulatory copeptide could account for their findings.

It is difficult to know the extent to which these rodent studies can be extrapolated to humans, and studies in primates and humans would provide more clarity. However, although these findings do suggest that rodent maturation can occur, despite alteration of kisspeptin signalling, in none of the models described was pubertal development wholly normal.

Not only has puberty been shown to be delayed due to pathology affecting the kisspeptin system, but evidence also suggests that puberty onset can be activated early due to mutations affecting it.

Teles and coworkers described an activating mutation affecting *KISS1r* responsible for development of precocious puberty in a proband (Teles *et al.* 2008). Identifying heterozygous substitution of cytosine for guanine in the region coding for the kisspeptin receptor, they subsequently used *in vitro* analysis to demonstrate that cells transfected with the mutated *KISS1r* had a slower rate of desensitisation and prolonged activation of the mutant *KISS1r* in response to kisspeptin. Importantly, the authors demonstrated the described system to be non-constitutive, explaining why rather than GnRH secretion being reduced due to permanent receptor desensitisation caused by excessive stimulation, GnRH release was instead increased with a reduction in the rate of *KISS1R* desensitisation and thus prolonged cellular action. Although this mutation was absent in other unrelated patients with precocious puberty, Silveira and coworkers also reported the finding of missense mutations being associated with central precocious puberty (Silveira *et al.* 2010).

In both animal and human studies, kisspeptin has been shown to be crucial for normal pubertal development. As people progress into adulthood, reproductive health becomes vital to ensure fertility. The effect of kisspeptin on post-pubertal adults has been extensively studied in both healthy males and females and those with reproductive problems.

Adulthood: kisspeptin administration in health

Kisspeptin in males

In 2005, first-in-man studies were performed looking at the effect of a 90-min intravenous infusion of kisspeptin-54 in

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healthy adult males (Dhillo *et al.* 2005). The study found that at an infusion rate above 0.25 pmol/kg·min, mean LH increased over time in a dose-dependent fashion, up to an infusion rate of 12 pmol/kg·min. Although effects were seen on FSH and testosterone, similar to other studies (Thompson *et al.* 2004, Navarro *et al.* 2005), the authors noted the effect of kisspeptin to be most marked on LH, suggestive of kisspeptin acting through GnRH whose action is known to preferentially affect LH secretion (Skorupskaite *et al.* 2014).

A single intravenous bolus of the C-terminal decapeptide of kisspeptin has also been demonstrated to induce LH secretion (Chan *et al.* 2011). Interestingly, kisspeptin amplified the LH response with the larger LH pulses stimulated by kisspeptin administration than endogenous LH pulses (amplitude kisspeptin 5.0 ± 1.0 vs endogenous 2.1 ± 0.3 mIU/mL; $P = 0.02$). The authors also noted that kisspeptin-induced LH pulses had a morphology strikingly similar to that seen when administering GnRH, providing supportive evidence that the effect of kisspeptin is GnRH mediated. Pulse interval was significantly different following kisspeptin bolus ($P < 0.03$), suggesting that kisspeptin was able to reset the hypothalamic clock driving GnRH pulse secretion (Chan *et al.* 2011). Importantly, this study showed that a single intravenous bolus dose of kisspeptin was capable of interrupting GnRH neuron activity, with sustained activation of GnRH neurons lasting approximately 17 min *in vivo*. Other lengths of the kisspeptin peptide have been shown to possess bioactivity in humans.

George and coworkers administered kisspeptin-10 as an intravenous bolus followed by a continuous infusion over 22.5 h (George *et al.* 2011). Bolus injection of kisspeptin-10 was found to increase LH in a dose-dependent manner ($P < 0.0001$), as well as FSH ($P = 0.012$), but not testosterone, whereas infusion of kisspeptin-10 resulted in sustained increases in LH and FSH concentration, but also caused a statistically significant rise in testosterone. Jayasena and coworkers reproduced these findings (Jayasena *et al.* 2011), showing that a single intravenous bolus of kisspeptin-10 was sufficient to stimulate a significant increase in LH (area under the curve (AUC) increase in LH with dose of 1 nmol/kg, $P < 0.01$, compared with saline, and with dose of 10 nmol/kg, $P < 0.001$, compared with saline) and result in a lesser rise in FSH.

Despite varying peptide lengths being used, it is apparent that different isoforms of kisspeptin when administered in several routes are able to stimulate gonadotrophin release in healthy men, in keeping with previous animal and pharmacological studies.

Kisspeptin in females

Animal models have found kisspeptin to affect gonadotrophin release in female subjects as well as males (Irwig *et al.* 2004, Navarro *et al.* 2004). Building on this, Jayasena and coworkers administered an intravenous bolus of kisspeptin-10 in women at both the follicular and preovulatory phases of the menstrual cycle (Jayasena *et al.* 2011), finding that circulating gonadotrophins increased. Interestingly, the females' response to kisspeptin was altered according to their menstrual cycle, with intravenous infusion of kisspeptin-10 failing to increase gonadotrophin levels in women in the follicular phase.

Similarly, Chan and coworkers administered kisspeptin(112–121) as a single intravenous bolus to women at varying stages of the menstrual cycle (Chan *et al.* 2012). All those women in the luteal and preovulatory phases were found to have robust LH responses following intravenous bolus administration of kisspeptin. Response in women in the follicular phase, however, was varied, with only half of those in the follicular phase exhibiting a clear response, despite increasing the dose of kisspeptin given. The authors hypothesised that greater endogenous kisspeptin secretion in the early follicular phase could account for this variation across the menstrual cycle.

Importantly, however, George and coworkers gave a single intravenous bolus of kisspeptin-10 and were able to stimulate gonadotrophin secretion in women in the early follicular phase (George *et al.* 2012*a*); a difference which in part could be accounted for by the relatively small numbers involved in the earlier studies and the fact that, here, an LH baseline was measured, meaning that subtle changes could be detected.

These findings support those of earlier studies using kisspeptin-54, which showed that women in the early follicular phase did respond to exogenous administration, given both subcutaneously and intravenously (Dhillo *et al.* 2007, Jayasena *et al.* 2013). Taken as whole, it appears that exogenous kisspeptin-10 and kisspeptin-54 are both able to stimulate LH secretion in women in the follicular phase (Dhillo *et al.* 2007, George *et al.* 2012*a*, Jayasena *et al.* 2013), although these effects are less obvious than at other times in the cycle (Jayasena *et al.* 2011, Chan *et al.* 2012), which may in part be due to increased endogenous kisspeptin secretion.

Having demonstrated the effect of kisspeptin in eliciting gonadotrophin release in females, more recent studies have aimed to investigate the factors that control kisspeptin release.

George and coworkers administered a bolus of kisspeptin-10 to women in the early follicular phase, as

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well as those taking sex steroid contraceptives and those who were post-menopausal and not receiving sex steroid hormone replacement therapy (George *et al.* 2012*a*). They found that kisspeptin-10 stimulated LH secretion in women in the follicular phase, those on progesterone contraceptives and those who were post-menopausal, with those in the post-menopausal group having the greatest ΔAUC LH ($P=0.01$). Kisspeptin also stimulated FSH release in all groups, although the change was only significant in post-menopausal women ($P=0.02$). Thus, the authors concluded that these findings supported the notion that sex steroid feedback occurs at the hypothalamus, as well as the pituitary gland, with kisspeptin having its maximal effect in sex steroid-deficient women (post-menopausal). It is notable that similar to previous studies, George and coworkers found LH to be preferentially stimulated by kisspeptin compared with FSH (George *et al.* 2012).

With studies in both animals and humans consistently showing kisspeptin to modulate and stimulate gonadotrophin secretion in adulthood, studies have looked into using it as a potential therapeutic agent in several reproductive disorders.

Kisspeptin administration in disease

Kisspeptin in hypogonadotrophic hypogonadism and HA

Hypogonadotrophic hypogonadism and HA may result from structural damage to the hypothalamus or conditions affecting hypothalamic function. Given that kisspeptin acts upstream of GnRH as evidenced by the action of kisspeptin antagonists inhibiting GnRH neuronal activity (Roseweir *et al.* 2009, Millar *et al.* 2010), it may be hypothesised that where hypothalamic neurons remain intact, kisspeptin could instigate the recovery of the hypothalamic–pituitary–gonadal axis.

Functional hypogonadotrophic hypogonadism observed in obesity and type 2 diabetes results from disruption in hypothalamic stimulation of the pituitary gland leading to reduced circulating gonadotrophins (George *et al.* 2010). In a study comparing five medication-controlled type 2 diabetic males with low testosterone levels with healthy age-matched controls, acute kisspeptin-10 administration elicited a robust increase in serum LH in both healthy men and those with type 2 diabetes (5.5 ± 0.8 to 14.5 ± 1.8 IU/L, $P=0.002$, and 4.7 ± 0.7 to 10.7 ± 1.2 IU/L, $P=0.02$, respectively) (George *et al.* 2012*b*). Administration of kisspeptin-10 infusion resulted in a significant increase in total LH secreted (mean LH 3.9 ± 0.1 to 20.7 ± 1.1 IU/L, $P=0.03$), LH pulsatility amplitude ($P=0.007$) and frequency ($P=0.05$). Kisspeptin-10 infusion was also found to increase serum testosterone, from 8.5 ± 1.0 at baseline to 11.4 ± 0.9 nmol/L, $P<0.005$. The relatively young age of participants in this study (33.6 ± 3 years) makes it difficult to generalise these findings to a wider diabetic population. However, it certainly suggests that kisspeptin has therapeutic potential in the treatment of functional hypogonadotrophic hypogonadism.

In HA, as well as circulating gonadotrophins being reduced, there is subsequent secondary amenorrhoea (Baranowska & Zgliczynski 1982, Nakamura *et al.* 1985), and the therapeutic use of kisspeptin in this setting has also been explored.

Circulating gonadotrophins increased in pre-pubertal rats starved for 72 h when kisspeptin-10 was administered centrally (Castellano *et al.* 2005) with a heightened response observed compared with those fed *ad libitum*. Pubertal activation, as measured by vaginal opening, was observed in 60% of malnourished female rats, following administration of kisspeptin-10. Building on these findings, clinical research in humans has also found kisspeptin to be able to restore gonadotrophin release where hypothalamic dysfunction has occurred due to reduced energy intake.

In the first study looking at the use of kisspeptin in an infertility model, kisspeptin-54, given as a twice daily subcutaneous injection, significantly increased serum LH levels in women with HA on the first day of administration ($P<0.001$) (Jayasena *et al.* 2009). Women with HA appeared to be more sensitive to the stimulatory effects of kisspeptin than their healthy counterparts, with an approximate four-fold increase in acute LH response compared with healthy women in the follicular phase. Interestingly, whereas animal studies described phenotypic changes consistent with restoration of reproductive health with kisspeptin in humans with HA, no such changes were seen. However, repeat administration of kisspeptin was associated with a reduction in response, suggestive of desensitisation.

Further studies have attempted to identify a dosing regimen which yields maximal therapeutic benefits. A twice weekly dose of subcutaneous kisspeptin-54 was given to ten women with HA at a dose of 6.4 nmol/kg (the same dose as used in previous studies) (Jayasena *et al.* 2010). Kisspeptin administration increased gonadotrophin release on day 1, although importantly a reduction in LH response was seen after 2 weeks of twice weekly kisspeptin injections, suggestive of tachyphylaxis or chronic desensitisation. Although gonadotrophin secretion was restored to an extent, again follicle size and endometrial thickness remained unchanged.

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Given the observed effect of desensitisation with chronic administration, continuous intravenous infusion of kisspeptin-54 was given to five patients with HA to elucidate the dose-dependent therapeutic window, which would restore LH pulsatility without causing desensitisation (Jayasena *et al.* 2014*c*). Although inter-patient variation was noted, the authors found that a dose of 0.10 nmol/kg/h increased mean pulsatile LH secretion by four-fold, and at doses of 0.01–0.30 nmol/kg/h, there was no evidence of desensitisation. Importantly, the effects of intravenous KP54 on LH were removed at 4 h after discontinuation.

In sum, kisspeptin-54, given subcutaneously and intravenously, appears to be able to restore LH pulsatility in women with HA. However, significant issues remain regarding administration, and there has yet to be evidence showing kisspeptin's ability to affect phenotype in humans with HA.

Kisspeptin in patients with NKB signalling deficiencies

As earlier described, kisspeptin neuron activity appears to be modulated by Dyn and NKB. A cohort of patients presenting with congenital hypogonadotrophic hypogonadism has been found to have inactivating mutations affecting the NKB ligand (TAC3) and its receptor (Tusset *et al.* 2012). In a study looking at four such patients, intravenous infusion of kisspeptin-10 resulted in an increase in LH secretion from baseline ($P<0.0001$). Interestingly, although the stimulated LH responses were slower and at a lower concentration than that observed in a healthy population, the fold stimulation (reported as mean 3.1-fold) was similar to that in healthy men receiving the identical continuous infusion (3.2-fold) in a previous study (Young *et al.* 2013). The authors hypothesised that the slower responses in LH could be accounted for by GnRH neuron atrophy and suggested that a priming dose might be necessary (Young *et al.* 2013). Similar to previous studies, kisspeptin administration increased LH pulse frequency, but the amplitude was lower than that expected in normal subjects, providing evidence that GnRH function is restored with greater ease than gonadotroph function.

Kisspeptin in hyperprolactinaemia

States of hyperprolactinaemia are associated with suppression of pulsatile LH secretion and reduction in GnRH release (Grattan *et al.* 2007), with subsequent infertility and amenorrhoea alongside symptoms related to hyperprolactinaemia. Many patients fail to tolerate current standard treatment (dopamine agonists) with reports of up to 20% discontinuing treatment due to side effects (Kaiser 2012). There therefore exists a clinical requirement for an alternative therapy. Given the body of evidence showing kisspeptin's role in restoring the hypothalamic–pituitary–gonadal axis, some have investigated the use of kisspeptin as a potential treatment for hyperprolactinaemia.

States of high prolactin have been shown to be associated with reduced *KISS1* expression (Sonigo *et al.* 2012, Araujo-Lopes *et al.* 2014), with researchers suggesting that this may be the underlying mechanism behind hyperprolactinaemia-associated hypogonadism (Araujo-Lopes *et al.* 2014).

Sonigo and coworkers, using daily intra-peritoneal administration of kisspeptin-10, were able to induce normal oestrus cycles in mice rendered hyperprolactinaemic and subsequently acyclic (Sonigo *et al.* 2012). They demonstrated that not only was ovulation restored, but the biochemical effects of prolactin on gonadotrophins were also reversed. The authors postulated that this indicated that kisspeptin neurons played a significant role in hyperprolactinaemic anovulation and that it may thus have therapeutic potential for those unable to tolerate dopamine agonists.

A small case series regarding the use of kisspeptin in hyperprolactinaemic patients has recently been presented (Binart 2015). Yet, these findings remain to be fully replicated in human studies.

Kisspeptin in *in vitro* fertilisation

In a natural menstrual cycle, LH peaks at day 14 resulting in ovulation, as well as oocyte maturation (Kol & Humaidan 2010). This process is crucial, allowing fertilisation to occur, and hence, it is imperative to replicate it in *in vitro* fertilisation (IVF).

Current standard IVF protocols often use human chorionic gonadotrophin (hCG) that acts on the ovarian hCG/LH receptor to stimulate follicular maturation (Castillo *et al.* 2014). However, whereas the physiological LH surge has a duration of approximately 48 h (Hoff *et al.* 1985), the half-life of exogenous hCG is double that of the endogenous LH surge, with studies suggesting that hCG may persist in the circulation for up to 7 days (Damewood *et al.* 1989). It is thought that the perseverance of hCG contributes to the development of OHSS, a potentially life-threatening condition that can affect 4% of all women

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undergoing IVF (Delvigne & Rozenberg 2002), but whose prevalence can increase by five-fold in at-risk populations (Wada *et al.* 1993).

Animal studies first suggested kisspeptin's ability to induce the LH surge necessary for ovulation and oocyte maturation. Transgenic mice null for kisspeptin or its receptor have been noted to lack the LH surge and subsequent failure of ovulation (Clarkson *et al.* 2008). Kisspeptin administration has also been found to induce ovulation in several mammals, including sheep (Caraty *et al.* 2007), musk shrews and rats (Matsui *et al.* 2004). Building on this, proof-of-concept studies were carried out in patients undergoing IVF. Using varying doses, a single subcutaneous injection of kisspeptin was used to trigger egg maturation in a standard superovulation and GnRH antagonist protocol. At all doses, rates of egg maturation were 75 – 85% (Jayasena *et al.* 2014*b*), with oocyte yield increasing with kisspeptin dose. Fertilisation occurred in 92% of all patients with biochemical pregnancy rates of 40% and clinical pregnancy rates of 23%. This seminal paper demonstrated for the first time the ability of kisspeptin to achieve oocyte maturation sufficient for oocyte fertilisation in humans.

Although OHSS is not common, its clinical consequences can be profound, with death being a potential outcome (Mathur *et al.* 2006). Current strategies to prevent OHSS include the use of GnRH agonist to trigger oocyte maturation in place of hCG. Even with this strategy, however, there have been case reports of OHSS, despite the absence of supplemental hCG for luteal support (Macklon *et al.* 2006). Implantation rates have also been reported to be reduced with GnRH agonist use, due to luteal phase insufficiency (Macklon *et al.* 2006). Having demonstrated kisspeptin's ability to act via endogenous pathways to stimulate an LH surge of a duration much closer to that of a physiological cycle, it was hypothesised that it might provide an alternative for triggering oocyte maturation in women at risk of OHSS (Abbara *et al.* 2015).

Abbara *et al.* (2015) performed a two-phase multi-dose randomised controlled trial of 60 women at high risk of OHSS (Abbara *et al.* 2015). A standard antagonist protocol was used, with a single injection of varying doses of kisspeptin-54 used to trigger oocyte maturation. Across all doses, there were only three cases of mild early OHSS and one case of mild late OHSS, with no patients requiring medical intervention. This is significant, given that the population were at high risk of OHSS where OHSS rates of 27% might have been expected (Jayaprakasan *et al.* 2012).

Oocyte maturation was achieved in 95% of patients, with embryo formation occurring in 90%. At all doses, biochemical pregnancy rate was 63%, with clinical pregnancy rate of 53% and live birth rates of 45%. Highest pregnancy rates were seen with a dose of 9.6 nmol/kg, achieving rates of 85% for biochemical pregnancy, 77% for clinical pregnancy and 62% live births. Although the numbers included in this study at the best achieving dose were small, its findings provide further evidence for the potential of kisspeptin in a therapeutic setting.

Further work is necessary to gain a greater understanding of the use of kisspeptin to trigger oocyte maturation in women at risk of OHSS; in particular, a large randomised controlled trial is required to compare kisspeptin with current standard triggers to assess how clinically useful it will be. However, early evidence appears promising.

Kisspeptin and ageing

Kisspeptin and the menopause

The utility of kisspeptin extends beyond that of the reproductive age. Post-menopausal women possess a hormonal profile characterised by low-circulating sex steroids and initially high gonadotrophins due to reduced negative feedback. Given this, it might be hypothesised that expression of the *KISS1* gene and its receptor might alter. Rometo and coworkers looked at pre- and post-menopausal women's hypothalamic specimens finding marked hypertrophy of neurons in the infundibular nucleus of post-menopausal women (Rometo *et al.* 2007). Interestingly, most of these were labelled with the *KISS1* probe.

These findings were replicated when the infundibular nucleus of ovarectomised monkeys was examined, with an increase in *KISS1* neurons noted and evidence of kisspeptin neuron hypertrophy seen. When oestrogen or oestrogen with progesterone was given to ovarectomised monkeys, *KISS1* mRNA-expressing neurons reduced to near undetectable levels, providing evidence that changes in *KISS1* expression occur due to the loss of ovarian oestrogen. Other studies have provided similar evidence that removal of sex steroids at the menopause affects *KISS1* gene expression (Navarro *et al.* 2004, Smith *et al.* 2005*a*, Rometo *et al.* 2007, Oakley *et al.* 2009).

Building on this, some have compared the response of post-menopausal women to those using hormonal contraception, as well as those in the follicular phase (George *et al.* 2012*a*). They found that post-menopausal

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women showed a brisk response to kisspeptin-10 bolus injection, with LH increasing from a baseline of 35.3±2.8 to 44.7±3.4IU/L at its peak ($P<0.005$). Interestingly, in contrast to the other groups, FSH also increased from a baseline value of 21.8±4.9 to a peak of 25.7±4.4IU/L ($P<0.04$). Compared with those in the follicular phase, they found that post-menopausal women's response to kisspeptin-10 was greater, whereas those using exogenous oestrogen and progesterone for contraception had only a minimal response. These results, whereby post-menopausal women were more responsive to exogenous kisspeptin than those in the follicular phase, suggested that although sex steroid feedback is likely to influence kisspeptin secretion, the relationship is a complex one.

Although it is not clear yet whether kisspeptin modulates menopausal symptoms, or whether its manipulation might have a therapeutic benefit, the above observations raise the possibility that it is important and thus might be able to be influenced to provide symptom relief.

Kisspeptin in the ageing male

The ageing process also affects the male reproductive axis, with increased gonadotrophins (Feldman *et al.* 2002), reduced LH pulses (Veldhuis *et al.* 2010) and reduced serum levels of free testosterone. Given that evidence has shown that sex steroids influence kisspeptin secretion, it may be expected that male kisspeptin neuron expression will alter with age.

Molnar and coworkers performed a series of immuno-histochemical investigations on male hypothalamic tissue taken post-autopsy (Molnar *et al.* 2012). In men older than 50 years of age, kisspeptin cell bodies had a 2.6-fold higher mean density ($P=0.004$) and that although a similar change was seen in NKB cell bodies, the change was much less marked (at only 1.5-fold). Fibre density also increased by 3.1-fold ($P<0.05$) in kisspeptin neurons.

Although the effects of kisspeptin in ageing populations remain largely unexplored, the kisspeptin neuron population in the infundibular nucleus clearly increases with time. Interestingly, however, in the same manner that sexual dimorphism exists with regard to response to kisspeptin administration, Hrabovszky and coworkers demonstrated that kisspeptin neuron population is markedly different between ageing males and females with a much greater effect observed in females (Hrabovszky *et al.* 2011). Although much remains to be known about the effect of ageing, the findings are interesting and corroborate existing knowledge regarding the influence of sex steroids on kisspeptin production.

Conclusion

Since its first description in relation to metastatic disease, our understanding of the role of the *KISS1* gene, its peptide product and subsequent receptor has increased and altered significantly.

Kisspeptin-producing neurons have consistently, across species, been demonstrated as existing in the hypothalamus and in particular the pre-optic region and infundibular nucleus in humans, corresponding to the arcuate nucleus in rodents. Their role here has been shown to act as a crucial gatekeeper of reproductive function at pivotal times in the human lifespan, down-regulating fertility at times of physical strain, such as over-exercise or weight loss, and controlling normal physiological development at puberty.

Evidence also suggests that their influence extends beyond that of puberty and the age of reproduction, with kisspeptin neurons being demonstrated *in utero* and in the immediate postnatal periods. This is further confirmed by the observation that administration of kisspeptins stimulates gonadotrophin release and can modulate reproductive pathology.

Looking to the future, much remains undescribed with regard to kisspeptin. However, as a group of peptides, they possess huge potential not only for therapeutic use but also for manipulation across the lifespan, with the ability to perhaps change outcomes in reproductive health.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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