# Role of kisspeptins in the control of the hypothalamic-pituitary-ovarian axis: old dogmas and new challenges

Suvi T. Ruohonen, Ph.D.,[a,b] Matti Poutanen, Ph.D.,[a,b] and Manuel Tena-Sempere, M.D., Ph.D.[a,b,c,d,e]

[a] Research Center for Integrative Physiology and Pharmacology, Institute of Biomedicine, University of Turku; [b] Turku Center for Disease Modeling, Turku, Finland; [c] Department of Cell Biology, Physiology, and Immunology, University of Córdoba; [d] Instituto Maimónides de Investigación Biomédica de Córdoba and Hospital Universitario Reina Sofia; and [e] CIBER Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Córdoba, Spain

In humans and other mammals, a hallmark of female reproductive function is the capacity to episodically release fertilizable oocytes under the precise control of a cascade of hormonal regulators that interplay in a cyclic manner within the hypothalamic-pituitary-ovarian (HPO) axis. Although the basic elements of this neurohormonal system were disclosed several decades before, a major breakthrough in our understanding of how the HPO axis is controlled during the lifespan came in the first decade of the 21st century, when the reproductive dimension of kisspeptins was disclosed by seminal studies documenting that genetic inactivation of the kisspeptin pathway is linked to central hypogonadism and infertility. Kisspeptins are a family of peptides, encoded by the Kiss1 gene, that operate via the surface receptor, Gpr54 (also called Kiss1r), to regulate virtually all aspects of reproduction in both sexes. The primary site of action of kisspeptins is the hypothalamus, where Kiss1 neurons engage in the precise control of the pulsatile release of GnRH to modulate gonadotropin secretion and, thereby, ovarian function. Nonetheless, additional sites of action of kisspeptins within the HPO axis, including the pituitary and the ovary, have been proposed; yet, the physiologic relevance of such extrahypothalamic actions of kisspeptins is still a matter of debate. In this review, we summarize the current consensus knowledge and open questions on the sites of action, physiologic roles, and eventual therapeutic implications of kisspeptins in the control of the female reproductive axis. (Fertil Steril® 2020;114:465–74. ©2020 by American Society for Reproductive Medicine.)

**Key Words:** Kisspeptins, Gpr54, GnRH, gonadotropins, ovulation



## FIRST THINGS FIRST: THE HYPOTHALAMIC-PITUITARY-OVARIAN AXIS

Ovarian maturation and its exquisite cyclic function during the reproductive lifespan fully rely on the coordinate action of the neurohormonal elements of the hypothalamic-pituitary-ovarian (HPO) axis (1). As is the case for other neuroendocrine axes, the HPO axis is a hierarchic system, in which a scarce neuronal population located in the basal forebrain and producing the decapeptide GnRH, operates as a major conduit for the brain to control the reproductive system. Thus, GnRH is released in a pulsatile manner to the hypothalamic-hypophyseal portal circulation to reach the anterior pituitary, where GnRH pulses act on gonadotropes, to elicit the secretion of both gonadotropins, LH and FSH. These, in turn, are released to the systemic circulation to reach the ovary, where, acting in concert on different cellular components, they promote ovarian maturation and production and release of female gametes, as well as the secretion of sex steroids and other gonadal hormones of peptidergic nature (2).

An essential aspect of the functionality of the HPO system is the characteristic secretory modes of their upstream elements, namely, GnRH and gonadotropins (1). Indeed, acquisition of a mature pattern of pulsatile secretion of GnRH, which occurs at puberty, is mandatory for dictating appropriate secretory profiles of LH and FSH needed to ensure ovarian maturation and function (3). Perturbation of such pulsatile patterns, e.g., by continuous exposure to GnRH analogues, results in receptor desensitization and suppression of gonadotropins and, thereby, ovarian function, a feature

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Reprint requests: Manuel Tena-Sempere, Department of Cell Biology, Physiology, and Immunology, Faculty of Medicine, University of Córdoba, Avda. Menéndez Pidal s/n. 14004 Córdoba, Spain (E-mail: fi1tesem@uco.es).

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that has been exploited therapeutically in hormone-dependent disorders (4). Moreover, deregulation of GnRH pulsatility is seemingly implicated in various reproductive pathologies, such as polycystic ovary syndrome (PCOS) and hypothalamic amenorrhea (5).

Realization of these fundamental pathophysiologic roles has prompted active investigation of the mechanisms whereby GnRH neurons are capable to release, in a synchronized and timely manner, pulses of GnRH; a phenomenon that is driven by a complex circuit of neuronal and nonneuronal afferents to GnRH neurons (6–9), which form the GnRH pulse generator (10). This functional hub is able to integrate a wide range of regulators of ovarian function, from environmental cues (e.g., light cycle and nutritional inputs) to endogenous signals (e.g., metabolic hormones). In this network, Kiss1 neurons, producing kisspeptins, have recently been pointed out as a major component, with key roles in dictating GnRH pulsatility (11) and, thereby, cyclic ovarian function, as summarized in the following sections.

In addition, the elements of the so-called Kiss1 system have been detected at other levels of the HPO axis, where they may operate as local modulators of gonadotropin secretion at the pituitary and/or ovarian function In addition, uterine and gestational actions of kisspeptins have been described. Yet, the physiologic relevance of these extrahypothalamic actions of kisspeptins remains debatable and largely undefined. In this minireview, we summarize the current consensus knowledge and open questions about the roles of kisspeptins in control of the female reproductive axis. For a summary of the major biologic effects and sites of action of kisspeptins in the HPO axis, see also Table 1.

## THE BOSS ALWAYS WORKS FOR SOMEONE ELSE: EMERGENCE OF KISSPEPTINS IN REPRODUCTIVE PHYSIOLOGY

Despite the indisputable role of GnRH as the major output pathway whereby the brain controls the reproductive axis (12), compelling evidence gathered in recent decades has documented that GnRH neurons themselves are devoid of the main receptors for key regulators of the HPO axis, such as estrogens and leptin, just to mention two paradigmatic examples. This feature illustrates that a substantial component of the central regulation of the reproductive axis takes place upstream from GnRH neurons, so that integration of different regulatory inputs occurs at the level of neuronal and nonneuronal afferents of this neuronal population, which dictate the activation (or, eventually, inactivation) of the ultimate effector, GnRH.

Kisspeptins, a family of peptides encoded by the Kiss1 gene, initially regarded to be metastasis suppressors, were first recognized as gatekeepers of the reproductive axis in late 2003, when inactivating mutations of the gene encoding their receptor, Gpr54, were reported in patients affected of isolated central hypogonadism (13, 14). Short after the disclosure of the reproductive dimension of kisspeptins, the mechanism(s) and site of action whereby the Kiss1 system activates the reproductive axis began to be actively investigated. Compelling evidence conclusively demonstrated that the ability of kisspeptins to potently stimulate gonadotropin secretion, which has been documented in a wide variety of species, including humans, involves a primary action at the hypothalamic level, where kisspeptins are produced by discrete neuronal populations to operate as major synaptic excitatory input on GnRH neurons (1, 6).

The contention that kisspeptins elicit gonadotropin secretion via a GnRH pathway was initially suggested by the fact that blockade of GnRH actions, by the use of a pharmacologic antagonist, completely suppressed the gonadotropin-releasing actions of kisspeptins in vivo. In addition, in situ hybridization in rat brain sections showed that a majority (>75%) of GnRH neurons coexpress the mRNA encoding the kisspeptin receptor (Gpr54) and that kisspeptin is able to activate GnRH neurons, as measured by c-Fos induction and electrical firing of GnRH neurons (6). In addition, the capacity of kisspeptins to elicit GnRH secretion was documented both ex vivo, with the use of rat hypothalamic explants, and in vivo, where central infusion of kisspeptin was shown to induce a marked rise of GnRH levels in the cerebrospinal fluid of sheep. As a whole, these data convincingly pointed out that kisspeptins operate primarily on hypothalamic GnRH neurons to induce GnRH secretion, which in turn drives LH and FSH release from the pituitary.

The advent of more incisive techniques for neuronal monitoring and manipulation has allowed confirming and refining those initial observations. Thus, by using an elegant combination of fiber photometry and optogenetic approaches, Clarkson et al. recently documented that populations of Kiss1 neurons located in the hypothalamic arcuate nucleus (ARC) play a fundamental role in the generation of GnRH pulses (11) as an essential driver for the pulsatile secretion of gonadotropins. ARC Kiss1 neurons have been shown to display discrete calcium bursts, which perfectly coincide with LH secretory pulses. In addition, whereas optogenetic activation of ARC Kiss1 neurons evoked LH pulses, their inhibition, with the use of hyperpolarizing optogenetic tools, suppressed LH pulsatility (11). These functional studies are concordant with data from genetically modified mouse models, showing that kisspeptin actions solely at the level of GnRH neurons are sufficient to attain puberty and grossly maintain fertility (15). Altogether, this evidence unambiguously demonstrates that the effects of kisspeptins occur primarily at the level of GnRH neurons to centrally activate the HPO axis.

Despite the consensus on the indispensable role of these direct actions of kisspeptins, compelling evidence has also suggested that part of their central modulatory effects may derive from the ability of kisspeptins to indirectly modulate GnRH neurosecretion, via intermediary afferents. Thus, blockade of fast synaptic transmission, to globally eliminate ionotropic glutamatergic and GABAergic inputs, caused a decrease of GnRH neuronal responses to kisspeptin (16). Moreover, besides their direct postsynaptic effects on GnRH neurosecretion, kisspeptins operating presynaptically have been shown to increase glutamatergic and GABAergic transmission to GnRH neurons (17). These findings point out that at least part of the kisspeptin effects on GnRH neurons might be indirectly mediated via activation of glutamate and/or

GABA afferents to GnRH neurons. In favor of this possibility, more recent work with a mouse model engineered to preserve kisspeptin signaling selectively in GnRH neurons, but not elsewhere, revealed subtle, albeit detectable indirect actions of kisspeptins in the central modulation of the GnRH-gonadotropin axis (18). Admittedly, however, these indirect actions are modest and possibly less relevant than the direct effects of kisspeptin on GnRH neurons.

## ESSENTIAL ROLES OF KISSPEPTINS IN SEX STEROID FEEDBACK AND GONADOTROPIN SECRETORY PATTERNS

Proper function of the adult HPO axis critically relies on appropriate secretory patterns of GnRH and, thereby, gonadotropins. In contrast to males, where only tonic, pulsatile secretion occurs, in females two different modes of GnRH/gonadotropin secretion take place: the pulsatile and the surge modes (1, 19). The latter is responsible for triggering the ovulation and will be reviewed in the next section. The pulsatile mode is more predominant across the ovarian cycle and is shaped, to a large extent, by the negative feedback actions of ovarian steroids at the hypothalamic-pituitary unit. This pulsatile secretory pattern, which is responsible for driving follicular maturation and hormone production by the ovaries (12), is dictated to a large extent by the oscillatory activity of Kiss1 neurons located in the ARC in different mammalian species, or its equivalent infundibular area in humans (1, 11).

The tonic mode of secretion of GnRH is defined by a discrete burst of hormone release to the portal circulation, interspaced by periods of (very) low GnRH concentrations. Because the secretion of both LH and FSH is elicited by GnRH, but their secretory patterns diverge partially, it has been proposed that the frequency of GnRH pulses is critical for encoding preferential secretion of LH (high-frequency pattern) or FSH (low-frequency pattern) (20). Other parameters, such as the magnitude of hormone peaks and the threshold levels, contribute also to define the secretory profiles of gonadotropins, so that changes especially in circulating LH are thought to reflect similar changes in the portal patterns of secretion of GnRH.

As mentioned above, a major mechanism whereby pulsatile secretion of GnRH is homeostatically controlled is via the negative feedback actions of ovarian steroids, which contribute to keeping LH and FSH levels at check along the ovarian cycle (21). An apparent conundrum regarding negative feedback control was that GnRH neurons are devoid of estrogen receptor (ER) $\alpha$, which is responsible for mediating the major inhibitory effects of ovarian $E_2$ on the gonadotropic axis (12). This paradox was solved by the demonstration that Kiss1 neurons in the ARC do express ER$\alpha$ and are tonically repressed by estrogen, so that conditions of high $E_2$ levels are associated with inhibition of Kiss1 expression in the ARC (12). This provided a plausible pathway for transmitting the negative feedback actions of estrogen on GnRH.

This ARC population of Kiss1 neurons has been the subject of active investigation in the past decade. After the demonstration of its key role as target and transmitter for the negative feedback actions of estrogens, much excitement was caused by the finding that ARC Kiss1 neurons coexpress other transmitters with important functions in the central control of the HPO axis. Thus, in 2009, compelling evidence was presented for the colocalization of the tachykinin neurokinin-B (NKB) and the endogenous opioid dynorphin (Dyn) in a substantial fraction of ARC Kiss1 neurons in rodents and sheep (22, 23), a population that was named KNDy because of the co-expression of Kiss1, NKB, and Dyn (24). Contemporary to this finding, inactivating mutations of the genes encoding NKB (TAC3) or its receptor (TACR3) in humans were found to cause a state of central hypogonadism similar to that associated to inactivating mutations of the kisspeptin pathway (25). This, together with the realization that, to a variable extent, KNDy neurons are also found in the human hypothalamus, reinforced the interest for the role of this neuronal population in the control of the HPO axis also in humans. Functional analyses conducted in preclinical models led to the proposal of an oscillatory network within KNDy neurons, in which NKB and Dyn would operate as autoregulatory signals, modulating the output of kisspeptin onto GnRH neurons in a reciprocal manner. Thus, while NKB stimulates kisspeptin release via KNDy neurons, therefore inducing GnRH secretion, Dyn seemingly operates as an inhibitory input, suppressing kisspeptin secretion, and thereby GnRH pulsatility (26). Therefore, NKB and Dyn would act, in concert with other signals converging on Kiss1 neurons, in a yin-yang fashion, to shape kisspeptin pulses, which in turn dictate the generation of GnRH pulses as described above.

## KEY FUNCTION OF KISSPEPTINS IN THE CENTRAL CONTROL OF OVULATION: THE PREOVULATORY SURGE

Besides the tonic pattern of secretion described above, a massive discharge of gonadotropins, called the preovulatory surge, occurs periodically at mid-cycle to induce ovulation in adult females (1). This key event in female reproduction is driven by the surge mode of GnRH secretion, in which escalating levels of GnRH are detected over a period of hours in the portal circulation. This preovulatory surge of GnRH occurs in a timely fashion, which in rodents corresponds to the afternoon of proestrus (i.e., the phase preceding ovulation at estrus) and in women takes place in the later follicular phase of the menstrual cycle. Generation of this preovulatory surge critically relies on a switch from a predominant negative feedback to a positive feedback of estrogen, in which the increasing levels of circulating $E_2$ coming from the dominant follicles convey a stimulatory signal to GnRH neurons to enhance, rather than suppress, GnRH secretion (21). The cellular and molecular basis of such a dynamic and timely switch, which occurs only in the adult female, had remained to a large extent an enigma until the discovery of the key roles of specific populations of Kiss1 neurons in this phenomenon.

The first evidence for a putative role of Kiss1 neurons in estrogen positive feedback came from rodent studies, which documented that, whereas Kiss1 mRNA levels in the ARC are suppressed by estrogen, Kiss1 gene expression in a more rostral area of the hypothalamus, corresponding to the

anteroventral periventricular nucleus (AVPV), was reduced by ovariectomy and increased by estrogen replacement (27). Notably, the AVPV had long been known to act as a hypothalamic area involved in the positive feedback of estrogen. Thus, these initial observations strongly suggested the participation of AVPV Kiss1 neurons, which express ER$\alpha$ as well, in the positive feedback of $E_2$ on gonadotropin secretion during the preovulatory period (21). In good agreement, it was later demonstrated that, in the female rat, AVPV Kiss1 mRNA levels increase during the window of the preovulatory surge, whereas immunoneutralization of central kisspeptin or selective blockade of kisspeptin actions with the use of a specific antagonist blocked the preovulatory LH surge in cyclic rats (28, 29).

Although the connection of AVPV Kiss1 neurons with the preovulatory surge and estrogen positive feedback has been solidly documented in rodents, the role of equivalent populations, distinct from ARC/infundibular Kiss1 neurons, in humans and other mammals is still under debate. Admittedly, a region equivalent to AVPV is not found in primates or sheep, but Kiss1 neurons in the preoptic area have been described in these species. In addition, functionally different subpopulations of Kiss1 neurons within the ARC have been described in sheep, which might differentially contribute to mediate negative and positive feedback effects of estrogen. For example, Kiss1 neurons in the caudal portion of the ARC may collaborate with preoptic area Kiss1 neurons to mediate positive feedback in sheep (30). In humans, there is a lack of functional evidence for a specific subpopulation of Kiss1 neurons mediating the positive estrogen feedback to induce the preovulatory surge. Nonetheless, it is plausible that distinct Kiss1 neuronal pathways may participate in the generation of the surge mode of GnRH secretion in women (1). In fact, pharmacologic studies have revealed commonalities in other aspects of kisspeptin effects in the control of the HPO axis between women and rodents. For example, in both rat and human females, the efficiency of kisspeptin to elicit LH secretion changes across the ovarian cycle, it being maximal at the preovulatory stage (31, 32). It is worth noting that, in addition to changes at the hypothalamic level, an increase in GnRH signaling in the pituitary also occurs during the cycle (33, 34), which seems to play a major role in the generation of the preovulatory surge in women.

## PITUITARY ACTIONS OF KISSPEPTINS: WHAT IS THEIR PHYSIOLOGIC RELEVANCE?

While the predominance of the hypothalamic actions of kisspeptins in the control of the HPO axis is undisputed, fragmentary evidence has suggested the possibility of additional sites of expression and action at the pituitary level, where kisspeptins have been proposed to directly regulate gonadotrope function. Thus, initial in vitro analyses documented the capacity of kisspeptin-10 to stimulate pituitary LH secretion (35, 36). Yet these responses were modest in magnitude and clearly lower than those evoked by GnRH, despite the need of higher concentrations kisspeptin (35). In addition, kisspeptins have been shown to directly activate LH$\beta$ and FSH$\beta$ gene expression in murine primary pituitary cells and the L$\beta$T2 gonadotrope cell line (37). Furthermore, $Ca^{2+}$ responses in gonadotropes have been reported after kisspeptin stimulation in vitro in a variety of species, including rat (38), ovine (39), porcine (40), and bovine species (40, 41). Time- and dose-dependent LH responses have been detected also in primary cultures of pituitary cells from female baboons (42), which closely correspond to different aspects of human physiology. In addition, detectable concentrations of kisspeptins have been found in the hypophyseal portal circulation in the sheep, and secretion of kisspeptins at the level of the median eminence, with capacity to reach the portal system, has been documented in the rhesus monkey (43). Altogether, these data provided the basis for potential direct actions of kisspeptins of the control of the gonadotropic axis at the pituitary level.

In addition, Kiss1 and Gpr54 mRNAs, and their corresponding peptides, have been shown to be expressed at the rat pituitary (38, 44), with detectable levels in gonadotropes (44). This pituitary expression seems to be hormonally regulated: estrogens, acting via ER$\alpha$, enhance Kiss1 but reduces Gpr54 mRNA levels, whereas GnRH selectively enhances Gpr54 expression at the pituitary (44). Along the same lines, kisspeptin-positive cells have been found in the intermediate and anterior lobes of the rhesus monkey pituitary (45), although no evidence for the actual localization of kisspeptins in primate gonadotropes has been presented to date (45).

The consistency of the above findings, however, has been challenged by other studies that could not detect any effects of kisspeptins directly at the rat pituitary (46, 47), although differences in the experimental settings, including the age of the animals tested, might partially explain the apparent discrepancies across studies. In addition, kisspeptin levels in the portal circulation in the sheep did not change during relevant reproductive states, such as the preovulatory surge (39), which casts further doubts on the physiologic relevance of such pituitary effects of kisspeptins in the control of the HPO axis. Altogether, although direct pituitary actions of kisspeptins may contribute to the fine-tuning of the female gonadotropic axis, the potential physiologic relevance of such actions remains debatable and requires further investigation.

## DIRECT KISSPEPTIN ACTIONS IN THE OVARY: FACTS AND HYPOTHESES

In addition to potential pituitary effects, the possibility of additional, peripheral actions of kisspeptins in the control of reproduction has been suggested by a number of studies documenting the expression of Kiss1 and Gpr54 in the gonads, including the ovaries (48–50). However, it must be stressed that the eventual physiologic relevance of kisspeptin signaling in the gonads remains debatable. As mentioned in previous sections, direct kisspeptin actions in GnRH neurons appear to be sufficient to complete puberty and attain fertility (15, 18), and global Gpr54-null mice and humans can be forced to ovulate if appropriately primed with gonadotropins (14, 51). However, rodent models with ablation of kisspeptin actions elsewhere than in GnRH neurons present modest but detectable alterations

of the reproductive axis and display premature reproductive aging (18). Moreover, rescue of ovulation in global Gpr54-null mice is quantitatively incomplete and requires intensive gonadotropin priming (51). Therefore, actions of kisspeptins downstream from gonadotropins (e.g., at the ovarian level) might contribute to the fine-tuning of reproductive function.

Regarding the female reproductive axis, ovarian expression of the elements of the Kiss1 system has been demonstrated in different mammals, including rodent (rat, mouse, hamster), porcine, bovine, and primate ovaries (48, 49). The latter group includes marmoset monkeys and humans (52). Of note, Kiss1 mRNA expression in the rat ovary changes according to the stage of the cycle, with maximum levels at the preovulatory phase (49). This activation is caused by the ovulatory surge of gonadotropins. In turn, inhibition of prostaglandin synthesis, which is known to severely perturb ovulation, caused a marked drop of ovarian Kiss1 mRNA levels and prevented the capacity of ovulatory doses of hCG to induce Kiss1 expression in the rat ovary (52). Altogether, these data are suggestive of a role of locally born kisspeptins in the control of ovarian functions, whose physiologic importance has yet to be elucidated.

Local kisspeptin signaling in the ovary has been implicated in a variety of relevant functions, which include the control of steroidogenesis (53), follicular maturation, ovulation, and ovarian senescence (48). Intra-ovarian infusion of a kisspeptin antagonist resulted in delayed puberty and perturbed estrous cyclicity in rats, without changes in circulating LH levels (54). In addition, not only was the pattern of ovarian expression of Kiss1 severely perturbed in rat models of disrupted ovulation (52), but also blockade of local kisspeptin signaling by intra-ovarian infusion of a kisspeptin antagonist in adult female rats reduced the number of large (type III) follicles and corpora lutea, the latter being a marker of ovulation (55). Conversely, direct ovarian injection of kisspeptin caused the opposite effect (55). Altogether, these data support a discernible role of local kisspeptins in the control of follicular dynamics and ovulation.

However, the relative importance of such direct ovarian actions remains controversial, because they are subordinated to the dominant central effects of kisspeptins in the control of the GnRH/gonadotropin system. In fact, the inherent difficulty to tease apart central versus local actions of kisspeptins has shadowed the relevance of ovarian kisspeptin signaling that, despite being globally dispensable for ovulation, is likely to play a role in follicular dynamics and oocyte survival, with potential impact in the precise modulation of ovulatory efficiency and, eventually, ovarian aging (51, 56). Thus, Gpr54 heterozygosity, which results in decreased ovarian expression of Gpr54 mRNA, in face of preserved (if not increased) gonadotropin secretion, caused late-onset ovarian failure (51). In addition, signaling via the neurotropin receptor NTRK2 in the oocyte requires preserved kisspeptin signaling to promote oocyte survival and prevent premature ovulatory failure (56). This evidence, together with the fact that the oocyte expresses Gpr54 in a number of species, including rodent, canine, and porcine species (56–58), strongly suggests that direct kisspeptin actions in the oocyte may contribute to modulate follicular survival and ovulation. This contention is solidly supported by our findings in a novel mouse line engineered to lack Gpr54, and thus direct kisspeptin actions, in the oocyte, which displays distinctive features of progressive premature ovarian insufficiency.

## KISSPEPTIN ROLES IN THE UTERUS AND PREGNANCY

In addition to local expression and function in the gonads, kisspeptins are reportedly expressed in the uterus (59), where they have been implicated in the control of endometrial gland formation and placentation, key phenomena for reproductive success. Mice with congenital ablation of Kiss1 or Gpr54 suffer from severe uterine hypoplasia and absence of endometrial glands (60), which may be mainly due to the hypogonadal state of these animals caused by the the lack of central stimulatory actions of kisspeptins. Yet, experimental evidence suggests that part of this hypoplastic phenotype might derive from the lack of kisspeptin effects directly at the uterus. In detail, with the use of genetically modified murine models with global or conditional ablation of kisspeptin signaling, it has been documented that, while uterus growth is largely dependent on the estrogenic input driven by the central activity of the HPO axis, endometrial adenogenesis (i.e., the process of endometrial gland generation) is severely compromised in conditions of preserved estrogen levels but lack of peripheral kisspeptin signaling (61). Considering the key role of endometrial glands in the local production of factors essential for uterine receptivity and embryo implantation, such local actions of kisspeptins at the endometrial level might be relevant in achieving maximal reproductive efficiency.

In addition, kisspeptins have long been associated with different aspects of human placentation and gestation, although the actual physiologic roles of kisspeptins during pregnancy remain ill defined. An exhaustive recapitulation of such gestational roles of kisspeptins is beyond the scope of this review, but it is worth mentioning that the elements of the Kiss1 system are known to be expressed in human endometrium and placenta (62) and that kisspeptins have been suggested to participate in the control in human placentation (62, 63). Thus, kisspeptin signaling seems to operate as a repressor of human trophoblast migration and invasion (64), but has also been suggested to operate as a promoter of embryo implantation (62). Of note, kisspeptins and Gpr54 seem to be appropriately located at the fetal-maternal interface to modulate placental invasion. Kiss1/kisspeptins are highly expressed in the syncytiotrophoblast in normal human placenta (64), whereas Gpr54 is present in the villous and invasive extravillous human cytotrophoblasts (64, 65), thus providing the basis for putative autocrine and paracrine regulation of invasion by trophoblast cells. Altogether, these data suggest a putative function of kisspeptins in human placentation. In addition, the circulating levels of kisspeptins dramatically increase during human gestation (66), with a ~1,000-fold increase in the first trimester, and up to a ~7,000-fold increase in the third trimester (66). The physiologic

## TABLE 1

**Major biologic effects and sites of action of kisspeptins at different levels of the female reproductive axis. For specific references, see the corresponding sections in the text.**

| Site of action | Distribution and biologic effect |
|---|---|
| Hypothalamus | |
| Distribution | Kiss1 neurons are found in the hypothalamic ARC/infundibular region in mammals of both sexes, including rodents and primates; a set of ARC Kiss1 neurons coexpress neurokinin B and dynorphin and are termed KNDy |
| | In the female, a second population of Kiss1 neurons is found in the AVPV in rodents and the preoptic area in sheep and primates |
| Actions on GnRH neurons | Kisspeptins potently stimulate firing of GnRH neurons and GnRH secretion in mammals, including rodents, sheep, and primates |
| | ARC Kiss1/KNDy neurons are involved in mediating the negative feedback of sex steroids and are an essential component of the GnRH pulse generator |
| | AVPV Kiss1 neurons play a major role in generation of preovulatory surge of gonadotropins and ovulation (mostly documented in rodents) |
| Non-GnRH neuron actions | Primary actions of kisspeptins on brain targets other than GnRH neurons have been suggested, but the physiologic relevance of such non-GnRH actions has yet to be fully characterized |
| Pituitary | |
| Distribution | Kiss1/kisspeptin and Gpr54 have been found in rat pituitary gonadotropes. Kisspeptins have detected in the monkey pituitary as well, but colocalization in gonadotropes is unclear in primates |
| Effects in gonadotropes | Kisspeptins can directly stimulate LH secretion by rat pituitary explants ex vivo, although some reports have failed to detect such direct stimulatory actions |
| | Kisspeptins induce transcriptional activation of LH$\beta$ and FSH$\beta$ gene in pituitary cells |
| | Kisspeptins elicit $Ca^{2+}$ responses in gonadotropes in a variety of species, including rat, ovine, porcine, bovine, and primate species |
| Ovary | |
| Distribution | Kiss1/kisspeptin and Gpr54 have been shown to be expressed in rodent (rat, mouse, hamster), porcine, bovine, and primate ovaries. Gpr54 expression has been documented in oocytes |
| | Kiss1 expression in the rat ovary is cyclic and hormonally regulated, with peak levels preceding ovulation being driven by the preovulatory surge of gonadotropins |
| Biologic effects | Local kisspeptin actions have been involved in different ovarian functions, including modulation of steroidogenesis, ovulation, and ovarian senescence; the physiologic relevance, however, remains ill defined |
| | Blockade of local kisspeptins in the rat ovary delays puberty onset and reduces ovulatory efficiency in adulthood, as denoted by decreased number of corpora lutea |
| | Haplo-insufficiency of Gpr54, which reduces ovarian Gpr54 expression, results in premature ovarian failure in mice; progressive premature ovarian insufficiency is also found in a model of conditional ablation of Gpr54 from oocytes |
| Uterus | |
| Distribution | Elements of the Kiss1 system are expressed in the mouse uterus, including the luminal and glandular epithelia on the day of implantation |
| Biologic effects | Absence of kisspeptin signaling causes severe uterine hypoplasia and absence of endometrial glands |
| | Uterine hypoplasia due to global elimination of kisspeptin signaling is mainly due to the lack of central effects of kisspeptins, which results in low estrogenic input to the uterus |
| | Proper endometrial gland formation in the mouse uterus is not dependent on central kisspeptin signaling and requires peripheral actions of kisspeptins, possibly at the level of the uterus |
| Placenta and gestation | |
| Distribution and levels | Kiss1/kisspeptins are highly expressed in the syncytiotrophoblast in normal human placenta, whereas Gpr54 is present in the villous and invasive extravillous human cytotrophoblast |
| | Plasma levels of kisspeptins dramatically increase in human gestation, with a >1,000-fold increase in the first trimester, and up to a ~7,000-fold increase in the third trimester |
| Biologic actions | Kisspeptins have been suggested to participate in the control in human placentation, acting as repressor of human trophoblast migration and invasion |
| | Local kisspeptins may promote embryo implantation in mice |
| | Placentation and pregnancy can progress despite the absence of kisspeptin signaling |
| | The putative role of increased plasma levels of kisspeptins during gestation remains unknown; they might contribute to hormonal and metabolic adaptations during pregnancy |

*Note:* ARC = arcuate nucleus; AVPV = anteroventral periventricular nucleus; FSH = follicle-stimulating hormone; GnRH = gonadotropin-releasing hormone; LH = luteinizing hormone.

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consequences of such elevation remain an enigma, however, although it has been suggested that it might contribute to the metabolic adaptations of pregnancy.

Of note, few women who are infertile due to inactivating mutations of the GPR54 gene have been reported to give birth to healthy children after ovulatory induction (67). This would dismiss an indispensable role of kisspeptin signaling at the maternal side for completion of human pregnancy. It must be stressed, however, that the particular characteristics of placentation of these patients could not be evaluated in detail; therefore, it is possible that the absence of kisspeptin signaling might have caused modest alterations of placental morphology or function that, even if not being incompatible with successful gestation, may affect optimal organ

**TABLE 2**

**Potential clinical applications of kisspeptins and related factors in reproductive medicine.**

| Medical condition | Potential medical application |
|---|---|
| Infertility | |
| IVF | Peripheral administration of kisspeptins has been shown to induce ovulation in different mammals, and subcutaneous injection of kisspeptin can induce egg maturation and ovulation in protocols of IVF |
| | The reported protocols of kisspeptin stimulation do not clearly improve the efficiency of current procedures of gonadotropin stimulation and require pretreatment with recombinant FSH; are new analogues needed? |
| Prevention of OHSS | Kisspeptin stimulation likely evokes a more physiologic gonadotropin stimulation and is less prone to cause the most serious complication of IVF, i.e., OHSS |
| | Women at risk of OHSS do not commonly display such adverse complication after kisspeptin stimulation, despite application of a second dose of kisspeptin to extend the duration of LH secretion |
| PCOS | |
| Pathophysiology | Fragmentary evidence suggests alterations of hypothalamic expression of Kiss1 in preclinical models of PCOS |
| | Inconclusive evidence has pointed out alterations of circulating levels of kisspeptins in women with PCOS |
| Treatment | Repeated injections of kisspeptin-54 induced gonadotropin responses and rescued ovulation in preclinical models of PCOS, but with incomplete efficacy |
| | A pilot study in anovulatory women with PCOS showed that treatment with kisspeptin-54 can induce gonadotropin responses in patients with PCOS, but ovulation was rescued in only a fraction of treated women |
| Hormone-dependent conditions | |
| Endometriosis | Data from preclinical models suggest that kisspeptin analogues (antagonists or agonists, via desensitization) may cause suppression of ovarian function without reaching castration levels; no evidence from clinical studies yet |
| Uterine fibroids | Data from preclinical models suggest that kisspeptin analogues (antagonists or agonists, via desensitization) may cause suppression of ovarian function without reaching castration levels; no evidence from clinical studies yet |
| Hot flushes | Antagonists of NKB, a peptide coexpressed with Kiss1 in KNDy neurons, are novel pharmacologic tools for the control of menopausal hot flushes; NKB analogues are currently in clinical trials to test for efficacy and safety |
| Biomarkers | |
| Gestational diseases | Low circulating levels of kisspeptin have been proposed as biomarker of gestational alterations, such as intrauterine growth restriction and preeclampsia |
| | Low circulating levels of kisspeptins, together with suppressed levels of miR-324-3p, were recently proposed as biomarker of ectopic pregnancy |
| Risk of abortion | Decreased circulating levels of kisspeptins have been proposed as a putative predictor of miscarriage risk |
| Gestational tumors | Circulating levels of kisspeptins increase in gestational trophoblastic neoplasia and decrease with treatment |

*Note:* Conditions and applications are predicted based on preclinical and clinical research; no kisspeptin-based protocols are currently in routine practice. FSH = follicle-stimulating hormone; IVF = in vitro fertilization; KNDy = Kiss1/neurokinin B/dynorphin; LH = luteinizing hormone; NKB = neurokinin B; OHSS = ovarian hyperstimulation syndrome; PCOS = polycystic ovary syndrome.

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physiology. Along the same line, placentas from global Kiss1- or Gpr54-null mice have been shown to display grossly preserved structure and basic function, as evaluated by stereologic studies and analyses of amino acid and glucose transport in placentas from Gpr54 and/or Kiss1 knockout mice (68). Though direct extrapolation of these findings to humans must be made with caution, these data suggest that congenital lack of kisspeptin signaling at either the maternal or the fetal side is not incompatible with completion of gestation, even if distinct functions of kisspeptins in the control of embryo implantation, placentation, and gestation have been documented by clinical and experimental studies.

## CLINICAL APPLICATIONS OF KISSPEPTINS: WHERE ARE WE; WHERE DO WE GO?

Given the paramount importance of kisspeptins in the control of HPO axis, and their capacity to potently activate gonadotropin secretion in humans and other mammals, kisspeptin analogues (both agonists and antagonists) have been explored as potential pharmacologic tools for the management of various reproductive disorders. Likewise, changes in kisspeptin levels have been proposed as putative markers for improved diagnosis of some conditions. While the clinical use of kisspeptins, either for diagnostic or therapeutic purposes, has yet to be consolidated, some illustrative examples along these potential medical applications of kisspeptins are discussed here and summarized in Table 2.

A number of studies in various animal species have documented that peripheral administration of kisspeptins can evoke ovulation (46, 69, 70). Of particular interest, subcutaneous injection of kisspeptin has been shown to induce egg maturation and ovulation in protocols of in vitro fertilization (IVF) in women, thus paving the way for the use of kisspeptins in the protocols of ovarian stimulation in IVF techniques (69). In principle, it is arguable that kisspeptin stimulation might evoke a more physiologic gonadotropin stimulation than exogenous gonadotropin priming, therefore reducing the potential of off-target and side-effects. In fact, the protocols of kisspeptin administration to induce oocyte maturation are less prone to cause the most serious complication of IVF, ovarian hyperstimulation syndrome (OHSS) (71). Of note, even women at risk of OHSS do not commonly display such an adverse complication after kisspeptin stimulation, despite application of a second dose of kisspeptin to extend the duration of LH secretion (72). Admittedly, however, the reported protocols of kisspeptin stimulation do not improve the efficiency of current protocols of gonadotropin stimulation and require pretreatment with

recombinant FSH (69). In this scenario, the use of longer-acting analogues of kisspeptins might pose an advantage for IVF procedures, which has yet to be clinically proven.

Along the same lines, the possibility that kisspeptin-based treatments might improve the management of common anovulatory conditions, such as PCOS, has been recently addressed. PCOS is a prevalent endocrinopathy, commonly associated with oligo/anovulation. Even though it affects a notable fraction of the human female population in reproductive age, the treatments for PCOS remain mostly symptomatic and are of moderate efficacy when conception is desired, they being linked with potentially life-threatening complications, such as OHSS. Thus, kisspeptins might provide a therapeutic advantage for induction of ovulation in PCOS women. In a recent study, we reported the effects of administration of kisspeptin-54 in a pilot exploratory cohort of anovulatory women with PCOS (73). Our data showed that administration of kisspeptin-54 twice daily for 21 days elicited LH responses in five of the seven women, but only two presented growth of a dominant follicle with subsequent ovulation. In the same study, the ability of repeated injections of kisspeptin-54 to induce LH and FSH responses and to cause ovulation was evaluated in three different preclinical models of PCOS (73). While kisspeptin administration consistently induced LH and FSH responses, albeit with differences in magnitude across the rat models, efficiency of this treatment in terms of ovulatory induction was variable, and it evoked ovulation only in models of postnatal androgenization, but not in those of continuous exposure to high androgen levels. Altogether, this combination of preclinical and clinical data demonstrates that kisspeptin administration in anovulatory preclinical models and in women with PCOS can stimulate reproductive hormone secretion and ovulation, albeit with incomplete efficacy, thus arguing for the need of personalized management of anovulatory dysfunction in women with PCOS, some of whom may benefit from kisspeptin-based treatments.

Development of kisspeptin antagonists and realization of the coexpression of kisspeptins with other neuropeptides with key roles in the control of reproductive function, such as NKB in KNDy neurons, has led to the proposal of additional therapeutic uses of kisspeptin analogues or related compounds. Based on preclinical data, kisspeptin antagonists might be useful to prevent the preovulatory surge, decrease gonadotropin levels without achieving the castration range, and manage endocrine-dependent female reproductive disorders ranging from endometriosis to uterine fibroids. Admittedly, however, clinical data supporting these applications are still missing. On the other hand, demonstration that NKB produced by KNDy neurons might contribute to the generation of menopausal hot flushes has led to active clinical investigation of the potential utility of NKB receptor antagonists (74), which are now in clinical trials. In addition, evidence for the eventual use of NKB antagonists in normalizing GnRH/LH hypersecretion, seen in women with PCOS, has been proposed very recently (75).

Finally, because tissue Kiss1 and/or Gpr54 expression, as well as circulating kisspeptin levels, have been reported to change in some pathophysiologic conditions, the possibility that they might serve as biomarkers of disease has been explored, for example, in gestational pathologies. As described in the preceding section, blood levels of kisspeptins have been reported to dramatically increase during human gestation; accordingly, inappropriately low kisspeptin levels have been proposed as biomarker of gestational alterations, such as intrauterine growth restriction and preeclampsia (76, 77). Likewise, altered circulating levels of kisspeptins might serve as a putative predictor of miscarriage risk (78). In line with this possibility, a recent pilot study comparing women with viable intrauterine pregnancy versus women with confirmed spontaneous abortion suggested that kisspeptin levels during an early gestational window (weeks 6–10) might serve as a biomarker of pregnancy viability (79). In the same vein, we recently described that disproportionately low kisspeptin levels, together with decreased circulating levels of its regulator, miR-324-3p, might serve as putative biomarkers for accurate screening of ectopic pregnancy at early gestational ages (80).

## CONCLUSION

Discovery of the reproductive dimension of kisspeptins has revolutionized our understanding of the basic mechanisms responsible for the precise control of the female reproductive axis, kisspeptins now regarded as indispensable elements for the proper maturation and function of the HPO axis. Although realization of the fundamental reproductive roles of kisspeptin dates back only to late 2003, the progress in the field has been astonishingly rapid and has allowed us to decipher key aspects of kisspeptin physiology. Conclusive evidence has demonstrated that the primary site of actions of kisspeptins is the population of GnRH neurons in the hypothalamus, where kisspeptins mediate the feedback effects of ovarian steroids and are able to induce potent excitatory effects that are essential for normal puberty onset, proper pulsatile gonadotropin secretion, and ovulation, a contention that can be regarded as dogma in the field. In addition, as yet fragmentary evidence has been gathered over the past 15 years for additional actions of kisspeptins, not only on neuronal circuits other than for GnRH, but also at other reproductive tissues, such as the pituitary, the gonads, the uterus, and the placenta. We can consider these actions to be less striking and of modest magnitude and possibly subordinated to the central GnRH-centric effects of kisspeptins in the HPO axis. In any event, characterization of such peripheral actions of kisspeptins and their actual physiologic relevance can be considered as open challenges for reproductive physiology and medicine, and their elucidation will help to reveal the whole set of pathophysiologic, diagnostic, and therapeutic implications of kisspeptins in the context of female reproduction.

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