frontiers | Frontiers in Endocrinology

TYPE Review
PUBLISHED 22 August 2022
DOI 10.3389/fendo.2022.917464

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Experimental Endocrinology, a section of the journal Frontiers in Endocrinology

RECEIVED 11 April 2022
ACCEPTED 02 August 2022
PUBLISHED 22 August 2022

CITATION
Masumi S, Lee EB, Dilower I, Upadhyaya S, Chakravarthi VP, Fields PE and Rumi MAK (2022) The role of Kisspeptin signaling in Oocyte maturation.
Front. Endocrinol. 13:917464.
doi: 10.3389/fendo.2022.917464

The role of Kisspeptin signaling in Oocyte maturation

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Kisspeptins (KPs) secreted from the hypothalamic KP neurons act on KP receptors (KPRs) in gonadotropin (GPN) releasing hormone (GnRH) neurons to produce GnRH. GnRH acts on pituitary gonadotrophs to induce secretion of GPNs, namely follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are essential for ovarian follicle development, oocyte maturation and ovulation. Thus, hypothalamic KPs regulate oocyte maturation indirectly through GPNs. KPs and KPRs are also expressed in the ovarian follicles across species. Recent studies demonstrated that intraovarian KPs also act directly on the KPRs expressed in oocytes to promote oocyte maturation and ovulation. In this review article, we have summarized published reports on the role of hypothalamic and ovarian KP-signaling in oocyte maturation. Gonadal steroid hormones regulate KP secretion from hypothalamic KP neurons, which in turn induces GPN secretion from the hypothalamic-pituitary (HP) axis. On the other hand, GPNs secreted from the HP axis act on the granulosa cells (GCs) and upregulate the expression of ovarian KPs. While KPs are expressed predominantly in the GCs, the KPRs are in the oocytes. Expression of KPs in the ovaries increases with the progression of the estrous cycle and peaks during the preovulatory GPN surge. Intrafollicular KP levels in the ovaries rise with the advancement of developmental stages. Moreover, loss of KPRs in oocytes in mice leads to failure of oocyte maturation and ovulation similar to that of premature ovarian insufficiency (POI). These findings suggest that GC-derived KPs may act on the KPRs in oocytes during their preovulatory maturation. In addition to the intraovarian role of KP-signaling in oocyte maturation, *in vivo*, a direct role of KP has been identified during *in vitro* maturation of sheep, porcine, and rat oocytes. KP-stimulation of rat oocytes, *in vitro*, resulted in Ca^{2+} release and activation of the mitogen-activated protein kinase, extracellular signal-regulated kinase 1 and 2. *In vitro* treatment of rat or porcine oocytes with KPs upregulated messenger RNA levels of the factors that favor oocyte maturation. In clinical trials, human KP-54 has also been

10.3389/fendo.2022.917464

administered successfully to patients undergoing assisted reproductive technologies (ARTs) for increasing oocyte maturation. Exogenous KPs can induce GPN secretion from hypothalamus; however, the possibility of direct KP action on the oocytes cannot be excluded. Understanding the direct *in vivo* and *in vitro* roles of KP-signaling in oocyte maturation will help in developing novel KP-based ARTs.

KEYWORDS

Ovarian follicles, kisspeptin, kisspeptin receptor, GnRH, gonadotropin, oocyte maturation, assisted reproductive technologies

Introduction

Kisspeptins (KPs) are a group of neuropeptides encoded by the *Kiss1* gene (1–3). The preprotein of 145 amino acids is cleaved into peptides containing 54 (52 in rodents), 14, 13, and 10 amino acids (2) (Figure 1). All of these peptides share the carboxy terminal 10 amino acids (RF amide in primates and RG amide in rodents), which bind to a G-protein coupled receptor (GPR54, encoded by the *Kiss1r* gene, referred as KPR in this article) with similar affinity (2, 3). Mouse and rat KPRs are nearly 95% identical, and approximately 85% identical to human KPRs (4).

KP was initially identified in a human melanoma cell line, and was suggested to be a metastasis suppressor (1). It was named metastatin, due to its potential antimetastatic and antiangiogenic properties (5). Subsequent studies demonstrated that the primary role of KPs lies within the hypothalamic-pituitary-gonadal (HPG) axis for reproduction (6–8). Individuals carrying inactivating mutations in either the KP or KPR genes suffer from hypogonadotropic hypogonadism (6–8). On the other hand, activating mutations of the KPR resulted in precocious puberty (9). These findings suggest that KP signaling system is crucial for a normal functioning HPG axis. The roles of KPs and KPRs in the HPG axis or reproduction have also been examined in experimental mouse and rat models by knocking out the *Kp* or *Kpr* gene (7, 10). Loss of KPs or KPRs disrupted GPN secretion, which affected gonadal development, steroidogenesis, as well as gametogenesis in both sexes (6–8, 10). Remarkably, *Kpr* knockout (Kpr^{KO}) mouse models exhibited a more severe hypogonadism phenotype than the knockout of the *Kp* gene (Kp^{KO}) (7, 10).

Expression of KPs in hypothalamic KP neurons is regulated by gonadal steroid hormones (11). However, during the pre-pubertal period, expression of KPs occurs independent of gonadal steroids, which switches to gonadal steroid dependent at puberty (11). KPs act on the KPRs in gonadotropin (GPN) releasing hormone (GnRH) neurons to induce the expression of GnRH (11). GnRH induces pituitary gonadotrophs to release the GPNs, follicle stimulating hormone (FSH) and luteinizing hormone (LH) (12–14).

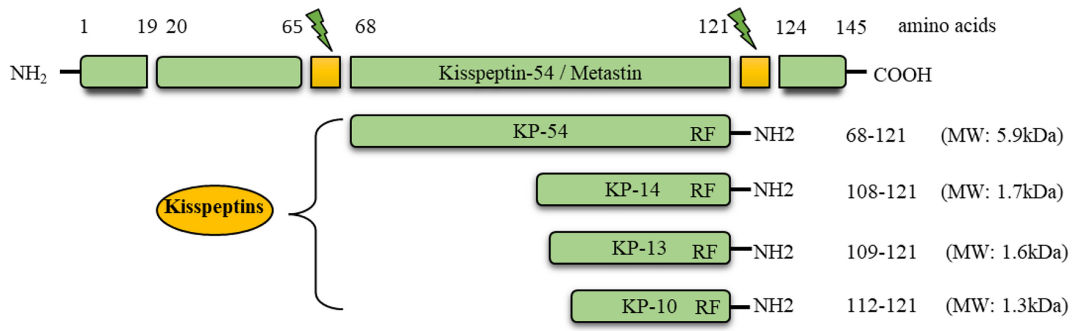

FIGURE 1
Conversion of prepro-kisspeptin into the active peptides. The prepro-kisspeptin is encoded by the *KISS1* gene in primates and encodes a 145 amino acid prepro hormone (MW: ~15.4 kDa). This polypeptide is cleaved into 54, 14, 13, and 10 amino acids. The C-terminal, 10 amino acid sequence is conserved in all the forms, and can activate the G-protein coupled receptor, GPR54 (KPR).

GPNs act on the gonads to promote specific hormonal and reproductive functions (6, 7, 15, 16). Thus, hypothalamic expression of KPs is essential for the gonadal development, onset of puberty, and maintenance of gonadal functions (6, 7, 12, 13, 15, 16).

Expression of KPs and KPRs has also been detected in several extrahypothalamic sites inside the brain as well as organs outside the brain (17–26). There have been various types of suggested functions for extrahypothalamic KPs in different organ systems (27–32). However, until recently, proven functions of extrahypothalamic KP-signaling has remained limited to pancreas, liver, and the ovary (33–36). KPs and KPRs are expressed in ovaries across species (27–32). KPs are expressed predominantly in rat granulosa cells (GCs), whereas KPRs are expressed mainly in rat oocytes (24). Intrafollicular KP levels rise with the stages of oocyte maturation and correlate with follicular estradiol concentration (37). KP-signaling has been found to be crucial for mouse oocyte survival (35, 36) as well as oocyte maturation and ovulation (35, 38). In women undergoing *in vitro* fertilization (IVF), administration of exogenous KPs or a KPR agonist have also been found to improve oocyte maturation (39–42). Additionally, several studies have demonstrated that treatment with KPs can increase *in vitro* maturation (IVM) of oocytes from different species (38, 43, 44). The purpose of the current review is to understand the role of hypothalamic and extrahypothalamic KPs in maturation of oocytes, *in vivo* and *in vitro*.

Hypothalamic kisspeptins

KP-signaling represents a regulatory link between the gonadal steroid hormones and the GPNs. GPNs are secreted from the anterior pituitary gland in response to GnRH, and act on the gonads (45). In response to GPNs, the gonads produce steroid hormones including estradiol, progesterone, and testosterone (46). The gonadal steroid hormones in turn regulate the secretion of GPNs from the HP axis (46). GnRH neurons in the hypothalamus that produce GnRH to regulate GPN secretion from the anterior pituitary lack receptors for gonadal steroid hormones (47). Instead, the GnRH neurons express KPRs, whereas the KP neurons in the hypothalamus possess receptors for gonadal steroid hormones (46, 47). Gonadal steroids act on KP neurons to regulate the expression of KPs, and KPs act on the KPRs expressed in GnRH neurons to induce GnRH production (46, 47). Although there have been many suggested functions for hypothalamic KPs, the primary role of hypothalamic KPs is to regulate the GnRH secretion from the GnRH neurons.

Hypothalamic kisspeptin mediated regulation of ovarian function

As aforementioned, hypothalamic KP-signaling is the key mediator of estrogen-induced GnRH and GPN secretion that regulates gonadal functions (48, 49). There are two major populations of hypothalamic KP neurons: one localized in the infundibular nucleus in primates (50, 51) and the other in the nuclei in the preoptic area (PeN) in ruminants (52–59) and primates (51, 60–63). The corresponding nuclei in rodents are located in arcuate [ARC] nucleus and the anteroventral periventricular [AVPV] nucleus respectively (64–68) (Figure 2).

While gonadal steroids negatively regulate KP expression from the infundibular/ARC nucleus, they positively regulate the KP neurons in the PeN/AVPV (69, 70). Estrogen receptor α (ERα) is the predominant estrogen receptor that regulates both positive and negative regulation of KP expression in hypothalamic KP neurons (64). The KP/Neurokinin B/dynorphin (KNDY) neurons serve as the GPN pulse generator (71). It was found that rescuing 20% of the KNDY neurons in the infundibular/ARC nucleus could recover the GPN pulse release in Kp^{KO} rats (71). Pulsatile secretion of KPs from the infundibular/ARC nucleus results in pulsatile secretion of GnRH in both sexes. In contrast, bolus secretion of KPs from the PeN/AVPV nucleus in response to elevated estrogen level in females leads to preovulatory GnRH and subsequent GPN surge. While pulsatile secretion of GPNs from infundibular/ARC is important for ovarian follicle development, the preovulatory GPN surge from the PeN/AVPV nucleus is essential for the final stages of oocyte maturation and ovulation (69, 70).

Sexual dimorphism in hypothalamic kisspeptin

Both the quantity and transcriptional activity of hypothalamic KP neurons are sexually dimorphic. Studies in human autopsy samples found that females have significantly higher numbers of KP synthesizing fibers in the hypothalamus compared to males (72). In addition, studies in mouse models found a sex difference in KP expression starting from fetal life, females always showing significantly higher levels (73). The sexually dimorphic feature is more prominent in the PeN/AVPV nucleus compared to the infundibular/ARC nucleus. A10-fold, female-dominance in the numbers of KP neurons is found in the PeN/AVPV (65). This prominent PeN/AVPV KP system in females is important for generating female-specific preovulatory GnRH and GPN surge (65, 74). An increased level

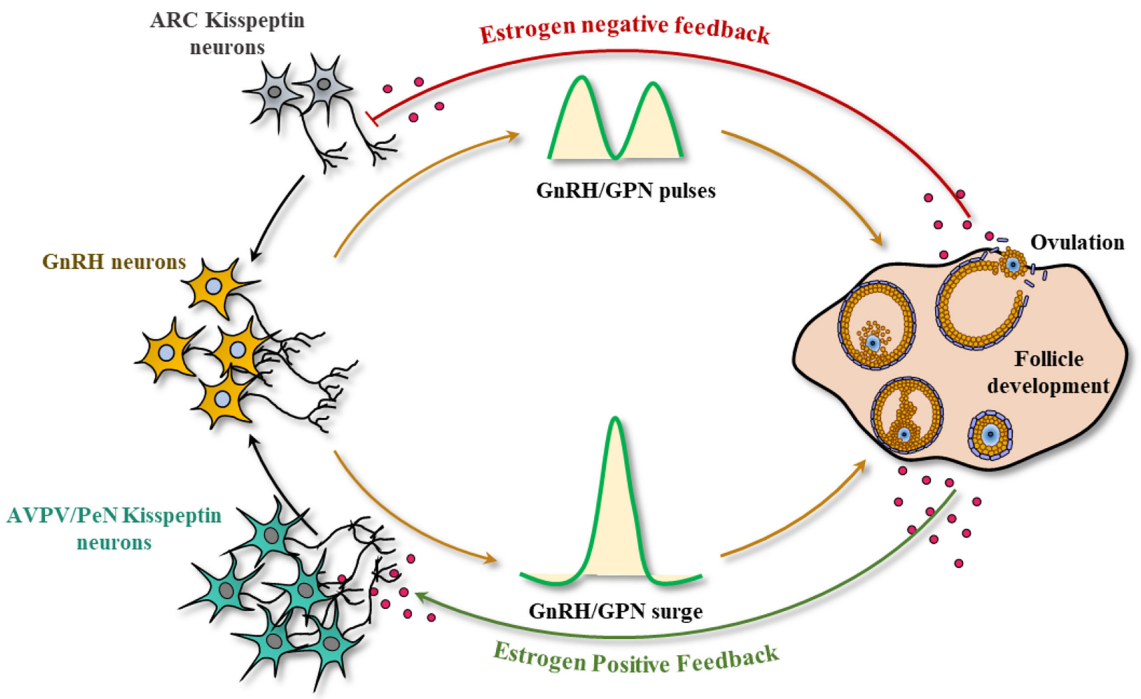

FIGURE 2
Kisspeptin signaling in the hypothalamic-pituitary-gonadal axis. In most mammals, there are two major sites of kisspeptin (KP) expression in the hypothalamus: the infundibular nucleus, and the nuclei in preoptic area (PeN) (arcuate nucleus (ARC) and anteroventral periventricular nucleus (AVPV) in rodents). Small amounts of estrogen synthesized from the ovary results in the activation of KP neurons in the infundibular/ARC nucleus, modulating the pulsatile secretion of GnRH and gonadotropins (GPNs). In contrast, an increased level of estrogen during the follicular phase (analogous to proestrus stage) activates the KP neurons in PeN/AVPV and inhibits the infundibular/ARC neurons, generating the GnRH/GPN surge, enabling oocyte maturation, and ovulation.

of estrogens increases KP expression in PeN/AVPV nuclei and results in a preovulatory GPN surge, which is essential for oocyte maturation, ovulation, and formation of the corpus luteum (75).

Hypothalamic kisspeptins and oocyte maturation

Although oocytes express KPRs, a widely accepted role of KPs in oocyte maturation is indirect, through induction of GnRH and GPN response from HP axis. KPs induce pulsatile GPN secretion from the infundibular/ARC nucleus, which results in FSH-induced ovarian follicle development to the antral state (69). Further development of the antral follicles to preovulatory Graafian follicles, maturation of oocytes, and ovulation are mediated by the LH surge induced by large amount of KPs released from the PeN/AVPV nucleus (69). Recent studies emphasize the importance of KPRs in oocytes for induction of oocyte maturation (35, 76). But it remains unknown whether hypothalamic KPs reach the ovarian follicles and act on the KPRs in the oocytes. On the other hand, the preovulatory GPN surge results in intraovarian KP expression, which may also act on the KPRs expressed in oocytes. The role of GC-derived KPs in oocyte maturation, however, has not been proven.

Extrahypothalamic kisspeptins

It is well-established that KPs and KPRs play a critical role in regulation of GPN secretion from HP axis (6, 7, 10). Recent studies demonstrated a more expansive role for KP-signaling in various extrahypothalamic organs both inside and outside the brain (12, 13, 29). Several studies have found that the functions of extrahypothalamic KPs are also vital for regulation of metabolism and reproduction (33–36).

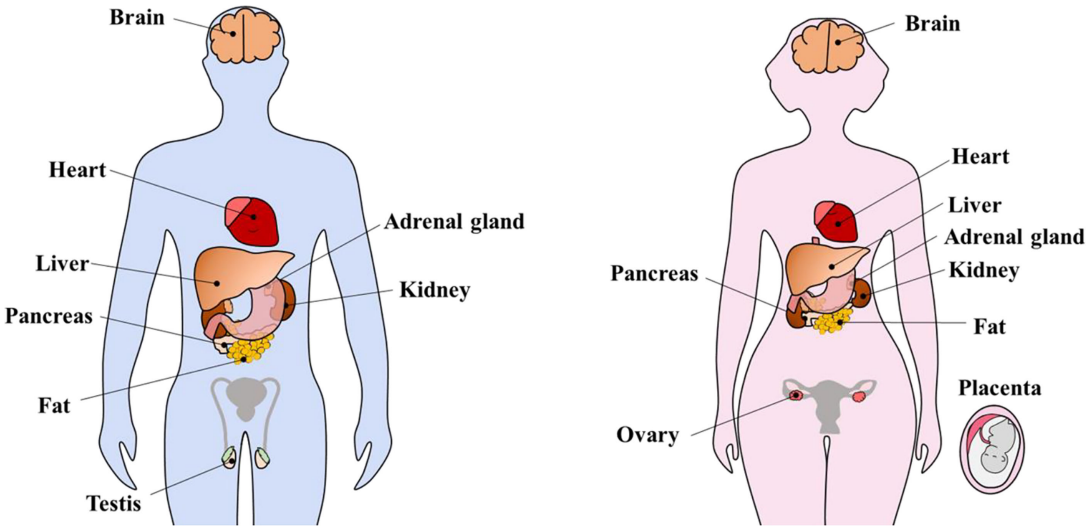

FIGURE 3
Extrahypothalamic kisspeptins and kisspeptin receptors. Kisspeptins (KPs) and kisspeptin receptors (KPRs) are expressed in several extrahypothalamic sites inside the brain as well as peripheral organs including heart, liver, adrenal gland, pancreas, kidney, fat, testis, and ovary. During pregnancy, KP expression increases tremendously, in part due to placental expression.

Kisspeptin signaling in the pituitary gonadotrophs

The role of KPRs and KP-signaling in hypothalamic GnRH neurons regulating GnRH secretion and reproductive function in both sexes is widely recognized (6, 10, 48, 49, 77, 78). KPRs are also detected in the pituitary gland, but their roles remain largely unclear (79, 80). It was shown that KPs can activate KPRs in a pituitary gonadotroph cell line LβT2 and induce protein kinase C-dependent expression of FSHβ and LHβ (80). In contrast, a recent study has demonstrated that pituitary-specific knockout of KPRs reduced the FSH level in male mice, but not the LH level or testicular function (81). Neither GPN levels in female mice nor the reproductive function in male or female mice was affected following the loss of KPRs in pituitary gland (81). Thus, the clinical importance of KPRs in the pituitary is still under investigation.

Extrahypothalamic kisspeptins and kisspeptin receptors outside the brain

KP and KPRs have been detected in extrahypothalamic organs with a multitude of functions. It has been shown that KPs/KPRs are expressed in the liver (17, 82), pancreas (83, 84), adipose tissue (17–19), testis (17, 20, 21), ovary (22–24), adrenal gland (85), heart (86), uterus (87–89) and placenta (25, 31, 88) (Figure 3). Peripherally administered KP can act on hypothalamic GnRH neurons to induce GPN secretion (61, 90–92). Based on this finding, we can assume that KP produced in extrahypothalamic tissues may have a direct impact on hypothalamic GnRH neurons and GPN secretion, and *vice-versa*. Future studies will determine if hypothalamic or extrahypothalamic KPs play such a role.

Role of extrahypothalamic kisspeptins inside and outside the brain

The major function of KP-signaling in the brain involves regulation of reproductive functions (48, 91). In addition to reproductive neuroendocrinology, KPs may serve diverse neurological functions inside the brain (93). While administration of exogenous KP enhanced memory (94), loss of KP-signaling has been associated with a reduced anxiety-related behavior in mice (95). Estrogen driven KP-signaling regulates glutamate neurotransmission to the infundibular/ARC nucleus, which controls feeding behavior (96, 97). In addition, KP-signaling regulates the pathophysiology in several extrahypothalamic organs outside the brain including liver, pancreas, and heart (84, 97–100). It regulates insulin secretion from the pancreas and maintains glucose homeostasis (33), lipid metabolism in the liver and control of non-alcoholic fatty changes (34), as well as oocyte maturation and ovulation in the ovaries important for female fertility (35, 36). KP-signaling has been suggested to play a role in spermatogenesis and activation of sperm (101). KPs can modulate intracellular Ca^{2+} influx, sperm motility, sperm hyperactivation, and the acrosome reaction in the

human spermatozoa, that is critical for fertilization and IVF outcomes (32, 102). IVF rates were decreased after the treatment of sperm with KP antagonist KP234, which suggest the importance of KP-signaling in fertilization process (102). Further studies are required to determine if treatment of sperm with KPs can increase IVF. KPs and KPRs may also be involved in the regulation of cancer metastasis, vascular dynamics, implantation of embryos, and placental physiology, none of which are not yet understood clearly (27–32). Despite all these suggested functions, the only understood ones are limited to insulin secretion (100), metabolism, and ovarian folliculogenesis (33, 35).

Extrahypothalamic kisspeptins and oocyte maturation

The ovary is one of the extrahypothalamic sites of KP and KPR expression (27–32). The *Kp* gene is expressed predominantly in rat GCs, whereas the *Kpr* gene is found predominantly in rat oocytes (24, 38). *Kp* mRNAs are expressed within the ovaries in response to the preovulatory GPN surge (22, 24, 103), and KP concentration increases in follicular fluid during oocyte maturation (37). Taken together, it was hypothesized that intrafollicular KPs can induce KP-signaling in oocytes and promote *in vivo* oocyte maturation (24, 38, 104). Very recently, an elegant study from the Tena-Sempere lab has presented evidence that KP-signaling in mouse oocytes is essential for follicle development and survival of mature ovarian follicles (35). The research group has also demonstrated that loss of KP-signaling in oocytes leads to premature ovarian insufficiency (**POI**) in oocyte-specific Kpr^{KO} mice (35). Thus, the role of KPs in regulating female fertility can involve organ systems well beyond the HP axis.

Kisspeptin signaling in the ovary

KPs are known inducers of GPNs in the HP axis (48). But within the ovary, GPNs act as inducers of KP expression from the GCs (24, 38). While the hypothalamic *Kp* gene is regulated by ERα (105), ovarian *Kp* expression is regulated by ERβ (24). GPNs act on the GPN receptors (FSHR and LHCGR), on follicular theca-interstitial cells (**TCs**) and GCs, which ultimately promote oocyte maturation (106). Ovarian KPs may also act on KPRs in oocytes to induce follicle development and oocyte maturation (35, 36). Nevertheless, both hypothalamic and ovarian KP signaling indirectly or directly act to induce oocyte maturation. The findings discussed in the following sections were obtained from rodent studies.

Expression of kisspeptin and kisspeptin receptors

Kp and *Kpr* genes are expressed within the ovaries of diverse animal species including fish (107, 108), birds (109), rodents (22, 110, 111), canines (112, 113), cattle (114, 115), and primates (116). There has been some variation in reports regarding the relative expression of KPs or KPRs in oocytes and somatic cells (28). While some reports showed detection of KPs and KPRs in all ovarian cell types, others have reported that *Kp* mRNA is predominantly expressed in GCs, and KPRs are expressed in the oocytes (24, 38, 117). A marked upregulation in *Kp* gene expression was observed after the injection of human chorionic gonadotropin (hCG) into pregnant mare serum gonadotropin (PMSG) primed rats (24). The expression of the *Kp* gene in GCs was also dependent on the presence of ERβ (24). As expected, oocytes did not show the upregulation in *Kp* gene expression as they lack GPN receptors, and theca-interstitial cells (TCs) lacked *Kp* gene expression due to low levels of ERβ (24, 117–119). Indomethacin treatment that inhibits prostaglandin synthesis and prevents ovulation, also inhibited hCG-induced expression of the *Kp* gene, which suggests an essential role for cyclooxygenase and/or prostaglandins in *Kp* gene regulation (22, 116). Although *Kp* gene expression in hypothalamic KP neurons is independent of progesterone receptor (PGR), secretion of KPs from the KP neurons is regulated by PGR (120). Further studies are required to determine if PGR plays any role on intraovarian KP expression or secretion.

Gonadotropin secretion and steroidogenesis

Expression of KPs in GCs and KPRs in oocytes suggest that KP-signaling may represent bidirectional signaling between the GCs and oocytes, for controlling follicle development, steroidogenesis, oocyte maturation, and ovulation (24). Hypothalamic KPs regulate the secretion of GPNs, which act on gonadal cells to regulate steroidogenesis. It has been shown that low serum KP levels is associated with a lower levels of serum estrogen and progesterone (121). A low level of KPs represses GnRH release from hypothalamus, decreases GPN secretion from pituitary gonadotrophs that ultimately affects the synthesis of progesterone and estrogen in follicular TCs and GCs (122). As previously stated, these findings indicate that KPs of extrahypothalamic origin, including ovarian-derived KPs, may have a significant effect on hypothalamic GnRH production and GPN secretion. In contrast, results with a mutant mouse model suggests that KP-signaling in the hypothalamic GnRH neurons is sufficient for the regulation of the hypothalamic pituitary ovarian (HPO) function (123). In contrast, a later study using the same mutant mouse model

demonstrated that hypothalamic GnRH-specific expression of KPRs in global Kpr^{KO} mice still suffer from abnormal ovarian ultrastructure and premature ovarian ageing (124). In another study, $Er\beta^{KO}$ mouse ovaries were found to synthesize low levels of estrogens and the preovulatory GPN peak was remarkably diminished (125). Neither administration of exogenous estrogen could induce a natural GPN surge nor exogenous GPNs could mediate follicle maturation and ovulation in $Er\beta^{KO}$ mice, but exchange transplantation of wildtype ovaries could resolve those defects (125). These findings suggest that wildtype ovaries express a crucial factor that is absent in the $Er\beta^{KO}$ ovaries. Later, we identified that the ERβ-regulated crucial factor could be GC-derived KPs, which is absent in $Er\beta^{KO}$ GCs (24, 38, 104).

Follicle activation and follicle development

KP-signaling was found to be involved in both the initial and the cyclical recruitment of rat ovarian follicles (126). Administration of a KP antagonist, KP234, into rat ovaries increased primordial follicle activation (PFA) (126). Although the mechanism of increased PFA remains unclear, it was associated with decreased FSHR on preantral follicles that reduced the cyclic activation (126). However, administration of the KP antagonist decreased the numbers of mature follicles and ovulation, suggesting that KP-signaling plays a positive role in preovulatory follicle maturation (126). Ovarian expression of KPs increases during the preovulatory period (22), which correlates with the potential role KPs in preovulatory oocyte maturation. Administration of KPs in rats increases follicle maturation induced by hCG (116). In addition, Kpr^{KO} mice were observed to have arrested follicle development even in the presence of GPNs (36). In $Er\beta^{KO}$ rat ovaries, follicle development failed to progress beyond the antral stage, which is also associated with an absence of GPN-induced ovarian KP expression (127). It has been shown that KPs increase the number of type III follicles that originate from the preovulatory follicles and possess a large antral space (128–130). However, this response may in part be mediated by an indirect effect of KPs on the HP axis that induces GPNs.

Oocyte maturation

While KPRs are expressed in oocytes, surrounding GCs express KPs (24). A recent mutant mouse study has demonstrated an essential role for KPRs in oocytes for oocyte maturation and ovulation (Figure 4) (35). A low level of KP is expressed in the ovary in the basal condition, which is

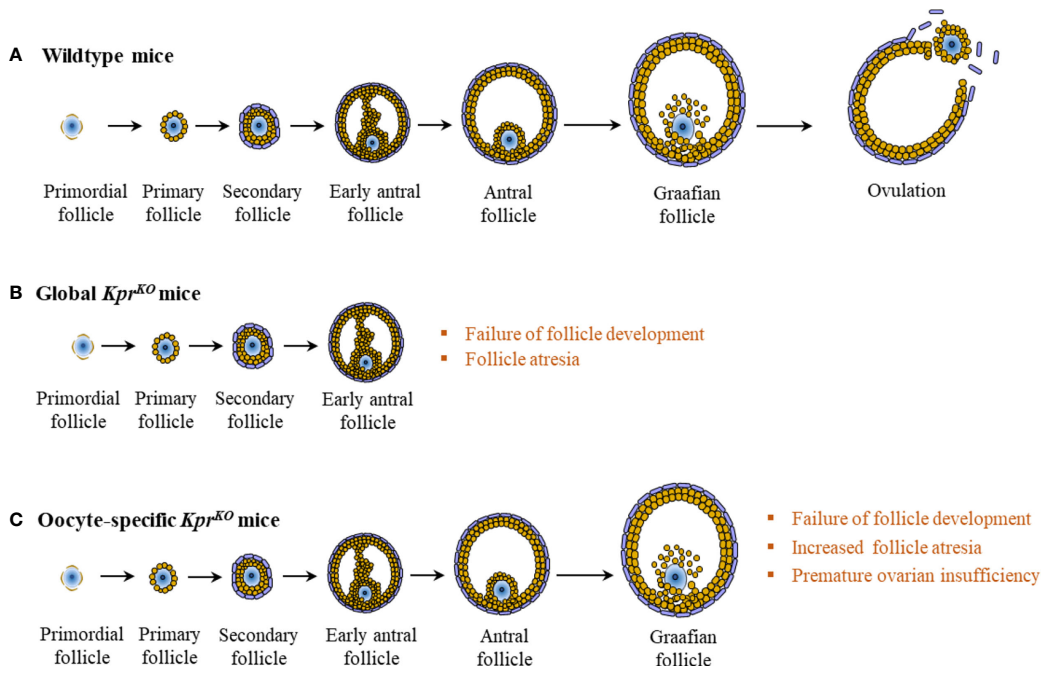

FIGURE 4
Intraovarian kisspeptin signaling. In wildtype mice, normal development of the ovarian follicle leads to ovulation **(A)**. However, in global Kpr^{KO} mice, follicles fail to develop beyond the secondary or the early antral stage **(B)**. In contrast to wildtype or global Kpr^{KO} mice, oocyte specific Kpr^{KO} mouse ovaries display markedly increased follicular atresia at late stages of follicle development, resulting in progressive failure of ovulation and premature ovarian insufficiency at 4 to 6 months of age **(C)**.

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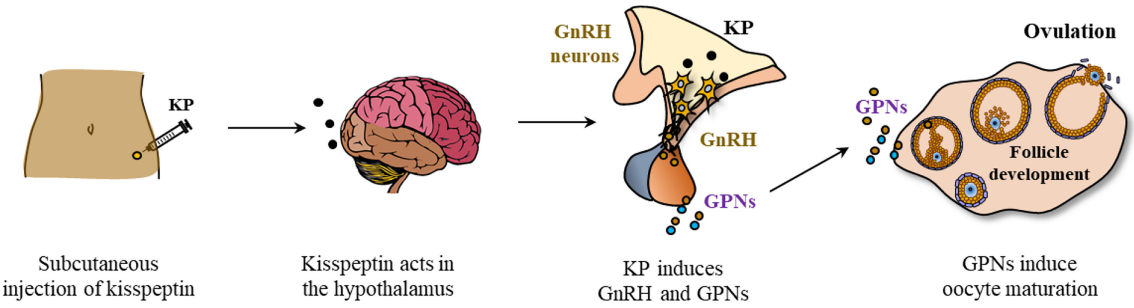

FIGURE 5
Kisspeptin induced oocyte maturation in patients undergoing *in vitro* fertilization. Exogenous kisspeptin (i.e., KP-54) administered at peripheral sites (subcutaneously) can reach the GnRH neurons in the hypothalamus and stimulate GnRH expression. GnRH acts on the pituitary gonadotrophs to induce gonadotropins (GPNs, LH and FSH) that act on the ovarian follicles to induce oocyte maturation and ovulation.

upregulated during the preovulatory period in late proestrus of rats (22). Basal levels of KPs in GCs is upregulated by PMSG-induced activation of FSHR, which is further upregulated by hCG-induced activation of LHCGR (104, 127). In human ovaries, intrafollicular KP levels were found to be increased with the progress in follicle development and maturation (37, 131).

These expression patterns of KPs and KPRs suggest that KPs produced by the GCs act on the KPRs expressed in oocytes to mediate a physiological response during the preovulatory oocyte maturation (24). It is likely mediated by KP-activated protein kinases like ERK1/2 and selective mRNA degradation to increase the expression of necessary proteins (38).

Ovulation

Over the past two decades, KPs have been recognized as the most potent activator of GnRH and GPN secretion in mice (49), rats (14, 132), sheep (48), goats (133), cattle (134), and humans (135). Hypothalamic KPs are essential for the preovulatory GPN surge, which is required for follicle maturation and ovulation. The autocrine and paracrine functions of KPs were further observed in an experiment in which oocyte-specific Kpr^{KO} mice failed to ovulate (35, 136). Although the expression patterns of ovarian KPs and KPRs suggest a potential role in preovulatory follicle maturation and ovulation, this has not yet been determined experimentally. During ovarian aging, fewer follicles are recruited for activation and maturation, decreasing the number of healthy antral follicles available for ovulation (137, 138). Despite a reduced number of follicles, the ratio of corpora lutea to antral follicles increases with advanced age, indicating that ovulation is more efficient (137, 138). This is associated with an increased level of KPs in aging females, which might be due to reduced inhibition of infundibular/ARC nuclei due to a lower level of estrogens (137, 138). An elevated level of KPs leads to increased GPN secretion that improves follicle maturation and ovulation. Administration of the KP antagonist, KP234 not only affects follicle development, but also disrupts oocyte maturation and ovulation (138). Loss of KPRs in mouse oocytes also disrupted oocyte maturation and ovulation (35, 36). Moreover, several recent studies have demonstrated that KP stimulation can induce IVM of oocytes (38, 43), which supports a potential *in vivo* role of KP in oocyte maturation. However, further studies are necessary to prove the physiological role of direct intraovarian KP-signaling in oocyte maturation.

Kisspeptin and *in vitro* fertilization of oocytes

Based on the positive results in animal experiments following peripheral administration of KPs, KP-54 (54 amino acid form of human KP) was tested for its role in IVF. In several studies, administration of exogenous KP-54 was found to be a good option for inducing oocyte maturation (39, 40, 139, 140). A single dose of KP-54 injection could trigger oocyte maturation in women undergoing IVF (39) (Figure 5).

Circulating LH, FSH, and progesterone levels were elevated following the administration of exogenous KP-54 to women undergoing IVF (39). There was a dose-dependent increase in mature oocyte yield with kisspeptin dose, although both 6.4 and 12.8 nmol/kg are likely to represent doses near the top of the dose-response curve as LH rises were similar (39). In an *in vitro* study, treatment of granulosa lutein cells with KP-54 significantly increased the expression of GPN receptors (141). However, it needs to be determined whether KP-54 can also upregulate the expression of GPN receptors on GCs *in vivo*.

hCG is administered to achieve the final stages of oocyte maturation for IVF, but this carries some adverse effects. hCG can cause severe complications like ovarian hyperstimulation syndrome (OHSS) due to its prolonged half-life and potent

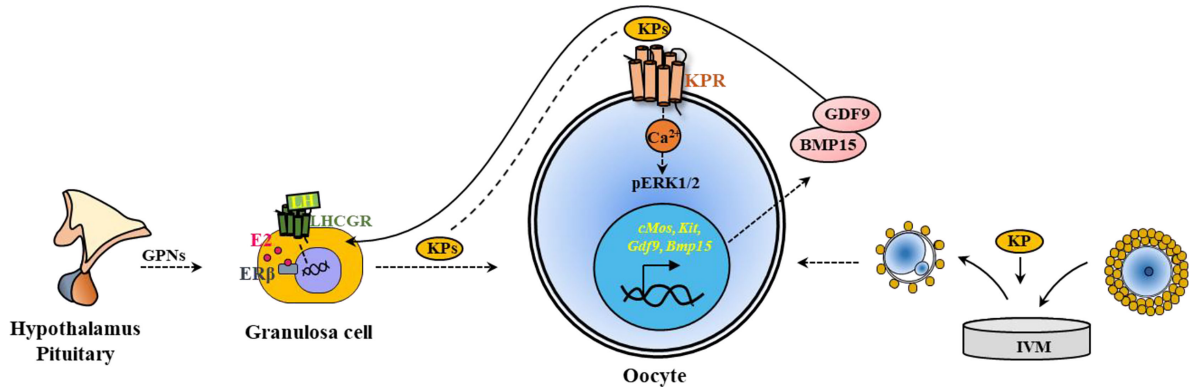

FIGURE 6
Kisspeptin induced *in vivo* and *in vitro* maturation of oocytes. Kisspeptins (KPs) mediate the maturation of ovarian follicles acting through hypothalamic-pituitary induction of gonadotropins (GPNs). GPNs act on granulosa cells and induce KP expression in the ovary. KPs can also act on the KPRs in the oocytes to induce oocyte maturation. Based on this strategy, KPs are included in the *in vitro* maturation (IVM) media to induce Ca^{2+} release and activate ERK1/2 in the oocytes. During IVM, KPs also upregulate the expression of oocyte genes like *cMos, Kit, Gdf9* and *Bmp15*, which improve oocyte maturation.

stimulation of LHCGR. hCG induced excessive VEGF production in the ovaries plays a crucial role in the pathophysiology of OHSS (142, 143). KPs induce endogenous GnRH and GPNs, which may not stimulate ovarian production of excessive VEGF (40, 143). Moreover, KPs have been shown to inhibit ovarian VEGF expression (143, 144), that may alleviate the side effects of hCG administration. A recent study has found that a second injection of KP-54 could improve oocyte maturation in women at high risk of OHSS (139). This finding suggests that KP-54 might be considered a safe choice for women undergoing ARTs, especially those who have a higher risk for developing OHSS. However, further studies are required to compare the relative risks of hCG induced OHSS with that of the GnRH agonist and KP-54.

More recently, a KPR agonist oligopeptide, MVT-602, has been administered to healthy women as well as women with polycystic ovary syndrome (PCOS) or hypothalamic amenorrhea (HA) and compared the effects with that of KP-54 (42). In healthy women, MVT-602 induced LH secretion was similar to that of KP-54, but the effect exhibited a prolonged duration (42). While PCOS patients responded similarly to MVT-602 and KP-54, HA patients had an early response to MVT-602. These findings indicate that MVT-602 possesses a considerable therapeutic potential comparable to KP-54 (42).

Kisspeptins and *in vitro* maturation of oocytes

IVM of oocytes is an advanced laboratory technique used for ARTs, where oocytes are collected before complete maturation. The partially mature oocytes are cultured *ex vivo* for further maturation. Once the *ex vivo* maturation is completed, the oocytes are fertilized *in vitro* and developing embryos are transferred to the uteri of the recipients. IVM is considered for younger females who have a larger follicle reserve. It requires minimal hormone administration, which reduces side effects including OHSS. IVM is recommended for patients with PCOS, as they are more susceptible to OHSS with IVF treatments. It is also very useful for women recovering from cancer, as cancer cells may get stimulated by the exposure to the hormones used in IVF.

Both hypothalamic and intraovarian KPs have the potential to target maturation of oocytes, as they express KPRs. Hypothalamic KPs are known to act through GPN secretion, ovarian KPs can act directly on the oocytes and activate KPRs (Figure 6).

On the other hand, the oocytes express KPRs, and KPs have been tested for a role in IVM of oocytes. Several studies have demonstrated that supplementation of IVM media with KPs increase the rate of maturation of rat (38), sheep (43), and sow (44) oocytes (Figure 6).

KPRs are expressed in the oocytes and respond to KPs resulting in oocyte maturation (24, 38). Activation of KPRs augment intracellular Ca^{2+} release and activate mitogen-activated protein (MAP) kinases, extracellular signal-regulated kinase 1 and 2 (ERK1/2) in rat oocytes (38), similar to that was observed in hypothalamic neurons and luteal cells (145, 146). However, *in vitro* treatment of rat oocytes did not activate AKT (protein kinase B), which is also important for ovarian follicle activation (38). Studies have shown that inclusion of FSH in the oocyte culture media increase the expression of KPRs and augment KP-induced IVM of oocytes (44). IVM of both rat and sow oocytes was associated with upregulation of oocyte genes crucial for differentiation of GCs, and maturation of oocytes (38, 44). KP-10 treatment of rat and sow oocytes

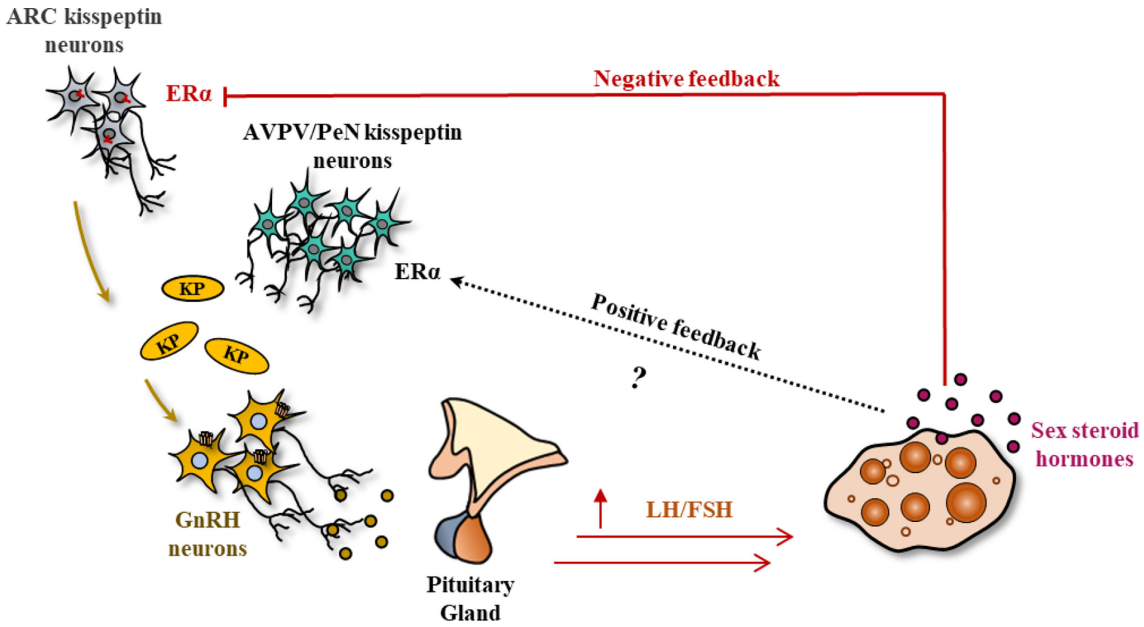

FIGURE 7
Polycystic ovary syndrome. Polycystic ovary syndrome (PCOS) is caused by increased levels of gonadotropins (GPNs, LH/FSH) due to the overstimulation by hypothalamic kisspeptins (KPs). Although the KP-response from the ARC nucleus is increased, it is suspected that PCOS lacks the estrogen induced KP surge from the PeN/AVPV nucleus required for generating the preovulatory GPN surge (unlike Figure 2). Lack of the preovulatory GPN surge in PCOS leads to a lack of follicle maturation and a failure of ovulation.

upregulated the relative expression of *cMos, Kit, Gdf9* and *Bmp15*, which are important for oocyte maturation (38, 44). As gene transcription is minimal during oocyte maturation, such differential expression of genes is likely due to selective degradation of mRNAs other than *cMos, Kit, Gdf9* and *Bmp15* (38, 44) (Figure 6).

KP-10 has been tested for IVM of rat, sheep, and sow oocytes (38, 43, 44). However, neither KP-10 nor KP-54 has been tested for IVM of human oocytes. In a recent study, the effects KPR agonist MVT-602 have been tested in cell lines and brain slices (42). MVT-602 induced inositol monophosphate (IP1) and Ca^{2+} signaling was comparable to that of human KP-54, however, the action potential firing of GnRH neurons in brain slices was longer than that of KP-54 (42). These findings suggest that both KP-54 as well as MVT-602 have a considerable potential for use in IVM of human oocytes.

Ovarian diseases linked to kisspeptins

Dysregulation in KP signaling disrupts the HPO axis of neuroendocrine signaling, and negatively impacts ovarian function and fertility (28). Recent studies have linked several ovarian diseases to abnormal KP-signaling (7, 147, 148). PCOS, a common gonadal and metabolic disease, is associated with an elevated level of circulating KPs (7, 147–151). Some of the symptoms of PCOS include; endothelial dysfunction, elevated inflammatory markers, hormonal imbalances, and irregularities in the menstrual cycle (Figure 7) (152).

PCOS is commonly associated with high LH, but low FSH levels and decreased synthesis of estradiol as compared to their healthy counterparts (149, 150, 153). Exogenous KP treatment can also induce GPN response and rescue ovulation in a subset of PCOS patients (154). However, it is not yet determined whether any defects in intraovarian KP-signaling is linked to failure of follicle maturation in PCOS. Studies also suggest a potential link between PCOS and hypothyroidism, which is a common endocrine disorder (155, 156). It has been shown that maternal hypothyroidism can reduce the KPs and KPRs expression in placenta (157). Thus, it will be clinically important to know if thyroid hormones also regulate KP and KPR expressions within the ovary.

Development of precocious puberty has been linked to hypothalamic KP signaling (158). An aberrant gain of KP expression or KPR signaling may lead to precocious puberty (159). KPs have been proved to be a major therapeutic remedy in assisting women with reproductive and infertility issues (40–42, 139, 160). However, hypothalamic amenorrhea, which is associated with deficiency in GPN release and low KP levels, does not respond well with exogenous KP-54 administration.

Despite an initial positive response, high dose KPs result in desensitization after a few weeks (161).

Summary and conclusions

While the hypothalamic KPs regulate GPN secretion (6, 7, 10), GPNs activate KP secretion in the ovary (22, 24). During the last 25 years, KP/KPR studies have been focused primarily on the hypothalamic KP-signaling. The hypothalamic role of KP-signaling is well accepted but the role of ovarian KP/KPR remains largely unknown. Recent studies have emphasized the importance of KP-signaling in several extrahypothalamic sites (35, 38). However, the majority of extrahypothalamic functions remain unclear. Administration of KP-54 (54 amino acid human KPs) to women undergoing *in vitro* fertilization was found to trigger maturation of oocytes (39). Patients at high risk of OHSS have been successfully treated with KP-54 that can replace hCG (40). A recent study has also evaluated a KPR-agonist, MVT-602, which showed promising results similar to that of KP-54 (41, 42).

In most instances, oocyte maturation effects of exogenous KPs or the KPR-agonist have been attributed to the induction of hypothalamic GnRH and pituitary GPNs (39, 40, 42). However, KPs and KPRs are also expressed in the ovaries, which have been shown to play an essential role oocyte maturation and ovulation (24, 35, 38). Nevertheless, it remains undetermined if exogenous KPs or KPs of hypothalamic or extraovarian origins can act on the KPRs in oocytes. Oocytes express KPRs and supplementation of IVM media with KPs was found to increase the rate of IVM (38, 43, 44). KP stimulation during IVM increased Ca^{2+} release, MAP kinase (ERK1/2) activation, and upregulation of oocyte genes that promoted oocyte maturation (38, 43, 44). Moreover, FSH has been demonstrated to upregulate the expression of KPRs and augment KP-induced IVM of oocytes, which offers an opportunity to include both FSH and KPs in IVM media (44).

Despite the promising results of KP-induced *in vivo* and *in vitro* oocyte maturation, the growth of blastocysts and trophoblast outgrowth was reduced by incubation with kisspeptin in an *in vitro* study (44). Studies employing intraovarian kisspeptin administration or antagonism, reveal that kisspeptin has a role in reducing acquisition of FSH receptors and increased corpora lutea, signifying an important role in follicular development and ovulation during reproductive aging (138). Most importantly, further studies are required to evaluate whether KP stimulated mature oocytes maintain their normal fertilization competence and developmental potential. We emphasize that elucidating the direct *in vivo* or *in vitro* roles of KPs or KP agonists in oocyte maturation will help develop novel KP-based strategies to improve the ARTs.

Author contributions

This review article and illustrations were prepared by SM, EL, ID, and SU. M.A.R planned structure of the article and edited the content. VC and PF reviewed the manuscript and made valuable corrections. PF editing the revised manuscript and language correction. All authors approved the contents of the revised manuscript.

Funding

This work was supported in part by the COBRE (P30 GM122731), K-INBRE (P20 GM103418), and NIH R21 HD105095 grant funding.

Acknowledgments

The authors acknowledge Nathan Gaid, a Summer Trainee in Fields lab, for reading the revised manuscript and suggesting some grammatical correction.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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