Regulation of Pregnancy: Evidence for Major Roles by the Uterine and Placental Kisspeptin/KISS1R Signaling Systems

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Abstract

Several studies provide strong evidence suggesting that in addition to central kisspeptin/KISS1R signaling, the peripheral uterine- and placental-based kisspeptin/KISS1R signaling systems are major regulators of pregnancy. Specifically, the evidence suggests that the uterine-based system regulates embryo implantation and decidualization, while both the uterine- and placental-based systems regulate placentation. Uterine kisspeptin and KISS1R regulate embryo implantation by controlling the availability of endometrial glandular secretions, like leukemia inhibitory factor, which are essential for embryo adhesion to the uterine epithelium. As for decidualization, the data suggest that decidualized stromal cells express KISS1R and secrete kisspeptin-inhibiting decidual cell motility and thereby indirectly regulate embryo and placental invasion of the uterus. Similarly, for placentation, placental kisspeptin and KISS1R negatively regulate extravillous trophoblast migration and invasion and thereby directly control placental invasion of the uterus. Having recognized a significant role for the uterine- and placental-based kisspeptin/KISS1R signaling systems regulating pregnancy, the future looks promising for the development of kisspeptin and KISS1R as prognostic and diagnostic markers of pregnancy disorders and the use of kisspeptin as a therapeutic agent in the prevention and treatment of conditions such as recurring implantation failure, recurrent pregnancy loss, and preeclampsia.

Keywords
- kisspeptin
- KISS1R
- implantation
- decidualization
- placentation

A large number of women are unable to become pregnant or carry a pregnancy to term and the underlying reasons are both diverse and complex. In the absence of male factors of infertility, chromosomal abnormalities in the embryo and uterine anatomical abnormalities such as fibroids, it is recognized that uterine and/or placental molecular defects arising at any point during pregnancy can lead to pregnancy failure.[1–3] The kisspeptin/KISS1R signaling system is now well established as a potent regulator of reproduction through its direct actions along the hypothalamic–pituitary–gonadal (HPG) axis[4–7] and indirect actions at other sites such as the hippocampus[8–10] and amygdala,[8,11,12] where it regulates sexual behavior, and the liver[13,14] and pancreas,[13–15] where it regulates energy status. The diagnostic and therapeutic value of kisspeptin and KISS1R is well recognized and continues to be the focus of intense investigations.[16–23] The objective of this review is to present the evidence that uterine and placental kisspeptin and KISS1R regulate pregnancy and to discuss the significance of such evidence. Pregnancy is initiated with the fertilization of the egg and ends with the delivery of the fetus. The intervening period is marked by several highly regulated events that impact directly on whether a pregnancy becomes established and goes to term. This review will focus on three major intervening events: implantation, decidualization, and placentation.

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Uterine Kisspeptin and KISS1R Regulate Embryo Implantation

Embryo Implantation: An Overview

Embryo implantation is the process where the blastocyst attaches to the uterine epithelium and invades the underlying stroma cells to an extent that is species specific. Implantation is a highly regulated process and occurs only when the preimplantation embryo has reached the blastocyst stage and when the uterus acquires a receptive state, two events that occur approximately at the same time. Implantation involves three distinct steps: apposition, adhesion, and penetration or invasion. In the mouse, uterine receptivity is achieved on the fourth day after mating (D4 of pregnancy) and embryo implantation begins on the evening of D4 and is completed by the morning of D5. In women, the uterus achieves a receptive state in less than a week after ovulation and it exhibits maximum receptivity to an implantation event between D20 and 24 of the menstrual cycle. The period in which the embryo can implant in the receptive uterus is referred to as the "window of implantation (WOI)" and in mice, the WOI opens following the nidatory surge of E2 and expression of glandular *Lif* on the morning of D4 of pregnancy.[24–26] In women, an equivalent E2 surge has not been reported, but the expression of cytokine genes, such as *LIF*, is also likely involved in regulating the opening of the WOI.[27] In both mice and women, the factors that lead to the closure of the WOI, thereby ushering in the refractory (nonreceptive) phase, are not well understood but at least one study suggests that in mice this might involve the expression of insulin-like growth factor 1 (IGF1) on D5 of pregnancy,[28] while in women it might involve tumor necrosis factor-α (TNFα).[27]

A major cause of female infertility is embryo implantation failure. Among patients undergoing assisted reproductive technology (ART), implantation failure (as determined by the lack of increased serum beta human chorionic gonadotropin [β-hCG] levels approximately 2 weeks after embryo transfer) is referred to as recurrent implantation failure (RIF) after three failed in vitro fertilization (IVF) attempts with morphologically high-grade embryos.[29] A large number of molecules, of which the majority are under E2 and/or P4 regulation, have been demonstrated to regulate implantation of the embryo in the receptive uterus.[30,31] Among these molecules are kisspeptin and its receptor, KISS1R. Kisspeptins and KISS1R are expressed in the nonpregnant and pregnant uterus of humans and several laboratory and domestic animal species,[32–42] and in the pregnant mouse, the uterus expresses a functional kisspeptin/KISS1R signaling system on the day of uterine receptivity and implantation.[34]

Uterine Kisspeptin and KISS1R Regulate Trophoblast Adhesion

In independently generated mouse lines bearing null mutations of *Kiss1* or *Kiss1r*, it was determined that some ovarian follicles developed to the preantral stage but failed to undergo final maturation and ovulation.[4,43,44] The Babwah Laboratory considered this was in part due to reduced follicle-stimulating hormone (FSH) levels coupled to the lack of the luteinizing hormone (LH) surge and demonstrated that following a period of E2 priming, the administration of FSH (in the form of pregnant mare serum gonadotropin) and LH (in the form of hCG) fully rescued follicular development and ovulation.[37] The eggs were fertilization competent and when the *Kiss1* or *Kiss1r* knockout (KO) females were mated to wild-type (WT) males, the preimplantation embryos developed normally.[37] However, while the hatched blastocysts (heterozygous for either *Kiss1* or *Kiss1r*) could be implanted into the uterus of a WT female, they could not in the KO female, despite E2 priming and normal circulating levels of P4.[37] Upon closer examination it was observed that the blastocyst had undergone successful apposition in the implantation crypt, but there was no evidence of attachment (adhesion) of the trophectoderm to the luminal epithelium.[37] In support of this finding it was later determined that kisspeptin treatment increases the adhesion of KISS1R-expressing human trophoblast cells and hatched mouse blastocysts to collagen.[45] Thus, a disruption in the uterine kisspeptin/KISS1R signaling system would account for the lack of blastocyst adhesion to the uterine epithelium.

In rodents, it has been demonstrated that endometrial glandular sections are essential for successful implantation and that the absence of some secretions disrupts implantation. One of the glandular secretions shown to be critical for implantation is leukemia inhibitory factor (LIF).[46,47] Following this discovery, LIF was also shown to increase the adhesion of primary human endometrial epithelial cells to fibronectin and collagen.[48] We therefore tested whether endometrial glands in the *Kiss1*$^{-/-}$ mice lacked LIF in the period preceding embryo implantation and observed that this was indeed the case as determined by immunofluorescence analysis of LIF levels.[37] We then administered LIF to pregnant mice on the day of uterine receptivity and were able to partially rescue *Kiss1*$^{+/-}$ embryo implantation in the uterus of the pregnant *Kiss1*$^{-/-}$ female. This finding initially led to the suggestion that kisspeptin/KISS1R signaling lies upstream of glandular LIF expression, but further investigations determined this was not the case.[36]

Uterine Kisspeptin and KISS1R Regulate Endometrial Adenogenesis and Gland Function

Uteri from the adult female *Kiss1* and *Kiss1r* null mice are thin and thread-like due to diminished E2 levels resulting from hypogonadotropic hypogonadism. These uteri are also almost completely devoid of endometrial glands. E2 priming after weaning restores uterine growth and development but fails to fully rescue gland development or adenogenesis, suggesting that an aspect of gland development is independent of ovarian E2 but dependent on uterine kisspeptin/KISS1R signaling.[36] In addition to the reduced adenogenesis it was also observed that the rescued glands expressed visibly reduced levels of FOXA2, as determined by immunohistochemistry (IHC). As it was reported that FOXA2 regulates endometrial gland development and function,[49] we hypothesized that the rescued glands were not fully functional and that in addition to LIF, they were not producing and/or secreting other substances required for implantation. We therefore sought to determine for glandular protein expression of secreted phosphoprotein 1 (SPP1) by IHC, as well as mRNA expression of *Prss28* (encodes protease,

serine 28), *Prss29* (encodes protease, serine 29), *Spink3* (encodes serine peptidase inhibitor, Kazal type 3), and *Ttr* (encodes transthyretin) and found that their levels were significantly reduced in the uterus of the pregnant *Kiss1*$^{-/-}$ female, suggesting that the rescued glands were in fact not functional.[36]

In our initial characterization of the female *Kiss1* and *Kiss1r* null mice, we suggested that kisspeptin/KISS1R signaling lies upstream of glandular LIF expression and it was the lack of LIF that blocked implantation.[37] While it is correct that a lack of LIF blocked implantation, our subsequent study[36] led us to realize that diminished LIF levels is only an indirect consequence of having nonfunctional glands. This conclusion is based on the findings that FOXA2 expression is diminished in rescued glands, and that E2-rescued glands exhibit diminished expression of additional molecules (LIF, SPP1, *Prss28*, *Prss29*, *Spink3*, and *Ttr*) implicated in gland function.[46,47,49–54] Thus, we concluded that kisspeptin/KISS1R signaling positively regulates both gland development and function.

Overall, our studies suggest roles for the uterine kisspeptin/KISS1R signaling system in regulating gland development and function and embryo implantation. These studies were conducted in the whole body *Kiss1* and *Kiss1r* KO mouse following E2/gonadotropin therapy that partially restored ovarian function. The extent to which ovarian function was restored was not fully characterized and it remains possible that it is the persistent lack of some ovarian functions, rather than uterine, which blocks endometrial gland development and function. This important determination, however, requires the development of a uterine KO mouse deficient in kisspeptin signaling.

Clinical Evidence Supports a Role for Uterine Kisspeptin and KISS1R as Regulators of Embryo Implantation in Women

To date, there are no clinical studies directly demonstrating that the loss of uterine kisspeptin or KISS1R expression is associated with implantation failure. However, two recent studies from the Rehman Laboratories[55,56] revealed that in women undergoing intracytoplasmic sperm injection (ICSI), low serum kisspeptin levels (measured by enzyme-linked immunosorbent assay [ELISA]) were associated with implantation failure. In one study, they determined that women with unexplained infertility had significantly lower levels of serum kisspeptin (determined on the second day of the menstrual cycle prior to starting the ovarian downregulation protocol) when compared with those with male factor infertility and the group with lower kisspeptin levels exhibited reduced implantation events following ICSI.[55] In a second study, they found that following embryo transfer, serum kisspeptin levels (also measured by ELISA) were significantly lower in women who failed to exhibit a clinical pregnancy (β-hCG < 25 mIU/mL).[56] While these studies demonstrate an association between low kisspeptin levels and implantation failure following ICSI, they do not determine the source of this kisspeptin. A major source of circulating kisspeptins during human pregnancy is thought to be derived from the placenta,[57] but the source in the nonpregnant woman or during early pregnancy in the preimplantation period remains to be determined. While hypothalamic kisspeptin seems to be an unlikely source, more likely sources include the uterus[37] and the liver.[14] Thus, it remains a formal possibility that reduced uterine kisspeptin levels are associated with poor implantation rates.

Uterine Kisspeptin and KISS1R Regulate Endometrial Stromal Cell Decidualization

Decidualization and the Decidua: An Overview

Despite some differences in the temporal, spatial, and regulatory aspects of stromal decidualization in mice and humans,[58,59] the decidua is critical for the survival of the implanting embryo and subsequent conceptus in both species. Like implantation, decidualization is also a tightly regulated process largely under the control of E2 and P4. The decidua is composed of terminally differentiated uterine stromal cells, blood vessels, and local immune cells that include uterine natural killer (uNK) cells, macrophages, and T lymphocytes. Decidualized stromal cells store glycogen and along with small amounts of maternal blood derived from ruptured capillaries they provide glucose needed to support the growth of the conceptus during early pregnancy.[60] Decidualized stromal cells also express a wide array of growth factors and cytokines and thereby exert developmental effects on the conceptus.[61] Immune cells of the decidua protect the embryo from being attacked by maternal immune cells and together with decidualized stromal cells regulate the development of the trophoblast lineage. As a result, they regulate the depth of invasion of the implanting embryo and placenta, ensuring their adequate attachment to the uterus.[61,62] To regulate invasion, decidualized stromal cells must exhibit motility to accommodate the embryo and placenta[63,64] but eventually must act as a physical and biochemical barrier to prevent the villi from becoming too deeply attached in the uterus and in some cases even to adjacent organs like the bladder. An abnormally firmly attached placenta, a condition that affects 1 in 2,500 pregnancies, not only results in hemorrhage during pregnancy but is a major cause of postpartum hemorrhage and is associated with significant maternal morbidity and mortality.[65] As the pregnancy progresses, the placenta becomes more active in transporting oxygen and nutrients from the maternal blood to the developing fetus and as a result the role of the decidua in nourishing the conceptus diminishes rapidly. However, as the pregnancy progresses and approaches parturition, the decidua continues to act as an immunological barrier between the mother and fetus.[66] Given the critical roles of the decidua in ensuring a successful pregnancy, understanding the molecular regulation of stromal decidualization and decidual function remains important areas of research.

Uterine Kisspeptin and KISS1R Regulate Endometrial Decidualization in Mice

Zhang et al[38] showed that in the pregnant mouse uterus, both KISS1 and KISS1R mRNA levels and protein rose significantly shortly after embryo implantation, strongly suggesting roles in stromal decidualization. They next showed that using in vivo and in vitro mouse models of induced decidualization, *Kiss1* and *Kiss1r* uterine levels increased with progressive

decidualization, while in vitro the knockdown of *Kiss1* in stromal cells attenuated the expression of cyclin D3 and PR, mediators of decidualization.[38] Taken together, their data strongly suggest a role for the signaling system in mouse stromal decidualization. Interestingly, we demonstrated that in the E2/P4-primed pseudopregnant *Kiss1*$^{-/-}$ mouse, the uterus could be induced to undergo decidualization,[37] suggesting that while the uterine kisspeptin/KISS1R signaling system regulates decidualization the lack of such signaling can at least be partially compensated by other signaling pathways in the hormone-primed mouse. Furthermore, in the E2/gonadotropin-primed pregnant *Kiss1*$^{-/-}$ mouse, following LIF administration and the rescue of embryo implantation, decidualization of stromal cells continued and the decidua persisted into D10 of pregnancy, the final day of our observation.[37] It must be noted, however, that our examination of decidualization was limited to a histological assessment and despite the detection of decidualized cells we cannot rule out the possibility that there were molecular defects that disrupted decidual function. This possibility coupled to the findings of Zhang et al[38] provide strong impetus for studying the kisspeptin/KISS1R signaling system further as regulators of decidualization.

Decidual Kisspeptin and KISS1R Regulate Decidual Function in Humans

Since human decidualized stromal cells express both kisspeptin[40] and KISS1R,[41] it is possible that decidual kisspeptin/KISS1R signaling regulates decidual function in humans. This possibility is supported by the following data. Using primary cultures of endometrial stromal cells isolated from healthy female donors, Baba et al[40] demonstrated that the in vitro decidualization of the stromal cells resulted in the increased expression of kisspeptin. In an independent study, Wu et al[41] isolated decidualized cells directly from women undergoing elective surgical termination of normal pregnancies after 6 to 8 weeks of gestation and showed that these cells expressed KISS1R. They further demonstrated that kisspeptin, in a dose-dependent manner, inhibited the invasion and migration of these cells, while the KISS1R antagonist (kisspeptin 234), in a dose-dependent manner, stimulated invasion and migration.[41] Taken together, the data suggest that decidual kisspeptin/KISS1R signaling regulates decidual function in humans.

Expression of Decidual Kisspeptin, but Not KISS1R, Is Reduced in Women Exhibiting Recurrent Spontaneous Abortion

Recurrent spontaneous abortion (RSA) is defined as the miscarriage of two or more pregnancies before the 20th week of gestation and about a half of all cases are idiopathic. This led Wu et al[67] to investigate whether the expression of kisspeptin and KISS1R was altered in the decidua of women experiencing RSA. Using IHC, kisspeptin and KISS1R expression was assessed in the decidua from RSA patients ($n = 32$) having pregnancy loss in the first trimester, and healthy women ($n = 35$) undergoing an elective termination of their pregnancy during the first trimester. Kisspeptin expression was observed in 88.6% of the control samples but only in 53.1% of the RSA samples. KISS1R expression was detected in 40% of the control samples and 40.6% of the RSA samples. For each of the samples studied, there was a very high association between the expression of kisspeptin and KISS1R. While these results revealed that RSA is associated with decreased kisspeptin expression in the decidua, it was noted that the expression of kisspeptin nor KISS1R was detected in 100% of the control samples. The significance of this remains to be determined.

Interestingly, in an earlier study conducted on first trimester human placenta (6–10 weeks of gestation), the Desoye Laboratory[68] reported that the decidua was devoid of kisspeptin expression; however, the authors did not report on KISS1R expression. The reason for the discrepancy in kisspeptin expression between the two studies[67,68] is unclear, but it might be a technical issue, as the studies used different antibodies to detect kisspeptin or it could be due to sample size as the former study analyzed samples from over 30 women[67] while the latter study examined samples from five women.[68] In conclusion, while there is growing evidence that uterine kisspeptin/KISS1R signaling regulates stromal cell decidualization in both mice and humans and that decidual cells secrete kisspeptin which regulate decidual function, further studies are required to better appreciate the importance of this system under both healthy and diseased states.

Placental Kisspeptin and KISS1R Regulate Human Placentation

Human Placentation: An Overview

Placentation refers to the growth and formation of the placenta and the development of the uterine capacity to supply the blood required by the fetus. In humans, the blastocyst attaches to the uterine epithelium on D6 postfertilization, and on D7 the embryonic trophoblast differentiates into the syncytiotrophoblast and cytotrophoblast layers marking the start of placentation. Over the next few weeks, the embryo obtains its nourishment from the surrounding decidua and while this can support embryo development for a few weeks, continued embryo growth and survival eventually requires access to maternal blood. To understand the critical importance of maternal blood in sustaining a pregnancy, consider that in the nonpregnant state the uterine artery carries less than 1% of the cardiac output with a flow rate of 50 mL/min; however, toward the end of pregnancy, maternal plasma volume increases by approximately 40 to 50% and the uterine artery carries approximately 10% of the cardiac output with a flow rate of 600 to 700 mL/min.[69]

To ensure the demands for maternal blood are met, toward the end of the first trimester of pregnancy, extravillous trophoblasts (EVTs; fetal cells) invade and remodel the maternal spiral arteries as far as one-third into the myometrium resulting in large-bore, low-resistance vessels that can transport sufficient blood to the maternal–fetal interface where oxygen and other nutrients are taken up to nourish the fetus. A heightened period of remodeling occurs between the 10th and 12th weeks of gestation and

this is reflected by a steep rise in placental O_2 tension from less than 20 mm Hg at 10 weeks to greater than 50 mm Hg at 12 weeks.[70] In response to the increased blood flow, the fetus enters a period of rapid growth and development. The remodeling of the maternal spiral arteries is essential for a successful pregnancy and factors that reduce EVT invasion and subsequent remodeling of maternal spiral arteries result in preeclampsia (PE). PE is a multifactorial disorder of pregnancy that is diagnosed following new hypertension (blood pressure of $\geq$ 140/90 mm Hg) on two separate readings at least 6 hours apart and when presented after 20 weeks of gestation in conjunction with proteinuria ($\geq$ 300 mg) per 24 hours.[71] The disease remains a major clinical challenge and results in significant maternal morbidity and mortality, causing 10 to 15% of maternal deaths.[72–74]

Placental Kisspeptin and KISS1R Regulate Placentation

The kisspeptin/KISS1R signaling system is expressed in the human placenta[57] and plasma concentrations of kisspeptin (believed to be placenta derived) rise dramatically during the course of pregnancy[75] and in the third trimester are 7,000-fold greater than that in nonpregnant women,[57] suggesting an important role for this signaling system in regulating placentation.[76–79] This idea got its first powerful experimental support from Bilban et al,[68] who in 2004 reported that kisspeptin potently inhibited EVT migration. They determined that kisspeptin exerted this effect by inhibiting EVT matrix metalloproteinase (MMP)-2 proteolytic activity which then diminished the capacity of EVTs to degrade and invade the surrounding matrix. These findings were further supported by Francis et al[80] who demonstrated using a scratch-migration assay that kisspeptin inhibited trophoblast migration by downregulating the expression of genes encoding MMP-1, -2, -3, -7, -9, -10, and -14 and vascular endothelial growth factor A (VEGF-A), and upregulating the expression of genes encoding tissue inhibitors of metalloproteinases (TIMP)-1 and -3. In addition to modulating MMP and VEGF-A expression and function, we demonstrated that in a dose-dependent manner, kisspeptin triggered increased adhesiveness of human EVTs to type I collagen, a major component of the human placenta, and proposed that this was another important mechanism by which kisspeptin reduced EVT migration.[45] Healthy placentation requires the expression of genes that promote the migration of EVTs as well as genes that inhibit the migration of EVTs, and it is the net activity of both groups of genes that ensures there is balanced invasion and remodeling of the maternal spiral arteries by EVTs during placentation. These studies[45,68,80] support an important role for the kisspeptin/KISS1R signaling system in human placentation. Since then, other studies have described the association between placental kisspeptin and KISS1R (mRNA and protein) levels and circulating kisspeptin levels during both healthy pregnancies and pregnancies complicated by intrauterine growth restriction (IUGR)[81–83] and PE.[81,84–96] The rest of this review will focus on our understanding of the relationship between kisspeptin and KISS1R expression and the development of PE.

Placental Kisspeptin and KISS1R Expression Is Altered in Preeclamptic Pregnancies

One of the first studies that demonstrated a relationship between PE and *KISS1* mRNA levels was by Qiao et al.[84] This study assayed the expression of the MMP-9 and KISS1 by RT-PCR and Western blotting in 90 healthy placentas (30 from the first trimester, 30 from the second trimester, and 30 at term) and 40 placentas from pregnancies complicated with PE (15 were isolated from women exhibiting mild PE and 25 from severe PE). The authors reported that in the healthy placenta, expression of MMP-9 mRNA and protein was high in early pregnancy and decreased gradually as the pregnancy progressed. In contrast, the expression of KISS1 mRNA and protein increased as pregnancy progressed. When compared with placenta from preeclamptic pregnancies, they found that *KISS1* mRNA and MMP-9 protein expression was significantly lower than that observed in the healthy term placenta. The authors concluded that increased KISS1 expression is associated with reduced EVT invasion, a finding consistent with that of Bilban et al.[68] Importantly, their data also revealed that reduced KISS1 levels are associated with PE, a finding that would subsequently be verified through several independent studies, many of which are discussed next.

Following the 2005 study by Qiao et al,[84] other groups began investigating whether reduced KISS1 levels are associated with the development or severity of PE. In 2006, Farina et al[94] reported on their goal to identify predictive biomarkers of PE by assaying cell-free mRNA levels for seven genes from blood obtained from women who had normal and preeclamptic pregnancies. Among the seven genes was *KISS1*. Blood was obtained from six subjects with mild or severe PE ($n = 6$) with or without IUGR and matched controls ($n = 30$) were retrospectively examined for circulating mRNA markers. All pregnancies studied spanned gestation weeks 27 to 37. The results showed that among PE patients, *KISS1* expression levels were lower than those of controls. Despite the small sample size for the affected cohort, this finding led the authors to suggest that *KISS1* cell-free mRNA has the potential of being developed into a predictive biomarker of PE.

In 2009, Armstrong et al[81] hypothesized that circulating kisspeptin levels early in pregnancy would differ in women who subsequently develop PE. To test this, they conducted a retrospective case–control study and analyzed kisspeptin in maternal serum from women with pregnancies in the second trimester (specifically, 16–20 weeks of gestation) who subsequently developed PE ($n = 57$) and controls ($n = 317$) matched for duration of storage at −70°C. They found that while serum kisspeptin levels were significantly lower in women who subsequently developed PE, the reduction was only modest. Thus, Armstrong et al[81] concluded that kisspeptin levels in the second trimester might not be robust enough as a single screening marker in PE but might be useful when measured in combination with other markers. In a subsequent prospective cohort study designed to examine and compare pregnancy outcomes in lean and severely obese pregnancy, members of this group again reported that in early preeclamptic pregnancies, and in obese women who are at increased risk of developing PE, circulating kisspeptin

levels were reduced and therefore kisspeptin might prove to be a useful biomarker for the prediction of PE.[92] However, the authors again cautioned against using kisspeptin as a sole biomarker for universal screening, as they found that low circulating kisspeptin levels are difficult to measure, at least based on one widely available commercial kisspeptin ELISA.

To date, among the studies published on the relationship between kisspeptin levels and PE, only a few have also examined placental KISS1R expression in detail. One of these studies is by Cartwright and Williams.[91] In this 2012 study, using qPCR, Western blotting and IHC, KISS1 and KISS1R mRNA, and protein expression was determined on fetal placental tissue obtained from women undergoing elective surgical termination of early pregnancy ($n = 10$) and from women following Caesarean section at term following a normal pregnancy ($n = 10$) or a pregnancy complicated by PE ($n = 10$). Based on the expression analysis of both protein and mRNA, it was found that KISS1 expression was reduced in PE compared with that in normal term pregnancy, a finding that was consistent with earlier reports. However, it was found that both mRNA and protein KISS1R levels increased in PE compared with that in normal term pregnancy. Based on their data, the authors proposed an intriguing mechanism where the increased KISS1R expression might trigger augmented kisspeptin/KISS1R signaling in PE resulting in greater inhibition of trophoblast invasion, a major underlying cause of PE.

The second major study that characterized placental and circulating levels of KISS1 as well as placental levels of KISS1R under healthy and preeclamptic conditions was reported by Matjila et al[88] in 2016. The study was conducted on healthy patients ($n = 30$, mean age of 28.47 ± 0.87 years) and patients with preeclamptic pregnancies ($n = 19$, mean age of 25.00 ± 1.31) both undergoing elective caesarean delivery. At delivery, the placenta (derived from the fetal compartment), the placental bed and decidua parietalis (derived from the maternal compartment) and maternal and fetal cord blood samples were collected. Through a combination of techniques that included qPCR, immunofluorescence, and an ELISA to detect maternal and cord blood kisspeptin, the authors reported that in pregnancies complicated by PE, placental (fetal) kisspeptin expression was high while circulating serum kisspeptin levels were low. They also found that in the maternal tissues (placental bed and decidua parietalis), KISS1 mRNA and protein levels were very low in both healthy and preeclamptic pregnancies. As for *KISS1R* mRNA, expression was not different between maternal and fetal tissues of both healthy and preeclamptic pregnancies. The authors concluded that in preeclamptic pregnancies, the increased placental (fetal) kisspeptin expression would reduce trophoblast invasion of the maternal spiral arteries and this likely represents a mechanism underlying the development of the disease. They further suggested that the decreased circulating kisspeptin levels could be developed as a marker for placental dysfunction.

Comparing the studies conducted by Matjila et al[88] and Cartwright and Williams,[91] two interesting differences emerge. Cartwright and Williams[91] found that placental (fetal) kisspeptin levels were low, while KISS1R levels were high in preeclamptic pregnancies compared with healthy pregnancies at term. In contrast, Matjila et al[88] found that placental (fetal) kisspeptin levels were high in preeclamptic pregnancies, while KISS1R showed no change in expression. Several reasons could account for this discrepancy and these include different patient cohorts and reagents. Regardless of the reasons, it is clear from all the studies described so far that circulating kisspeptin levels are decreased in preeclamptic pregnancies and with the development of more robust detection systems, the quantification of circulating blood kisspeptin levels, especially in combination with other predictive markers, holds promise in predicting the development and severity of PE.

Summary

Overall, there is now compelling evidence from a growing number of independent studies to suggest that in addition to central kisspeptin/KISS1R signaling, the peripheral uterine- and placental-based kisspeptin/KISS1R signaling systems are major regulators of pregnancy. Specifically, evidence suggests that the uterine-based system regulates embryo implantation and decidualization, while both the uterine- and placental-based systems regulate placentation. Uterine kisspeptin and KISS1R regulate embryo implantation by controlling the availability of endometrial glandular secretions, like LIF, which are essential for embryo adhesion to the uterine epithelium. As for decidualization, the data suggest that decidualized stromal cells express KISS1R and secrete kisspeptin, and this kisspeptin could act in an autocrine/juxtacrine/paracrine manner to inhibit decidual cell motility and thereby indirectly regulate embryo and placental invasion of the uterus. Similarly, for placentation, placental kisspeptin and KISS1R negatively regulate EVT migration and invasion and thereby directly control placental invasion of the uterus. Thus far, the kisspeptin and KISS1R studies have examined placentation in isolation of decidualization, but in fact, decidua-derived kisspeptin, in a paracrine manner, might also directly control placental invasion of the uterus by regulating EVT differentiation and function. These exciting possibilities require further investigation.

As we explore and develop kisspeptin and KISS1R as prognostic and diagnostic markers of pregnancy disorders and even the use of kisspeptin as a therapeutic agent in the prevention and treatment of conditions such as RIF and PE, there is a need to proceed cautiously. Despite the recognition of kisspeptin and KISS1R as potent regulators of the neuroendocrine-reproductive axis over 15 years ago, this field of research remains challenged by the lack of certain commercially available robust tools and assays for studying kisspeptin and KISS1R in health and disease. For example, we still lack reliable antibodies against KISS1R and sufficiently sensitive enzyme immunoassays, radioimmunoassays, and mass spectrometry assays for quantifying low concentrations of both circulating and tissue-expressed kisspeptins. As a result, there is reason to believe that some of the discrepancies noted among various studies discussed in this review might be due to the use of reagents that are insufficiently robust and reliable.

Another area that needs to be developed further to advance our understanding of kisspeptin and KISS1R signaling in health and disease is the development of new animal models. This is particularly noteworthy when studying placentation. While researchers have relied heavily on the mouse and rat as laboratory models of human placentation, other small animal models, such as the guinea pig, better recapitulate EVT invasion and remodeling of the spiral arteries observed in women.[97] Thus, the guinea pig might prove to be an important addition to the research tools available to kisspeptin and KISS1R researchers.

Despite the limitations and challenges discussed earlier, we now recognize important roles for the uterine- and placental-based kisspeptin/KISS1R signaling systems in regulating pregnancy. Based on these roles coupled to technical advances being made in creating long-acting and potent kisspeptin analogs (such as the investigational kisspeptin analog, TAK-448[98–100]), the development of kisspeptin and KISS1R in the clinical treatment of female infertility, both at the central and peripheral levels, looks promising.

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