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The Role of Relaxin in Normal and Abnormal Uterine Function During the Menstrual Cycle and Early Pregnancy

Sarah A. Marshall, BSc, MSc[1], Sevvandi N. Senadheera, BSc, MSc, PhD[1], Laura J. Parry, BSc(Hons), MSc, PhD[1], and Jane E. Girling, BSc(Hons), PhD[2]

Abstract

The hormone relaxin is a 6-kDa peptide with high structural similarity to insulin. It is primarily produced by the corpus luteum during pregnancy but is also synthesized by other reproductive organs such as the uterus, decidua, and placenta. Relaxin binds to its receptor RXFP1, which has been localized to a wide variety of reproductive and nonreproductive tissues. The peptide's many uterotropic effects include stimulating uterine growth and vascularization, remodeling extracellular matrix components, and regulating vascular endothelial growth factor in preparation for implantation. Evidence also supports a role for relaxin in the systemic maternal vascular adaptations required for a healthy pregnancy. Diminished relaxin levels in early pregnancy are linked with increased risks of miscarriage and the development of preeclampsia. In addition to pregnancy, relaxin may also play a functional role in the uterus during the menstrual cycle, and modified relaxin activity may contribute to gynecological disorders such as uterine fibrosis and endometriosis. Despite over 75 years of research, we still have a limited understanding of relaxin's broad roles in the uterus, particularly as there are significant species differences in its synthesis and activity, which restricts the use of animal models for human-centric questions. Here, we review current knowledge regarding relaxin actions in the human uterus during the menstrual cycle and in early pregnancy, with a focus on its potential roles in various gynecological disorders, as well as the pregnancy disorders such as preeclampsia, recurrent miscarriage, and early pregnancy loss.

Keywords

relaxin, menstrual cycle, pregnancy, uterus

Introduction

In 1926, Dr Hisaw experimentally induced relaxation of the pubic ligament in virgin guinea pigs by injecting serum collected from pregnant animals; this mimicked the process seen in pregnancy.[1] Four years later, Hisaw and colleagues isolated a crude extract of the hormone responsible for the observations and termed it relaxin.[2] Since then, extensive research on relaxin and related hormones has demonstrated that it is a 6-kDa peptide with high structural similarity to insulin, thereby classifying it as a member of the insulin superfamily.[3] Like insulin, relaxin is synthesized as a 32-kDa preprohormone that consists of an N-terminal signal peptide, a B-chain, C-chain, and a COOH terminal A-chain.[4,5] Posttranslational modifications remove both the signal peptide and the C-chain via proteolytic digestion to produce a mature hormone. Two disulfide bonds covalently join the chains with an intradisulfide link in the A-chain and are crucial to the structure of the active heterodimer.[6,7] A highly conserved region in the B-chain, amino acids 13 to 20, is required for successful binding of the relaxin ligand to its receptor (Arg-X-X-X-Arg-X-X-Ile/Val).[8]

In humans, there are 3 relaxin peptides—relaxin-1, relaxin-2, and relaxin-3 (RLN1, RLN2, and RLN3). In all other species, there are 2 forms—relaxin-2 and relaxin-3. Relaxin-3 is the ancestral relaxin gene, and gene duplication events led to the evolution of the other variants.[9] In mammals, RLN3 is primarily expressed in the brain, with hypothesized functions in stress and appetite regulation.[10-12] In contrast, RLN1 is believed to have evolved more recently from a gene duplication event,[13,14] with RLN1 being primate specific.[15] Relaxin-1 is

[1] School of Biosciences, University of Melbourne, Melbourne, Victoria, Australia
[2] Department of Obstetrics and Gynaecology, Gynaecology Research Centre, The University of Melbourne and Royal Women's Hospital, Melbourne, Victoria, Australia

Corresponding Author:
Jane E. Girling, Department of Obstetrics and Gynaecology, University of Melbourne, Royal Women's Hospital, Cnr Grattan St and Flemington Rd, Parkville, Victoria 3052, Australia.
Email: jgirling@unimelb.edu.au

expressed in the human ovary, placenta, and decidua, although its function remains unknown.[16] This review will focus on the major circulating form of relaxin in humans, RLN2 (referred to as relaxin throughout this review).[17] Relaxin binds to and activates relaxin/insulin like family peptide receptor 1 (RXFP1) (formerly LGR7), the relaxin family peptide receptor. This is a leucine-rich G protein-coupled receptor (GPCR) containing an N-terminal low-density lipoprotein type A module that is responsible for ligand-activated cyclic adenosine monophosphate (cAMP) signaling.[18,19] Although relaxin also binds to a second structurally similar GPCR, RXFP2 (formerly LGR8) in vitro, there is no evidence that relaxin activates RXFP2 in vivo.[18] RXFP3 and RXFP4 are GPCRs that differ both structurally and functionally from RXFP1 and RXFP2[20] because they have relatively short N-terminal extracellular domains and bind with high affinity to RLN3.[21,22] Readers are referred to extensive reviews by Bathgate and colleagues, which discuss relaxin and related peptides in greater detail.[20,23]

In mammals, both relaxin and its receptor RXFP1 are expressed in a variety of tissues including, but not limited to, the uterus, ovary, mammary gland, placenta, and testis.[23] Although the main source of circulating relaxin during pregnancy is the corpus luteum, circulating levels of relaxin are divergent between species both in concentration and timing; there is also species diversity in the timing of placental relaxin production. In rats and pigs, relaxin reaches peak concentration in the last half of pregnancy, with a prepartum surge in both species.[24,25] In contrast, in pregnant women, circulating relaxin peaks toward the end of the first trimester and then remains consistent at intermediate levels for the remainder of gestation.[26-28] These variations reflect variable roles for relaxin among different species, with essential functions prepartum and during parturition in rodents and pigs that are not directly replicated in the autocrine/paracrine processes regulating human birth. It should also be noted that women have significantly lower levels of circulating relaxin (1-5 ng/mL)[29] than most other species (eg, 50-150 ng/mL in rats and pigs).[24,25] Relaxin also likely contributes to uterine remodeling in preparation for implantation, as well as the systemic vascular changes required for a healthy pregnancy in both animals and humans (Figure 1). Further to the roles of relaxin in pregnancy, there is also evidence of additional roles for relaxin and its receptor in the uterus during the human menstrual cycle. Our understanding of these roles is limited by the functional differences in the activity and actions of relaxin and its receptor RXFP1 among different species. Further, there are relatively few studies that have specially focused on relaxin in the uterus during nonconceptive menstrual cycles in human or other menstruating primates.[30-32]

To date, there are no obvious clinical conditions associated with a relaxin deficiency in pregnant women.[33,34] However, women with reduced circulating relaxin in early pregnancy have a history of recurrent miscarriage[35] and have an increased risk of developing preeclampsia.[36,37] In addition, few studies have considered the potential contribution of relaxin to gynecological disorders. This review first summarizes the current understanding of relaxin's actions during the menstrual cycle and in early pregnancy and then considers the known and potential contributions of relaxin to gynecological disorders and pregnancy-related disorders such as early pregnancy loss, recurrent miscarriage, and preeclampsia. Our discussions are largely restricted to the uterus and uterine-based pathologies.

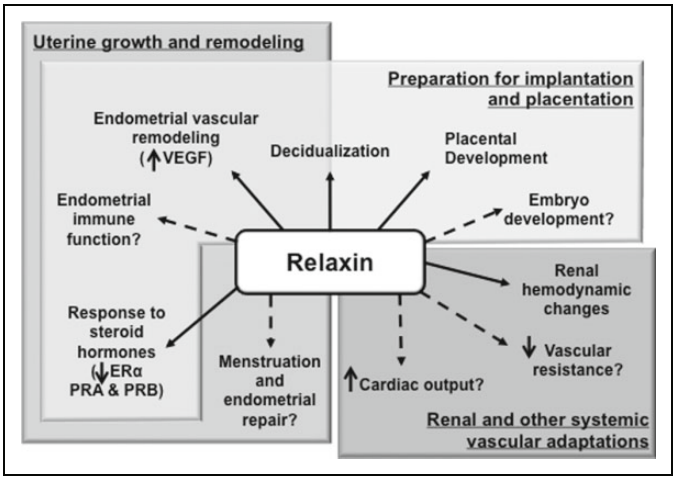

Figure 1. Diagram illustrating relaxin's hypothesized roles during the menstrual cycle and in early pregnancy. ERα indicates estrogen receptor α; ERβ, estrogen receptor β; VEGF, vascular endothelial growth factor.

Expression of Relaxin and Relaxin Receptors (RXFP1) During the Menstrual Cycle

Ovarian and Circulating Relaxin

Granulosa-derived luteal cells of the corpus luteum are the main source of ovarian relaxin and the major source of circulating relaxin in women.[38] Relaxin is also produced by the theca interna cells of follicles,[39] which are likely to be the primary contributors of the relaxin detected in follicular fluid.[40] RXFP1 messenger RNA (mRNA) has been detected in the corpus luteum of humans and monkeys[41] and specifically on granulosa and cumulus cells of pig antral follicles[42] and potentially in human granulosa cells of primordial, primary, and secondary follicles.[43] A variety of studies have suggested that circulating relaxin reaches its peak in the latter half of the luteal phase of the menstrual cycle with levels either low or nondetectable in other cycle stages.[29,44-47] In some studies, circulating relaxin was not detectable in all women.[45,47] More recently, serum relaxin was detected in all samples collected from women examined during the follicular, ovulatory, and luteal phases of the menstrual cycle, with no significant variation among menstrual phases.[48] These differences between studies are often attributed to the variation in sensitivity and possibly specificity of different assays, highlighting the need for appropriate validation of antibodies. Many early relaxin studies used the well-characterized R6 antibody that specifically recognizes the highly conserved receptor binding domain on the relaxin B-chain.[49] As the B-chain contains a number of identical amino acid residues common to other relaxin molecules, it is possible

that this antibody may cross-react with other relaxin-like peptides such as RLN3.[50] Despite the variations between studies, it can be concluded that relaxin is secreted during the menstrual cycle and is likely to peak during the luteal phase.

Relaxin concentrations increase 6 to 7 days after ovulation in human conceptive and nonconceptive cycles, but the highest relaxin levels are measured in the first trimester of pregnancy.[29] As detailed above, other species such as rats and pigs have the highest circulating levels of relaxin at the end of pregnancy.[24,25] Therefore, circulating relaxin may play a more critical role in early pregnancy adaptations in humans, with the interspecies differences in the timing of peak relaxin levels providing possible clues as to the hormone's functional roles. Considering the potential impact of relaxin on uterine function, variation in patterns of circulating relaxin and its interaction with the receptor RXFP1 may warrant further consideration, especially in the context of the various gynecological and pregnancy-related disorders discussed below.

Uterus

In addition to the ovary, relaxin is produced locally by uterine tissues. Relaxin mRNA is expressed in the endometrium, although it is generally low compared with early pregnancy, with little change during the menstrual cycle.[51,52] Cultured human endometrial stromal and epithelial cells also express relaxin mRNA and secrete relaxin protein into culture media.[53] Relaxin protein is detected in endometrial samples during all menstrual cycle stages, predominantly in luminal and glandular epithelial cells. By the late secretory stage, decidualized stromal cells also produce relaxin.[54] As relaxin is locally produced by a variety of intrauterine cells and tissues, it is likely that relaxin functions as a paracrine hormone to help prepare the nonpregnant uterus for pregnancy. Future studies need to differentiate between the effects of circulating endocrine relaxin versus local paracrine relaxin activity as a means to fully understand relaxin activity in uterine tissues and to provide insight as to why women with low to no circulating relaxin can still undergo successful pregnancies.

The human uterus is a known target for relaxin as both the relaxin receptor (RXFP1) gene and protein are expressed in uterine tissues during the menstrual cycle. *Rxfp1* mRNA is detected in both proliferative and secretory phase endometrial samples.[52,55-57] Although some studies report increased expression during the secretory phase relative to the proliferative phase,[51,52,55] others suggest constitutive expression throughout the menstrual cycle,[56,57] with considerable variation in expression among different patients.[57] In agreement with studies examining tissue expression, there was large variation in *Rxfp1* mRNA expression among isolated cell cultures derived from different patients.[57] *Rxfp1* was detected in cultured stromal fibroblasts, epithelial cells, and large granular lymphocytes isolated from endometrial tissues.[56,58] Untreated glandular cells had significantly higher *Rxfp1* expression than untreated stromal cells.[59] *Rxfp1* mRNA was also detected in human myometrial samples.[56] Primary human myometrial smooth muscle cells from nonpregnant women express *Rxfp1*, and relaxin stimulates cAMP production in these cells.[58,60] It should be noted, however, that there are multiple splice variants of *Rxfp1*[18]; most studies do not clarify which splice variant(s) they have amplified. Nor do they confirm whether the gene products observed represent functional *Rxfp1* isoforms. This means that different studies are potentially amplifying and quantifying "incorrect" *Rxfp1* mRNA. This in turn could be contributing to the variation recorded for *Rxfp1* mRNA in uterine tissues.

In the human uterus, there is similar variation in studies that report RXFP1 protein localization and binding of RXFP1, with no consensus on the predominant region expressing relaxin receptors. The common finding is that immunoreactive RXFP1 and relaxin-binding sites are localized in both the stromal and epithelial compartments of the human uterus. The majority of studies indicate that the density of RXFP1 protein is highest in the luminal and glandular epithelium of the endometrial functional layer throughout the menstrual cycle.[57,58] Glands in the endometrial basal layer were largely RXFP1 negative.[56] Similarly, the study by Krusche et al also noted that although the majority of stromal cells were RXFP1 negative, some individual scattered stromal cells stained intensely for RXFP1 in both menstrual and early proliferative stage samples; the specific type of cell with the intense staining was not determined.[56] In the marmoset monkey, Einspanier and colleagues reported that there was no binding of a biotinylated relaxin in the endometrium during the luteal phase, whereas relaxin binding was primarily concentrated to the superficial epithelium of the implantation area during the implantation period of a conceptus cycle.[61] There was also relaxin binding in superficial stromal tissue and glands. In addition to immunohistochemistry for RXFP1, relaxin binding was examined using quantitative autoradiography. A significant increase in relaxin binding was observed in the secretory phase of the menstrual cycle relative to the proliferative phase with binding largely in the endometrial glandular and luminal epithelium; binding was not observed in the myometrium.[51,62] Using a biotinylated relaxin, receptor staining was identified in the endometrial stroma during the proliferative phase, with increased intensity postovulation in the secretory phase.[63] Weak relaxin binding was also observed in the epithelial cells of endometrial glands. In the nongravid uterus, intensity was reduced. Staining in the myometrium staining was weak and did not vary throughout the cycle. In the early pregnant uterus, strong staining was observed in the decidual cells and in the area of maternal and fetal contact. The variation in RXFP1 localization could simply be due to differences in technique. However, as these studies have been performed in human and nonhuman primates, species variation is likely a contributing factor.

It is difficult to reconcile the variation in uterine localization of RXFP1 reported in various studies. The differences between studies may be explained by the variety of techniques used and issues associated with specificity of antibodies, biotinylated

molecules, and radiolabeled ligands, although all studies cited include appropriate negative controls. Although technical differences in the methods chosen for analysis will undoubtedly contribute to some of the variation, the multiple cell types within the endometrium and myometrium and the continual changes in the relative proportion of these cell types and their phenotype during the menstrual cycle must also be considered. Further, although most studies have examined samples collected during the proliferative and secretory phases of the cycle, fewer have analyzed relaxin and *Rxfp1*/RXFP1 in the menstrual phase samples. Considering the contribution of relaxin to various key processes associated with tissue and vascular remodeling, fibrosis, and hemodynamics, this is a significant knowledge gap. Regardless of the reported differences in RXFP1 localization, these studies are consistent with a role for endometrial relaxin in the secretory phase of the menstrual cycle and in the preparation for a successful pregnancy.

Decidua and Placental Tissues

Although the corpus luteum of the ovary is the primary source of serum relaxin during human pregnancy, relaxin is also expressed by the chorion, placental trophoblasts, and the basal plate of the placenta obtained from term elective cesarean and normal spontaneous vaginal delivery.[64] Relaxin was successfully purified from the human placenta and lengthened the pubic symphysis in mice confirming the bioactivity of the hormone.[65,66] Relaxin receptors are also localized in the placenta providing evidence that there are autocrine/paracrine relaxin-RXFP1 interactions within this tissue.

Functions of Relaxin During the Menstrual Cycle and in Preparation for Pregnancy

Relaxin is a pleiotropic hormone with functions in a broad range of processes including, but not limited to, angiogenesis (with interactions with key angiogenic factor vascular endothelial growth factor [VEGF]),[67] extracellular matrix remodeling (via matrix metalloproteinases [MMPs]),[68] bone remodeling, muscle regeneration,[69] vasodilation,[70] and maintaining cardiovascular function,[71] while capable of acting as an antifibrotic[72] and a cardiovascular protective agent.[73] The pleiotropic nature of relaxin and its actions on the uterus are illustrated by studies conducted using rhesus macaques (*Macaca mulatta*) and marmoset monkeys. As a model of early pregnancy, Goldsmith et al used ovariectomized, hormone-treated animals to examine the effects of relaxin on endometrial tissues.[30,31] Relaxin treatment increased uterine weights relative to controls but had no effect on endometrial thickness. In contrast, increased endometrial thickness was observed in relaxin-treated early pregnant marmoset monkeys,[61] along with an increased number of arterioles and total lymphocytes (neutrophils, uterine natural killer cells, macrophages). Relaxin treatment also increased endothelial cell proliferation in arterioles and capillaries in the endometrium of rhesus macaques mimicking the uterine remodeling associated with early pregnancy.[32] Relaxin treatment significantly inhibited endometrial estrogen receptor (ER) α but not ERβ protein levels and significantly inhibited progesterone receptor (PR) A and PRB levels.[30] Although relaxin is usually known as a collagenolytic and antifibrotic hormone,[74] the work of Goldsmith et al demonstrated that relaxin is a negative regulator of MMP expression in the uterus under this hormone regime. Relaxin treatment caused a decrease in proMMP and an increase in the MMP inhibitor TIMP-1 (TIMP metallopeptidase inhibitor 1); the specific consequences of these changes are not known. Most of the studies outlined above were designed to mimic early pregnancy; however, research designed to mimic the effects of relaxin in the time frame consistent with the menstrual cycle and with subsequent hormone withdrawal and menstruation has not been conducted. Such work would be of particular value considering the heavy or irregular menstrual bleeding described by some women being treated with relaxin during a clinical trial investigating the potential therapeutic use of relaxin for the treatment of scleroderma.[75,76]

Most studies investigating the mechanisms of relaxin action in uterine tissues have used nonprimate animal models (eg rat, pig) to demonstrate effects on growth, decidualization, and endometrial vascular remodeling.[28,34,77] These effects are mediated via direct or indirect effects on ERs, cAMP production (relaxin binds to RXFP1 to increase intracellular cAMP via G_s-mediated adenylyl cyclase, which inhibits phosphodiesterases that act to breakdown cAMP), various growth factors including VEGF, and extracellular matrix components (particularly MMPs); indirect effects are also likely due to relaxin's effects on uterine artery blood flow. Studies using human or nonhuman primate cells or tissues are consistent with relaxin having similar mechanisms of action in human uterine tissues (see above reviews for summary). Predictably, the response of cells to relaxin or other hormones in vitro is dependent on cell type, menstrual cycle stage, and/or hormone exposure.[59] For instance, the progestin medroxyprogesterone acetate (MPA) significantly increased RXFP1 mRNA expression in endometrial stromal cells, whereas relaxin treatment alone had no significant effect on RXFP1 mRNA expression.[59] In contrast, in endometrial epithelial cells, both MPA and relaxin increased RXFP1 mRNA expression. If glandular epithelial cells from the proliferative stage were cultured in vitro, relaxin treatment significantly reduced VEGF production; in contrast, VEGF production increased in epithelial cells derived from the secretory phase samples.[53] There was also a significant increase in VEGF expression in response to relaxin treatment in secretory phase–derived stromal cell cultures.[53]

It is apparent that relaxin contributes to the complex interactions regulating endometrial function during the menstrual cycle as the uterus prepares for pregnancy; however, the potential contribution of uterine relaxin to the process of menstruation and endometrial repair has not been considered. The relative contribution of ovarian versus locally produced relaxin to uterine function has not yet been determined. In addition, although it is likely that relaxin derived from the circulation or

the endometrial stroma has a role in vascular remodeling and decidualization, the role of epithelial-derived relaxin is less clear. To our knowledge, the possibility that relaxin is secreted into the uterine lumen has not been investigated nor the possibility that relaxin has a role in endometrial immune function. Further, studies have not yet addressed the interesting proposal by Anand-Ivell et al[78] that relaxin in semen may directly impact on the endometrium to immune desensitize the uterus prior to fertilization. Relaxin is secreted into the seminal fluid from the prostate gland and can influence sperm motility and the acrosome reaction.[79] Moreover, there has not been any research addressing the possibility that endometrial relaxin secreted by the luminal epithelium may influence sperm function.

In addition to its roles in adult females, relaxin has also been hypothesized to influence postnatal development of the female reproductive tract. Based on a fascinating set of studies in pigs, it was hypothesized that lactocrine signaling by relaxin present in colostrum and milk may influence development of the neonatal uterus, which is RXFP1 positive.[80,81] Relaxin is also present in the breast, colostrum, and milk of humans[82-84]; whether lactocrine signaling by relaxin has effects on the human uterus is unknown. While not directly related to the menstrual cycle, these studies further illustrate the pleiotropic nature of relaxin and the need for more careful studies of its role in uterine development and function.

Implantation

During the secretory phase of the menstrual cycle and in early pregnancy, uterine adaptations including angiogenesis, vascular maturation, and the initiation of decidualization support the development of a highly receptive uterus for implantation.[85] Both relaxin and its receptor RXFP1 are highly expressed in the uterus (RNA extracted from whole uteri) during the periimplantation period in marmoset monkeys, with increased expression of RXFP1 around the site of attachment.[61] It is unknown whether a similar pattern occurs in humans. In addition, relaxin significantly advanced the development of in vitro fertilization (IVF) embryos to blastocysts by at least 12 hours.[86] This provides evidence that relaxin plays a role in preimplantation embryo development in primates.

Inhibiting myometrial contractions in early pregnancy is thought to support implantation. However, evidence to date suggests that relaxin is not involved in inhibiting myometrial contractions in human pregnancy, but rather, this is a function of relaxin in rodents and pigs.[87,88] In the rat and pig, exogenous porcine relaxin induces quiescence of the myometrium by acting on smooth muscle cells to block both spontaneous and oxytocin-induced contractions.[88,89] Porcine relaxin did not inhibit human myometrial activity. These studies highlight the potentially diverse nature of the relaxin peptide in preparation for implantation in mammals.

Angiogenesis is the formation of new blood vessels from preexisting vasculature. It is fundamental to the menstrual cycle and early pregnancy and is stimulated by the exposure of endothelial cells to proangiogenic agents such as VEGF.[90] Relaxin is a known stimulant of VEGF in human endometrial stromal and glandular epithelial cells in a dose-dependent manner in vitro.[33,53,76] Relaxin also acts on connective tissue to modify expression of MMPs and their inhibitors (TIMPs). Human dermal fibroblasts treated with relaxin secreted increased levels of MMPs, while decreasing expression of TIMPs.[91] In the presence of collagen overexpression induced by cytokines, relaxin treatment still decreased collagen expression, demonstrating that relaxin can regulate connective tissue in dermal fibroblasts. Relaxin's ability to increase expression of VEGF and modulate MMP expression while inhibiting TIMPs suggests a mechanism by which relaxin can support endometrial vascularization in preparation for implantation. However, no studies have demonstrated a direct stimulatory effect of relaxin in the vascularization of the endometrium in women.

Relaxin is thought to be a key factor responsible for initiating decidualization in preparation for implantation. The hormone is produced by human decidual cells[92] and can induce decidualization of primary cultures of endometrial stromal cells.[93] As mentioned previously, relaxin induces production of cAMP, which is necessary for decidualization.[93-95] This leads to endometrial thickening and production of VEGF,[96] which are important steps in preparation for implantation. In virgin female mice, relaxin stimulated differentiation of endometrial stromal cells to cells resembling decidualized cells and the induction of laminin production, which is required for trophoblast adherence.[97] Infusion of human relaxin into nonpregnant rhesus monkeys induced an increase in the serum decidual cell marker proteins insulin-like growth factor binding protein-1 (IGFBP1) and prolactin.[98] Relaxin treatment of human decidual cells stimulated production of IGFBP1 and prolactin.[99] Rather than stimulating stromal cell growth, relaxin increases prolactin production.[100] Relaxin also increased mRNA expression and secretion of interleukin 11 via the cAMP/protein kinase A pathways,[101] which are known to be essential for decidualization in mice and are expressed by human endometrium during the secretory phase.[102]

Systemic Vascular Adaptations in Pregnancy

The maternal cardiovascular system undergoes dramatic changes throughout a healthy pregnancy. In the first trimester, the systemic and renal vascular resistance decreases in the maternal circulation, assisting cardiac output to increase by approximately 40% to accommodate the increased blood volume.[103-105] In addition, global arterial compliance increases in parallel with cardiac output to preserve end-diastolic blood pressure.[106] These adaptations increase blood flow (>10 fold) to the fetoplacental unit to maintain a healthy oxygen and nutrient supply to the fetus for successful growth and development.[107] Interestingly, the maternal cardiovascular changes that occur in pregnancy are also observed in the luteal phase of the menstrual cycle, although to a lesser degree[108]; this

supports the hypothesis that factors secreted by the corpus luteum may have an important role in early systemic changes of pregnancy.

Infertile women who conceive through egg donation, IVF, or fresh embryo transfer (with no measurable circulating relaxin) fail to undergo the normal renal adaptations to pregnancy compared to women with normal ovarian function by week 7 of gestation.[109] This information is consistent with a role for relaxin in maternal cardiovascular adaptations to pregnancy, as demonstrated in animal studies by Conrad and Davison.[104] Briefly, the treatment of nonpregnant rats with relaxin decreased systemic vascular resistance, increased global arterial compliance and cardiac output,[110] and increased glomerular filtration rate and effective renal plasma flow by 20% to 40%,[111] mimicking the changes seen in human pregnancy.

Less is known about relaxin's role in the adaptation of other vascular beds in early pregnancy, although a number of vascular phenotypes have been demonstrated in pregnant relaxin-deficient rodents. Uterine arteries of pregnant rats treated with a relaxin neutralizing antibody (MCA1) to remove circulating relaxin had stiffer vessel walls.[112] MCA1 treatment also increased renal and other systemic vascular resistance to levels similar in nonpregnant rats.[113] However, this model is limited as these rats can still produce relaxin locally in reproductive tissues. Studies in relaxin knockout mice have overcome this problem, demonstrating stiffer uterine arteries in aged relaxin deficient mice relative to their wild-type counterparts due to impaired uterine artery remodeling.[114] RXFP1 is expressed in rat abdominal aorta, uterine, mesenteric, femoral, and small renal arteries, with protein localized to both the smooth muscle and endothelial cells.[112,115-117] A high expression of this receptor has been observed during early pregnancy compared to late pregnancy in the uterine artery of rats.[112] Although arterial localization of RXFP1 implies a role of relaxin in these vascular beds, future studies are required to establish if relaxin, via RXFP1, is contributing to the regulation of the systemic vascular adaptations of early pregnancy.

Parturition

Historically, relaxin is known for its role in relaxation of the pubic symphysis during the parturition process in rodents. Evidence is less convincing in humans[118] and will not be discussed further here. Readers are referred to reviews,[28,77] including a Cochrane Review on the use of relaxin for preventing preterm birth.[119] Although there is no evidence that circulating relaxin facilitates delivery in humans, relaxin produced by the placenta may influence fetal membrane rupture, contributing to the induction of labor.[120,121] Relaxin is hypothesized to act locally via autocrine/paracrine signaling to increase the expression of MMPs in fetal membranes; this suggests that relaxin could be contributing to the degradation of extracellular matrix components in human pregnancy.[120] Increased expression of *Rxfp1*/RXFP1 was demonstrated in fetal membranes collected preterm from women undergoing cesarean section for medical reasons (approximately 34-36 weeks) in comparison to term tissue collected from women undergoing cesarean and natural birth (approximately 38-39 weeks).[122] It is also interesting to note that altered serum relaxin levels have been associated with spontaneous preterm birth.[123,124] More recent studies suggest that single-nucleotide polymorphisms in the promoter region of relaxin are linked with preterm birth and premature rupture of fetal membranes.[125,126] Together, these studies suggest a role for relaxin produced by fetal membranes in the preparation for parturition.

Relaxin and Gynecological Disorders

As a pleiotropic peptide hormone with key roles in reproductive function in women, it is important that we consider the potential contribution of relaxin to various gynecological disorders. Understanding the roles of relaxin and its receptor may inform diagnosis and prognosis; relaxin may even be useful as a therapeutic target. Unfortunately, very few studies have specifically addressed the role of relaxin in common uterine disorders; the information that is available is discussed below.

Abnormal Uterine Bleeding

Abnormal uterine bleeding is a term used to describe uterine bleeding patterns of abnormal frequency, regularity, duration of flow, or volume in nongravid women; there are also various clinical conditions known to cause abnormal bleeding symptoms, for example, ovulatory disorders, uterine fibroids (leiomyoma), malignancy, and coagulation disorders.[127] To our knowledge, no studies have specifically investigated the potential role of relaxin in abnormal uterine bleeding, although there have been limited studies examining the role of relaxin in some clinical conditions linked with menstrual bleeding problems (eg, uterine fibroids, see below). The possibility that relaxin could contribute to abnormal uterine bleeding is illustrated by the heavier or irregular menstrual bleeding reported by some women being treated with relaxin during a clinical trial investigating the potential therapeutic use of relaxin for the treatment of scleroderma.[75,76] Unscheduled uterine bleeding or spotting can also occur in women using various types of hormonal contraceptive therapy.[127] Whether relaxin has a role in causing this breakthrough bleeding has not been specially considered. However, various studies (discussed above) have demonstrated effects of exogenous steroid hormones on uterine tissues or cells, such as MPA causing an increase in RXFP1 in endometrial stromal cells.[59] These studies demonstrate the potential for hormonal contraceptive therapies to have a direct effect on relaxin activity in the uterus. Future studies that aim to determine the specific role of relaxin, either circulating or of uterine origin, in normal menstruation and abnormal uterine bleeding would contribute to our understanding of a significant gynecological problem.

Uterine Fibroids

Uterine fibroids (leiomyoma) are common, benign tumors of the uterine muscle wall (myometrium) that affect up to 80% of women by age 50.[128] Although many fibroids are asymptomatic, others cause menstrual bleeding abnormalities, such as heavy menstrual bleeding and/or pressure/pain symptoms; fibroids are also associated with various obstetric complications including miscarriage or preterm labor. These tumors are monoclonal in origin, enriched in extracellular matrix, and their growth is estrogen and progesterone dependent (see Bulun[129] and references therein). Various growth factors and cytokines have also been linked with their pathophysiology; transforming growth factor-β has received particular attention.[129]

Early studies of nonpregnant uterus by MacLennan et al demonstrated relaxin production by uterine fibroids but not myometrium.[130] The authors suggested that these benign tumors may be sites of relaxin storage or receptor activity and hypothesized a role for relaxin in the development of uterine fibroids. Other studies have considered relaxin receptor expression in fibroids. In contrast to the studies on relaxin production, RXFP1 mRNA and protein expression were downregulated in fibroid relative to adjacent myometrium (cycle stage not specified).[131] Consistent with a role of relaxin in fibroid activity, it has been reported that relaxin treatment of ELT-3 cells (derived from Eker rat leiomyoma cells), suppressed phosphorylation of SMAD2 by transforming growth factor β.[131] However, in vitro studies by Suzuki et al[132] are not consistent with the expression patterns reported by Li et al. Suzuki et al suggest a differential response of cultured human leiomyoma cells relative to normal myometrial cells with RXFP1 protein expression increased in leiomyoma cells relative to myometrial cells.[132] In addition, treatment with recombinant human relaxin increased the number of viable cultured cells, increased the number of proliferating cell nuclear antigen (PCNA)-positive cells, and decreased the TUNEL-positive cells in leiomyoma cells relative to myometrial cells.[132]

The impact of relaxin activity on uterine fibroids requires further research. Comprehensive studies examining the expression of relaxin and RXFP1 through the menstrual cycle in myometrium relative to uterine fibroids have not yet been conducted. If individual tumors are a local source of relaxin production, the potential impact of this peptide hormone on the surrounding vasculature would be of particular interest. Further, the potential interaction of relaxin and fibroids in early pregnancy would benefit from additional investigation.

Endometriosis

Endometriosis is a common debilitating gynecological disorder affecting 6% to 10% of reproductive-age women; key symptoms include pain (eg, menstrual period pain, chronic pelvic pain) and infertility.[133,134] The disorder is defined by the presence of estrogen-dependent ectopic lesions containing endometrial glands and stroma outside the uterus. Although several hypotheses have been suggested to explain the presence of these ectopic lesions, the etiology of the endometriosis remains unknown. No studies have yet addressed the possibility that relaxin and related peptides may contribute to the etiology of endometriosis. To our knowledge, the only research considering relaxin in endometriosis reports significantly lower expression of relaxin and RXFP1 in ectopic endometriotic lesions relative to matched utopic endometrium and endometrium from women without the disorder.[52,135] The impact of relaxin production by lesions on adjacent tissues has not been investigated. Endometriosis is a complex multifactorial disease; our understanding of its pathophysiology would benefit from knowing the potential contribution of relaxin and its receptors.

Gynecological Cancers

Relaxin has demonstrated roles in cancer progression and metastasis (cancer cell proliferation, cell invasion, angiogenesis).[136,137] Although there has been a particular focus on the interaction of relaxin with cancers of the prostate and breast, there is little research attention directed toward gynecological cancers, despite the known and hypothesized roles for this peptide in ovarian and reproductive tract function in nonpregnant cycles and during pregnancy.

It is likely that the role of relaxin in tumor progression will be cancer specific, and for this reason, studies examining specific gynecological cancers are warranted. This is highlighted by studies demonstrating differential effects of relaxin on growth of cells derived from human adenocarcinoma of the cervix relative to a human breast adenocarcinoma cell line (MCF-7).[138] Early studies demonstrated relaxin immunoreactivity in syncytiotrophoblast of normal placenta, as well as in hydatidiform mole, invasive mole, and choriocarcinoma; this study did not observe relaxin immunoreactivity in nontrophoblastic cancer (including secretory-type adenocarcinoma).[139] Serum relaxin is also detectable by radioimmunoassay in women with normal and molar pregnancy, invasive mole, choriocarcinoma, and persistent trophoblastic disease; the authors hypothesized that relaxin secretion is dependent on human chorionic gonadotropin from the trophoblastic tissues effecting production by the corpus luteum.[140,141] In studies using semiquantification of relaxin immunoreactivity in invasive endometrial carcinoma, strong relaxin immunoreactivity was associated with high-grade disease, increased depth of myometrial invasion, and reduced survival.[142] In 2 endometrial carcinoma cell lines, relaxin treatment increased migration and invasion but not proliferation; these effects were mediated by MMPs.[142]

It is apparent that the specific role of relaxin in various gynecological cancers deserves additional attention, particularly the source and impact of relaxin on disease etiology. The various ovarian cancers have not been examined, and as yet, endometrial cancer has received little research focus despite the association of relaxin with poor clinical outcome.[142]

Relaxin and Pregnancy-Related Disorders

Several studies have now postulated a link between relaxin and pregnancy-related disorders. Below is a summary of current information about relaxin and the common pregnancy disorders, miscarriage, recurrent pregnancy loss, and preeclampsia.

Recurrent Miscarriage and Early Pregnancy Loss

Women can undergo a successful pregnancy without circulating relaxin, but there are clinical implications if serum relaxin levels are lower than normal. In a recent study, women who had a miscarriage had the lowest circulating relaxin levels in a study of approximately 500 women.[143] Interestingly, among these women, those with the higher circulating relaxin levels had a longer duration of gestation. However, earlier studies that have correlated relaxin levels and miscarriage are conflicting. One study demonstrated an association between lower relaxin levels and miscarriage in women,[144] whereas another reported no such correlation.[145] It is important to note that these earlier studies used different radioimmunoassays to measure levels of relaxin, which may contribute to the varying results.

It has also been suggested that women with reduced circulating relaxin in early pregnancy have a higher prevalence of recurrent miscarriage.[35,146] Recurrent miscarriage may be influenced by the expression of VEGF and its receptors.[147] As relaxin is hypothesized to upregulate VEGF expression to support angiogenesis, reduced serum relaxin may result in lower bioavailability of VEGF. Infiltration of the placental bed and spiral arterioles by trophoblasts is an essential part of early pregnancy. Reducing vascular resistance within these spiral arterioles ensures adequate perfusion of the placenta. Disruptions to these essential processes can contribute to miscarriage and early pregnancy loss. However, evidence from human miscarriages suggested that trophoblast infiltration is not directly responsible for the increase in uterine blood flow as had previously been thought.[148] Instead, a reduction in uterine artery resistance actually begins in the luteal phase of the menstrual cycle before implantation, during a time when relaxin and other hormones are produced.[149,150] Jauniaux et al demonstrated that relaxin, in combination with estrogen and progesterone, may play a role in the development of the uteroplacental circulation to support early pregnancy development.[151] Failure of the endometrium to properly vascularize can also result in miscarriage.[152,153] Therefore, we postulate that relaxin in early pregnancy provides support to uterine vascular remodeling, which, when absent, contributes to pregnancy complications.

Preeclampsia

As mentioned previously, the maternal cardiovascular system undergoes dramatic changes to support a healthy pregnancy. Failure of maternal vasculature to adapt to early pregnancy can lead to complications such as preeclampsia (see recent review[154]), which is one of the leading causes of maternal and fetal morbidity and mortality with long-term health implications for the mother.[155]

Abnormal maternal vascular adaptations combined with reduced placental perfusion in gestation lead to the release of the antiangiogenic factors soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) from the hypoxic placenta.[156,157] These factors enter the maternal circulation and contribute to the widespread endothelial dysfunction and increased superoxide production observed in women with preeclampsia.[156] This leads to enhanced sensitivity to vasoconstrictors (eg, angiotensin II), with renal dysfunction and hypertension, and in severe cases of multiple organ failure. It is suggested that a widespread deficiency in the bioavailability of VEGF may be involved in the progression of this disease.[158] Soluble fms-like tyrosine kinase-1 inactivates circulating VEGF by competitively binding to the protein, preventing it from binding to the membrane-bound VEGF receptors. It is unclear if relaxin has any role in placental production of sFlt-1 and sEng. However, as mentioned previously, relaxin is known to enhance VEGF production[33,53,76] and has been demonstrated to attenuate vascular responses to angiotensin II in animal models.[159,160] In addition, women with ovarian failure have abnormal renal vascular adaptations to pregnancy compared to women with normal ovarian function by week 7 of gestation.[109,161]

Circulating relaxin levels are not significantly different between women with preeclampsia relative to those with normal singleton pregnancies.[162,163] However, there are 2 separate abstracts reporting that low levels of relaxin in the circulation are associated with an elevated risk of developing preeclampsia.[36,37] Both indicate that serum relaxin concentrations were not significantly different between preeclamptic women and controls but that women with circulating relaxin levels in the lowest quartile were more likely to develop preeclampsia.

It is important to note that circulating relaxin levels measured in serum only represent relaxin produced by the ovaries. Circulating levels do not account for relaxin produced locally by the decidua, placenta, and other maternal tissues. Therefore, any deficit in uterine and placental relaxin production is not necessarily reflected in the circulation. It is unclear if a deficiency of locally produced relaxin underlies issues with early vascular adaptations to pregnancy or low bioavailability of VEGF; these areas have not yet been investigated. Regardless, the pharmacological effects of relaxin on the vasculature, blood pressure, and renal function (summarized in Conrad and Davison[104]) hint at the exciting possibility that relaxin could be used therapeutically in the treatment of preeclampsia.

Summary and Conclusion

In this review, we have highlighted the pleiotropic activity of the peptide hormone relaxin in women's health and reproduction. Various studies have suggested a role for relaxin in both the menstrual cycle and pregnancy. However, significant gaps in our knowledge illustrate the need for further investigation to show that insufficient or abnormal relaxin activity contributes

to an increased risk for adverse outcomes in women. A full understanding of relaxin and its roles in reproductive health is a challenging issue made more difficult because of species differences and the limitations of animal models. Relaxin studies in nonhuman primates have suggested the relaxin may be supportive for both the establishment and maintenance of a successful pregnancy. Further investigations need to address whether and how relaxin may contribute to various gynecological disorders. These studies are of particular importance as it is possible that relaxin may be a useful therapy in reproductive health, especially in early pregnancy and particularly in those with low or absent circulating levels. In brief, we believe that relaxin may play crucial supportive roles in women, facilitating and contributing to uterine health and the complex development of pregnancy.

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