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Biological Roles of Uterine Glands in Pregnancy

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Abstract

All mammalian uteri contain glands in the endometrium that synthesize or transport and secrete substances essential for survival and development of the conceptus (embryo/fetus and associated extraembryonic membranes). This review summarizes information related to the biological roles of uterine glands and their secretions in blastocyst/conceptus survival and implantation, uterine receptivity, and stromal cell decidualization in humans and animal models. The infertility and recurrent pregnancy loss observed in the ovine uterine gland knockout (UGKO) model unequivocally supports a primary role for uterine glands and, by inference, their secretions present in uterine luminal fluid in survival and development of the conceptus. Further, studies with mutant and progesterone-induced UGKO mice found that uterine glands and their secretions are required for establishment of uterine receptivity and blastocyst implantation as well as stromal cell decidualization. Similarly in humans, uterine glands and their secretory products are likely critical regulators of blastocyst implantation, uterine receptivity, and conceptus growth and development during the first trimester. Circumstantial evidence suggests that deficient glandular activity may be a causative factor in pregnancy failure and complications in humans. Thus, an increased understanding of uterine gland biology is important for diagnosis, prevention and treatment of fertility and pregnancy problems in mammals.

Keywords

uterus; glands; blastocyst; implantation; decidualization; pregnancy

Introduction

Histotrophic nutrition can be defined as the provision of nutrients through secretions from the oviductal and uterine glands, and it represents the initial form of nutrition for the conceptus (embryo/fetus and associated extraembryonic membranes) of all species, prior to the establishment of the placenta.¹ Development and differentiation of most female reproductive tract organs are complete at birth; however, the uterus is not fully developed or differentiated at birth and lacks or has rudimentary glands.^{2,3} The glandular epithelium (GE) initially develops as invaginations of luminal epithelium (LE) that eventually form a network of coiled tubules that extend to the myometrium.⁴⁻⁷ The infertility and recurrent

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pregnancy loss observed in uterine gland knockout (UGKO) sheep and mice clearly demonstrate the necessity of uterine glands and, by inference, their secretions for early pregnancy success.⁸⁻¹⁰ The purpose here is to provide an overview of how uterine glands have established or potential biological roles in survival, development and implantation of the blastocyst/conceptus, uterine receptivity for implantation, and stromal cell decidualization in animal models and humans. Important, inadequate development and function of uterine glands may be a causative factor in pregnancy failure and complications in humans.

Uterine glands and early pregnancy in sheep

Ruminants (cattle, goats, and sheep) have a bicornuate uterus with a small common corpus.⁵ The endometrium of adult ruminants has a large number of aglandular caruncular areas, which are dense stromal areas covered by a simple LE, and intercaruncular areas that contain many hundreds of glands per cross-section of the uterine wall. In synepitheliochorial placentation found in ruminants, interdigitation of placental cotyledons with endometrial caruncles forms placentomes, which serve a primary role in fetal-maternal gas exchange and derivation of nutrients by the placenta for hemotrophic nutrition of the fetus. Of note, uterine glands undergo hyperplasia and continue to function throughout gestation in domestic animals for histotrophic nutrition of the fetus via specialized areas of the placenta termed areolae.

Establishment of pregnancy in domestic ruminants (i.e., sheep, cattle, goats) begins at the conceptus stage and includes pregnancy recognition signaling, implantation, and placentation.^{11,12} The morula-stage embryo enters the uterus on days 4 to 6 post-mating and then forms a blastocyst that contains an inner cell mass and a blastocoele or central cavity surrounded by a monolayer of trophoctoderm. After hatching from the zona pellucida (days 8 to 10), the hatched blastocyst slowly grows into a tubular or ovoid form and is then termed a conceptus (embryo/fetus and associated extraembryonic membranes).^{13,14} The ovoid conceptus of about 0.5 to 1 cm on day 11 begins to elongate on day 12 and forms a filamentous conceptus of 10 to 15 cm or more in length that occupies the entire length of the uterine horn ipsilateral to the corpus luteum. After day 16, the elongated conceptus begins the process of implantation and placentation that involves firm attachment and adhesion to the LE. Conceptus elongation involves exponential increases in length and weight of the trophoctoderm and onset of extraembryonic membrane differentiation, including gastrulation of the embryo and formation of the yolk sac and allantois that are vital for embryonic survival and formation of a functional placenta.

Uterine gland knockout (UGKO) sheep model

Uterine gland differentiation and development (or adenogenesis) in sheep is a uniquely postnatal event.^{2,3,5} The onset of neonatal uterine adenogenesis in sheep was found to be independent of the ovary but could be inhibited by exposure to progestins.^{15,16} This original observation by Frank “Skip” Bartol served as the foundation for the idea that prolonged exposure of neonatal ewes to a progestin would permanently inhibit uterine gland differentiation, thereby producing a UGKO phenotype in adults.³ Subsequently, exposure of

neonatal ewes to norgestomet for the first 8 weeks of life was found to permanently ablate the postnatal differentiation of uterine glands, resulting in a UGKO phenotype in the adult.¹⁷⁻¹⁹ Importantly, exposure of neonatal ewes to norgestomet did not affect development or function of brain, hypothalamic-pituitary-ovarian axis, ovary, or other Müllerian duct derivatives, including oviduct, cervix and vagina.^{18,20} Of note, the uteri of adult UGKO ewes were devoid of glands and lacked intercaruncular endometrial areas characteristic of normal ewes with reduced amount of LE.¹⁸ Adult UGKO sheep were completely infertile and exhibited recurrent pregnancy loss, thereby revealing an essential role for uterine glands and their secretions in survival and development of the conceptus.^{10,18,19} Morphologically normal blastocysts were present in the uterus of UGKO sheep after mating on day 9, but conceptuses were absent or severely growth-retarded when assessed on day 14 post-mating. The lack of conceptus elongation in UGKO ewes was hypothesized to result from the absence of specific secretions normally produced by the uterine glands.^{2,19} The UGKO sheep model is particularly useful for the study of early pregnancy, since the majority of natural pregnancy loss occurs during the second to third weeks of pregnancy in ruminants.

Uterine gland histotroph and pregnancy in sheep

Histotroph in the uterine luminal fluid (ULF) of mammals is a complex and rather undefined mixture of ions, amino acids, carbohydrates, proteins, lipids, and other substances that are that are selectively transported into the uterine lumen by the uterine epithelia from serum transudate as well as specific secretory products encoded by genes expressed in the LE and GE.^{2,12,21} Available evidence supports the idea that ovarian progesterone induces expression of a number of genes, specifically in the endometrial LE and/or GE, that are then further stimulated by factors from the conceptus (interferon tau or IFNT, prostaglandins, cortisol, and chorionic somatomammotropin hormone 1 (placental lactogen or CSH1) as well as the endometrium itself (prostaglandins, cortisol).^{22,23} The genes and functions regulated by these hormones and factors are hypothesized to trigger specific changes in the intrauterine histotrophic milieu that are necessary for conceptus elongation based on the phenotype of the UGKO sheep. Although ruminant blastocysts can be entirely derived *in vitro*, their quality is lower than *in vivo* and must be transferred into the uterus in order to elongate and form a filamentous conceptus.²⁴

Our knowledge of the GE transcriptome and ULF histotroph remains very incomplete in sheep as well as other ruminants. A mass spectrometry experiment found that a large number of proteins are present in the ULF of cyclic and pregnant day 16 sheep.²⁵ Candidate gene studies found that the uterine glands of early pregnant sheep express genes that encode for secreted factors (CTGF, GRP, WNT11), amino acid transporters (SLC1A1, SLC1A4, SLC1A5, SLC7A1, SLC7A2, SLC7A5, SLC7A8, SLC43A2), glucose transporters (SLC2A1, SLC2A5, SLC2A12, SLC5A1, SLC5A11), secreted migration and attachment factors (LGALS15, SPP1), a regulator of calcium/phosphate homeostasis (stanniocalcin one or STC1), secreted peptidases (CTSH, CTSL, CTSS, CTSZ), secreted protease inhibitors (CST3, CST6), and an immunomodulatory factor (SERPINA14; also known as uterine milk protein or uterine serpin). Many of those GE-expressed genes are also expressed in the LE, induced by ovarian progesterone, and are hypothesized to alter the ULF histotroph by

increasing select amino acids, glucose, cytokines and growth factors whose biological functions would support blastocyst survival and growth into an ovoid conceptus and elongation in sheep and cattle.^{12,22,23,26}

As noted previously, the uterine glands of sheep undergo a program of hyperplasia followed by hypertrophy during the first two months of pregnancy that seems dependent on temporal and spatial actions of hormones from the ovary (progesterone) and placenta (CSH1 and growth hormone or GH).^{2,23} Uterine gland morphogenesis during pregnancy allows for an increased output of secretory proteins, which are transported to the fetus by specialized areas of the placenta termed areolae, to provide histotrophic nutrition to the fetus throughout pregnancy. Little is known about gene expression in and secretory products of the uterine glands during pregnancy in sheep or other domestic animals.^{12,22,26}

Uterine glands and pregnancy in mice

Laboratory rodents (mouse and rat) have a long duplex uterus.⁵ The endometrium of the adult rodent uterus consists of a simple columnar LE surrounded by stromal cells containing slightly coiled glands lined by simple cuboidal GE cells. The endometrium typically contains only 10 to 20 glands in a cross-section of the uterine wall, and they are predominantly found in the antimesometrial area of the uterus and not tightly coiled as found in the uterus of domestic animals and humans.

Blastocyst implantation involves trophectoderm apposition, attachment, adhesion to the LE followed by penetration and growth of the trophectoderm into the decidualizing stroma.^{7,27-30} This complex process requires dialogue between an implantation-competent blastocyst and a receptive uterus. In mice, the endometrium becomes receptive to blastocyst implantation on day 3.5 of pregnancy or pseudopregnancy (day 0.5 = morning of a post-coital vaginal plug observation), but it is non-receptive by the afternoon of day 4.5. The implantation process is initiated by blastocyst attachment to the receptive LE on day 3.5. Recent evidence suggests that there are two separate uterine signals regulating blastocyst activation for implantation, one that primes the trophectoderm for attachment to the LE and another that initiates its motility, but the nature of those signals are not well defined.³¹ By day 5.5, the LE cells lining the implantation chamber and near the attached blastocyst undergo apoptosis allowing the motile trophectoderm to come into contact with stromal cells that are differentiating into decidual cells. Decidualization in the mouse uterus requires proliferation and differentiation of stromal cells into decidual cells and is required for successful pregnancy and formation of a functional placenta.^{29,32}

Mouse gene knockouts affecting uterine gland development and function

Secretory products of uterine glands have been known to be essential for blastocyst implantation in rodents for the past 20 years. Colin Stewart discovered that leukemia inhibitory factor (LIF) is expressed specifically by the uterine glands in response to the nidatory surge of estrogen from the ovary in mice.³³ Subsequently, LIF was found to be essential for blastocyst implantation in mice, as *Lif* null mice exhibited infertility due to a failure of blastocyst implantation.³⁴ Recently, a number of knockout (*lymphoid enhancer factor 1* or *Lef1*) and conditional knockout mice [catenin (cadherin associated protein), beta

1 or *Ctnnb1*; *forkhead box A2* or *Foxa2*; leucine-rich repeat containing G-protein-coupled receptor 4 or *Lgr4*; wingless-related MMTV integration site 4 or *Wnt4*; and *Wnt7a*] were found to lack uterine glands in the adult.³⁵⁻⁴¹ Many of those mice exhibit defects in blastocyst implantation and stromal cell decidualization and thus fertility, which can be attributed to the absence of LIF and other uterine gland-derived factors.

Progesterone-induced UGKO mouse model

In mice, uterine epithelial proliferation is important for gland development and growth into the stroma and almost completely blocked by progesterone.⁴² Paul Cooke found that progesterone treatment inhibited uterine adenogenesis in neonatal mice, resulting in an UGKO phenotype in the adult.⁹ Adult progesterone-induced UGKO (PUGKO) mice had normal female reproductive tract organs, with the exception of the uterus, and cycled normally but were infertile.^{9,43} The PUGKO mice were used to investigate the biological role of uterine glands in blastocyst implantation and stromal cell decidualization.⁸ Histological assessment of PUGKO uteri on day 5.5 and 8.5 post-mating found a hatched blastocyst apposed to an intact LE without evidence of implantation or stromal cell decidualization. Expression of several implantation-related factors, including *Lif*, were absent in the PUGKO uterus, whereas steroid hormone receptors and their regulated genes were not different. The defect in blastocyst implantation in PUGKO mice was attributed to inadequate uterine receptivity and/or blastocyst activation (trophectoderm attachment and motility) resulting from the absence of specific secretory products of the uterine glands.

Uterine gland secretions and histotroph in mice

A number of candidate gene profiling experiments and several microarray studies support the idea that the LE and GE have a distinct molecular signature and act differentially and synergistically to establish uterine receptivity, govern trophoctoderm attachment and motility for blastocyst implantation, and influence stromal cell decidualization.^{7,27,28,30,44-47} However, very little is known about the nature of the ULF or uterine gland secretions in mice.^{31,48}

Carbohydrates and 19 amino acids were measured in ULF of mice at estrus⁴⁸, but no similar data is available for those nutrients or other components of histotroph in the peri-implantation mouse uterus. In fact, LIF has not been documented in the ULF of mice. A number of transporters for amino acids and glucose are differentially expressed in the uterine LE and GE of the peri-implantation mouse uterus.^{46,47} Of note, amino acids (AA) play an important role in blastocyst activation for implantation.⁴⁹ Mouse embryos cultivated *in vitro* require a specific and defined culture medium containing AA. This embryonic requirement for AA is not simply nutritive, as AA exposure induces trophoctoderm motility through activation of mammalian target of rapamycin (mTOR)-dependent signal transduction cascades only after the embryo has reached the early blastocyst stage.⁴⁹ A recent study found that leucine and arginine, and in particular, uptake of leucine through the SLC6A14 amino acid transporter, are each required individually and together are sufficient to induce mouse blastocyst activation.³¹ Of note, human embryos can take up the amino acid leucine, and leucine is the only amino acid significantly depleted in culture medium by

human embryos (days 2 to 3 after insemination) that subsequently developed to the blastocyst stage.⁵⁰

Involvement of uterine glands in blastocyst implantation and stromal cell decidualization

Results with the PUGKO mouse model, along with those from conditional *Foxa2* and *Lgr4* mutant mice that have much reduced uterine glands^{35,41} and *Lif* null mice³⁴, support the hypothesis that uterine glands and, by inference, their secretions have important biological roles governing blastocyst implantation and decidualization.^{51,52} Many factors and pathways regulating implantation and decidualization have been identified in mice.^{7,27-30} Although LIF has a well-established role to establish receptivity of the LE to implantation⁵³, the role of many other genes expressed in the uterine glands has not been established (Table 1). Recent transcriptional profiling experiments indicate that GE of day 2.5 and 3.5 mouse uteri express many genes that encode for enzymes, transporters or secreted proteins that could regulate blastocyst implantation by modifying the ULF histotroph.^{46,47}

The concept that uterine glands secreted paracrine-acting factors into the stroma to promote decidualization is a relatively new concept based on mouse models that lack uterine glands. As expected due to a defect in blastocyst implantation, no evidence of stromal cell decidualization was found in the PUGKO mouse uterus on day 5.5 post-mating.⁸ Thus, an artificial model of decidualization was used that involves ovariectomy, hormone replacement and intrauterine administration of oil as a deciduogenic stimulus.⁸ Using that artificial model, the PUGKO mice exhibited a distinct lack of uterine stromal cell decidualization. Interestingly, LIF can substitute for nidatory ovarian estrogen at inducing both implantation and decidualization in hormonally prepared, ovariectomized bred mice that have glands in their uterus.⁵³ However, intrauterine LIF failed to rescue decidualization in ovariectomized PUGKO mice that lack uterine glands.⁸ Similarly, mice with a conditional ablation of *Foxa2*, *Lgr4* or *Wnt4* have a uterus with much reduced or absent glands and also exhibit defects in blastocyst implantation and stromal cell decidualization.^{35,36,41} The identities of uterine gland-derived factors that regulate stromal cell decidualization are not known, but one candidate is SPINK3 (serine peptidase inhibitor, Kazal type 3). Although *Spink3* mRNA is present only in the GE of the mouse uterus, SPINK3 protein was detected in the LE and decidual cells as well as glands of the pregnant mouse uterus.⁵⁴ That finding suggests that SPINK3 is secreted in both an apical and basal manner, as found for many other proteins secreted by polarized epithelia. Further, the LE and presumably factors it secretes upon LIF stimulation also has a role in stromal cell decidualization.⁵⁵ Thus, paracrine crosstalk between the GE and stroma, LE and stroma as well as exocrine crosstalk between the GE and LE and perhaps GE and trophectoderm may be necessary for successful stromal cell decidualization (Figure 1).

Uterine glands and pregnancy in humans

Humans have a simplex uterus with a single corpus.⁵ The endometrium is lined by a simple LE and contains many coiled tubular glands that radiate through endometrial stroma toward the myometrium. Adult human and primate endometria are divided into two functional

layers, the upper stratum functionalis containing large numbers of glands surrounded by loose stroma, and the lower stratum basalis consisting of bodies of glands and dense stroma. The endometrial functionalis is lost during menses. The basalis is organizationally and functionally dynamic but structurally stable and is not eroded during menstruation or following gestation. This tissue is the germinal compartment of the endometrium in women, providing stem cells from which the functionalis regenerates after each cycle or following gestation. Although initiated fetally, human uterine gland development is only completed postnatally, similar to domestic animals, and involves differentiation of GE from LE followed by radial development of coiled tubular glands through the stroma to the myometrium. This pattern of endometrial development is distinct from gland genesis in uteri of adult women and primates, where glands develop adluminally from the basalis during the proliferative phase after menses. The glands have a high density within the human uterus, and there are approximately 15 openings per square millimeter of uterine lumen surface in the non-pregnant state. Although secretions of uterine glands have been hypothesized for many decades to play an essential role in sustaining the conceptus prior to implantation^{56,57}, their potential role in post-implantation conceptus growth and development in humans has been largely ignored, until recently.^{51,52}

During the past decade, our understanding of development of the early human placenta has undergone a radical transformation by Graham Burton and his colleagues (Figure 2).^{58,59} The human conceptus undergoes interstitial implantation. Nidation of the blastocyst begins about day 7 and is complete by days 10 to 12 post-conception.^{1,51,60} Initial attachment of the blastocyst to the uterine LE takes place between the uterine gland openings.⁶¹ As the trophoblast of the blastocyst begins to attach and adhere to the epithelium, it begins to differentiate and form a syncytiotrophoblast that then penetrates between the LE cells into the stroma. The implanted conceptus becomes encapsulated within the superficial endometrium via a combination of invasion and proliferation of the surrounding decidualizing stromal cells.^{51,52} By the time implantation is complete, the chorionic sac is surrounded by a mantle of syncytiotrophoblast in which spaces representing the forerunners of the intervillous space (lacunae) are already present. As the syncytiotrophoblast enlarges, it erodes into the necks of the glands; consequently, connections between the lumens of glands and the developing intervillous spaces of the placenta can be observed as early as day 17 post-fertilization⁶² and persist throughout the first trimester.⁵⁹ The importance of uterine glands and histotrophic nutrition during the first trimester of pregnancy was only realized once it was recognized and documented that the maternal arterial circulation to the placenta is not fully established until 10-12 weeks of pregnancy (8-10 weeks post-fertilization).⁶³⁻⁶⁵ Indeed, the spiral arteries of the human uterus do not penetrate into the superficial third of the functionalis endometrium and are present in the mid-zone of the functionalis endometrium. Thus, the conceptus has to enlarge significantly before it can make contact with the tips of the spiral arteries and break into and plug them using endovascular trophoblast cells. Thus, the end of the first trimester represents a change from histotrophic to hemotrophic nutrition when the plugs of endovascular trophoblast dissipate and allow for directed blood flow into the intervillous spaces of the placenta.^{58,59} Prior to that time, the intervillous space is filled with secretions derived primarily from the uterine glands. Consequently, the relationship between decidual uterine glands and conceptus is equivalent

in humans and domestic animals, except that uterine gland secretions are intraplacental rather than intraluminal.⁵¹

Gland secretions in the uterine luminal fluid of humans

Uterine histotroph and gland secretions include amino acids, ions, carbohydrates (glucose), lipids, proteins (cytokines, enzymes, hormones, growth factors, proteases and their inhibitors, transporters, etc.) and likely other substances in the human.^{56,57,66-70} They are presumed to be important mediators of uterine receptivity, blastocyst implantation (trophoblast attachment, growth, and invasion), stromal cell decidualization, and conceptus growth.^{27,51,52,69} Deficient glandular activity, usually described as a “secretory phase defect”, is hypothesized to be a significantly underlying cause of early pregnancy failure in humans.^{52,71} Recent proteomic studies found that proteins in the ULF of fertile women change during the menstrual cycle and are altered in ULF of fertile as compared to infertile women.^{68,72,73} Aspiration or flushing and collection of ULF offers a minimally invasive means of studying uterine secretions and function in women and can be conducted before embryo transfer in IVF cycle without negatively affecting implantation rates.^{73,74} Indeed, this approach was recently used to determine that interleukin 1 beta (IL1B) and tumor necrosis factor (TNF) were key predictive markers of implantation in women undergoing IVF.⁶⁸ However, it is likely that a complex set of biomarkers is expected to provide the most sensitive and specific means for assessing uterine receptivity for conceptus implantation.^{56,57}

The development of the endometrium that occurs during the secretory phase of the cycle appears to be maintained into early pregnancy. The endometrium beneath the conceptus is 5-6 mm thick at 6 weeks gestational age (4 weeks post-ovulation), but is reduced to 1-2 mm by the end of the first trimester.⁶⁶ Histologically, the GE cells present in and near the decidua, termed decidual uterine glands, appear highly active during early pregnancy and resemble those of the early secretory phase of the cycle.^{59,66,75} They have a tall columnar phenotype, with accumulations of glycogen in the apical cytoplasm and lipid droplets towards their base. By the end of the first trimester, the GE cells are more cuboidal in shape and appear morphologically to be more quiescent. The changes in GE morphology correlate with concentrations of progesterone-associated endometrial protein (PAEP; also known as glycodeilin A), one of the principal components of the uterine histotroph, which peaks in the maternal serum and amniotic fluid of the conceptus towards the end of the first trimester.^{76,77} Thus, activity of the decidual uterine glands may decrease in concert with the onset of the maternal intraplacental circulation that occurs at the end of the first trimester.⁷⁸ Importantly, available evidence supports the hypothesis that uterine glands continue to function during the first trimester, deliver their contents into the intervillous space where they may play important biological roles in placental development, and are regulated by the ovary, trophoblast, and decidua (Figures 2 and 3).

Uterine gland secretions and conceptus nutrition during the first trimester

The importance of ULF or histotroph as a key provider of nutrition to the peri-implantation embryo has long been recognized in other mammalian species, such as domestic animals in

which the embryo undergoes a prolonged period of development before superficial implantation and placentation.^{2,21} Available evidence supports the idea that uterine glands also represent an important source of nutrients for the human conceptus during the first trimester.^{51,52,66} Indeed, adequate nutrition of the blastocyst/conceptus has long-term implications for growth of the fetus and health of the adult.^{79,80} Although amino acids and sugars are substantially important for early embryogenesis and conceptus growth in other species⁸¹, few studies have examined their transport systems in human endometrium and, particularly, the uterine glands.

Evidence that the glands may act as a source of nutrition for the human embryo is provided by the observation that the syncytiotrophoblast covering the surfaces of villi facing the endometrium contain accumulations of glycogen.⁵⁹ These accumulations are greatest close to the materno-fetal interface, suggesting a concentration dependent uptake by the trophoblast. The first trimester syncytiotrophoblast also phagocytizes maternal glycoproteins such as PAEP⁵⁹, which is expressed in the uterine glands but not placental tissues.⁸² Of note, little information is available on the genes expressed by and secretions produced by the uterine glands, particularly during the first trimester.

Uterine glands and placental development

Besides providing a source of nutrients, the uterine glands may play a more active role in regulating placental development of the human conceptus through the production of growth factors. A variety of growth factors have been identified within the GE, including epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and leukaemia inhibitory factor (LIF), and receptors for these factors are present on the placenta during the first trimester.⁶⁶ Growth factors, such as LIF and FGF, are key for maintenance of trophoblast stem cells.⁸³ Further, EGF treatment of villi from early gestation stimulated cytotrophoblast cell proliferation and enhanced secretion of chorionic gonadotropin (CG) and CSH1.⁸⁴ Intense proliferation of cytotrophoblast cells is observed during early pregnancy after implantation. The proliferation of cytotrophoblast cells is important, because it is essential for: (1) establishment of villous trees and maintenance of syncytiotrophoblast layer that performs nutrient exchange and secretes endocrine factors such as CG and CSH1; (2) generating a complete cytotrophoblastic shell that protects the conceptus during early pregnancy; and (3) providing a sufficient supply of extravillous trophoblast (EVT) cells that are pivotal in remodeling the spiral arteries. Thus, uterine gland secretion of growth factors into the intervillous spaces of the placenta may create a microenvironment that stimulates trophoblast cell proliferation and differentiation necessary for growth and development of the conceptus.

Hormones and factors regulating uterine glands during pregnancy

In the normal non-pregnant menstrual cycle, glycogen accumulations within the uterine glands begin to disperse around days 23-24, suggesting a decline in secretory activity.⁸⁵ Given that glycogen accumulations persist through at least 6 weeks of pregnancy, uterine gland secretory activity must be maintained during early pregnancy. Indeed, the glands present in and near the decidua are substantially larger than those in other areas of the

pregnant uterus. The idea that factors from the ovary and conceptus can influence uterine gland development and functions stems from work in domestic animals.⁸⁶ In sheep, the uterine glands undergo considerable hyperplasia between days 15 and 50 of gestation followed by hypertrophy to increase their surface area.² Indeed, sequential exposure of the uterus to estrogen, progesterone, interferon tau (IFNT), CSH1 and GH stimulated the expression of genes in the uterine glands that encode secreted proteins such as serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 14 (SERPINA14 also known as uterine milk proteins).⁸⁷ Interestingly, CSH1 from the binucleate trophoblast cells acts via prolactin receptors (PRLR) on the uterine glands, but the actions of CSH1 require ovarian progesterone and trophoblast-derived IFNT.^{87,88} Similarly, PRL also stimulates progesterone-induced uteroglobin expression in the glands of the rabbit uterus.⁸⁹

As originally proposed by Burton and colleagues^{51,52}, an equivalent mechanism may be present in the human uterus (Figure 3). Initially, progesterone from the ovary down-regulates expression of the progesterone receptor (PGR) that results in substantial changes and induction of gene expression in the uterine glands referred to as secretory transformation⁹⁰; the PGR continues to be expressed by the decidual cells, as progesterone effects are unequivocally required for stromal cell decidualization and function. Next, CG from the syncytiotrophoblast acts on the uterine glands as they contain abundant CG receptors (LHCGR).⁹¹ In essence, CG likely plays an equivalent role to IFNT in the sheep during early pregnancy. First, it is the pregnancy recognition signal produced by the conceptus that acts via LHCGR in the corpus luteum to maintain its function and thus progesterone secretion. Second, CG acts on the GE cells to increase their secretory function. For instance, CG can stimulate the expression and secretion of PAEP by the glands in the baboon uterus.^{92,93} Moreover, treatment of isolated GE cells with highly purified CG resulted in a time- and dose-dependent increase in levels of prostaglandin-endoperoxide synthase 2 (PTGS2 or cyclooxygenase-2) expression and PGE2 secretion⁹¹, which is important for stromal cell decidualization. In addition to CG, lactogenic hormones also may act on the uterine glands. One of the major progesterone-regulated secretory products of decidual cells is PRL, and the PRLR is abundant in the uterine glands during the secretory phase and early pregnancy.⁹⁴ Thus, two lactogenic hormones (PRL from the decidua and CSH1 from the syncytiotrophoblast) likely stimulate the uterine gland secretory functions in concert with CG. In turn, the uterine gland secretions regulate development and/or function of the placenta and perhaps decidua. In this way, the conceptus has a direct impact on the supply of nutrients and other factors it receives from the mother.^{51,52}

Uterine glands and stromal cell decidualization

Decidualization is a process of morphological and biochemical differentiation in which the endometrial stromal cells become rounded, polyploid and secrete proteins including PRL and insulin-like growth factor binding protein one (IGFBP1).²⁹ In women, decidualization is initiated during the mid-late secretory phase by ovarian progesterone and is independent of the presence of an implanting blastocyst; however, the decidua of pregnancy is only formed following implantation. During pregnancy, decidualization extends to the basalis endometrium and is a critical regulator of trophoblast invasion and placenta formation.⁹⁵

Recent evidence suggests that paracrine crosstalk between the GE and stroma may be needed for optimal stromal cell decidualization in humans.⁹⁶

Peptide hormones and prostanoids implicated in promoting decidualization of stromal cells in the human include CG, corticotropin releasing hormone (CRH), relaxin (RLX), and PGE2 that signal via cAMP, which is a critical signaling molecule important for stromal cell decidualization.⁹⁷ A number of studies have found that locally and temporally produced products of the endometrium, such as cytokines and growth factors [interleukin-11 (IL11), RLX, PGE2, activin A, CRH, and LIF], progress or enhance *in vitro* progesterone-induced decidualization of endometrial stromal cells from humans and/or mice.^{98,99} In women, the LE and GE maximally express LIF during the mid-secretory phase of the menstrual cycle.¹⁰⁰ Further, treatment of human endometrial GE cells with CG increased expression of PTGS2 and PGE2 secretion.⁹¹ Determination of factors produced by uterine glands and their potential actions on stromal cells and decidual cells is an important area for future studies in the human.

Conclusions and Future Directions

Available evidence strongly supports the hypothesis that uterine glands and their secretions in histotroph have important biological roles in pregnancy. Some of the cellular events and molecular pathways involved in uterine gland development and function have been identified through animal models. In humans, however, a significant knowledge gap remains regarding how the uterine glands and intraplacental histotroph support early pregnancy as well as the impacts of deficient glandular function on pregnancy success and complications.

It is increasingly clear that histotroph from the uterine glands is important for blastocyst survival, growth and implantation in humans^{56,57} and may also be involved in pregnancy loss and complications such as miscarriage, preeclampsia and fetal growth retardation.^{52,58} Defective development of uterine glands during the cycle as well as deficient secretory activity, usually described as a luteal phase defect, has been speculated to be a cause of early pregnancy failure in humans; however, there is little but circumstantial evidence to support that hypothesis. Miscarriage and preeclampsia may result from deficient glandular activity causing an incomplete cytotrophoblastic shell, inadequate trophoblast proliferation, or abnormal decidual function. In summary, a more comprehensive understanding of the biological roles of uterine glands in in blastocyst/conceptus growth and implantation, uterine receptivity, and uterine decidualization is needed to generate new knowledge essential for understanding implantation failure and pregnancy loss and to improve pregnancy success in humans and domestic animals.

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Figure 1.

Hypothesis on the biological roles of uterine glands in blastocyst activation, uterine receptivity to blastocyst implantation, and stromal cell decidualization during pregnancy in mice. See text for detailed description of hypotheses and supporting data.

Figure 2.

Schematic illustrating the relationship of the conceptus to the uterine glands during the first trimester of pregnancy in humans. A) The blastocyst attaches to the luminal epithelium and then implants into the superficial functionalis endometrium between the openings of the uterine glands. B) The implanted conceptus begins to grow and the syncytiotrophoblast invades into the superficial capillaries as well as the uterine glands, releasing the contents of both into the lacunae that will form the intervillous spaces. C) As the conceptus enlarges, the syncytiotrophoblast begins to reach the tips of the spiral arteries of the endometrium. It is essential that the cytotrophoblastic shell is fully developed by this stage in order that sufficient endovascular extravillous trophoblast cells (EVT) are available to plug the spiral arteries near the conceptus. Complete plugging of the arteries in the periphery of the normal placenta is necessary to allow the EVT to remodel them prior to the onset of maternal circulation at the end of the first trimester. Drawn by Carolyn Allen and adapted from Burton and coworkers.⁵²

Figure 3.

Hypothesis on the interrelationships of the ovarian corpus luteum, conceptus trophoblast, uterine glands, and decidual cells during early pregnancy in humans. See text for detailed description of hypotheses and supporting data. Adapted from Burton and coworkers.⁵²

Table 1

Genes expressed in the glandular epithelia of the mouse uterus and effects of mutation on pregnancy outcomes

Symbol	Name	Expression ¹	Null/Conditional Phenotype	Reference
<i>Cdh1</i>	E-cadherin	LE and GE (GD1-4), stroma (GD5-8)	Embryonic lethal, Implantation defect (conditional)	101,102
<i>Clca3</i>	chloride channel calcium activated 3	LE and GE (peaks on GD1)	Viable and fertile	103,104
<i>Cxcl15</i>	chemokine (C-X-C motif) ligand 15	GE, LE	Viable and fertile	105,106
<i>Fnbp1</i>	formin binding protein 1	GE (GD4-5)	N/A	44
<i>Foxa2</i>	forkhead box A2	GE (neonatal and adult)	Embryonic lethal, Implantation defect (conditional)	35,107
<i>Gulo</i>	gulonolactone (L-) oxidase	GE>>LE (GD4-5)	Viable and fertile	44,108
<i>Ihh</i>	Indian Hedgehog	LE and GE (peaks on GD3-4)	Embryonic lethal, Implantation defect (conditional)	109
<i>Il6st</i>	interleukin 6 signal transducer	GE (GD3-5) Decidua (GD7)	Viable and fertile	110,111
<i>Klf5</i>	Kruppel-like factor 5	LE and GE (GD1-5) Decidua (GD5-8)	Embryonic lethal, Implantation defect (conditional)	112
<i>Lgr4</i>	leucine-rich repeat-containing G protein-coupled receptor 4	GE, LE	Implantation defect	41
<i>Lif</i>	leukemia inhibitory factor	GE>>Stroma (GD4)	Implantation defect	34
<i>Ltf</i>	lactotransferrin	LE and GE (GD1-2)	Viable and fertile	113,114
<i>Lyz2</i>	lysozyme 2	Stroma, GE (GD3-5)	Viable and fertile (knock-in)	44,115
<i>Msx1</i>	homeobox, msh-like 1	LE and GE (peaks on GD4 and declines)	Embryonic lethal, Subfertile (conditional)	116,117
<i>Msx2</i>	homeobox, msh-like 2	LE and GE (peaks on GD4)	Viable and fertile, Infertile (double conditional)	116,117
<i>Prss28</i>	protease, serine, 28	GE (GD5-8)	N/A	118
<i>Prss29</i>	protease, serine, 29	GE (GD5-9)	N/A	119
<i>Ptgs1</i>	Prostaglandin-endoperoxide synthase 1	LE and GE (peaks on GD4)	Viable, delayed parturition	120,121
<i>Sh3tc2</i>	SH3 domain and tetratricopeptide repeats 2	GE (GD4-5)	Viable and fertile	44,122
<i>Slc23a2</i>	solute carrier family 23 (nucleobase transporters), member 2	GE	Postnatal lethal	44,123
<i>Spink3</i>	serine peptidase inhibitor, Kazal type 3	GE (onset GD4)	Postnatal lethal	54,124
<i>Sult1d1</i>	sulfotransferase family 1D, member 1	GE>>LE (GD3-4)	N/A	44
<i>Tro</i>	Trophinin	LE and GE (peaks between GD4 and 6)	Viable and fertile	125
<i>Ttr</i>	transthyretin	GE only (peaks on GD4)	Viable and fertile	126,127

¹ GD, gestational day; GE, glandular epithelium; LE, luminal epithelium